Octopamine fuels fighting flies

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The neural basis of aggression is poorly understood. A study in this issue used genetic scalpels to dissect the circuitry of the fly brain and identified a small cluster of octopaminergic neurons that can make a fly fighting mad.

Aggression is an innate behavior that helps to ensure that the fittest animals survive. Aggression among conspecific animals typically favors individuals that can most effectively protect and utilize limited resources such as mates or food. In effect, more aggressive males mate more often and generate more aggressive offspring, which outcompete less aggressive rivals. But how is such a behavioral trait inherited? Insights into this question have arisen from studies of the genetic model organism, the fruit fly *Drosophila melanogaster*. *Drosophila* is an excellent genetic model system for studying aggression. Males exhibit robust, stereotypical aggressive behaviors in fights over food and mates. Aggressive and nonaggressive fly strains can be bred[1]. So what is going on inside a fighting fly's mind that regulates its aggression? In this issue, Zhou and colleagues report the identification of a small population of octopaminergic neurons that are essential for aggressive behaviors[2].

Aggressive behaviors in *Drosophila* were first described by Sturtevant in 1915 and characterized in greater detail by Jacobs in the 1960s and Hoffman in the 1980s (reviewed in ref. 3). A renaissance of fighting fly research arose from studies in recent years[4,5]. One group streamlined and simplified the behavioral setup by designing a simple food-containing 'arena' in which two flies would fight for access to food. They described and quantified the aggressive traits observed in fighting flies. Both male and, to a lesser degree, females fight with different stereotyped behaviors. Aggressive males exhibit such behaviors as holding, lunging (rearing up and snapping down on the opponent), boxing (both flies rear up and strike with forelegs) and tussling (both flies wrestle over each other). Aggressive females instead exhibit shoving and head-butting behaviors.

The sexual dimorphism of fighting behaviors is genetically controlled by the transcription factor *fruitless*, a master regulator of sexual behavior[6]. *fruitless* is alternatively spliced to yield a male-specific form, which is expressed in ~2% of a male fly's neurons. Male flies that are engineered to lack the male version of *fruitless* fight like females. Female flies that express the male version of *fruitless* fight like males, even though they still look like females. These results indicate that there are neurons in the brain that, when masculinized or feminized, will alter the fighting behavior of a fly.

How do we identify neurons that regulate fighting? A good starting point is the well-characterized neurotransmitters that have already been shown to influence aggressive behaviors. The study by Zhou *et al.*[2] focuses on octopaminergic neurons. Octopamine can trigger complex aggressive behaviors in invertebrates. It elicits a submissive posture in lobsters, but an aggressive stance in crickets (reviewed in ref. 7). Octopamine is derived from the amino acid tyrosine, which is decarboxylated by tyrosine decarboxylase (TDC) to yield tyramine. Tyramine is in turn hydroxylated by tyramine-β-hydroxylase (TβH) to yield octopamine (**Fig. 1a**). Mutation of TβH completely abolishes all octopamine in the animal, while increasing the amount of tyramine. TβH mutant flies indeed show reduced levels of aggression[8–10].

How do octopamine levels determine aggression? In particular, are TβH and octopamine required for the initial development of aggression circuitry or are they necessary only in adult flies to induce aggression? To address this question, Zhou *et al.*[2] took advantage of a temperature-sensitive variant of dynamin driven by TDC2-Gal4 to silence specifically octopaminergic (and tyraminergic) neurons in adult flies. At the permissive temperature, when octopaminergic neurons function, the flies behaved normally. However, at the restrictive temperature, when octopaminergic neurons are silent, the flies displayed decreased aggression. Thus, octopaminergic neuron signaling is required in the adult, but not during development, to stimulate aggressive behaviors (see also ref. 10).

Aggression is highly sensitive to social rearing[11]. Indeed, Zhou *et al.*[2] found that flies that were isolated from birth were, on average, 5–10-fold more aggressive than flies that were raised in the presence of even just one other fly. Do octopamine levels influence such socially induced aggressive behaviors? Zhou *et al.*[2] examined the effects of increasing octopamine levels in the less aggressive socially reared flies. Feeding socially reared flies an octopamine agonist, or selectively activating octopaminergic (and tyraminergic) neurons, led to levels of aggression that were indistinguishable from those of flies raised in isolation. On the other hand, the already highly aggressive isolated flies showed no further increase in aggression when octopamine signaling was increased. Thus, it appears as though octopamine signaling in isolated flies is already saturated.

