**Brief Communication**

**Rapid and persistent suppression of feeding behavior induced by sensitization training in *Aplysia***

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In *Aplysia*, noxious stimuli induce sensitization of defensive responses. However, it remains largely unknown whether such stimuli also alter nondefensive behaviors. In this study, we examined the effects of noxious stimuli on feeding. Strong electric shocks, capable of inducing sensitization, also led to the suppression of feeding. The use of multiple training protocols revealed that the time course of the suppression of feeding was analogous to that of sensitization. In addition, the suppression of feeding was present only at the time points in which sensitization was expressed. These results suggest that, in *Aplysia*, noxious stimuli may produce concurrent changes in neural circuits controlling both defensive and nondefensive behaviors.

[Supplemental material is available for this article.]

Sensitization is an elementary form of learned fear in which defensive responses are enhanced following exposure to aversive stimuli (for review, see Carew and Sahley 1986; Brunelli et al. 1997; Kandel 2001; Fioravante et al. 2008). Great progress in understanding the mechanisms of sensitization has been made using the marine mollusk *Aplysia californica* (for review, see Kandel 2001; Hawkins et al. 2006; Byrne et al. 2009). In *Aplysia*, strong electric shocks applied to the body wall, which mimic the attack of a predator (Watkins et al. 2010), increase defensive responses, including withdrawal reflexes of the gill, tail, and siphon, and escape locomotion (Stoper and Carew 1988; Kandel 2001; Byrne et al. 2009). When paired with stimuli such as seawater of altered salinity or pH, strong electric shocks also enhance other defensive responses, including inking and respiratory pumping (Levy and Susswein 1999).

In order to budget its responses following exposure to aversive stimuli, an organism is also required to change the expression of nondefensive behaviors (Kavaliers and Choleris 2001). Interestingly, whereas modifications of defensive responses have been extensively described in *Aplysia* and other animal models (e.g., Carew and Sahley 1986; Cleary et al. 1995; Frost et al. 1998; Kandel 2001; Zaccardi et al. 2001), the effects of noxious stimuli on nondefensive behaviors have been only marginally explored. Characterizing the effects of noxious stimuli on both defensive and nondefensive behaviors is critical to fully appreciate the means by which encountered stimuli differentially influence the animal’s behavioral repertoire.

Early evidence of the effects of noxious stimuli on nondefensive responses in *Aplysia* was provided by Kupfermann and Pinsker (1968), who reported a reduction of feeding following the delivery of strong electric shocks. However, the effectiveness of the same training protocol to induce changes in defensive responses was not analyzed. Therefore, the goal of this study was to explore whether behaviors of different nature (defensive and nondefensive) were concurrently altered by exposure to noxious stimuli in *Aplysia*. In particular, we examined the ability of different protocols of noxious stimulation to induce both sensitization of a defensive reflex and changes in a nondefensive behavior: feeding.

As readouts of a defensive response and feeding, we used the duration of the tail-induced siphon withdrawal reflex (TSWR) and the occurrence of bites, respectively. The TSWR consists of a contraction of the siphon in response to a brief, mild current pulse delivered through a pair of electrodes implanted into one side of the tail (Supplemental Fig. S1A1; see Supplemental Material for details). The duration of the TSWR from the onset of the contraction to the onset of relaxation of the siphon was used as a measure of reflex strength (e.g., Goldsmith and Byrne 1993; Cleary et al. 1998; Wainwright et al. 2002; Antzoulatos et al. 2006; see Supplemental Material for details). Bites consist of rhythmic movements of the radula (Kupfermann 1974; Susswein et al. 1976; Brema et al. 2002), which are controlled by the activity of a well-characterized neural circuit (for review, see Elliott and Susswein 2002; Cropper et al. 2004). Bites were elicited and counted during a testing period (Supplemental Fig. S1B1; see below and Supplemental Material for details).

Three previously established training protocols were employed to analyze the effect of noxious stimuli on TSWR and feeding: (1) single-trial training, (2) brief shock treatment, and (3) long-term sensitization training (see Supplemental Material for detailed description of the protocols).

