A NEURONAL SWITCH FOR APPROACH/AVOIDANCE IN COST-BENEFIT DECISION

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DISSERTATION
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ABSTRACT

Animals make decisions by integrating their internal state, external sensory stimuli, and previous experiences. The neuronal bases of these decisions are accessible in the predatory sea-slug *Pleurobranchaea*, which has a very simple nervous system. Orienting and avoidance responses are dependent on the appetitive state, such that very hungry animals may attack noxious stimuli and satiated animals will avoid appetitive stimuli. A multifunctional turn network is active in response to the unilateral application of the stimulus, promoting the appropriate turn response.

The goal of my study was to answer the following questions:

1) How does the nervous system encode the appetitive state of animal?

2) How does the animal’s appetitive state affect the turn network to drive approach or avoidance?

3) How does the turn network compute the orientation of the turn?

We hypothesize that corollary outputs from the feeding network modulate the configuration of the premotor network that mediates turns, toggling the turn choice from avoidance to orienting. Consequently, electrophysiological recordings from the isolated central nervous system of animals demonstrated the conservation of donor appetitive state. As animal’s appetitive state (readiness to feed) was conserved in the fictive turning decisions of the isolated nervous system, and the decision to orient or avoid was a function of spontaneous rhythmic activity in the feeding motor network. In addition, manipulating the feeding network activity modified the fictive turn motor output in response to the unilateral sensory stimulus. Activation and suppression of the feeding network by driving feeding command neurons or stimulation of
feeding nerve promoted approach and avoidance, respectively. Thus these results confirmed that the excitation state of the homeostatic feeding network manifested the animal’s readiness to feed and controlled expression of approach vs. avoidance turn response to the stimulus. A simple possible model suggested that corollary outputs from the feeding to the turn network changed the configuration of the premotor turn network and thereby switching the turn output. Electrophysiological observations were found to fit to this model.

Bilaterally paired serotonergic neurons of the turn CPG network critical to turning output were asymmetrically active during turns appropriate to turn direction. These neurons, necessary for avoidance, were inhibited during fictive feeding, consistent with behavioral observations in intact animals. Bath addition of serotonin, an endogenous regulator of arousal and appetitive state, converted animal’s choice of turn response to the stimulus from avoidance to orienting. Serotonin was also found to excite the feeding network. Thus serotonin as a neuromodulator encodes the animal’s hunger state, and toggles animal’s turn decision by changing its internal state.

These findings demonstrate the neuronal mechanisms of appetitive state dependence of behavioral choice processes. Results outline the framework for a simple neuronal circuit model for the cost benefit decision in approach/avoidance behavior, which could be applicable to the modeling of decision-making processes in more complex systems.
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CHAPTER 1

OVERVIEW

1.1 Neuroethology and decision making

Animals make moment-to-moment decisions to respond to the changing environment. The neuronal mechanisms of animal behaviors have been studied with the use of various recording methods. Each individual animal species may exhibit distinct behavioral patterns, but they are based on the same ethological principles. Their decisions aim at minimizing risk (e.g. predator), and maximizing benefit (e.g. nutrition). They must localize stimuli and express appropriate behavioral output. For instance, a strong noxious stimulus may evoke escape behavior. Animals localize the noxious stimuli or predator and may respond to it by fleeing in another direction. Escape circuit mechanisms are well identified in many animals such as cockroach (Camhi, 1988), crayfish (Krasne, 1969), lamprey (McClellan and Grillner, 1983), and sea-slug (Willows, 1969; Jing and Gillette, 1995).

In moment-to-moment decision making, animals also choose a specific behavior over others by integrating internal state, external cues, and memory. Interacting neuronal networks allow animals to choose the appropriate behavior over others in response to the stimulus. When the animal is exhibiting escape behavior, for example, other behavioral networks, such as feeding or egg laying networks, are shut off because of the inhibitory signal from the escape network. Thus, behaviors are hierarchically organized. Escape behaviors are dominant behaviors that override others by suppressing them. Similar phenomena occur when the animal is presented with food. Feeding inhibits withdrawal behaviors (Kovac and Davis, 1977). Switch like
mechanisms in the nervous system allow animals to choose appropriate behaviors (Kovac and Davis, 1977). Recently, presynaptic inhibitory switch mechanisms were identified in the leech, *Hirudo medicinalis*. The animal’s local bending behavior was presynaptically inhibited at the sensory neuronal level (Gaudry, 2009; Kemenes, 2009). However, decision making that specifically involves the approach/avoidance turn choice has not yet been researched in detail.

In the predatory sea-slug *Pleurobranchaea californica* (Figure 1.1), animals may orient to or avoid a chemotactile stimulus dependent on animal’s readiness to feed: hungry animals may attack noxious stimuli and non-hungry animals may even avoid food stimuli. Two neural networks, the turn and feeding networks, are involved in this behavioral decision (Figure 1.2). Computational modeling and previously identified neuronal connections allowed us to speculate that the signal from the feeding network to turn interneurons may act as a switch between approach and avoidance turn choices. In chapter 2, I have shown that the turn network exhibits approach or avoidance as functions of the excitatory states of feeding network, which can be modified electrophysiologically. Spontaneous activities manifested in rhythmic feeding motor nerve activities may encode the animal’s appetitive state, which in turn, affects the turn decisions. In chapter 3, I have shown the directional coding mechanisms in the turn network that computes the turn motor output. Directional coding mechanisms in the nervous system allow animals to move to and from the cue with precise angle. In vertebrate animals, it has been known that the sensory map of the tectum allows animals to locate the sensory stimuli and in turn respond to it (Knudsen, 1984; Keeton, 1979). Similar mechanisms also exist in invertebrates. Asymmetrical activities in left and right neurons allow animals to turn to the specific direction (Kristan et al, 2005). In *Pleurobranchaea*, I showed that asymmetric activities in bilaterally symmetrical turn
interneurons encode the direction of the turn. These results described in Chapter 2 & 3 elucidate the detailed cellular mechanisms of turn decision that is adapted to the states of feeding network.

Behaviors can be chemically modified. Applying a specific neurotransmitter to the isolated nervous system or injecting one to animals may evoke a specific behavioral pattern. For example, injection of serotonin to crayfish causes slow abdominal flexation (Livingstone et al., 1980). In *Aplysia, Pleurobranchaea*, and leech, serotonin has been known to evoke the food arousal response (Palovcik, 1982; Gaudry, 2009). The level of serotonin in the feeding network neurons indicates the hunger state in *Pleurobranchaea* (Hatcher, 2007). In chapter 4, I have shown that turn decision making based on the internal state in *Pleurobranchaea* may involve serotonin. Serotonin bath application to the isolated nervous system resulted in switching the fictive turn output from avoidance to orienting in response to unilateral oral veil nerve stimulation.

In conclusion, these results elucidate the cellular level of turn decision making mechanisms in *Pleurobranchaea*. Animal’s appetitive state manifested in the feeding network toggles the switch between avoidance and approach, and asymmetric activities of bilaterally symmetrical turn interneurons encode the direction of the turn output. Moreover, studies also focused on the spontaneous activities in the nervous system, which were also found to be informative in order to examine the animal’s readiness to feed. These results would be applicable to more complex models and bridge the gap between the microscopic and multicellular levels of economical decision making studies.
1.2 References


1.3 Figures

Figure 1.1 *Pleurobranchaea*

The gastropod mollusk *Pleurobranchaea* is a predatory sea-slug inhabiting the coastal Californian waters at depths of 10-200 m. The sizes of the animals range up to 30 cm. The ventral foot functions in forward locomotion. The oral veil and rhinophores function as primary sensory organs. Lateral body wall muscles located laterally on the body wall mediate turns. The orienting turn is usually slower and consists of twisting the foot by contracting the body wall muscles ipsilateral to the stimulus. Avoidance turn is more vigorous and higher speed of turn is achieved by raising the front portion of the foot and contracting the body wall muscle contralateral to the stimulus.
**Figure 1.2 Behavioral choice in *Pleurobranchaea***

The figure below outlines behavioral choice in *Pleurobranchaea*. Food and chemotactile signals can be aversive or appetitive, depending on the internal state of the animal (hunger state). Appetitive state is manifested in feeding network activity, where satiation suppresses feeding and hunger promotes active feeding responses. The animal’s avoidance response can be switched to approach, depending on the state of the feeding network.
CHAPTER 2
A NEURONAL SWITCH OF APPROACH/AVOIDANCE

2.1 Abstract

The internal state (readiness to feed) of sea slug, *Pleurobranchaea*, was conserved in the isolated central nervous system (CNS). Isolated CNS from donor animals with either high or low feeding thresholds expressed avoidance or orienting fictive turn motor output, respectively, in response to brief unilateral stimulation of chemotactile sensory nerves. Animals’ feeding thresholds also corresponded with frequency of spontaneous rhythmic activity in the feeding motor nerve.

Bilaterally paired serotonergic turn network interneurons were necessary for fictive turns. These neurons were inhibited during fictive feeding, consistent with suppression of avoidance turning during active feeding in the intact animal. Moreover, activating a feeding command neuron to increase excitation of the feeding network switched fictive turn output from avoidance to orienting, while hyperpolarizing it switched the turn from orienting to avoidance.

Thus synaptic inputs from the feeding network account for the transition between approach and avoidance. The feeding network embodied appetitive state and toggled approach-avoidance decision by configuring the response symmetry of the premotor turn network. A resulting model suggests a basic cost-benefit decision module from which to consider evolutionary elaboration of the circuitry to serve more intricate valuation processes in complex animals.

Some of the materials found in Chapter 2 were previously published (Hirayama and Gillette, 2012) with permission to reprint.
List of abbreviations

CPG: central pattern generator
CNS: central nervous system
LOVN: large oral veil nerve
iLBWN: ipsilateral lateral body wall nerve
cLBWN: contralateral lateral body wall nerve
R3: retractor nerve 3
SGN: stomatogastric nerve
PCp: phasic paracerebral feeding command neuron
As: serotonergic A cluster neuron
2.2 Introduction

Animals must make fundamental decisions for survival and homeostatic control. In foraging behavior, for example, animals choose to either orient themselves toward or avoid the food source based on both their internal state and sensory stimuli (Figure 2.1). Other behavioral choices include escaping from predators or noxious stimuli. This decision making is based on cost-benefit and risk assessments to optimize reward. However, such decision making mechanisms that generate and control behaviors are not well understood, neither on the cellular nor functional level.

