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Change in excitability of a putative decision-making neuron in *Aplysia* serves as a mechanism in the decision not feed following food satiation

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Abstract

Although decision making is a ubiquitous function, the understanding of its underlying mechanisms remains limited, particularly at the single-cell level. In this study, we used the decision not to feed that follows satiation in the marine mollusk *Aplysia* to examine the role of putative decision-making neuron B51 in this process. B51 is a neuron in the feeding neural circuit that exhibits decision-making characteristics *in vitro*, which bias the circuit toward producing the motor programs responsible for biting behavior. Once satiated, *Aplysia* decided not to bite for a prolonged period of time (24 h) when presented with a food stimulus that normally elicits feeding in non-satiated animals. Twenty-four hours after satiation, suppressed feeding was accompanied by a significant decrease of B51 excitability compared to the control group of unfed animals. No differences were measured in B51 resting membrane properties or synaptic input to B51 between the satiated and control groups. When B51 properties were measured at a time point in which feeding had recovered from the suppressive effects of satiation (i.e., 96 h after satiation), no difference in B51 excitability was observed between satiated and control groups. These findings indicate that B51 excitability changes in a manner that is coherent with the modifications in biting resulting from food satiation, thus implicating this neuron as a site of plasticity underlying the decision not to bite following food satiation in *Aplysia*.

Keywords

Aplysia; decision making; excitability; feeding; nonsynaptic plasticity; satiation

In recent years, great progress has been made toward understanding the neural bases of decision making, such as the discovery of specialized “decision-making neurons”, the

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Conflict of interest

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activity of which is correlated with the making of decisions (reviewed by [1,2]). Characterizing the properties of individual decision-making neurons within known neural circuits would further elucidate the logic of the process of decision making. However, the complexity of decision-making circuits in humans and other vertebrates, as well as the lack of detailed information about the biophysical underpinnings of individual decision-making neurons, has made this depth of analysis difficult to achieve [3,4]. Furthermore, data directly relating the biophysical properties of decision-making neurons to the execution of the downstream behaviors are limited. In this study, we analyzed the role of a putative decision-making neuron (B51) in the elementary decision not to feed that follows satiation in the marine mollusk *Aplysia*.

B51 is a premotor neuron [5–7] in the neural circuit underlying the consummatory phase of feeding (i.e., the sequence of all-or-nothing bites [8]), which is the component of the feeding response that leads to the ingestion of food and can be modulated by internal and external stimuli (e.g., [9–13]). B51 is active during ingestive buccal motor programs (BMPs) [7,14] that underlie the cycles of protraction, closure and retraction of the radula generated during bites [15,16]. Specifically, this neuron exhibits intrinsically-generated bursts of action potentials (i.e., plateau potentials) [5,17] that occur during the radula retraction/closure phase of ingestive BMPs [7,14].

In vitro, B51 exhibits decision-making features that enable it to switch in an all-or-nothing fashion from an inactive sub-threshold state to an active state in which plateau potentials correlate with the expression of ingestive BMPs [14,18]. When B51 is artificially depolarized above threshold during the occurrence of a BMP, the nature of that BMP is likely to be ingestive [14]. Conversely, when B51 is artificially hyperpolarized to prevent the occurrence of plateau potentials, the type of BMPs elicited is not ingestive [14]. Therefore, although not essential for the elicitation of BMPs (a function attributed to other neurons known as pattern-initiating neurons, such as B31/32 and B63 [6,7]), B51 operates as if it “decides” whether or not the feeding neural circuit executes ingestive BMPs and, consequently, bites *in vivo* [7, 14].

The ability of B51 to influence the selection of a specific motor program *in vitro* offers the unique opportunity to examine the decision-making functions of this neuron in behavioral states that modulate feeding. Indeed, previous work has identified B51 as a locus of plasticity following associative and nonassociative forms of learning that alter feeding. In particular, B51 excitability is increased following an operant reward learning protocol that enhances feeding [17] and is decreased after the presentation of aversive external stimuli that suppress feeding [19]. However, it remains unknown whether B51 is also a site of plasticity underlying changes in the animal’s internal state that influence its decision to feed. Therefore, in this study, we manipulated the animal’s internal state using food satiation and analyzed the role of B51 in the decision not to feed associated with this behavioral state.