We first employed a single-trial training, consisting of one 10-sec train of electric shocks, delivered to the lateral body wall (60-mA maximal intensity, 500-msec impulses, 1 Hz) (Supplemental Fig. S1A1,A2). The single-trial training induces sensitization, which manifests as an increased duration of the TSWR on the side of the animal that received the training (Byrne et al. 1991; Fernandez et al. 2003). The effects of the single-trial training were examined at 15 min, 2 h, and 24 h after treatment in trained and untrained (control) animals (Supplemental Fig. S1A2,B2). We initially characterized the time course of the sensitization of the TSWR induced by the single-trial training. For each time point, the change in TSWR duration (i.e., [post-test TSWR duration] - [pretest TSWR duration]) was calculated to assess modifications.
in the reflex strength due to treatment (e.g., Goldsmith and Byrne 1993; Cleary et al. 1998; Antzoulatos et al. 2006). Data were compared between trained and untrained animals at each time point (Wainwright et al. 2002; Antzoulatos et al. 2006; Miniaci et al. 2008), using the Mann-Whitney U-test. All values are reported as means ± SEM. The change in TSWR duration was significantly greater in trained animals as compared to untrained controls at both 15 min (trained: 3.75 ± 2.14, n = 8; untrained: 0.97 ± 0.13, n = 9; P < 0.05) (Fig. 1A1) and 2 h after treatment (trained: 6.16 ± 2.78, n = 8; untrained: 1.28 ± 0.37, n = 9; P < 0.05) (Fig. 1A2). However, by 24 h after treatment, the change in TSWR duration was no longer significantly different between trained and untrained animals (trained: 1.66 ± 0.73, n = 7; untrained: 1.84 ± 0.59, n = 7; P = 0.81) (Fig. 1A3). These results indicate that the single-trial training produced sensitization of the TSWR. Sensitization with a similar temporal profile has been previously reported following a training protocol consisting of five shocks spaced 1 sec apart (Sutton et al. 2002).

In a separate group of animals, we next examined whether the single-trial training altered the expression of a nondefensive behavior: feeding (see Supplemental Material for details). Feeding was assessed by counting the number of bites generated during a 5-min testing period (i.e., biting test), in which animals were placed in a solution of aquarium seawater containing seaweed extract (Supplemental Fig. S1B1). The seaweed extract provides a tonic chemical stimulus, which readily elicits bites (see Supplemental Material for details; Brembs et al. 2002). Biting tests were conducted before (pretest) and 15 min, 2 h, and 24 h after treatment (post-tests) (Supplemental Fig. S1B2). Treatment-induced changes in feeding were analyzed as a difference in the number of generated bites (i.e., bites during post-test minus bites during pretest) (Lechner et al. 2000; Lorenzetti et al. 2006).

The difference in bites was compared between trained and untrained animals at each time point, using the Mann-Whitney U-test. Trained animals showed a significant decrease in the number of bites compared to untrained controls at both 15 min (trained: −10.67 ± 1.94 bites, n = 15; untrained: −1.60 ± 2.84 bites, n = 15; P < 0.05) (Fig. 1B1) and 2 h after treatment (trained: −12.47 ± 2.29 bites, n = 15; untrained: −3.73 ± 2.81 bites, n = 15; P < 0.0) (Fig. 1B2). However, by 24 h after treatment, the difference in bites was no longer significantly different between trained and untrained animals (trained: −1.80 ± 2.36 bites, n = 15; untrained: −1.13 ± 2.45 bites, n = 15; P = 0.95) (Fig. 1B3). These results indicate that the single-trial training produced a suppression of feeding (Fig. 1B1,B2) with a time course that paralleled the sensitization produced by the same protocol (Fig. 1A1,A2).