Addiction and eating disorders are problems in human choice, for which the neuronal mechanisms and circuits are only imperfectly understood. Decision making mechanisms in higher organisms are hard to access due to their behavioral complexity and nervous systems with considerably greater number of cells. In contrast, relatively simple molluscan nervous systems have been used as an informative model for analyzing various behaviors. For instance, feeding behaviors in *Aplysia* (Kupfermann, 1974; Teyke et al., 1990), *Clione* (Norekian, 2006), *Lymnaea* (Staras et al., 1999), and *Pleurobranchae* (Kovac, et al., 1983) have been well studied. Especially in *Aplysia* and *Lymnaea*, the network topology and functionality of the neurons involved in feeding behavior are well known (Kupfermann, 1974; Benjamin and Rose, 1979). A number of cells identified across these different species are likely homologous and may play critical roles in the regulation of feeding behavior (Elliott and Susswein, 2002). In our model of *Pleurobranchae californica*, past studies showed that feeding can suppress ongoing behaviors, such as avoidance turn (Jing and Gillette, 2003) or withdrawal behavior (Kovac and Davis, 1977). Orienting and avoidance responses in the predatory sea-slug *Pleurobranchae* depend on its
internal state (Gillette et al., 2000), such that very hungry animals may attack noxious stimuli and satiated animals will avoid appetitive stimuli.

In *Pleurobranchaea*, the neuronal circuits for the feeding network (Kovac et al., 1983; London and Gillette 1984) and turning network (Jing and Gillette, 2003) are well characterised. The turn motor network is a small bilateral network of identified neurons asymmetrically active during turns (Jing and Gillette 2003). Fictive turning motor output can be elicited in the isolated CNS by electrical stimulation of the Large Oral Veil Nerve (LOVN) of the cerebropleural ganglion and recorded in the Lateral Body Wall Nerves (LBWNs) of the pedal ganglia. Behavioral observations and computational modeling imply that feeding network activity can invert the turning motor network’s state from avoidance to orienting (Yafremava, et al., 2007). Thus, the neuronal basis of behavioral choice mechanisms are accessible in the animal’s simple nervous system. *Pleurobranchaea* provides excellent preparations for dissecting the circuit mediating approach vs. avoidance choice.

We investigated the mechanism that toggles avoidance vs. orienting turn decisions dependent on the animal’s internal state. We found that the donor animals’ readiness to feed was conserved in the turning decisions of the isolated nervous system. CNS's from low and high feeding threshold donors expressed fictive orienting or avoidance, respectively, in response to brief stimulation of sensory nerves. Moreover, the decision to orient or avoid was a function of activity in the feeding motor network, which regulated activity in critical neurons of the turning motor network. The animal’s readiness to feed, as measured in feeding thresholds, correlated with the spontaneous cyclic activity level of the feeding motor network. Artificially exciting the feeding network converted fictive avoidance to orienting. Activating feeding command neuron or driving stomatogastric nerve (connecting gut) switched
the choice of fictive turn from avoidance to orienting. Most recent results demonstrated that activating a specific pair of corollary discharge neurons in the buccal ganglion (previously found by Kovac and Davis, 1977) also altered the turn decision from approach to avoidance.

Thus, the feeding network embodied appetitive state and toggled approach-avoidance decision by configuring response symmetry of the premotor turn network. These results are consistent with a neuronal model of decision making for approach vs. avoidance mediated by corollary outputs from the feeding network to the turn premotor network that can flip the symmetry of the turn network from avoidance to orienting.

2.3 Methods

Specimens of Pleurobranchaea Californica (10-800 g) were obtained from Monterey abalone (Monterey, CA) and kept in circulated artificial seawater at 14 °C until use.

Feeding thresholds

Appetitive state, or behavioral readiness-to-feed, in Pleurobranchaea is controlled by sensation, nutritional state, learning, reproductive condition and health. Readiness-to-feed is quantitated in terms of feeding thresholds measured as the minimal concentrations of appetitive stimuli that elicit proboscis extension and active biting (Gillette et al., 2000; Davis and Mpitsos, 1971). Feeding thresholds were measured as previously described biting (Gillette et al., 2000; Davis and Mpitsos, 1971) in responses to betaine (trimethylglycine; Sigma-Aldrich) solutions in seawater + 10 mM MOPS at pH 8.0 applied in 1.5 ml volumes to the oral veil with a hand-held
Pasteur pipette over 10 seconds in a series of ascending concentrations from $10^6$ to $10^{-1}$ M. Feeding thresholds measured were those concentrations at which animals showed proboscis extension and biting. When specimens failed to respond to the highest concentration ($10^{-1}$ M) the next highest value, $10^0$, was assigned for proboscis extension and $10^1$ for biting threshold, since biting threshold is normally higher than proboscis extension threshold. Tests began with a control sea-water application assigned a value of $10^{-7}$. These conventions assign conservative finite values to essentially infinitely high or low thresholds. Data were presented as the logarithms of the dilutions; thus, $10^{-1}$ is -1.0 and so on (Davis et al., 1974). Data were then analyzed with non-parametric statistic tests that best accommodate the threshold conventions described above. In the laboratory population, thresholds are somewhat skewed to the high end as result of the sample population being largely larger reproductive animals, an artifact of both trapping methods and seasonality.

**Isolated central nervous system (CNS) preparation**

Immediately after measuring the feeding threshold, a specimen was anesthetized by cooling to 4 °C for 15 -30 min. Then the CNS, consisting of cerebropleural, pedal, and buccal ganglia (Figure 2.1), was isolated by dissection under the microscope, while constantly being covered with saline. Saline was composed of (in mM) 460 NaCl, 10 KCl, 25 MgCl$_2$, 25 MgSO$_4$, 10 CaCl$_2$, and 10 MOPS (3-morpholinopropane-1-sulfonic acid) buffer (pH7.5). The isolated CNS was kept at 4 °C until recording. For recording, it was fixed on a Sylgard dish filled with saline and temperature-controlled at 11 °C. There, connective tissues were removed with caution
such as not to damage ganglia and nerves. Finally, the outer as well as inner sheath of the ganglia were removed.

**Electrophysiology**

For intracellular recordings, connective tissues were removed over neuron cell bodies. Intracellular and extracellular recordings and nerve stimulation were made with 3 M KCl-filled glass micropipettes and polyethylene suction electrodes, respectively. Data were acquired with Power Lab software (AD Instruments). Fictive turns were induced by brief, unilateral stimulation of one of the bilateral pair of Large Oral Veil Nerves (LOVNs; 15 hz, 2 msec pulse duration; Jing and Gillette, 2003). Fictive feeding was induced by driving $PC_P$ feeding command neurons (Gillette et al., 1982) or by stimulating stomatogastric nerve (SGN) at 2-4 hz, 2 msec duration pulses; Davis and Mptitsos, 1971). Fictive feeding was recorded from buccal ganglion nerve root 3 as bursts of fictive radular retraction activity. Data were captured and analyzed with Chart 5 Pro (AD Instruments). Fictive turn events were characterized by comparing mean spike frequencies in bilateral lateral body wall nerves (LBWNs), which are motor outputs for the turn network (Jing and Gillette, 2003). Spikes were counted after selecting a threshold level above spontaneous noise. Spike frequencies were normalized to spike counts for 20 seconds prior to the stimulus event and plotted in 2-3 second bins. The non-parametric Mann-Whitney U-test was used for statistical analyses. The p values were calculated by comparing the spike counts in ipsilateral and contralateral LBWNs for 30 seconds from the first steep inflection following the initial peak. The initial peak corresponds to a fictive withdrawal (Jing and Gillette, 2003) preceding the turn, as in intact animals. Criterion for assigning “fictive avoidance” vs. “fictive
orienting” to LBWN activity was a significant difference with at least $p<0.05$ for bilateral spike counts; in fact, for all avoidance results, $p$ values were $<0.0001$; and for orienting, $p$ values ranged $p<0.01$ to 0.0001.

2.4 Results

Appetitive state and choice of turn are conserved in isolated CNS

Previously, it was shown that unilateral stimulation of nerves innervating the oral veil induced fictive turn motor output, recorded in the nerves of the lateral body wall muscles, shown in higher spike activity in contralateral or ipsilateral body wall nerves LBWNs for avoidance and orienting turns, respectively (Jing and Gillette, 2003). Here we observed fictive orienting turning in isolated CNS’s specifically from relatively low feeding threshold donors, and either fictive avoidance or null responses in those from high threshold donors (Figure 2.2, A). Feeding thresholds were measured in the intact animal as betaine dilutions induced proboscis extension and biting responses. We compared behavioral thresholds and fictive turn responses of 40 CNS donors. Of them, 11 CNS’s showed fictive orienting to large oral veil nerve (LOVN) stimulation, 12 showed no clear turn response and 17 expressed fictive avoidance turns (Figure 2.2, A). Donor feeding thresholds of the fictive orienting CNS’s were significantly lower than donor thresholds of the non-turning or fictively avoiding CNS’s. A clear transition existed in donor feeding thresholds and avoidance/null responding vs. orienting preparations. In intact animals high feeding thresholds are characteristically associated with either active avoidance or no reactions to food stimuli (Gillette et al., 2000).
Figure 2.2 B and C illustrate fictive avoidance and orienting in enhanced motor activity of the lateral body wall nerve (LBWN) of the side contralateral or ipsilateral to the stimulus, respectively. In the intact animal this shortens that side of the body relative to the other, resulting in a turn away or toward the stimulus to the oral veil (Jing and Gillette, 2003). LBWN activity differences tended to be less marked in orienting than avoidance, which is consistent with the intact animal’s behavior where aversive turns are typically greater in amplitude and more stereotypic in postural involvement, as might be expected of an escape-like behavior (Gillette et al., 1991). These results confirm that the donor animal’s turn choice, as measured by its feeding threshold, is conserved in the isolated CNS’s fictive turn choice, as measured by its motor nerve activity levels.

