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Effects of internal and external factors on the budgeting between defensive and non-defensive responses in *Aplysia*

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ABSTRACT

Following exposure to aversive stimuli, organisms budget their behaviors by augmenting defensive responses and reducing/suppressing non-defensive behaviors. This budgeting process must be flexible to accommodate modifications in the animal's internal and/or external state that require the normal balance between defensive and non-defensive behaviors to be adjusted. When exposed to aversive stimuli, the mollusk *Aplysia* budgets its behaviors by concurrently enhancing defensive withdrawal reflexes (an elementary form of learning known as sensitization) and suppressing feeding. Sensitization and feeding suppression are consistently co-expressed following different training protocols and share common temporal domains, suggesting that they are interlocked. In this study, we attempted to uncouple the co-expression of sensitization and feeding suppression using: 1) manipulation of the animal's motivational state through prolonged food deprivation and 2) extended training with aversive stimuli that induces sensitization lasting for weeks. Both manipulations uncoupled the co-expression of the above behavioral changes. Prolonged food deprivation prevented the expression of sensitization, but not of feeding suppression. Following the extended training, sensitization and feeding suppression were co-expressed only for a limited time (i.e., 24 h), after which feeding returned to baseline levels as sensitization persisted for up to seven days. These findings indicate that sensitization and feeding suppression are not interlocked and that their co-expression can be uncoupled by internal (prolonged food deprivation) and external (extended aversive training) factors. The different strategies, by which the co-expression of sensitization and feeding suppression was altered, provide an example of how budgeting strategies triggered by an identical aversive experience can vary depending on the state of the organism.

1. Introduction

Organisms respond to environmental stimuli by budgeting the expression of their responses to optimize their behavioral output [1]. This principle, originally postulated by Charles Sherrington [2], applies to all animals, including humans. If more energy is required to perform a certain action, such as escape locomotion to avoid a predator, that energy is no longer available for any other actions, such as foraging, growing, or mating [3]. In the most basic form, the behavioral responses made by all organisms can be classified as defensive (i.e., those that increase the survivability of the organism when exposed to danger) and non-defensive (i.e., those that maintain the life of the organism when not exposed to a direct threat) [3,4].

It seems logical for an organism to enhance defensive behaviors at the expense of non-defensive behaviors when threatened, because if they fail to defend themselves they most likely will not survive [5,6]. Indeed, the hierarchical model of behavioral choice, in which defensive responses override other behaviors, including feeding, to prevent predation, was originally shown at the ethological level [7,8] and was later validated at the cellular level [9,10]. There are circumstances, however, in which this choice becomes more complicated, such as when the organism is extremely hungry, or when it is challenged with extreme threat of predation making foraging dangerous for prolonged periods of time. Faced with such situations in which avoiding feeding for prolonged periods could also result in death, an organism must assess the risks associated with foraging to determine if nutritional gain outweighs predation risk [1,5,11,12].

Characterizing how internal and external stimuli differentially modulate the balance between defensive and non-defensive responses over time is, therefore, critical to understand the means by which experience generates and maintains the complex adaptive behavioral output. However, although the effects of aversive experience on defensive and non-defensive responses have been individually studied in vertebrates and invertebrates [3,4,13–15], the relationship between these changes is not yet fully understood. Moreover, previous work on behavioral budgeting primarily describes the interactions between

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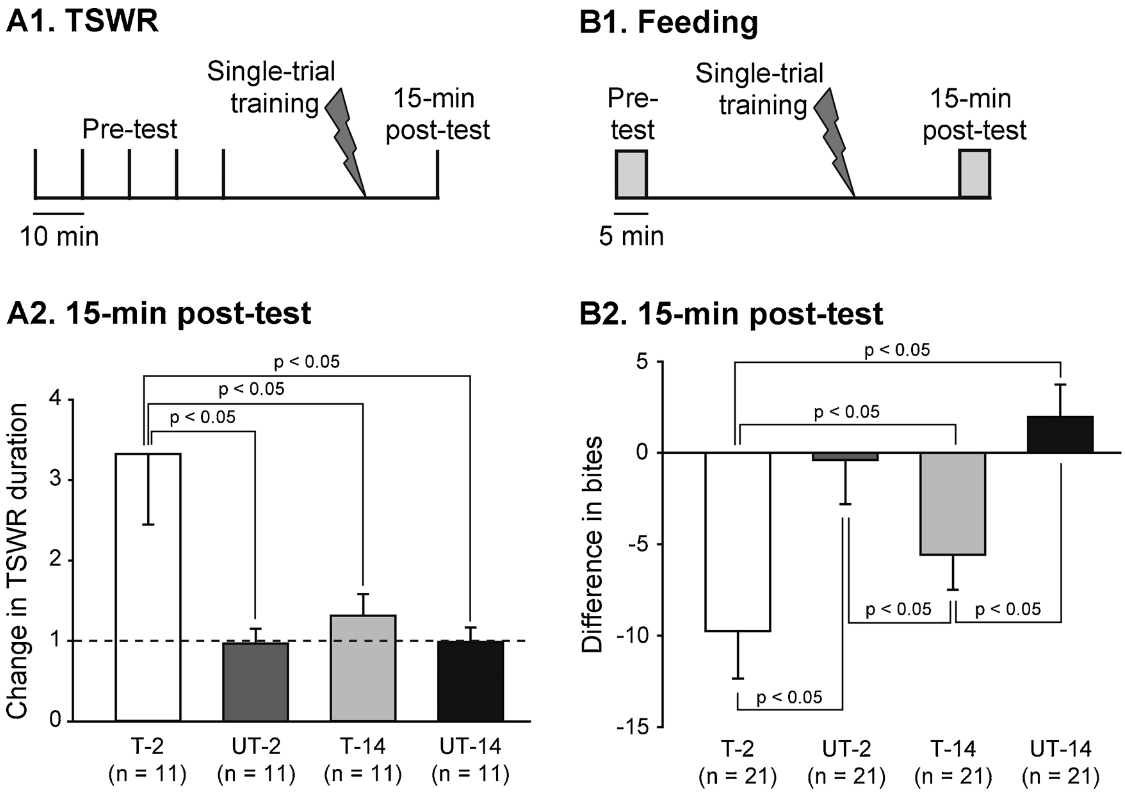