In the next series of experiments, we explored whether the expression of sensitization was a requirement for the occurrence of the suppression of feeding. As a training protocol, we employed a brief shock treatment (BST), which does not induce sensitization within 25 min of the treatment (Antzoulatos et al. 2006). BST consisted of a 2-sec train of AC electric shocks, delivered to the lateral body wall (60-mA maximal intensity, 500-msec impulses, 1 Hz) (Supplemental Fig. S2A1). Using two series of experiments, we examined the effects of BST on the TSWR and feeding, respectively. Confirming previous results (Antzoulatos et al. 2006), BST did not induce sensitization 15 min after training (change in TSWR duration, trained: 0.92 ± 0.04, n = 7; change in TSWR duration, untrained: 1.14 ± 0.17, n = 8; P = 0.28) (Fig. 2A1). Similarly, feeding was not significantly altered 15 min after BST (difference in bites, trained: 0.50 ± 2.83 bites, n = 8; difference in bites, untrained: −2.33 ± 1.05, n = 9; P = 0.63) (Fig. 2B1). Interestingly, 2 h after BST treatment, both sensitization (change in TSWR duration, trained: 3.80 ± 1.38, n = 7; change in TSWR duration, untrained: 1.24 ± 0.23, n = 8; P < 0.05) (Fig. 2A2) and suppression of feeding (difference in bites, trained: −5.75 ± 3.20 bites, n = 8; difference in bites, untrained: −0.67 ± 1.14, n = 9; P < 0.05) (Fig. 2B2) were observed.

These findings indicate that, following BST, feeding was not changed at a time point in which sensitization was not observed (15 min) (Fig. 2A1,B1) but was suppressed at the time point in which sensitization was expressed (2 h) (Fig. 2A2,B2), further demonstrating the temporal relation between these two behavioral changes. The concurrent presence/absence of both sensitization and suppression of feeding at different time points suggests a mechanistic relation between these two behavioral changes.

In Aplysia, extended training with repeated presentations of noxious stimuli produces enduring forms of sensitization (e.g., Pinsker et al. 1973; Frost et al. 1985; Cleary et al. 1998; Sutton et al. 2002; Wainwright et al. 2002; Khabour et al. 2004). Does extended training also induce long-lasting suppression of feeding? To address this question, we used a training paradigm that induces sensitization of the TSWR persisting for at least 24 h (long-term sensitization; LTS) (Cleary et al. 1998; Khabour et al. 2004). In this series of experiments, TSWR and feeding were measured in

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Single-trial training produced sensitization of the TSWR and suppression of feeding with similar time courses. (A) The single-trial training produced sensitization of the TSWR, which was observed at 15 min (A1) and 2 h (A2) but not at 24 h after treatment (A3). (B) The single-trial training produced suppression of feeding, which was observed at 15 min (B1) and 2 h (B2) but not at 24 h after treatment (B3). In this and in the following figures, data from trained and untrained animals were expressed as mean ± SEM and were compared at each time point using the Mann-Whitney U-test. Statistical significance was set at P < 0.05.
Figure 2. A brief shock treatment (BST) produced delayed sensitization of the TSWR and suppression of feeding 2 h after treatment. (A) BST did not produce sensitization of the TSWR at 15 min post-treatment (A1) but induced sensitization at 2 h post-treatment (A2). (B) BST did not suppress feeding at 15 min post-treatment (B1) but induced a suppression of feeding at 2 h post-treatment (B2).