**Feeding activity in CNS reflects the appetitive state**

The excitation state of the feeding motor network was monitored in the buccal ganglion nerve root 3 (R3). During active feeding, fast rhythmic bursts can be seen in R3, which innervates the retractor musculature of the buccal feeding apparatus in the intact animal. During non-feeding, slower spontaneous rhythmic activities occur in R3. Recording of the R3 nerve showed spike bursts whose frequencies varied directly with the donors’ readiness to feed (Figure 2.3, A). Quantitative analysis of rhythmic bursts in the isolated CNS demonstrated R3 burst activity was an approximately linear function of donor feeding thresholds on a log-log plot (Figure 2.3, B). In the absence of sensory input, such spontaneous activity is likely to reflect the intrinsic excitatory state of the feeding network. These results showed the conservation of the animal’s appetitive state in the excitation state of the feeding motor network in the isolated CNS.
Initiation of fictive feeding suppresses fictive avoidance

Fictive avoidance was elicited by LOVN stimulation. Fictive feeding was driven by stimulating the Stomatogastric Nerve (SGN), resulting in rhythmic feeding output in buccal R3 (Figure 2.4, A). The bilateral As2/3 serotonergic turn network neurons were previously identified to be necessary for turning output, as their inhibition suppresses the turn (Jing and Gillette, 2003). The As2/3 neurons were confirmed to be activated during the avoidance turn (Figure 2.4, A). In addition, they were found to be suppressed in response to the initiation of feeding (Figure 2.4, B). Fictive avoidance activity was again recognized to be associated with the higher activity level in cLBWN (Figure 2.4, A bottom; p<0.0001, Mann-Whitney U Test), but was not observed while As2/3 neuron was suppressed due to feeding.

Activation of fictive feeding toggles the approach/avoidance switch

Paracerebral neurons (PCps) act as feeding command neurons in *Pleurobranchaea californica* (Gillette, et al., 1978). Characteristics of these feeding command neurons were studied in detail previously. Cyclic bursts in PCps are phase-locked with the animal’s feeding rhythm (Gillette, et al., 1978). They are sufficient and necessary for the induction of feeding, thus identifying PCps as command neurons (Gillette, et al., 1978). The feeding network includes PCps, interneurons in the cerebropleural ganglion that provide inhibitory inputs to PCps, and retractor/protractor oscillatory neurons in the buccal ganglion (Figure 2.5). The PCps can be activated by the presence of food in the intact animal, and can also be recruited to follow the feeding rhythm when the stomatogastric nerve (SGN) was stimulated (Gillette et al., 1982). If
the animals are conditioned for food-avoidance, enhanced activity of inhibitory interneurons (Int-2) to food stimuli results in suppression of PCps (London and Gillette, 1986). Moreover, activation of escape swimming inhibits these feeding command neurons, thus overriding feeding activity (Jing and Gillette, 1995).

However, the role of PCps in the avoidance/approach turn choice has not been studied so far. Here we have found that the activity level in PCps switches between avoidance and approach. The CNS from donor animals with high feeding thresholds demonstrated fictive avoidance in response to the unilateral LOVN stimulus initially (Figure 2.6, A). Once the PCp was activated, rhythmic bursts were seen in R3 and the fictive turn response to the stimulus was now switched to orienting (Figure 2.6, B). Hyperpolarization of PCp abolished the rhythmic bursts in R3, thereby switching the fictive turn response to avoidance (Figure 2.6, C). It was also noteworthy that activation of PCp subthreshold to induce fictive feeding (slower bursts were seen in R3) restored the orienting turn again (Figure 2.6, D). Similar results were found for the stimulation of the stomatogastric nerve (SGN) of the buccal ganglion, which drives rhythmic protraction/retraction feeding cycles in *Pleurobranchaea* and other opisthobranch gastropods (Davis and Mpitsos, 1971). When SGN stimulation excited the feeding network in five fictively avoiding CNS’s, the responses to LOVN stimulation also converted from avoidance (Figure 2.7, A) to orienting (Figure 2.7, B). SGN stimulation caused weak rhythmic bursts in the feeding motor nerve R3. This increase in feeding network excitation was sufficient to switch the fictive turn response.

In addition, most recent results demonstrated that activating corollary discharge neurons (Kovac and Davis, 1977) could also switch the fictive turn response (Figure 2.8). Corollary Discharge (CD) neurons of the buccal ganglion provide inhibitory input to the premotor network.
in order to inhibit withdrawal and are thus involved in the feeding vs. withdrawal behavioral choice (Figure 2.8, A; Kovac and Davis, 1977). We speculated that the same neurons could also be involved in the approach/avoidance turn choice. Consequently, we found that activating CD neurons caused the induced turn response to change from avoidance (Figure 2.8, B) to orienting (Figure 2.8, C). Furthermore, hyperpolarizing CD neurons caused the induced turn response to revert to avoidance (Figure 2.8, D) and driving CD neurons promoted orienting (Figure 2.8, E).

Thus these results above clearly showed that the excitation state of the feeding network manifested the choice of avoidance vs. approach.

2.5 Discussion

Appetitive control of Approach/avoidance decision

The turn behavioral choice to the stimulus is conserved in the isolated CNS. The decision to approach/avoid depends on the internal state, previous experience and the external stimuli applied to the donor animals. In particular, this study demonstrated that appetitive state, measured behaviorally in readiness-to-feed, was a direct function of the excitation of the homeostatic feeding network. The appetitive states of intact animal donors were conserved in their isolated CNS’s and reflected as rates of spontaneous rhythmic output of the feeding network. CNS’s from donor animals with low feeding thresholds showed orienting turns, while those with high feeding thresholds exhibited avoidance or null turn responses (Figure 2.2). These results matched previous behavioral studies showing that satiation exchanges orienting turn responses for avoidance or null responses (Gillette et al., 2000). The marked linearity of the
double logarithmic plot for CNS donor feeding thresholds vs. spontaneous burst frequency (Figure 2.3, B) directly related responsiveness for appetitive stimuli to the feeding network excitation state. The identity of the appetitive state was further confirmed by inducing the switch from avoidance to orienting by artificially adding and subtracting excitation in the feeding network via feeding command neuron activity (Figure 2.6) and SGN stimulation (Figure 2.7).

The control of the turn response by the excitation state in the feeding motor network predicts two aspects of the choice mechanism at the neuronal level: 1) Corollary outputs from feeding to turn network must exist that promote the orienting turn, and 2) The default organization of the turn network circuitry is likely to be that for avoidance turns. In default mode, turn interneurons ipsilateral to the sensory stimulus are activated and the consequent asymmetric network response drives contralateral, avoidance turn output (Jing and Gillette, 2003). However, in low feeding threshold animals, corollary output from the feeding network would reconfigure the premotor turn network into its alternative activity mode of orienting, for ipsilateral output. These corollary outputs may resemble or actually be the same as those found by Kovac and Davis (1977; Figure 2.8). Recording demonstrated that activating corollary discharge (CD) neurons modified the induced turn response from avoidance to orienting.

**Spontaneous cyclic feeding activities indicate the animal’s motivational state**

The spontaneous activities in the R3, a feeding motor nerve, were correlated with the animal’s readiness to feed. More cyclic activities were observed in hungry donors than in non-hungry donors. Similar to the turn choice, therefore the state of the feeding network is also conserved in the CNS.
The importance of studying spontaneous behaviors and activities is well documented in *Drosophila*. Non-linear intrinsic activity may provide the variability in behaviors and play a role in operant behavior (Maye et al., 2007). Motivation builds up during spontaneous activity (Lorenz, 1981). The animal’s motivation (hunger state) is preserved and manifested as spontaneous activities in the feeding network. Our results clearly demonstrated that state of the spontaneous activities (excitation state) of the feeding network account for the choice of turn to the stimulus.

**Behavioral choice between feeding and turns**

Our results demonstrate that activation of the feeding network inhibits turning, indicating that inputs to the premotor interneurons from the feeding network may flip the switch from avoidance to orienting by reconfiguring the activity of turn interneurons. These corollary outputs resemble or may actually be the same as those identified by Kovac and Davis (1977) that suppress withdrawal motor output during feeding (Figure 2.8, A). The output from the bilateral feeding neurons in the buccal ganglion suppresses the animal’s withdrawal by inhibiting the withdrawal neurons. We suggest that these same outputs also inhibit the premotor network interneurons.

Similar mechanisms are observed in the case of escape swimming vs. feeding in *Pleurobranchaea*. From behavioral observations, escape swimming is known to suppress feeding, thus initiation of escape swimming overrides ongoing feeding. In the nervous system, escape swimming is controlled by A1 swimming interneurons, homologous to the C2 neurons in *Tritonia diomedea* (Willows and Hoyle, 1969). Bursting activity in A1 corresponds to the fictive
swimming rhythm, the dorso-ventral swimming motor patterns in the intact animal. Activation of A1 inhibits PCps, thus suppressing the ongoing feeding (Jing and Gillette, 1995; Gillette and Jing, 2001). In the case of feeding vs. turns, the cyclic feeding activities initiated from the buccal feeding center may provide the inhibitory feedback to the turn network interneurons, thereby suppressing the turns.

**Modeling Basic Cost-Benefit Decision**

These and previous observations point to the feeding network as a final integration site for sensation, internal state and memory in cost-benefit decision. The effects of learning and satiation were found to be expressed at strategic sites in the feeding network, where they controlled responses to aversively learned odors and appetitive stimuli (Davis et al., 1983; London and Gillette, 1986). In naïve hungry animals food stimuli induce network excitation, culminating in rhythmic feeding motor output (Gillette et al., 1982). However, in odor-avoidance trained and partially satiated animals the feeding network is inhibited, thereby raising the feeding threshold to appetitive stimuli by a factor of 100-1000 (Davis et al., 1983). In these cases, active feeding can be released by increasing the stimulus strength or by providing excitation directly to the feeding network via PCp feeding command neurons. The excitation state of the feeding network thus integrates the contributions of learning and motivation into its responsiveness to sensory input, and so embodies the appetitive state in the animal’s moment-to-moment regulation of its readiness to feed.

