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# Octopamine indirectly affects proboscis extension response habituation in *Drosophila melanogaster* by controlling sucrose responsiveness

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ABSTRACT

Octopamine is an important neurotransmitter in insects with multiple functions. Here, we investigated the role of this amine in a simple form of learning (habituation) in the fruit fly *Drosophila melanogaster*. Specifically, we asked if octopamine is necessary for normal habituation of a proboscis extension response (PER) to different sucrose concentrations. In addition, we analyzed the relationship between responsiveness to sucrose solutions applied to the tarsus and habituation of the proboscis extension response in the same individual. The *Tyramine-β-hydroxylase* (*Tβh*) mutant lacks the enzyme catalyzing the final step of octopamine synthesis. This mutant was significantly less responsive to sucrose than controls. The reduced responsiveness directly led to faster habituation. Systemic application of octopamine or induction of octopamine synthesis by *Tβh* expression in a cluster of octopaminergic neurons within the suboesophageal ganglion restored sucrose responsiveness and habituation of octopamine mutants to control level. Further analyses imply that the reduced sucrose responsiveness of *Tβh* mutants is related to a lower sucrose preference, probably due to a changed carbohydrate metabolism, since *Tβh* mutants survived significantly longer under starved conditions. These findings suggest a pivotal role for octopamine in regulating sucrose responsiveness in fruit flies. Further, octopamine indirectly influences non-associative learning and possibly associative appetitive learning by regulating the evaluation of the sweet component of a sucrose reward.



## 1. Introduction

In insect learning assays, a sucrose solution is frequently used as unconditioned stimulus and reward (Scheiner, 2004; Scheiner et al., 2004; Schwaerzel et al., 2003; Burke et al., 2012; Scheiner et al., 2013). The sucrose stimulus comprises two components, a sweet and a nutrient component. Recent studies demonstrated that both components can be dissociated in insect associative memory (Burke and Waddell, 2011; Fujita and Tanimura, 2011). Interestingly, the evaluation of the sweet component alone correlates with individual learning performance in non-associative and associative learning performance (Scheiner et al., 1999, 2004, 2005; Scheiner, 2004). Individuals which place a high value on a sucrose solution which they can taste with their antennae (honey bees) or front tarsus (fruit flies), learn faster to associate a sucrose reward with an odor (Scheiner et al., 2001b, 2013) but need longer to habituate the proboscis extension response to sucrose (Scheiner, 2004; Scheiner et al., 2004; Cevik and Erden, 2012). Importantly, only the sweet component of a sucrose solution plays a role in non-associative learning, since animals are not allowed to imbibe the solution and therefore do not evaluate the nutrient component.

The biogenic amine octopamine is an important neurotransmitter, neuromodulator and neurohormone in insects (Evans, 1980; Roeder, 1999; Scheiner et al., 2006). Its physiological functions range from aggression in crickets (Stevenson et al., 2005), over bioluminiscence in glowworms (Rigby and Merritt, 2011), to sucrose responsiveness and appetitive learning in honey bees and fruit flies (bees: Hammer and Menzel, 1995; Scheiner et al., 2002; Behrends and Scheiner, 2012; fruit flies: Schwaerzel et al., 2003; Burke et al., 2012). In addition, it is involved in numerous behaviors in fruit flies such as the olfactory startle response (Scholz, 2005), ethanol tolerance (Scholz et al., 2000, 2005) and olfactory ethanol preference (Schneider et al., 2012). Importantly, in bees as in fruit flies,

*Abbreviations:* AL2 cluster, antennal lobe 2 cluster; PER, proboscis extension response; SRS, sucrose response score; *Tβh*, *Tyramine-β-hydroxylase*; VM cluster, ventral median cluster.

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octopamine plays a decisive role in mediating the sweet component of the sucrose reward in appetitive learning and memory (Hammer and Menzel, 1995; Burke et al., 2012). Octopamine further appears to indirectly affect non-associative and associative learning in insects by modulating the individual's evaluation of sucrose stimuli used as unconditioned stimuli and rewards. Bees with a low responsiveness to sucrose measured at their antennae perform poorly in associative learning (Scheiner et al., 1999, 2001a,b, 2005). But when they are treated with octopamine, they place a higher value on the sweet component of a sucrose solution (Scheiner et al., 2002) and perform better in associative learning (Behrends and Scheiner, 2012).