In *Aplysia*, food satiation produces a robust suppression of feeding that lasts for at least 24 h [8,20]. To capture the contribution of B51 to the suppression of feeding, we used a behavioral testing procedure that utilizes a seaweed extract (SWE) to isolate the consummatory phase of feeding during which B51 is selectively active. SWE provides a

tonic chemical stimulus that triggers the elicitation of bites [13,17] with minimal or no expression of the appetitive phase of feeding, which includes head-up posture and head waving [8,20] (see Supplementary Methods for details). Bites were counted during 5-min tests of SWE exposure, conducted prior to (pre-test) and 24 h after satiation (post-test; Fig. 1A). During the satiation treatment, animals were fed pieces of seaweed until they failed to ingest the seaweed for two consecutive 90-s periods of continuous stimulation [20]. Animals in the control group were handled identically except that they were not fed. For each animal, the difference in bites (bites measured during post-test minus bites measured during pre-test) was used as the index to assess changes in feeding resulting from treatment [13,19]. Differences in bites were compared using the Mann-Whitney *U* test [13,19]. In all experiments, data were collected by an investigator who was unaware of the treatment history of the animal. Additional detailed information about testing, satiation and control procedures are provided in the Supplementary Methods.

Twenty-four hours after treatment, the satiated group exhibited a significant decrease in the number of bites (-15.0 ± 2.3 bites, $n = 9$) compared to the control group (8.8 ± 4.1 bites, $n = 8$; $p < 0.05$; Fig. 1B). Notably, bites were reduced to zero 24 h after treatment in all satiated animals, regardless of their pre-test values (bites pre-test: 15.0 ± 2.3 bites, $n = 9$; bites post-test: 0.0 ± 0.0 bites, $n = 9$). In controls animals, bites slightly increased 24 h after treatment (bites pre-test: 12.4 ± 2.0 bites, $n = 8$; bites post-test: 21.1 ± 3.6 bites, $n = 8$). These findings indicate that the satiation treatment produced a robust and lasting suppression of feeding.

Although these experiments selectively targeted the effects of satiation on the consummatory phase of feeding, additional findings revealed that satiation does not alter the appetitive phase of feeding (Supplementary Fig. 1). The lasting duration of suppressed feeding is ideal to analyze the role of B51 in the decision not to feed as it allows one to isolate the buccal ganglion (the part of the *Aplysia* nervous system that contains the feeding neural circuit [6,7]) and analyze B51 activity while the induced behavioral changes persist [12,19]. Therefore, the next experiment analyzed whether food satiation altered the activity of B51 24 h after treatment.

Two groups of animals were first examined at the behavioral level by testing feeding prior to and 24 h after treatment (satiation or control; Fig. 2A). Twenty-four hours after treatment, the satiated group exhibited a significant decrease in the number of bites (-20.1 ± 2.6 bites, $n = 19$) compared to the control group (-2.9 ± 1.6 bites, $n = 18$; $p < 0.05$; Fig. 2B), indicating that satiation produced a robust, lasting suppression of feeding in this cohort of animals. Immediately following the 24-h post-test, buccal ganglia were prepared for measurement of B51 properties (Fig. 2A), using previously-described methodologies [18,19] (see also Supplementary Methods for details). Briefly, the buccal ganglion was removed from the buccal mass with one of the two buccal nerves 2,3 (denoted as n.2,3) [16] and was desheathed to expose the soma of B51 [5,14,17]. B51 membrane properties (resting potential, input resistance and excitability) were measured using standard two-electrode current-clamp technique. B51 excitability was assessed by determining the threshold to elicit a plateau potential (i.e., burst threshold) [14,18,19]. Ten minutes after determining the burst threshold, satiation-induced changes in the synaptic inputs to B51 were evaluated by stimulating n.2,3 for 2 min. This stimulation initially evokes a primarily inhibitory complex

postsynaptic potential (cPSP) in B51 [19] and then leads to the recruitment of B51 into the feeding neural circuit [14] (Suppl. Fig. 2A; see also Supplementary Methods for details). Membrane and synaptic properties of B51 were compared between satiated and control animals using the Mann-Whitney *U* test [18,19].