Collectively, these results can be summarized in a simple model (Figure 2.9). In it, the excitation state of the homeostatic feeding network controls a switch between avoidance and
approach responses (Figure 2.9, A). Sensory signals are processed through integrative sensory networks for Incentive and Deterrence to promote orienting or avoidance, dependent on the homeostatic feeding network activity (Figure 2.9, B). Active avoidance and the feeding network are reciprocally inhibitory. The simple design extends an earlier model proposed to explain effects of satiation on the approach/avoidance decision (Gillette et al., 2000). The excited homeostatic network is shown to simply to exhibit a continuum of excitatory states culminating in active feeding (cf. Gillette, 2008). The model can be further elaborated on the basis of new observations and is markedly accessible to computational simulation.

Not explicitly shown in the model is the sea-slug’s ability for associative learning of odor value. Moreover, the feeding network also exchanges outputs with conflicting neuronal networks, whose interactions mediate decision in escape swimming (Jing and Gillette, 1995) and local withdrawal vs. feeding (Kovac and Davis, 1977; Kovac and Davis, 1980), and which also manifest effects of behavioral conflict (London and Gillette, 1986; Kovac and Davis, 1980; Davis and Gillette, 1978; London and Gillette, 1984; Kovac et al., 1986). Incorporating these interactions may eventually result in a more complete model of animal behavior.

**Economic decision making**

The PCp activity is known to be an indicator of the animal’s motivational state and to be modifiable by learning. In food avoidance learning, previously active PCp was found to be suppressed as a result of the training (London and Gillette, 1984; London and Gillette, 1986; Kovac et al., 1986). Our findings explain the connection between this PCp suppression and the
according turn choice: inactive PCp prevents the feeding oscillator from providing feedback to the turning network, thus triggering that network to choose avoidance instead of orientation.

The inhibitory input from the feeding network can modify the state of the premotor network. More specifically, the outputs from the feeding network provide the presynaptic inhibition to the premotor network neurons, acting as a switch between avoiding and orienting. Our model is a good demonstration of economic decision making, namely of how the organism decides when and where to obtain a reward (Rowland et al., 2008; Schultz, 2004; Watson, 2008). Animals choose the behavior in a way to maximize utility (nutrition necessary for the reproduction), and their central nervous system preserves the decision and computation between risk and payoff. Mechanisms of information processing in the simple molluscan nervous system may provide the clues for understanding more complex decision making processes in humans.
2.6 References


2.7 Figures

Figure 2.1 Central nervous system (CNS) of *Pleurobranchaea californica*

The CNS of *Pleurobranchaea californica* is composed of a cerebropleural ganglion, two bilateral pedal ganglia, and a buccal ganglion. Pedal ganglia provide the motor outputs for locomotion, escape swimming as well as avoidance turn. Fictive turning motor output can be elicited in the isolated CNS by electrical stimulation of the Large Oral Veil Nerve (LOVN) of the cerebropleural ganglion and recorded in the Lateral Body Wall Nerves (LBWNs) of the pedal ganglia. In some experiments the feeding network was excited by driving a $PC_p$ feeding command neuron.

The buccal ganglion is involved in the generation of cyclic feeding patterns, which are composed of retraction and protraction. Motor output for the retraction can be recorded from Retractor nerve 3 (R3). Stomatogastric nerves (SGN) innervate gut and stimulation of SGN elicits fictive feeding.
**Figure 2.2 Fictive turn direction was a function of donor feeding thresholds.**

Hunger states of CNS donors were determined by measuring the feeding thresholds for the lowest concentrations of the appetant betaine (trimethylglycine) inducing proboscis extension and biting. *A:* CNS’s of high threshold donors responded to sensory LOVN stimulation with fictive avoidance turns (N=17) or null responses (N=12), while orienting turns (N=11) characterized CNS’s from animals with lower feeding thresholds (p<0.001 for both biting and proboscis extension thresholds, two-tailed Mann-Whitney U Test). Transition from avoidance/null to orienting responses occurred at thresholds of 10⁻³ M for proboscis extension and between 10⁻¹ M and 10⁰ M for biting. *B&C:* Fictive turn direction was represented in differing relative spike rates of the LBWNs (Jing and Gillette, 2003) following LOVN stimulation (bar). In the two representative experiments shown, an avoidance turn (*B*) was seen in higher spike rates in LBWN contralateral to the stimulated nerve, while an orienting turn (*C*) was shown in higher relative activity in the ipsilateral LBWN (p<0.0001 in both cases, two-tailed Mann-Whitney U Tests). The initial post-stimulation peaks corresponded to fictive withdrawal preceding the fictive turn response (solid arrow; Jing and Gillette, 2003), as indicated.
Figure 2.2 (Cont.)

B

C

LOVN stim

Time (s)

Avoidance

Fictive
withdraw

Fictive turn

p<0.0001

Relative spike freq.
(normalized)

Avoidance

iLBWN
cLBWN

LOVN stim

Time (s)

Orienting

p<0.0001

Relative spike freq.
(normalized)

Orienting

iLBWN
cLBWN

LOVN stim

Time (s)
Figure 2.3 Conservation of donor appetitive state in the isolated CNS.

A: Spontaneous burst frequency recorded from nerve R3 was less in the isolated CNS’s of high-threshold donors than in those of low threshold animals. B: R3 burst frequency was an approximately linear function of donor feeding thresholds on a log-log plot (N=25; $R^2=0.54$ and 0.59 for proboscis extension and biting, respectively; Least Squares fit). Three high threshold donor CNS’s did not show burst patterns in R3 and were excluded here.
Figure 2.4 Fictive avoidance was suppressed by initiation of fictive feeding

Fictive avoidance was elicited by LOVN stimulation (solid bar; 4 V, 2 msec duration pulses at 15 hz for 3 sec). Fictive feeding was driven by stimulating the Stomatogastric Nerve (SGN; dashed bar; 4 hz) and rhythmic feeding output was observed in buccal R3 (B top). The As2/3 turn network neuron, necessary to turning output, was activated during the avoidance turn (A), but became suppressed during feeding (B). Fictive avoidance activity was recognized as the higher activity in cLBWN (A; p<0.0001, Mann-Whitney U Test), but was not observed while the As2/3 neuron was suppressed by feeding (B).
Figure 2.5 Feeding network model

Feeding network is composed of distributed oscillator in buccal and cerebropleural ganglion. The feeding oscillator, similar to those found in the locomotor half center model, is composed of retractor and protractor neurons (shown as “R” and “P” in the figure, respectively) that are activated alternatively. PCNs are feeding command neurons necessary for the feeding rhythm and provide the input to the excitatory feeding oscillator. I2 and I1 are inhibitory neurons that modify the excitatory state of PCNs.
Figure 2.6 Activation of feeding command neuron switched the fictive turn preference from avoidance to orienting

A fictive turn event is shown in (A). Stimulation of LOVN caused increased activity in the contralateral lateral body wall nerve (cLBWN), corresponding to fictive avoidance. Notice that no spontaneous feeding activity is seen on the feeding retractor motor nerve, R3 (A). Turn events were identified by measuring the mean spike frequency in bilateral LBWNs. In each experiment, the spike frequency was normalized to the baseline frequency (before the stimulation event) and plotted in 3 bin. Increased spike frequency was seen in cLBWN (P<0.0001, two-tailed Mann-Whitney U-test), corresponding to fictive avoidance.

Activation of the feeding command neuron, PCp (Phasic Paracerebral neuron), is known to induce feeding. Rhythmic feeding motor patterns were seen on the R3 nerve of the buccal ganclion. LOVN stimulation now caused fictive orienting (B) as observed by increased spike frequency in ipsilateral Lateral body wall nerve (iLBWN; P<0.005).

Hyperpolarization of the PCp abolished bursting activity in the feeding nerve R3 and caused the induced turn response to revert to avoidance (C). Increased contralateral lateral body wall nerve (LBWN) activity corresponded to fictive avoidance (p<0.001).

Releasing PCp activity caused restored feeding nerve activity and promoted fictive orienting in response to large oral veil nerve (LOVN) stimulation (D). Increased ipsilateral LBWN activity corresponded to fictive orienting (p<0.001; N=4). Initial peak right after the stimulation corresponded to fictive withdrawal (dashed arrow) preceding the fictive turn response (solid arrow).
Figure 2.7 Feeding motor activities subthreshold to active feeding switched turn to orienting

Increasing feeding network excitation by stomatogastric nerve stimulation (4 hz, 2 msec duration pulses) to drive slow rhythmic bursting in the buccal nerve R3 switched the avoidance turn (A) to orienting (B; N=5). Fictive avoidance (A) corresponded to higher spike frequency in contralateral LBWN (cLBWN) (p<0.0001, Mann-Whitney U Test), while fictive orienting (B) was indicated by higher activity in ipsilateral LBWN (iLBWN) (p<0.0001). Such significant differences were observed in all experiments.

A

B

Relative spike freq. (normalized)

0 1 2 3

LOVN stim Time (s)

Relative spike freq. (normalized)

0 1 2 3

LOVN stim Time (s)
Figure 2.8 Activating corollary discharge neurons of buccal ganglion switched turn responses

A: Behavioral choice model presented by Kovac and Davis (1977). The output from corollary discharge (CD) neurons on the buccal ganglion, which are part of the feeding network, serves as the inhibitory input to the withdrawal command interneurons (WCN) on the cerebropleural ganglion. Therefore feeding inhibits withdrawal. B: The CNS of a donor animal with high feeding threshold exhibited fictive avoidance turn in response to large oral veil nerve (LOVN) stimulation (bar). Increased contralateral lateral body wall nerve (cLBWN) activity corresponded to fictive avoidance (p < 0.001, Two-tailed U test). C: Penetration of the Corollary Discharge (CD) neurons of buccal ganglion caused the induced turn response to change to orienting. Increased ipsilateral LBWN (iLBWN) activity corresponded to fictive orienting (p < 0.0001). D: Hyperpolarization of the CD caused the induced turn response to revert to avoidance (p<0.001). E: Driving CD activity promoted fictive orienting (p<0.01).
Figure 2.8 (Cont.)

