Does starvation-resistance in flies without octopamine explain differences in sucrose preference and learning?

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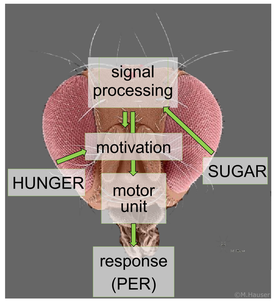

Introduction

Biogenic amines are involved in a multitude of biological processes. Hence, experimental manipulations of biogenic amines always affect multiple traits including sensation, motivation, learning or motor control. Drosophila melanogaster is an ideal model system to dissect the neuronal subpopulations mediating the affected functions. In flies without octopamine, appetitive classical conditioning is impaired as well as sucrose preference, motor control of walking, aggression and many other traits. Here we present the first experiments towards identifying the octopaminergic subpopulations underlying this multitude of effects. To exclude locomotor deficits in the sucrose preference tests of mutant flies, we use the proboscis extension response (PER), and show that the thresholds for eliciting a PER with sucrose is increased in flies without octopamine. These PER thresholds are dependent on the motivation of the fly, induced by starvation. Flies lacking octopamine also show a reduced response to starvation, leading to increased survival. Therefore, we also study the rate at which the flies decrease their hemolymph trehalose concentration with starvation.

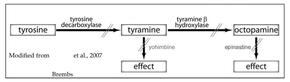

Fig. 1: The Synthesis of Octopamine
Tyrosine is converted to tyramine by the tyrosine hydroxylase. Tyramine is the precursor of octopamine and synthesized by tyramine beta hydroxylase. In flies this enzyme can be mutated and the transmitter levels are shifted: The octopamine level is abolished whereas the tyramine level is increased.

Octopamine-less flies show lower sugar preference

in T-maze-assay

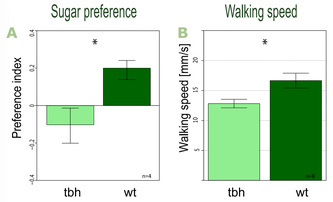

Fig. 2: tbh mutants prefer sugar in a lower level may be because of their reduced walking speed.
A The preference for sugar in a T-maze is abolished in *tbh* mutant flies.
B *tbh* mutants walk slower in Buridan's paradigm.

PER assay can detect slight difference in sugar motivation

Wild type

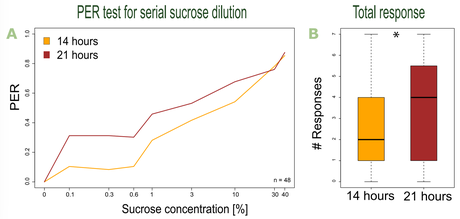

Fig. 3: Shorter starvation decreases sucrose responsiveness in wild type flies.
A Wild type CantonS flies that are starved longer respond in a higher level to a serial dilution of sucrose.
B The pooled response level shows a difference with a starvation time difference of 7 hours.

Octopamine-less flies survive longer under starvation

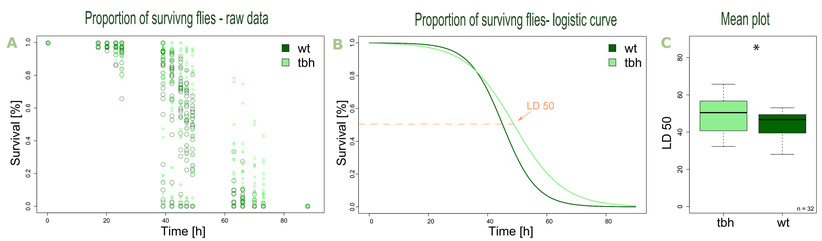

Fig. 4: Survival rate and LD 50.
A Percentage of alive wild type and mutant flies in relation to starvation time. B Logarithmic curves of survival rate of *tbh* mutants compared to wt control.
C The timepoint when 50% of the flies are dead is significantly later in the *tbh* mutants compared to wild type. In general, females survive longer than males. Here gender data are pooled.

Assays

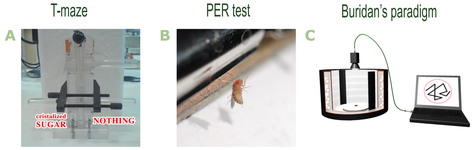

Fig. 5: T-maze, PER-test, Buridan's paradigm
A Flies can be trained for one stimulus and presented a choice point in a T-maze. B The tethered fly is presented sucrose to their legs and Proboscis Extension Reflex can be tested. C In a round illuminated arena flies with cut wings are allowed to freely walk between two stripes.

Octopamine-less flies are less sugar responsive

Mutants

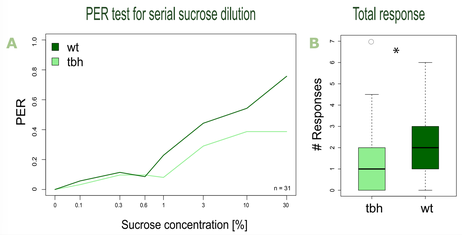

Fig. 6: tbh mutants show a decreased sucrose responsiveness.
A *tbh* mutants start extending the proboscis at a higher concentration and the response level stays lower.
B The total number of responses is significantly lower in *tbh* mutants compared to wild type. They behave like being shorter6starved.

Octopamine-less flies walk differently

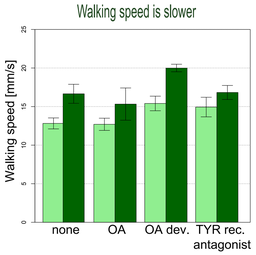

Fig. 7: Walking speed is slower in tbh mutant flies.
Speed measured in flies walking in the Buridan arena is reduced when octopamine is lacking. The speed increases in both, mutants and control, when octopamine is applicated during development. Blocking tyramine receptors rescues the mutant phenotype to wild type speed. Acute octopamine treatment had no effect.

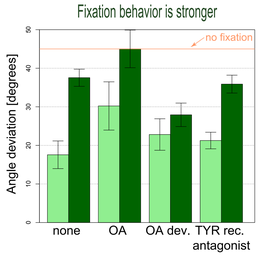

Fig. 8: Stripe fixation is stronger in tbh mutants.
When the angle between the walking direction and the stripe is measured octopamine-less flies are more directly walking than wild type. Acute octopamine treatment could decrease the fixation in both, mutants and wild type. Octopamine treatment during development raised fixation in wild type but made *tbh* behavior more randomly.

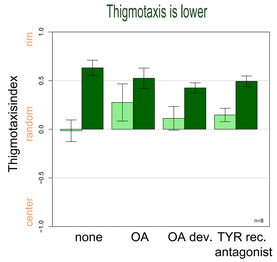

Fig. 9: Thigmotaxis is decreased in octopamine-less flies.
The walking *tbh* mutants show less thigmotaxis behavior than wild type. They become more rim oriented with acute octopamine treatment. Octopamine given during development made wild type less thigmotaxic.

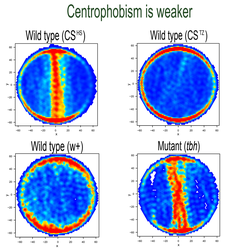

Fig. 10: Occupancy plots.
In these plots it is shown where in the arena the fly is located during the experiment - the warmer the colour the more often. Centrophobic behavior is very variant in wild types. Octopamine-less mutants are different to their genetic control.

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