Twenty-four hours after treatment, B51 burst threshold was significantly greater in the satiated group (19.0 ± 2.4 nA, $n = 11$), compared to the control group (10.9 ± 1.6 nA, $n = 8$; $p < 0.05$; Fig. 2C1,C2). No significant differences in resting potential (satiated: -59.7 ± 1.5 mV, $n = 14$; control: -60.0 ± 2.3 mV, $n = 11$; $p = 1.00$; Fig 2D1) or input resistance (satiated: 2.0 ± 0.3 M Ω , $n = 12$; control: 2.0 ± 0.2 M Ω , $n = 8$; $p = 0.22$; Fig. 2D2,D3) were observed between the satiated and control groups.

Next, to provide a comprehensive characterization of the effects of satiation on the synaptic input to B51, two approaches were used: 1) analysis of the cPSP evoked by the initial 2 s of n.2,3 stimulation and 2) analysis of the synaptic drive (i.e., inhibitory input followed by a graded excitatory potential; Suppl. Fig. 2A) that culminates with the onset of the plateau potential during B51 recruitment into the feeding neural circuit. The analysis of the n.2,3-evoked cPSP revealed that neither the peak amplitude (satiated: -9.4 ± 1.3 mV, $n = 10$; control: -9.3 ± 0.9 mV, $n = 12$; $p = 0.92$, Fig. 2E1,E2) nor the area of the cPSP (satiated: 13.5 ± 2.5 arbitrary units, $n = 10$; control: 12.2 ± 1.8 arbitrary units, $n = 12$; $p = 0.67$) differed between satiated and control animals. The analysis of the synaptic drive during B51 recruitment revealed that the peak amplitudes of the inhibitory (satiated: -6.4 ± 0.6 mV, $n = 11$; control: -5.7 ± 0.7 mV, $n = 10$; $p = 0.55$, Suppl. Fig. 2B1) and excitatory synaptic inputs (satiated: 18.5 ± 1.3 mV, $n = 11$; control: 16.9 ± 1.2 mV, $n = 10$; $p = 0.38$, Suppl. Fig. 2B2) did not differ between satiated and control animals. Similarly, no difference was found in the areas of the inhibitory (satiated: 250.7 ± 94.4 arbitrary units, $n = 11$; control: 158.4 ± 33.5 arbitrary units, $n = 10$; $p = 0.53$; Suppl. Fig. 2C1) and excitatory synaptic inputs (satiated: 253.7 ± 46.8 arbitrary units, $n = 11$; control: 267.7 ± 61.3 arbitrary units, $n = 10$; $p = 0.92$; Suppl. Fig. 2C2) between satiated and control animals. To assess the effects of satiation on the balance between excitatory and inhibitory inputs, we also examined the [excitatory/inhibitory] area ratio for each satiated and control preparation. No difference was found in the [excitatory/inhibitory] area ratio between satiated (3.0 ± 1.2 ; $n = 11$) and control preparations (3.1 ± 1.0 , $n = 10$; $p = 0.82$).

The lack of change in B51 resting properties as well as synaptic inputs both at the onset of nerve stimulation and during B51 recruitment into the feeding neural circuit indicate that the increase in burst threshold reflects a decrease in excitability that occurs intrinsically to the cell, thus implicating B51 as a site of plasticity underlying the long-term suppression of feeding produced by satiation.

If the decrease in B51 excitability plays a role in the decision not to bite following satiation, it should be observed only when biting is suppressed and should be absent once biting has recovered from the suppressive effect of satiation. To address this issue, it was first necessary to determine at which time point feeding resumed from satiation. A pilot study revealed that feeding is resumed 96 h after the satiation treatment (see Supplementary Results for details). Consequently, the next experiment explored whether differences in B51

membrane properties and synaptic input between satiated and control animals were observed 96 h after satiation.