B

LOVN stim

Relative spike freq.
(normalized)

Time (s)

C

LOVN stim

Relative spike freq.
(normalized)

Time (s)

D

Hyperpolarization

Relative spike freq.
(normalized)

Time (s)

E

Relative spike freq.
(normalized)

Time (s)
Figure 2.9 Models showing the mechanism of turn behavioral choice in *Pleurobranchaea*

A model for cost-benefit decision in a simple forager summarizes appetitive regulation of approach-avoidance. *A*: The excitation state of the homeostatic (feeding) network controls expression of orienting vs. avoidance via corollary outputs to the directional turn motor network. The transition is sub-threshold to active feeding. *B*: Sensory inputs are processed in the integrating sensory networks for Incentive and Deterrence, which in turn promotes excitation of feeding and avoidance, respectively. Excitation in the homeostatic feeding network suppresses avoidance behavior and promotes transition to approach from avoidance. Active avoidance and satiation are inhibitory to appetitive state in the homeostatic network, while homeostatic network activity also suppresses Deterrence input (cf. Kovac and Davis, 1977). Except where noted with negative signs, all arrows represent excitatory effect.
CHAPTER 3

ASYMMETRIC ACTIVITY OF BILATERALLY PAIRED TURN
NETWORK DETERMINES THE DIRECTION OF TURN

3.1 Abstract

In many animals mapping of sensory input into the brain allows individuals to localize the direction and angle of a stimulus and respond to it precisely. In *Pleurobranchaea*, the bilaterally paired serotonergic neurons of the turn central pattern generator (CPG) network, critical to turning output, were asymmetrically active during turns appropriate to the turn direction. In whole animal preparations, the previously identified As2/3 turn interneurons and the A4 avoidance turn command neuron located ipsilateral to the appetitive stimulus application locus were activated, while contralateral turn interneurons were suppressed. Similarly, As2/3 turn interneurons contralateral to the turn direction had higher activity levels both for avoidance and orienting turns in the isolated nervous system preparations. These results demonstrate that the asymmetrical activities of multifunctional locomotor interneurons account for the directional coding in *Pleurobranchaea*.

Figure 3.1 found in Chapter 3 was previously published by Jing and Gillette (2003). Figure 3.2, 3.3 & 3.4 were the unpublished works by Hatcher N, who generously allowed using his materials.
3.2 Introduction

Animals direct themselves in response to the chemotactile sensory stimuli. Approach/avoidance turn behavioral choice is essential for animal’s survival. Especially in animals that are at risk of predation, optimizing the rate of successful foraging through the precise approach/avoidance directional coding is essential for reproduction. Computational mechanisms in the nervous system allow animals to make cost-benefit decisions for approach or avoidance of appetitive or noxious stimuli with precise angles. In vertebrate organisms, such as owls and lamprey, there is a space map in the tectum that precisely codes the sensory receptive field, and in turn provides the turn output (Knudsen, 1984; du Lac and Knudsen, 1990; Saitoh et al., 2007).

However, the mechanisms of turn computation at the single neuronal level have not yet been studied in detail. In the predatory sea slug, Pleurobranchaea californica, a bilaterally symmetrical turn network has been studied well. A pair of turn command neurons as well as several serotonergic turn interneurons control the avoidance turn in response to a noxious stimulus.

Now we have found that the same network is responsible for the orienting turn as well. Asymmetric activity in the pair of serotonergic turn neurons in fact determines the orientation of the turn. Electrophysiological recordings from isolated central nervous system and whole animal nervous system have shown the correlation between the asymmetric activities in turn interneurons and the direction of the turn. Moreover, these results fit a previous turn computational model (Yafremava, thesis). A chemotactile stimulus applied to the large oral veil of the animal was processed in the peripheral chemotactile sensory neurons. The angle of the
turn was proportional to the loci of the stimulus application. Furthermore, the turn angle was a function of the activities in the bilateral chemotactile nerves. Combined with the previous studies, our results will demonstrate how the sensory information is processed and computed at the interneuronal level and how the asymmetry in the turn network determines the orientation of the turn.

3.3 Methods

**Whole animal preparation**

The animal was placed in a clear chamber containing artificial seawater (11°C). Several fish hooks tied to a thread each were pierced into the dorsal head region of the specimen. The other end of each thread was fastened to an edge of the chamber. This setup allows the animal to move its posterior body freely while stabilizing the brain’s position for electrophysiological recording. A small incision was made posterior to the rhinophores to reveal the cerebropleural ganglion. Then a small black wax-coated platform was inserted just underneath the cerebropleural ganglion and the latter was fixed to the platform with small pins. Connective tissues around A cluster cells of the cerebropleural ganglion were carefully removed for recording.

**Electrophysiological methods**
Intracellular and extracellular recordings were taken using KCl-filled glass micropipettes and polyethylene suction electrodes, respectively. To make electrodes for intracellular recording, glass micropipettes were pulled (David Kopf Instruments, Needle/Pipette puller model 720) and kept in distilled H₂O until further use. To finish the electrodes, the pulled micropipettes were filled with 3 M KCl, and their tips were dipped in black ink for better contrast. AgCl-coated Ag wire connected to the amplifier was placed inside of the micropipette. The tip impedances were measured and typically found to range from 10-20 MΩ. Another AgCl-coated Ag wire was placed inside of the recording chamber as a ground electrode. Electrodes for extracellular recording were made by pulling the polyethylene tube over a flame. Then the tip was connected to the syringe for suction. AgCl-coated Ag wires were then placed. All the electrodes were connected to the amplifier (A-M systems Inc, model 1700) and electrophysiological data were displayed on an oscilloscope (Tektronix, model 5115). Data were acquired with Power Lab (AD Instruments, model 8/30). Fictive feeding and avoiding behaviors were introduced by stimulating stomatogastric nerve (STG) and large oral veil nerve (LOVN), respectively (frequency, 2-4 pps for STG, 15 pps for LOVN; delay, 2 ms; duration, 2 ms; voltage, 1-5 V).

**Data analysis**

Recorded data were analyzed numerically using Chart 5 Pro (AD Instruments). Fictive turn events of motor outputs were identified by counting the mean spike frequency (spike counts per second) in bilateral lateral body wall nerves (LBWNs), which are the motor outputs for the turn network. Each spike frequency was normalized to the baseline frequency (before the
stimulation event) and plotted in 1-3 bin. A non-parametric test (Mann-Whitney U-test) was used for statistical analysis.

3.4 Results

Asymmetric turn network activities in whole animal preparation

The turn network in *Pleurobranchaea californica* has been studied in detail. A-cluster neurons in the cerebropleural ganglion were known to regulate escape swimming as well as avoidance turn (Figure 3.1, A&B). The turn network is composed of bilaterally symmetrical As2/3 neurons coupled with avoidance turn command neurons, A4s. A4s, in turn, project to the contralateral motor nerve to process avoidance turn (Figure 3.1, A).

We investigated the roles of these turn interneurons in both avoidance and orienting turns. Preliminary data suggested that there is differential activity between left and right turn interneurons during the turn (Hatcher, unpublished data). In the reduced animal preparation (similar to the isolated CNS preparation except that oral veil of the animal is intact and attached to the brain), the unilateral application of the appetitive stimulus, betaine, to the tentacle induced orienting turn. Increased motor nerve activity was observed in the ipsilateral lateral body wall nerve (Figure 3.2), which corresponds to fictive orienting. Furthermore, the unilateral application of betaine activated contralateral As2/3 neurons (Figure 3.3, A) but inhibited ipsilateral cells (Figure 3.3, B) during the orienting turns in the whole animal preparation. Previously identified turn interneurons, A4, are known to have command ability for avoidance, but the role of A4 interneurons in orienting has not yet identified. In whole animal preparation,
one observation showed that the unilateral application of betaine lowered the activity level of the ipsilateral A4 turn command interneuron (Figure 3.4).

**Differential activities in left and right As turn interneurons**

Similar to previous observations in whole animal preparations, our recordings from the isolated CNS showed that asymmetric activities in bilateral As2/3 in response to the unilateral stimulation account for the direction of the turn. Specifically, recordings from animals with varying feeding thresholds showed that As2/3 located contralateral to the turn direction was activated more than the ipsilateral set (Figure 3.5; p<0.005, Mann-Whitney U Test). These results applied to both avoidance (Figure 3.5, A&B) and orienting turns (Figure 3.5, C&D). During the avoidance turn, As2/3 cells located contralateral to the turn direction were activated about 2 fold more than ipsilateral ones (Figure 3.5, A&B). During orienting turn, As2/3 cells located ipsilateral to turn direction were suppressed, but not the contralateral cells (Figure 3.5, C&D). These results fit to the previously demonstrated computational model (Yamremava, 2006). This computational model applying the Hodgkin-Huxley neurons predicted that adding presynaptic inhibitory inputs to As2/3 neurons could flip the turn network output from avoidance to orienting.

**3.5 Discussion**

Asymmetric activity in turn interneurons, As2/3
Previous studies suggested that the turn direction is precisely encoded by turn interneurons in the animals. The study in other organisms such as owl (Knudsen, 1984) showed there is a spatial representation of a sensory receptive field in the tectum. Stimulation of the population of neurons on the optic tectum demonstrated that the direction and speed of the head movement are aligned with the sensory representations of visual and auditory space (Du Lac and Knudsen, 1990), but directional coding of turns at the single neuronal level has not been fully studied. Our results showed that asymmetrical activities in bilateral turn interneurons in *Pleurobranchaea* account for the direction of turn, which corresponds to our previous results, showing that turn activities are computed precisely through the turning motor network (Yafremava, et al., 2007). The recording made from whole animal preparations showed that the turn directions and angles depend on the site and intensity of stimulus application. Moreover, the activities of turn motor nerves were dependent on the angle of the turn in both orienting and avoidance. Our results demonstrated that the turn interneurons contralateral to the direction of turn exhibited higher activity both during orienting and avoidance.

In addition, the firing activities of turn motor nerves were distinct between during avoidance and orienting: turn motor nerves were activated considerably stronger during avoidance than orienting (Table 3.1, p<0.05, Mann-Whitney U-test). This also corresponded to the differential firing frequencies in As2/3 turn interneurons during avoidance/orient: contralateral As2/3 neurons fired more actively during avoidance than orienting. This is consistent with the distinct natures of the turns: avoidance is a fast and vigorous response, which usually lasts about 20 seconds involving a greater angle of turn, while orienting is a slower response that lasts sometimes more than 40 seconds and involves a smaller angle of turn.
The study also demonstrates that approach/avoidance turn is encoded by a single set of turn interneurons, showing the multifunctional nature of turn neurons. Previous studies demonstrated that these serotonergic turn interneurons are also part of the escape swimming network as well as involved in locomotion (Jing and Gillette, 1995; Gillette and Jing, 2001). Thus, dependent on the inputs to the turn network as well as the state of the feeding network, this versatile network can generate approach, avoidance, locomotion, or escape swimming responses. This circuit is expected to be similar to one found in Tritonia, where a single set of neurons controls a complex motor circuit (Frost and Katz, 1996).