Fig. 1. In Experiment 1A, prolonged food deprivation prevented the expression of sensitization but not the expression of feeding suppression following single-trial training. The TSWR (A1) and feeding (B1) were measured prior to, and 15 min after training. Sensitization was absent in T-14 animals but was observed in T-2 animals (A2). Conversely, feeding suppression was expressed in both T-14 and T-2 animals (B2). In this and in the following figures, values are expressed as mean ± SEM. Statistical significance was set at $p < 0.05$.

different behaviors and their underlying neural networks in close temporal proximity to stimulus exposure [9,16–20], with little focus on how the budgeting is maintained over time.

In this study, we investigated the process of behavioral budgeting and its maintenance by using a robust learning-induced concurrent modulation of defensive and non-defensive behaviors in the mollusk *Aplysia californica,* which persists from minutes to days depending on the amount of exposure to aversive stimuli [21,22]. In *Aplysia*, exposure to noxious electric stimuli, which mimic the attack of a predator [23], induces a nonassociative enhancement of defensive responses (e.g., withdrawal reflexes and escape locomotion), known as sensitization [24,25], and a concurrent suppression of feeding [21,22,26]. We have consistently observed that feeding is suppressed when sensitization is expressed, but it is unchanged at time points in which sensitization is not expressed [21,22]. The consistent co-expression of sensitization and feeding suppression suggests a strong relationship between these behavioral changes, possibly indicating an underlying mechanistic link.

The goal of this study was to challenge the behavioral budgeting induced by aversive stimuli by attempting to uncouple the co-expression of sensitization and feeding suppression in two different ways. In the first experiment, we examined the effects of a regimen of prolonged food deprivation on the co-expression of sensitization and feeding suppression. Would this regimen shift the balance of behavioral budgeting to favor feeding behavior over defensive withdrawal? With the second experiment, we challenged the co-expression of sensitization and feeding suppression by presenting aversive stimuli using a training protocol that induces sensitization lasting for several weeks (Fig. 3A) [27–29]. Would feeding remain suppressed as long as sensitization is expressed, despite the metabolic strain this would put on the animal? Or, would the behavioral balance shift to allow the organism to feed despite still being in a state of defensive arousal?

2. Materials and methods

General methodologies are described in Sections 2.1–2.4 and experimental designs specific to Experiments 1 and 2 are described in Sections 2.5 and 2.6. In all experiments, the experimenter performing the behavioral tests was unaware of the training history of the animals.

2.1. Animals

Adult *Aplysia californica* (120–190 g) were obtained from South Coast Bio-Marine (San Pedro, CA), and were individually housed in two aquaria (Aquatic Enterprises Inc., WA) of continuously circulating 15 °C aquarium seawater (Instant Ocean) on a 12-h light/dark cycle. Before entering experiments, animals were fed one strip of dried seaweed (3 × 19 cm; 0.5 g; Emerald Cove® Organic Pacific Nori; Great Eastern Sun, Asheville, NC) three times a week [21].

2.2. Measurement of TSWR

At least one week prior to behavioral testing, the posterior portions of the parapodia (i.e., wing-like extensions of the body wall surrounding the mantle cavity) were surgically removed bilaterally (i.e., parapodectomy) to permit full visualization of the siphon withdrawal [29–31]. Prior to the parapodectomy, animals were anaesthetized by placing them under ice for an amount of time (about 20 min for most of the animals tested) sufficient for the animal not to respond to tactile stimuli delivered to the rhinophores [29–31]. This procedure allowed us to remove the parapodia without contractions or the release of ink and/or opaline [21,31]. In the rare cases in which animals secreted ink and/or opaline during or after parapodectomy, they were excluded from the study (7% of animals). The duration of the TSWR was used as a measure of the reflex's strength and was assessed using previously-