Feeding was examined 24 h and 96 h after treatment in satiated and control animals (Fig. 3A). At 24 h, the satiated group exhibited a significant decrease in the number of bites (-17.9 ± 1.2 bites, $n = 42$) compared to the control group (3.3 ± 1.4 bites, $n = 40$; $p < 0.05$; Fig. 3B1), confirming that the satiation treatment effectively suppressed feeding in this cohort of animals. At 96-h post treatment, bites were no longer significantly different between the satiated (-2.1 ± 1.8 bites, $n = 42$) and the control group (1.8 ± 1.4 bites, $n = 40$; $p = 0.11$; Fig. 3B2), indicating that feeding had recovered from the suppressive effects of satiation.

Immediately following the 96-h post-test, buccal ganglia were prepared for measurement of B51 properties (Fig. 3A). B51 burst threshold was not significantly different between the satiated (13.8 ± 1.2 nA, $n = 33$) and the control groups (14.7 ± 1.4 nA, $n = 34$; $p = 0.96$; Fig. 3C1,C2), indicating that B51 excitability was no longer altered by food satiation 96 h after treatment. Moreover, no significant difference was found between satiated and control animals in resting potential (satiated: -58.4 ± 1.0 mV, $n = 34$; control: -56.5 ± 3.6 mV, $n = 34$; $p = 0.53$; Fig. 3D1), input resistance (satiated: 2.2 ± 0.1 M Ω , $n = 34$; control: 2.4 ± 0.1 M Ω , $n = 33$; $p = 0.22$; Fig. 3D2,D3), peak amplitude (satiated: -7.4 ± 0.8 mV, $n = 26$; control: -6.2 ± 0.6 mV, $n = 27$; $p = 0.30$; Fig. 3E1,E2), or area of the n.2,3-evoked cPSP (satiated: 10.4 ± 1.4 arbitrary units, $n = 26$; control: 8.8 ± 0.9 arbitrary units, $n = 27$; $p = 0.51$).

Our results indicate that changes in B51 excitability contribute to the decision not to bite induced by satiation in *Aplysia*. Because of the all-or-nothing nature of the intrinsically-generated plateau potential in B51 [5,17,18] and its role in motor-program selection [7,14], a decrease of B51 excitability may directly translate into a reduced likelihood of expression of ingestive BMPs and the consequent suppression of feeding observed *in vivo* following satiation. Combined with previous evidence about the modulation of B51 activity by different forms of learning [7,12,19], our findings further support the view that B51 excitability changes coherently with the modulation of feeding induced *in vivo*. Increased excitability sustains increased biting, as occurs in operant reward learning [7,17], whereas decreased excitability sustains reduced biting, as observed following exposure to aversive stimuli [19] and food satiation (Fig. 2 in this study).

The absence of changes in resting membrane potential and input resistance suggests that the satiation-induced decrease in B51 excitability may involve modifications of voltage-dependent channels (reviewed by [21]). Because voltage-dependent Na⁺ and K⁺ channels, rather than Ca²⁺ channels, contribute to the genesis and maintenance of B51 plateau potential [5], changes at the level of these channels likely play a role in the satiation-induced reductions of B51 excitability. Future experiments using selective pharmacological blockers for voltage-dependent Na⁺ and K⁺ channels may characterize the biophysical substrates of the decreased B51 excitability produced by satiation.

Although B51 plays a pivotal role in deciding whether or not a bite is elicited, we expect that other neurons in the feeding neural circuit are altered by satiation. Putative additional

sites of satiation-induced plasticity may include pattern-initiating neurons in the buccal ganglion, such as B31/32 and B63, as well as cerebral command-like interneurons, which have the ability to drive the feeding neural circuit (reviewed by [6]).

In summary, the modulation of B51 excitability represents, at least in part, a nonsynaptic state-dependent mechanism by which the *Aplysia* feeding neural circuit makes the decision whether or not to bite in response to a food stimulus. Work in various invertebrate models has revealed a key role for synaptic plasticity in elementary forms of decision making (e.g., [22–24]). By showing that changes in neuronal excitability can also contribute to an elementary decision in *Aplysia*, our findings add an additional dimension to the current understanding of the mechanisms underlying decision making in an invertebrate system, and may ultimately be instrumental in further understanding the process of decision making in more complex organisms.