Finally, the model demonstrating the asymmetric activity of turn network during approach/avoidance is depicted in Figure 3.6. As previously shown in Chapter 2, animals exhibit avoidance/approach dependent on the excitation state of the feeding network. We speculated that inhibitory output from the feeding network to the premotor network may change the configuration of the premotor network, thus toggling the turn. In high feeding threshold animals, a sensory stimulus applied to the right oral veil is processed through right turn interneurons and activation of right turn interneurons results in avoidance turn to the left (Figure 3.6, A, left). In low feeding threshold animals, corollary outputs from the feeding oscillator network (probably same as those found in Kovac and Davis, 1980) suppress part of the premotor network neurons, causing the activation of left turn interneurons to result in orienting (Figure 3.6, A, right). A simulation of the switching action that mediates sensory input to bilateral As2/3 interneurons of the turn network resembled the experimentally recorded responses (Figure 3.6, B; constructed with AnimatLab software).
3.6 References


3.7 Figures and table

Figure 3.1 Turn network model showing the serotonergic turn interneurons and turn command neuron, A4

The turn network is composed of bilateral serotonergic turn interneurons (As1-3) and the turn command neurons A4s. The turn command neuron A4 projects to the contralateral motor nerve, lateral body wall nerve (LBWN) and promotes avoidance turn once excited. Bilateral serotonergic turn interneurons (As1-3) are electrically coupled to the corresponding counterpart (A). The structures and localization of these serotonergic A-cluster neurons have been identified (B). Modified from Jing and Gillette, 2003.
**Figure 3.2 Unilateral application of betaine induces orienting in whole animals**

Unilateral application of betaine ($10^{-1}$ M) to the tentacle induced orienting turn in reduced animal preparations. Increased spike activity appeared in ipsilateral lateral body wall nerve (iLBWN) corresponds to orienting turn. Modified from Nathan Hatcher’s unpublished data.
Figure 3.3 Unilateral feeding stimulus resulted in asymmetric activity in bilateral As2/3 turn neurons

Application of betaine ($10^{-1}$ M) to the right tentacle caused right orienting turn in whole animal (A). Corresponding to the orienting turn, the activity in the left As2/3 neuron was increased right after the application of betaine (B). Conversely, application of betaine to the left tentacle caused left orienting turn and lowered the activity in the left As2/3 neuron. Modified from Nathan Hatcher’s unpublished data.

A

![Right Orienting Turn](image)

B

![Left As2/3 Activity](image)
Figure 3.3 (Cont.)

C

D

10^{-1} M Betaine

Left As2/3

25 mV

5 sec
Figure 3.4 Asymmetric activity in turn command neuron during orienting

A4 is known as an avoidance turn command neuron. However the role of A4 in orienting has not been identified yet. Unilateral application of betaine ($10^{-1}$ M) to the tentacle in whole animal resulted in lowering the activity in the ipsilateral A4 (bottom), not the contralateral A4 (top). Modified from Nathan Hatcher’s unpublished data.
Figure 3.5 Turning and network symmetry: As cells contralateral to turn direction are more active

Fictive avoidance was elicited by LOVN stimulation (solid bar; 2.5 V, 2 msec duration pulses at 10 hz for 2 sec). Activity of the As2/3 turn network neurons (Jing and Gillette, 2003) was higher in cells contralateral to the turn direction. During the right avoidance turn, the left As2/3 neuron fired twice as frequently (A; mean spike freq. =1.11 hz) as during a left avoidance turn (B; mean spike freq. =0.55 hz). Similar observations were made for fictive orienting. The right As2/3 neuron was active during the left orienting turn in response to the left LOVN stimulation (C; mean spike freq. = 0.74 hz), but not during the right orienting turn in response to the right LOVN stimulation (D).
Figure 3.5 (Cont.)

A

LLOVN stim

Relative Spike Freq. (Normalized)

Time (s)

0 6 12 18 24 30 36 42 48 54 60 66 72

LLOVN stim

Relative Spike Freq. (Normalized)

Time (s)

0 6 12 18 24 30 36 42 48 54 60 66 72

B

RLOVN stim

Relative Spike Freq. (Normalized)

Time (s)

0 3 6 9 12 15 18 21 24 27 30 33 36 39

C

Left As Cell

Right LBWN

Left LBWN

D

RLOVN stim

Relative Spike Freq. (Normalized)

Time (s)

0 6 12 18 24 30 36 42 48 54 60 66 72

LLOVN stim

Relative Spike Freq. (Normalized)

Time (s)

0 3 6 9 12 15 18 21 24 27 30 33 36 39

Turning and network symmetry: As cells contralateral to turn direction are more active
Figure 3.6 A simple hypothetical neuronal network model demonstrating approach/avoidance coding mechanism

A. Left: By default the turn network is configured to exhibit an avoidance turn response to unilateral input. The turn network model is modified from Jing and Gillette (2003). Higher neuron activity is indicated by darker color. Corollary outputs from feeding network modify the configuration of the turn network. In high feeding threshold animal, a sensory stimulus applied to the right oral veil is processed through right switch neurons (Sw). Sw neurons are expected to have a suppressing effect on the contralateral projection. Activation of right turn interneurons results in an avoidance turn to the left. In low feeding threshold animal, corollary outputs from the feeding oscillator network (probably same as those found in Kovac and Davis, 1980) suppress part of the Sw neurons, causing a bias toward the activation of left turn interneurons, leading orienting. Right: Corollary outputs from the feeding network acting as a simple dyadic neuronal switch can change the turn network response from avoidance to orienting. B: A simulation of the switching action of the switch dyads mediating sensory input to bilateral As2/3 interneurons of the turn network, constructed with AnimatLab software.
Figure 3.6 (Cont.)

A

High feeding threshold

AVOIDANCE

Low feeding threshold

ORIENTING

B

LAS2/3

LAS2/3 during feeding

RAS2/3

RAS2/3 during feeding
Table 3.1 Differential turn motor nerve activity during avoidance/orienting

Mean relative spike frequencies in corresponding turn nerves were counted and compared. Turn motor nerve exhibited higher spike frequencies during avoidance compared to orienting.

**Motor nerve mean relative frequency (Hz) after stimulation (N=4)**

<table>
<thead>
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<th>Period after Stimulus</th>
<th>Avoidance</th>
<th>Orienting</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MEAN</td>
<td>SEM</td>
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</table>

**U test results**

<table>
<thead>
<tr>
<th>Avoidance</th>
<th>Orienting</th>
<th>U</th>
<th>P (two-tailed)</th>
<th>P (one-tailed)</th>
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</thead>
<tbody>
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<td>93</td>
<td>6720</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
CHAPTER 4
SEROTONERGIC MODULATION OF CHOICE OF TURN

4.1 Abstract

Serotonin is an intrinsic modulator of the animal’s general food arousal state (Palovcik, 1982) and its levels in the feeding network neurons change with animal’s hunger state (Hatcher et al., 2008).

Previously we reported that effects of an animals’ readiness to feed were conserved in the fictive turn decision of the isolated central nervous system (CNS) and the homeostatic feeding network controls a switch between avoidance and approach decision (Hirayama and Gillette, 2012).

We have investigated actions of serotonin (5-HT) on the fictive turn decision in response to the sensory stimulus. In intact animals 5-HT acts to reduce feeding thresholds and augment general arousal state. Avoidance/approach turn choice to the unilateral repetitive stimulus application was modified by 5 μM 5-HT injection. In isolated CNS, bath application of 5-HT stimulated fictive feeding and altered the fictive turn response from avoidance to orienting in response to the unilateral sensory nerve stimulus. These results combined with previous results that the feeding network excitation state manifests animal’s appetitive state (Hirayama and Gillette, 2012), suggesting that 5-HT changes the fictive turn preference by enhancing the feeding network excitability.

Materials found in Figure 4.3 are works by Hatcher N.
4.2 Introduction

From worms to mammals, 5-HT (5-hydroxytryptamine) modulates the animals’ appetitive states. In mammals, hypothalamic serotonin regulates the eating behavior (Leibowitz and Alexander, 1998). In Caenorhabditis elegans, feeding behaviors as well as fat regulation were controlled by serotonin (Srinvasan et al., 2008). Similarly, in gastropod molluscs, it has been shown that 5-HT influences the animal’s appetitive state in Pleurobranchaea and Aplysia (Palovocik et al., 1982). In Aplysia, the serotonergic metacerebral neuron (MCC) regulates the feeding-induced arousal by modifying the feeding central pattern generator (CPG) for biting (Rosen et al., 1989). In Pleurobranchaea, a recent study demonstrated that 5-HT concentration in the homologous neuron was 4 fold higher in hungry donors than satiated (Hatcher et al., 2008). Thus the level of 5-HT in feeding network manifested the hunger state of the animal.

It is reasonable to think that serotonin can affect the motivational state of the animals, and thereby change the behavioral decision dependent on animal’s readiness to feed. However, few studies have been done about serotonin mediated cost-benefit decision making.

Previously, we reported that the animals’ readiness to feed was conserved in the fictive turn decision in the isolated CNS. The CNSs from less hungry and hungry animals respond to unilateral oral veil nerve stimulation with the contralateral motor output for an avoidance turn and the ipsilateral motor output for orienting, respectively. The excitation state of the feeding network tripped a neuronal switch between avoidance and orienting. We took advantage of this simple system that demonstrates avoidance/approach decision to sensory stimuli, and expanded investigation to the modulatory roles of serotonin on cost-benefit based decision making.
Consequently, injection of serotonin (5-HT) or its precursor 5-hydroxytryptophan (5-HTP) was found to both lower the feeding threshold and to alter the turn decision to the noxious stimulus in intact animals. Moreover, bath application of serotonin onto the isolated central nervous system acted to excite the feeding network and thereby switched the fictive turn output from avoidance to orienting in response to the electrical stimulus. The results suggest a general model in which serotonin mediates cost-benefit based behavioral decision by adjusting the excitation state of the homeostatic network.

