Genetic dissection of octopamine action in reward-related behavior and motor control in *Drosophila*

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Biogenic amines in insects

coordination of physiological processes and behaviour

Universität Regensburg

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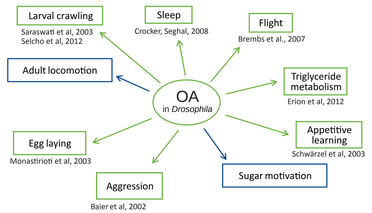
Introduction

Octopamine acts as neurohormone, neuromodulator and neurotransmitter, contributing to the control of the animal physiology and behavior. What cellular processes are at play in order to coordinate those different actions?

Many roles of octopamine:



We use flies mutant for the enzyme tyramine-ß-hydroxylase *tßh*. These mutants lack octopamine but their levels of tyramine are increased.

Octopamine synthesis affected by the *tßh* mutation:

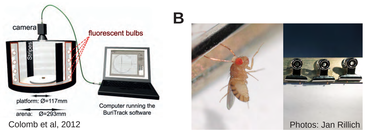
Wild type: TYR —TDC→ TA —TßH→ OA

Mutant: TYR —TDC→ TA —~~TßH~~→ ~~OA~~

Walking behavior was tested in Buridan's paradigm (in **A**). Flies with clipped wings were placed in a round illuminated arena with two opposite stripes. Average walking speed and the angle between the fly's walking direction and the center of the stripe was calculated as a measure of stripe fixation.

We tested sugar response in a proboscis extension response test (PER, in **B**). After starvation flies were glued to hooks and sugar was presented to their legs. The total number of responses is shown as box plots representing the median as midline and 25%/75% as box bounderies and 95%/5% as whiskers.

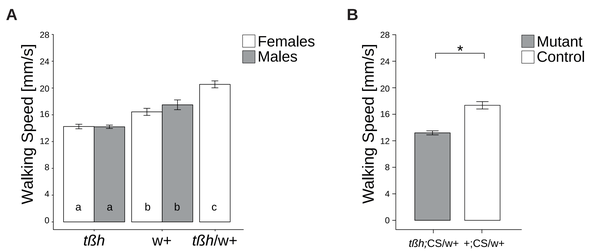
Trehalose and glucose (sugar) content in extracted hemolymph was measured photometrically after enzymatic convertion of trehalose into glucose.



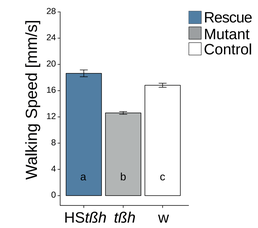
Colomb et al, 2012

Results

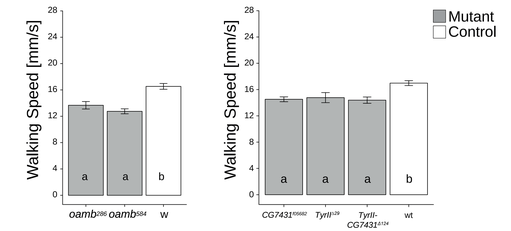
Walking speed



1 A. *tßh* mutants walk more slowly in Buridan's paradigm. The phenotype is inverted in heterozygous mutants (n=12, ANOVA: p<0.025, TukeyHSD). **B.** The mutant phenotype persists when tested in a different genetic background (n=34, T-test p<0.025).

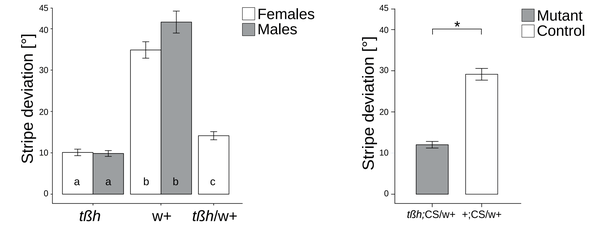


2. Temperature-dependent ubiquitous expression of *tßh* immediately before testing increases the mutants' walking speed (n=19, ANOVA: p<0.025, TukeyHSD).

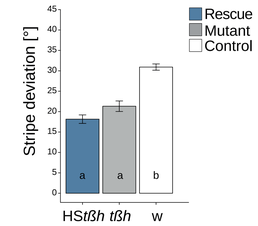


3 A. The *oamb* octopamine receptor mutants show a decreased walking speed (n=17-18, ANOVA: p<0.025, TukeyHSD). **B.** Different mutants for the tyramine receptor TyrII also exhibit reduced walking speed (n=23-24, ANOVA: p<0.025, TukeyHSD).