Growing attention is being given in recent years to the functional relevance of changes in excitability as a mechanism underlying neurobiological mechanisms, including dendritic integration, activity-dependent neuronal plasticity and memory storage (reviewed by [21,25]). Our results provide further validation of the role of nonsynaptic plasticity as a ubiquitous cellular mechanism underlying fundamental brain functions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Research Highlights

- We examined the mechanisms of the decision not to feed after satiation in *Aplysia*
- The decision not to feed was accompanied by decreased excitability of neuron B51
- The decrease of B51 excitability was not observed once feeding had resumed
- Changes in B51 excitability contribute to the decision not to feed after satiation

Fig 1.

Food satiation suppressed feeding 24 h after treatment. (A) Protocol for testing the effects of satiation on feeding. Feeding behavior (biting) was measured prior to (pretest) and 24 h after treatment (post-test) in satiated and control animals. (B) Satiation induced a suppression of feeding 24 h after treatment. In this and in the following figures, data from satiated and control animals are expressed as mean $\pm$ SEM, and compared using the Mann-Whitney U test. Statistical significance was set at $p < 0.05$.

Fig. 2. Satiation reduced B51 excitability through an increase in burst threshold 24 h after treatment. (A) Protocol for testing the effects of satiation on feeding and for the subsequent analysis of B51 properties. (B) Satiation induced a suppression of feeding 24 h after treatment. After the 24-h post-test, animals were sacrificed and B51 membrane properties (C, D) and synaptic input (E) were measured. (C1) Sample traces of B51 burst threshold from satiated and control animals. (C2) Summary data illustrate that satiation significantly increased B51 burst threshold. (D) Satiation did not affect B51 resting membrane properties.

(D1) B51 resting potential was not altered by satiation. (D2) Sample traces of input resistance from satiated and control animals indicating that this membrane property was not affected by satiation. (D3) Summary data illustrate that B51 input resistance did not differ between satiated and control animals. (E) Satiation did not affect the synaptic input to B51. (E1) Sample traces of n.2,3-evoked cPSP in B51 from satiated and control animals. The shaded region underneath each trace indicates the area of the cPSP over 2 s of n.2,3 stimulation. Individual stimuli are indicated with arrows below the recordings. (E2) Summary data illustrate that the peak amplitude of the cPSP did not differ between satiated and control animals.

Fig. 3.

The satiety-induced decrease in B51 excitability was no longer measured once feeding resumed 96 h after treatment. (A) Protocol for testing the effects of satiety 96 h after treatment. Feeding behavior was measured prior to (pre-test) and at two post-test time points after treatment (24 h and 96 h) in satiated and control animals. (B) Feeding was suppressed 24 h after treatment (B1), but recovered 96 h after treatment (B2). After the 96-h post-test, animals were sacrificed and B51 properties were examined (A). (C1) Sample traces of B51 burst threshold from satiated and control animals. (C2) Summary data illustrate that B51

burst threshold did not differ between satiated and control animals. (D) B51 resting membrane properties did not differ between satiated and control animals 96 h after treatment. (D1) B51 resting potential did not differ between satiated and control animals 96 h after treatment. (D2) Sample traces of input resistance from satiated and control animal measured 96 h after treatment. (D3) Summary data illustrate that B51 input resistance did not differ between satiated and control animals 96 h after treatment. (E) The synaptic input to B51 also did not differ between satiated and control animals 96 h after treatment. (E1) Sample traces of n.2,3- evoked cPSP in B51 from satiated and control animals 96 h after treatment. The shaded area underneath each trace indicates the area of the cPSP over 2 s of n.2,3 stimulation. Individual stimuli are indicated with arrows below the recordings. (E2) Summary data illustrate that the peak amplitude the cPSP did not differ between satiated and control animals 96 h after treatment.