4.3 Methods

Feeding thresholds

Animal feeding thresholds were measured as the minimum concentrations of appetitive stimuli that elicit proboscis extension and biting responses (Davis and Mpitsos, 1971; Gillette et al., 2000). Each animal was put into an aquarium filled with seawater and kept a few minutes for habituation. Betaine (N,N,N-trimethylglycine; Sigma-Aldrich) solutions were used as appetitive stimuli as previously described (Davis et al., 1980; Mpitsos and Collins, 1975; Davis and Gillette, 1978; Gillette et al., 2000). Betaine solution in seawater + 10 mM MOPS at pH 8.0 applied in 1.5 ml volumes to the oral veil with a hand-held Pasteur pipette over 10 seconds in a series of ascending concentrations from $10^{-6}$ to $10^{-1}$ M. The thresholds at which animals elicited proboscis extension and bite (opening and closing of mouth) were recorded. In case in which the animals failed to respond to the highest concentration ($10^{-1}$ M) the next highest value, $10^{0}$, was assigned for proboscis extension and $10^{1}$ for biting threshold (biting threshold is normally higher
than proboscis extension threshold). A control sea-water application was assigned a value of $10^{-7}$. These conventions assign conservative finite values to essentially infinitely high or low thresholds. Data were presented as a function of logarithm of the dilutions; thus, $10^{-1}$ corresponds to -1.0 and so on (Davis et al., 1974). Data were then analyzed with non-parametric statistical tests that best accommodate the threshold conventions described above.

**Behavior**

After measuring the feeding threshold, $10^{-2}$ M taurine (2-aminoethanesulfonic acid) was unilaterally applied to the tentacle of the animal and avoidance/orienting turn response was observed. Taurine acts as a deterrent chemosensory stimulus and $10^{-2}$ M taurine induces avoidance response in non-hungry animals (Gillette et al., 2000). Avoidance/Orienting response was defined as the animal’s turning movement directly away or toward the stimulus application site by contracting the body wall muscles. Animal volume was measured by seawater replacement and the amount of hemolymph was estimated. Freshly prepared 5-hydroxytryptamine creatine sulfate (5-HT; Sigma-Aldrich) in *Pleurobranchaea* saline (see below) was injected into the dorsal surface close to the tail of the animal using 25 gauge syringe needles. Feeding thresholds and turn response to unilateral taurine application were measured again 12 minutes after the injection. 12 minutes duration had previously been found to be a time at which robust effects of 5-HT were measurable (Gillette R, Personal communication).
Electrophysiological recording

Isolated CNS Preparation

Shortly after measuring the feeding threshold, the animals were anesthetized by cooling to 4 °C. The CNSs, consisting of cerebropleural, pedal, and buccal ganglia, were dissected out and pinned in a Sylgard dish under Pleurobranchaea saline (in mM) 460 NaCl, 10 KCl, 25 MgCl2, 25 MgSO4, 10 CaCl2, and 10 MOPS buffer at pH 7.5 and 12-13 °C.

Electrophysiology

For intracellular recordings, connective tissues were carefully removed. Intracellular and extracellular recordings and nerve stimulation were made with 3 M KCl-filled glass micropipettes and polyethylene suction electrodes, respectively. Data were acquired with Power Lab software (AD Instruments). Fictive turns were induced by brief, unilateral stimulation of one of the bilateral pair of Large Oral Veil Nerves (LOVN; 15 hz, 2 msec pulse duration; Jing and Gillette, 2003). Data were captured and analyzed with Chart 5 Pro (AD Instruments). Fictive turn events were characterized by comparing mean spike frequencies in bilateral lateral body wall nerves (LBWNs), which are motor outputs for the turn network (Jing and Gillette, 2003). Spikes were counted after selecting a threshold level above spontaneous noise. Spike frequencies were normalized to spike counts for 20 seconds prior to the stimulus event and plotted in 2-3 second bins. The non-parametric Mann-Whitney U-test was used for statistical analysis. The p values were calculated by comparing the spike counts in ipsilateral and contralateral LBWNs for 30 seconds from the first steep inflection following the initial peak. The initial peak corresponds to a fictive withdrawal (Jing and Gillette, 2003) preceding the turn,
as in intact animals. Criterion for assigning “fictive avoidance” vs. “fictive orienting” to LBWN activity was a significant difference with at least p<0.05 for bilateral spike counts (Hirayama and Gillette, 2012).

For serotonin bath application, 5-HT (5-50 μM final concentration) was directly applied to the recording chamber with pipette.

4.4 Results

**Serotonin lowers the feeding threshold and affects the choice of turn in intact animals**

Previously it was shown that injection of serotonin (5-hydroxytryptamine; 5-HT) or its precursor, 5-Hydroxytryptophan (5-HTP), lowered animal’s feeding thresholds (Moroz and Gillette, unpublished). Effects of 5-HT were observed as early as 12 minutes after the injection and those of 5-HTP were seen 10 hours after injection. Therefore feeding threshold as well as the turn response to taurine (deterrent stimulus) were measured 12 min after the injection of 5 μM (final concentration) of 5-HT. Results showed that biting feeding thresholds measured from 8 animals were significantly lowered after injection of 5-HT in intact animals (Figure 4.1). The effect of serotonin injection on feeding thresholds lasted over 40 minutes. Two control injections of saline resulted in no significant difference.

Turn responses to taurine were also altered 12 min after the injection of 5 μM (final concentration) of 5-HT (Figure 4.2). Unilateral application of 10⁻² M taurine (in seawater) normally induces avoidance response in high feeding threshold animals (Gillette et al., 1991; Gillette et al., 2000). Among 14 animals tested, 11 animals avoided the taurine stimulus and 3
animals showed null (no turn) response initially. However, 12 minutes after the injection of serotonin, avoidance turn response was not observed upon stimulus (N=4) or orienting turn was elicited upon stimulus (N=8), and only 2 animals showed avoidance (Figure 4.2).

Modulatory effect of 5-HT on the feeding network

Effects of 5-HT on the feeding network were tested. Resulting feeding network activity was concentration-dependent, with lesser and more variable effects at the lower concentrations of 5 µM. We used this low concentration to compare the sensitivity to 5-HT of CNS from 5 hungry and 5 non-hungry animals. Extracellular recordings were made from the buccal retractor motor nerve R3 (Davis et al., 1973). In the CNS from both hungry and non-hungry animals 5-HT significantly raised average spike frequency (Figure 4.3A, top; p<0.05, 1-tailed t-test). However, there were no robust differences in 5-HT stimulation of motor activity between the CNS of hungry and satiated donors (p>0.75; 2-tailed t-test). Furthermore, 5µM application of 5-HT activated the retractor feeding motor neuron (MN) on the buccal ganglion (Figure 4.3A, bottom).

50µM 5-HT application also drove the metacerebral giant neuron (MCG) (Figure 4.3B, top) by providing the inward current (Figure 4.3B, bottom; Hatcher NG, PhD thesis). MCGs are known as serotoninergic feeding interneurons bilaterally located on the cerebropleural ganglion and to innervate feeding network neurons located on buccal ganglion to elicit feeding motor patterns (Gillette and Davis, 1977).
Modulatory effect of 5-HT on the choice of turn

Previous findings indicated that animals’ appetitive states were conserved in the approach/avoidance turn decision in the isolated CNS and the modifiable excitation level of the feeding network determined the animal’s approach/avoidance decision (Hirayama and Gillette, 2012). Thus, we intended to investigate whether serotonin accounts for this appetitive state dependent turn decision. Extracellular recording of the isolated CNS showed that applying 5-HT affected fictive turn responses in Pleurobranchaea. Specifically, the CNS from donor animals with high feeding threshold (above 10⁻¹M betaine threshold) responded with fictive avoidance to the unilateral sensory nerve stimulus initially. However, upon bath application of 5μM 5-HT, the same CNSs showed fictive orienting to the stimulus (Figure 4.4; N=6). Increased overall nerve activities were observed both in turn and feeding motor nerves right after the application of 5-HT (Figure 4.5; Table 4.1; N=5) and gradually declined over 10-20 minutes. The turn switch response was observed 5 minutes after the application of 5-HT.

4.5 Discussion

Serotonin acts as a modulator of internal state, switching the homeostatic network state

Internal state (hunger state) is represented in the concentration of various neuromodulators in the nervous system of animals. In Aplysia and Pleurobranchaea, the level of 5-HT in feeding network neurons is an indicator of the hunger state (Hatcher et al., 2007). In other animals like Caenorhabditis elegans and Drosophila, neuropeptide F (homologue of human neuropeptide Y) is also known to act as a food arousal factor (de Bono and Bargmann,
1998; Shen and Cai, 2001). In mammalian systems orexin seems to have a functional similarity, controlling the appetite and food arousal activity (Gillette, 2006). We expected that modulators including serotonin from feeding network may determine the appetitive state dependent choice of turn outputs. We demonstrated that serotonin modifies the internal state by lowering the feeding threshold, and in turn, switching the turn output from avoidance to orienting in response to the unilateral sensory stimulus. Moreover, our results suggested that changing available serotonin level altered the activity of the feeding network. When the isolated CNS was bathed in serotonin, the feeding motor nerve and motor neurons were excited. Thus, serotonin modifies the state of the homeostatic feeding network, leading to the modulation in animal’s appetitive behavior. Finally, the fictive turn response recorded from the isolated CNS was also altered by serotonin application. Combined with our previous study that the activity level of feeding network manifests the appetitive state and outputs the appropriate turn choice (Hirayama and Gillette, 2012), our current results suggest that serotonin mediates the hunger state dependent turn choice by changing the activity level in the homeostatic network. The model summarizing this behavioral choice mechanism was illustrated in Figure 4.6. Here the animal’s appetitive state is based on the animal’s sensations (incentive and aversion), internal state (hunger state), and learning (not explicitly shown). In hungry animals, readily available higher level of 5-HT accounts for the excitatory state of the homeostatic feeding network, and in turn controls the appetitive behavior. The activity level of this homeostatic feeding network manifests the animal’s appetitive state and therefore toggles the switch between avoidance and orienting in response to the sensory stimulus.
Mechanism of neuromodulatory effect of serotonin on the feeding network

Our current study on the serotonergic feeding network interneurons, MCG (metacerebral ganglion) cells, showed that serotonin enhanced the activity of MCG by providing the inward current (Figure 4.3; Hatcher NG, unpublished). MCGs are highly conserved feeding interneurons existing in other gastropod including *Aplysia* (Kupfermann et al., 1979) and *Lymnaea* (Yeoman et al., 1996). They similarly project axons to the buccal ganglion cells and control feeding and other consumatory behaviors by targeting part of the feeding pattern generating neurons. Previous works demonstrated that elevated level of 5-HT was seen in the soma of MCG in hungry animals (Hatcher et al., 2007). Thus higher level of readily releasable serotonin from MCG to the feeding network contributes to the excitatory state of the homeostatic network. Figure 4.3 showed that 5-HT provided slow inward current. One possible mechanism of serotonin’s neuromodulatory action is through the cAMP dependent Na+ current. cAMP dependent Na current exists in many molluscan feeding network neurons including MCG (Sudlow et al., 1993, Potgieter et al., 2010) and may contribute to the potentiation of the feeding motor network. Thus the enhanced neuromodulatory effect of 5-HT accounts for the boosted excitability of the feeding network in the CNS of hungry donor animals.