Here we ask in how far responsiveness to the sweet component of a sucrose solution (i.e. the "sucrose responsiveness") and habituation of the proboscis extension response depend on octopamine signaling in the *Drosophila* brain. We address this question from two different perspectives. (1) We investigate individual sucrose responsiveness and habituation in octopamine-less *Tβh* mutants, and (2) we test whether pharmacological or genetic "rescue" of sucrose responsiveness can restore habituation in octopamine-less fly mutants. The *Tβh* mutants lack Tyramine β-hydroxylase, the enzyme catalyzing the final step of octopamine synthesis and are therefore devoid of octopamine (Monastirioti et al., 1996). Females of this *Tβh* mutant are sterile, because octopamine is required for egg deposition (Monastirioti, 2003). In addition, *Tβh* mutants were recently shown to have an abnormal olfactory ethanol preference (Schneider et al., 2012). Based on our honey bee data, we hypothesized that the lack of octopamine in *Tβh* mutants would lead to a reduced sucrose responsiveness and thus to a faster habituation of the PER, because habituation depends on individual sucrose responsiveness (Scheiner et al., 2004; Cevik and Erden, 2012). To further study the role of octopamine in nutrition-related behavioral decisions, we compared sucrose intake and sucrose preference of *Tβh* mutants and respective controls. To test whether the lack of octopamine in *Tβh* mutants modulates sucrose responsiveness through changes in metabolism, we compared the survival of these mutants and controls under normal and starved conditions.

## 2. Materials and methods

### 2.1. Drosophila melanogaster lines

Flies were raised on standard *Drosophila* food at 25 °C with a 10 h light/14 h dark cycle. Only five-to-six-day-old male flies were tested. The following lines were used: $w^+$, $T\beta h^{nM18}$ and the respective $w^+$ background (Schneider et al., 2012); $w^{1118}$, $T\beta h^{nM18}$ and the respective control $w^{1118}$ (Monastirioti et al., 1996); $w^{1118}$, $T\beta h^{nM18}$, *UAS-Tβh* (Schneider et al., 2012); $w^{1118}$; *NP7088*-GAL4 (Busch et al., 2009). The strain $w^+$, $T\beta h^{nM18}$ was generated via recombination with Canton S from the Scholz lab. A non-recombinant $w^+$ allele was used as a control for the same recombination.

### 2.2. Food deprivation and octopamine application

Earlier behavioral experiments with fruit flies have shown that sucrose responsiveness strongly depends on duration of food deprivation (Scheiner et al., 2004). We therefore investigated sucrose responsiveness after 2 h and after 24 h of food deprivation in the first experiment. We expected that possible differences in sucrose responsiveness should be larger after 24 h of food deprivation than after 2 h of food deprivation. In all subsequent experiments, we used a 24 h period of food starvation. Approximately 50–80 flies spent this time in a vial containing 5 ml of 1% agar dissolved in water to avoid dehydration. Flies treated with octopamine during this time either received a mixture of agar and octopamine dissolved in green food coloring (Monastirioti et al., 1996, final octopamine concentration in tube: 2.86 mg/ml octopamine) or a mixture of agar and food coloring. The food coloring was used to ensure that only flies actually imbibing octopamine were tested for PER. These flies were easily recognizable by their colored stomach. Food coloring had no effect on sucrose responsiveness in flies, as was tested in preliminary experiments. When octopamine was applied for shorter periods than 24 h, octopamine was dissolved in food coloring as before and a few droplets of this solution were applied on the surface of the agar in the tubes. Care was taken not to wet the flies in the tube with the solution. Surfaces were regularly checked and when they became dry, octopamine solution was added again as before to allow the flies to freely imbibe octopamine solution during the starvation period.

### 2.3. Measuring sucrose responsiveness

After the starvation period of either 24 ± 0.5 or 2 ± 0.5 h, each fly was caught individually and placed in a pipette tip (0.5–20 μl, Th. Geyer) whose end was cut off (Fig. 1). One leg of the fly protruded out of the pipette tip. The tarsus was touched with a toothpick moistened with water or one of the following sucrose concentrations: 0.1% ($0.3 * 10^{-2}$ mol/l), 0.3% ($0.9 * 10^{-2}$ mol/l), 1% ($0.3 * 10^{-1}$ mol/l), 3% ($0.9 * 10^{-1}$ mol/l), 10% (0.3 mol/l), 30% (0.9 mol/l) (weight/volume). This corresponds to a logarithmic series of −1; −0.5; 0; 0.5; 1; 1.5 (log%), which has proved very sensitive for similar tests in honey bees and fruit flies (Scheiner et al., 2001c, 2002; Scheiner, 2004; Belay et al., 2007). For each experiment, one of seven different sequences of applying water and the different sucrose concentrations was selected (Table 1). This pseudo-randomized order, which has been used successfully before (Scheiner et al., 2004; Belay et al., 2007), was chosen to minimize experimental bias by the sequence of applied stimuli. Each stimulus (water or sucrose solution) was presented once to each individual. For each fly we recorded whether a specific stimulus concentration elicited proboscis extension (Fig. 1). Only flies which did not respond to stimulation with water were analyzed to prevent experimental bias by thirst.

### 2.4. Habituation of proboscis extension

Habituation was tested 2–12 min after sucrose response scores were determined. For habituation of the proboscis extension

**Fig. 1.** The PER assay. A fly is fixed in a cut-off pipette tip with one front leg protruding from the holder. The front tarsus is stimulated with a toothpick moistened with sucrose solution to induce the extension of the proboscis. The assay was used to determine sucrose responsiveness and habituation.