The differential effect of noxious stimuli on the TSWR (augmented [Figs. 1A, 2A, 3A]) and feeding (suppressed [Figs. 1B, 2B, 3B]) in Aplysia is consistent with the view of a central arousal system that optimizes behavioral outputs through the concurrent modulation of defensive and nondefensive responses (Walters et al. 1981). The analogous temporal dynamics exhibited by the two behavioral modifications would support the hypothesis of a modulator(s) that, released by noxious stimuli, concomitantly alters the neural circuits controlling the TSWR and feeding. The neurotransmitter serotonin (5-HT) is a potential candidate as a biochemical link connecting the noxious stimuli to the two observed behavioral modifications. 5-HT is released into the hemolymph and into the neuropil following strong electric stimulation of the body wall (Levenson et al. 1999) or after peripheral nerve shock (Marinesco and Carew 2002). Notably, 5-HT plays a key role in mediating changes in the withdrawal reflexes circuits underlying sensitization in Aplysia (e.g., Brunelli et al. 1976; Walters et al. 1983; Glanzman et al. 1989; Sugita et al. 1997; Levenson et al. 1999; Marinesco and Carew 2002; Marinesco et al. 2004a,b). 5-HT also contributes to the modulation of the feeding at both behavioral and cellular levels (e.g., Weiss et al. 1978; Kupfermann and Weiss 1982; Alexeeva et al. 1998; Levenson et al. 1999; Kabotynski et al. 2000), although its modulatory role in the feeding neural circuit is less clear. Indeed, 5-HT appears to be capable of both increasing and decreasing the activity of the feeding neural circuit. For example, the serotonergic metacerebral cells (MCCs) mediate aspects of food-induced arousal (Weiss et al. 1978; Kupfermann and Weiss 1982; Rosen et al. 2004a,b). 5-HT also contributes to the modulation of the feeding at both behavioral and cellular levels (e.g., Weiss et al. 1978; Kupfermann and Weiss 1982; Alexeeva et al. 1998; Levenson et al. 1999; Kabotynski et al. 2000), although its modulatory role in the feeding neural circuit is less clear. Indeed, 5-HT appears to be capable of both increasing and decreasing the activity of the feeding neural circuit. For example, the serotonergic metacerebral cells (MCCs) mediate aspects of food-induced arousal (Weiss et al. 1978; Kupfermann and Weiss 1982; Rosen et al. 2004a,b).
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M.W. from the Texas Research Development Funds. A.A. and K.K. were supported by the DOD reference


M.W. from the Texas Research Development Funds. A.A. and K.K. were supported by the DOD/NSF Research Experiences for Undergraduates grant 0453329. M.S.-J. was supported by NIH EARD grant 5G11HD046353-05. We thank Drs. Douglas Baxter, Leonard Cleary, and Gregg Phares for helpful comments on an earlier draft of the manuscript. We also thank Max Odem and Harris Weisz (Texas A&M University-Corpus Christi) for conducting some of the behavioral experiments using the BST protocol.

This work was supported by start-up funds from the College of Science and Technology to R.M. and by a grant to R.M. and M.W. from the Texas Research Development Funds. A.A. and K.K. were supported by the DOD/NSF Research Experiences for Undergraduates grant 0453329. M.S.-J. was supported by NIH EARD grant 5G11HD046353-05. We thank Drs. Douglas Baxter, Leonard Cleary, and Gregg Phares for helpful comments on an earlier draft of the manuscript. We also thank Max Odem and Harris Weisz (Texas A&M University-Corpus Christi) for conducting some of the behavioral experiments using the BST protocol.

Acknowledgments

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1989), whereas application of 5-HT to the isolated buccal ganglion reduces the excitability and the plateau-like potential of pattern-initiation neuron B31/32 (Susswein and Byrne 1988; Kabotyanski et al. 2000). Albeit complex, the role of 5-HT as a component of the training-induced biochemical pathway leading to changes in different neural circuits is intriguing and warrants future investigation.

The expression of the suppression of feeding in a long-term form (Fig. 3) provides the framework for the characterization of its underlying cellular mechanisms, as it would allow us to identify the cellular sites of plasticity within the feeding neural circuit, while the effects of LTS training persist. Modifications at the level of the decision-making neuron B51, which is critical to bias the feeding neural circuit to generate the neurophysiological activity associated with bites (Plummer and Kirk 1990; Nargeot et al. 1999a,b; Mozzachiodi and Byrne 2010; Nargeot and Simmers 2011), may contribute, at least in part, to the suppression of feeding produced by LTS training. In addition, pattern-initiating neurons B31/32 and B63 (Susswein and Byrne 1988; Hurwitz et al. 1997) are two other putative loci of plasticity that may play a role in the suppression of feeding observed following LTS training.

In conclusion, our findings lay the foundation for a mechanistic analysis of the effects of noxious stimulation on the neural circuits underlying nondefensive behaviors, which, combined with the current knowledge of the cellular underpinnings of sensitization, would help understand how aversive experience shapes the animal's behavioral repertoire.


*Received October 22, 2011; accepted in revised form February 16, 2012.*
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*Learn. Mem.* 2012 19: 159-163
Access the most recent version at doi:10.1101/lm.024638.111

Supplemental Material  http://learnmem.cshlp.org/content/suppl/2012/03/14/19.4.159.DC1.html

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