The homologue of serotonergic networks exists in other nudibranch mollusks with divergent behaviors (Newcomb and Katz, 2007). In *Tritonia*, serotonin modifies the swimming network both as a fast neurotransmitter and as a slower neuromodulator (Katz, 1998). Thus serotonin’s actions as part of the large serotonergic network may be multifunctional and control varying behavioral choices.
Serotonin is involved in behavioral choice

Recently, the behavioral choice mechanism between feeding and the shortening response to tactile stimulation was reported in medicinal leech Hirudo (Kemenes, 2009; Gaudry and Kristan, 2009). Interestingly the feeding dominance over the shortening response was mediated with serotonin. According to the authors, serotonin presynaptically inhibits the mechanosensory transmission that leads the shortening response, causing the suppression of shortening. Although the mechanical details may be different, similar mechanisms can exist in Pleurobranchaea. Our system, however, may explain the dual decision making mechanisms in which the feeding network interacts with the turn network, making the feeding vs. turn decision, and the turn network is in turn asymmetrically activated, leading the approach/avoidance decisions.

Considering all these results, it is reasonable to suggest that serotonin is one of the modulators that account for feeding mediated behavioral switching. Future research may reveal mechanical details of these neuromodulatory effects originating from the feeding network and their actions on the turn network. Our simple models may become the basis of more complex cellular level of decision making where hunger (internal) state modifies the choice of behaviors.
4.6 References


4.7 Figures and Table

**Figure 4.1 5-HT significantly lowers the animal’s feeding threshold**

Betaine feeding thresholds for proboscis extension and biting responses were measured 12 minutes after the injection of 5-HT (5 μM final hemolymph concentration). Serotonin, but not saline, injection lowered the animal’s feeding thresholds (N=8; p<0.05 for biting, 2-tailed Mann-Whitney U-test).
Figure 4.2 5-HT affected the choice of turns to noxious stimulus in intact animals

Responses to the unilateral application of the deterrent stimulus were compared after the injection of 5μM 5-HT to the intact animals. Among 14 animals tested, 11 animals showed avoidance and 3 animals showed null response (no turn) to the unilateral application of taurine (10^{-2} M) to the tentacle. 12 minutes after injection of serotonin, avoidance turn response was not observed upon stimulus (N=4) or orienting turn was elicited instead (N=8), and only 2 animals showed avoidance.
Figure 4.3 5-HT enhanced the feeding motor pattern and excited the feeding network neurons

A: Patterned motor output recorded from R3 feeding motor nerve was enhanced (top) and retraction motoneuron (MN) was driven (bottom) by 5μM 5-HT application. 5-HT significantly raised the average spike frequency in R3 recorded from the CNSs both hungry (N=5) and non-hungry (N=5) donors (p<0.05, 1-tailed t-test). There were no robust differences in R3 activity between the CNSs of hungry and non-hungry donors (p>0.75; 2-tailed t-test). B: 50μM 5-HT application drove the feeding interneurons, Metacerebral giant neuron (MCG), and caused the inward current. MCG is known as serotonergic feeding network interneuron that innervates the feeding network neurons in the buccal ganglion (Gillette and Davis, 1977). Modified from Hatcher NG, thesis.
Figure 4.4 5-HT changed the fictive turn preference from avoidance to orienting in non-hungry donors

5-HT (5 μM final concentration) application to the isolated CNS changed fictive turn preference from avoidance to orienting in non-hungry donors (Betaine biting thresholds were above $10^{-1}$ M CNS (N=4). Higher spike frequency observed in the contralateral lateral body wall nerve (cLBWN) in response to the unilateral large oral veil nerve (LOVN) stimulation corresponded to fictive avoidance (A; p<0.0001, Mann-Whitney U Test). 5 minutes after 5-HT application, higher spike frequency occurred in the ipsilateral lateral body wall nerve (iLBWN) in response to the LOVN stimulation corresponding to fictive orienting (B; p<0.0001).
Figure 4.5 5-HT contributes to overall increase in feeding motor nerve activities

Overall increase in nerve activities was observed right after the application of 5µM 5-HT application. Feeding motor nerve R3 was excited and showed bursting activities (N=5). These increased nerve activities gradually decreased over 10-20 minutes after the application of 5-HT.
**Figure 4.6 Appetitive state is regulated by modifying 5-HT levels and feeding network activity state**

Results predict that animal’s appetitive state (readiness to feed) is modifiable by changing the level of available 5-HT and the homeostatic feeding network excitation state. 5-HT is abundant in the feeding network *Pleurobranchaea*, and its concentration changes with the animal’s hunger state (Hatcher et al., 2008). Injecting serotonin switched the animal’s approach/avoidance preference to the deterrent stimulus by lowering feeding threshold in intact animals (Figure 4.1&4.2), and 5-HT bath application switched the fictive turn responses of the isolated CNS (Figure 4.4). Furthermore, 5-HT was found to excite the feeding network in the isolated central nervous system (Figure 4.3). Previous evidence suggested that excitation state of this homeostatic feeding network controls the appetitive behavior (Hirayama and Gillette, 2012). Thus the model summarizes 1) the animal’s appetitive state is embodied in the feeding network and regulated by animal’s internal (hunger) state and sensory inputs (incentive/aversion), 2) a readily available higher concentration of 5-HT in hungry animals activate the feeding network and, in turn, 3) regulates the approach/avoidance choice behavior by reconfiguring the directional motor network.
Figure 4.6 (Cont.)

[Diagram showing the relationships between internal (hunger) state, 5-HT, sensory input, incentive, aversion, feeding, satiation, suppression, avoidance, orienting, and directional motor network.]
Table 4.1 Feeding motor nerve was activated after 5-HT bath application

R3 feeding motor nerve recorded from isolated central nervous was activated immediately after the application of 5μM 5-HT. Frequencies of cyclic bursting activities were increased (table below). These increased nerve activities gradually decreased over 10-20 minutes after the application of 5-HT.

<table>
<thead>
<tr>
<th></th>
<th>Pre 5-HT</th>
<th>Post 5-HT</th>
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<tbody>
<tr>
<td><strong>Frequency of bursts</strong></td>
<td></td>
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<tr>
<td>in R3 (Hz)</td>
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<tr>
<td>Mean</td>
<td>0.00520</td>
<td>0.00913</td>
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<tr>
<td>SEM</td>
<td>0.00842</td>
<td>0.01992</td>
</tr>
<tr>
<td>Mean</td>
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<td>0.03448</td>
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<tr>
<td>SEM</td>
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<td>0.01825</td>
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<tr>
<td>Mean</td>
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<td>0.01418</td>
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<tr>
<td>SEM</td>
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<td>0.01919</td>
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<tr>
<td>Mean</td>
<td>0.00053</td>
<td>0.00425</td>
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(P<0.01, two-tailed U test)
CHAPTER 5

CONCLUSION

In summary, the current study demonstrated that approach/avoidance turn decision-making was based on the excitatory state of the feeding network and on asymmetric activities in the turn network interneurons that code the direction of turns. Spontaneous cyclic activities in the feeding motor nerves expressed the appetitive state of the animals. The homeostatic feeding network state therefore encoded the motivational state of the animal and adapted the threshold necessary for the transitioning from one type of behavioral output to the other in response to the sensory stimulus. The isolated central nervous system retained the donor appetitive state, thereby expressed the appropriate choice of turn to the stimulus.

Results also indicated that the level of available serotonin in the nervous system altered the turn behavioral decisions: higher levels of serotonin increased the animal’s readiness to exhibit appetitive behaviors. This study demonstrated that adding serotonin to the nervous system augmented the state of the feeding network. Thus serotonin may be one of the modulators that convey information about the animal’s general internal (hunger) state to mediate cost-benefit based behavioral decisions.

These behavioral observations and electrophysiological recordings agree with the computational model of turn choice in the nervous system. Our simplest model demonstrated that 1) corollary output from the feeding network to the premotor network altered the configuration of the turn network to promote orienting turn response to the stimulus, and 2) the turn network is readily organized to exhibit avoidance in default. This simple model may well be the basis of economic decision-making where the nervous system computes the risk and
payoff to choose the appropriate behavioral output. The default turn network state allows animals to avoid the stimulus when the risk is high or animals are in non-consummatory state. On the other hand, in the condition where animal is in need of foraging, animals choose to exhibit orienting by modifying the configuration of premotor network via the activated homeostatic feeding network. A possible advantage of this reconfiguration could be that it allows the animal’s body orientation to display ongoing adjustment in order to keep pointing to the prey in case of changing external influences.

Further research may identify specific interneurons that bridge the feeding and the turn network and incorporate these in the model for completion. We suggest that these decision-making mechanisms, demonstrated in the simple system of *Pleurobranchaea californica*, provide insights into the economic decision-making processes in more complex systems including mammals.