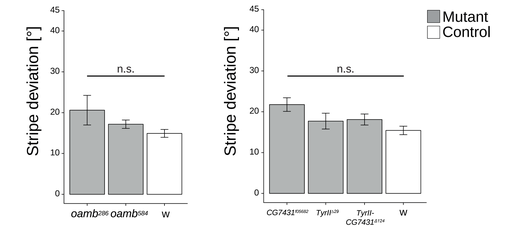
Fixation



4 A. *tßh* mutants show stronger stripe fixation in Buridan's paradigm. Heterozygous flies show an intermediate phenotype (n=12, ANOVA: p<0.025, TukeyHSD). **B.** The mutant phenotype persists when tested in a different genetic background (n=34, T-test p<0.025).

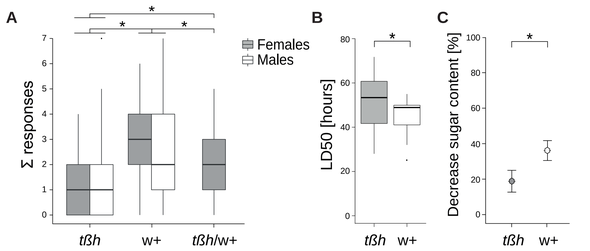


5. Temperature-dependent ubiquitous expression of *tßh* immediately before testing does not rescue the reduced stripe deviation in mutants (n=19, ANOVA: p<0.025, TukeyHSD).

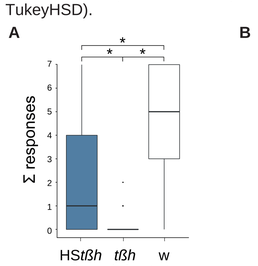


6 A and B. Mutants for the *oamb* receptor and different tyramine receptor mutants show no phenotype in stripe deviation (n=17-18 for oamb and n=23-24 for Tyr-rec, ANOVA: p=ns).

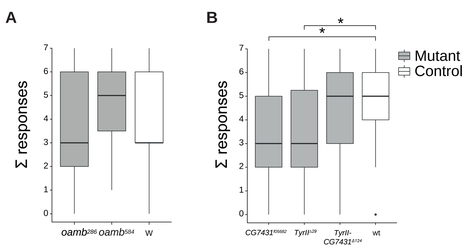
Sugar motivation



7 A. *tßh* mutants show a decreased total number of responses to a serial dilution of sucrose after the same starvation time. Heterozygous mutants seem to have an intermediate phenotype (n=36-40,Wilcoxon-test p<0.05). **B.** *tßh* mutants survive longer in starvation conditions (n=16,MannWhitneyU-test p<0.05). **C.** Hemolymph glucose/trehalose decreases more in wild type than in *tßh* mutants during starvation (n=12,T-Test p<0.05).



8 A. Temperature-dependent ubiquitous expression of the *tßh* gene increases the sucrose response when expression is induced 3h before testing (n=39-49, MannWhitneyU: p<0.01). **B.** Inducing *tßh*-expression during starvation but not before the test does not rescue the mutants' sucrose reponse phenotype (n=11-16, Mann WhitneyU, p=ns).



9 A. Mutants for the *oamb* receptor do not differ from controls in their sucrose response (n=29-31, MannWhitneyU: p=ns).
B. Different mutants for the tyramine receptor TyrII are impaired in sucrose response (n=24-40, MannWhitneyU: p<0.05).

Conclusions

Mutants without octopamine but with increased level of tyramine walk more slowly in Buridan's paradigm compared to wild type. This phenotype is independent of the genetic background and can be rescued by inducing *tßh*-expression immediately before testing. Both octopamine and tyramine receptor mutants are impaired in walking speed, suggesting that both neurotransmitters are involved in the control of this behavior.
Octopamine-lacking mutants show a stronger fixation behavior towards stripes in Buridan's paradigm. Although that phenotype is independent of the genetic background it is not rescued by ubiquitously expressing *tßh*. None of the investigated octopamine or tyramine receptor mutants show any modification in their fixation behavior.

tßh mutants show reduced starvation-dependent sucrose responsiveness and delayed mortality under starvation conditions. Both phenotypes may be explained by an action of the amines on metabolic rate. However, sucrose responsiveness is increased when *tßh* is expressed before test but not when rescued only during starvation. Tyramine receptor mutants show reduced sucrose responsiveness, suggesting an involvement of both tyramine and octopamine.

To do

- Neuron-specific rescue *of tßh* mutation
- Activation and silencing of aminergic neurons
- Direct link between octopamine and starvation signal: *tßh* overexpression, anatomical comparison between starved and fed animals
- Retest sugar response after adapting starvation level

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