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COMMENTARY
Drosophila mutants lacking octopamine exhibit impairment in aversive olfactory associative learning (Commentary on Iliadi *et al.* (2017))

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Despite the myriad of advances over the last decades, understanding the substrates of learning remains a key goal of modern neuroscience. Indeed, the subtleties and complexities of learning have ensured that achieving this goal is not a simple task. The same brain region can mediate both positive- (appetitive) and negative-valence (aversive) learning, different circuits within such regions can take precedence over others depending on motivational state or metabolic condition, and even the same neurotransmitters can promote different types of learning. The fruit fly, *Drosophila melanogaster*, has long been used to dissect the molecular mechanisms and circuits that underlie learning. Flies exhibit robust appetitive and aversive learning via training to associate odours with positive or negative stimuli (Busto *et al.*, 2010). In concert with the awesome power of fly genetics, this has allowed for elegant dissections of the mechanisms that underlie learning (Aso *et al*., 2014).

One of the dichotomies facing fly learning and memory researchers has involved the roles of dopamine and octopamine (the invertebrate homologue of norepinephrine). Early work posited a clear distinction between the two: dopamine mediates aversive conditioning while octopamine is required for appetitive conditioning (Schwaerzel *et al.*, 2003). Recent work, however, highlighted a duality for invertebrate dopamine (Krashes *et al.*, 2009; Burke *et al.*, 2012; Liu *et al.*, 2012), revealing evolutionary conservation with the mammalian gestalt (Bromberg-Martin *et al.*, 2010). Octopamine remained associated only with appetitive memory despite contrary evidence in honeybees (Agarwal *et al.*, 2011). In this issue of *EJN*, Iliadi and colleagues (2017) revisit the octopamine side of the dichotomy with fresh eyes. They demonstrate that established null mutants for tyramine β-hydroxylase (*TβH*), the enzyme necessary for synthesizing octopamine from tyramine (thus, mutants completely lack octopamine), show defects in aversive olfactory learning – flies failed to negatively associate an odour that had been paired with a noxious stimulus. These defects are ameliorated by restoring *TβH* to octopaminergic/tyraminergic neurons, demonstrating a clear role for *TβH* in those cells.

How can the same *TβH* mutation yield contrasting data? Iliadi and colleagues used two elements to reveal these defects. First, the authors use an expert grasp of fly genetics to carefully control for the *TβH* mutation (separating mutants from stocks used to maintain the null mutation to combat variability) and a precise excision as a control, maintaining the genetic background. This expanded rigour led to the second element: an inherently improved performance. Performance in a behavioural assay is an index (the PI) that ranges from 0 to 100, where 0 represents complete failure (every fly failed to associate the odour with the stimulus), and 100 represents complete success (every fly correctly avoided the stimulus based on the odour). Previously, control flies scored a PI around 50 (Schwaerzel *et al.*, 2003; Yarali & Gerber, 2010; Kim *et al.*, 2013), demonstrating that half the time, flies associated the odour with the stimulus. As such, *TβH* mutant scores of 40 were never statistically different from controls. In the present study, the carefully constructed control flies have a new baseline, scoring a PI of 70. Now, when *TβH* mutants score 40, the change is statistically significant, thus revealing a phenotype.

Do these new data vault octopamine into the echelons of molecules that regulate both appetitive and aversive behaviour? Iliadi and colleagues have opened the door to such a world, but the field must be cautious in walking through. While the phenotype here is evident, given the breadth of previous examples (Schwaerzel *et al.*, 2003; Yarali & Gerber, 2010; Burke *et al.*, 2012; Liu *et al.*, 2012), the baseline of controls should be considered. Previous backgrounds may have contributed non-specific interference to behavioural performance, decreasing indices to ~ 50 (i.e. the defect was always there but the flies were already impaired so it did not show up); the new study may have alleviated that issue. But genetic background can go both ways. The new enhanced performance may be accurate, but may also result from genetic interactions between a background mutation and functional *TβH*. This synergy is lost when *TβH* is mutated, manifesting as a behavioural deficit. If other (non-*TβH*) mutations in that same background affect behaviour as expected, this may be ruled out. In any case, the authors underscore a clear need for careful background control and measured rigour in behaviour.

Technicalities notwithstanding, the core question remains: does octopamine mediate aversive behaviour? Iliadi and colleagues conclusively show that *TβH* mutants who completely lack octopamine are defective in aversive learning. While this points towards octopamine, the issue needs further exploration. Learning deficits can be rescued by feeding flies octopamine (Schwaerzel *et al.*, 2003), alleviating the need for the fly to synthesize octopamine itself. Iliadi and colleagues could not rescue the observed defects with octopamine feeding, perhaps hinting that the issue may be more complex than a lack of octopamine. To further explore, neuronal silencing is needed: if octopamine regulates aversive behaviour, then silencing these octopaminergic neurons should recapitulate the learning deficit. Given the advent of new, neuron-subtype-specific drivers (Pfeiffer *et al.*, 2008), the precise octopaminergic circuit can even be readily studied (Burke *et al.*, 2012). This would cement

octopamine as the true culprit and further advance the case by identifying subcircuits. Similarly, blocking octopaminergic signalling using octopamine receptor mutants (Burke *et al.*, 2012; Kim *et al.*, 2013) should also phenocopy learning deficits. Connecting this behaviour to a specific receptor (or group of receptors) would further still advance our understanding of how the same neurotransmitter can regulate very different modes of learning, deepening the link between aversion and octopamine.

If not octopamine, though, what could cause these defects? In *TβH* mutants, flies cannot convert tyramine to octopamine, resulting in threefold enhanced levels of tyramine (Iliadi *et al.*, 2017). The authors demonstrate rescue of the learning deficits by expressing *TβH* in the octopaminergic AND tyraminergic neurons: not only does this re-enable octopamine synthesis from tyramine, but also it (presumably) alleviates the elevated levels of tyramine in these neurons. If excess tyramine causes altered signalling from tyraminergic neurons, thus occluding certain behaviours, this could explain the phenotypes seen in the *TβH* mutant. It might also explain why feeding flies octopamine did not rescue the aversive learning defect: in such a case, the elevated tyramine would be causative, not the absence of octopamine. But this remains to be tested. In all, however, with an eye towards genetics and elegant background control, Iliadi and colleagues offer strong new evidence that *TβH* is a more complex mutant than realized. The defects in aversive behaviour are clear, and plant the tantalizing seed of a hypothesis that octopamine, like dopamine, can mediate positive- and negative-valence behaviours. Future work will determine, however, whether this seed will grow into a full-fledged member of octopamine's garden.

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