There are approximately 120 octopaminergic neurons in the fly brain with clusters in regions of the protocerebrum, fan-shaped body, central complex, optic lobes, subesophageal ganglion (SOG) and antennal lobe[12] (**Fig. 1a**). Which of these 120 neurons are responsible for regulating aggressive behaviors? By carrying out a series of elegant genetic experiments, and taking advantage of a few lucky transgene expression patterns, Zhou *et al.* were able to identify just 2–5 of these 120 neurons as the source of octopamine-mediated aggressive behaviors.

To map the responsible neurons, the authors asked the following question: to which neurons does TβH need to be added to rescue wild-type aggressive behavior in a TβH mutant animal (which shows low levels of aggression)? Again the authors used the TDC2-GAL4 driver, this time to express TβH in 'octopaminergic' neurons of the TβH mutant. In fact, this driver does not express in all octopaminergic neurons—it lacks expression in the octopaminergic neurons of the protocerebrum, fan-shaped body, central complex and optic lobes. It does label the octopaminergic neurons in regions of the antennal lobe and SOG. Remarkably, restoring expression of TβH in these two populations was sufficient to fully rescue the aggressive behaviors (**Fig. 1b**). Thus, the source of octopamine-mediated aggressive behavior lies in these two populations, totaling about 20 neurons.

To narrow this further, the authors next expressed TβH in TβH null mutant flies using a GAL4 driver that is expressed in cholinergic neurons, cha-GAL4. This driver also labeled a subset of octopaminergic neurons (it is unclear whether these neurons are both octopaminergic and cholinergic or if cha-Gal4 is simply ectopically expressed). It restored TβH activity in all antennal lobe octopaminergic neurons, but only in some of the SOG octopaminergic

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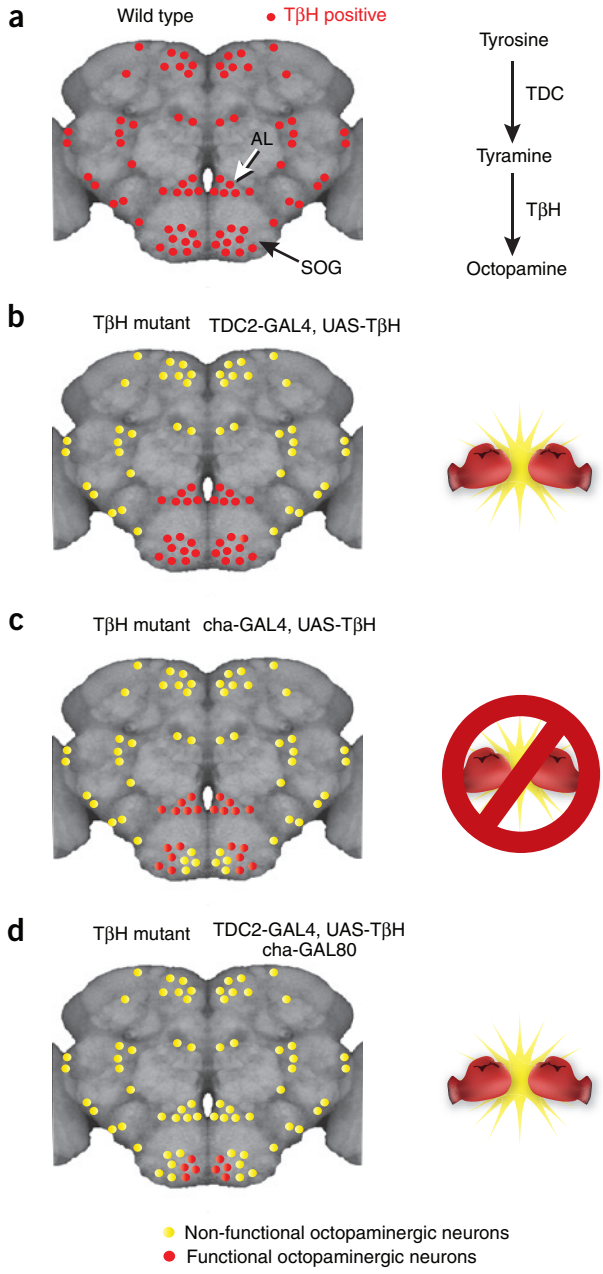

Figure 1 Identifying the neurons required for octopamine-mediated aggression. (**a**) Left, in the fly brain there are ~120 octopaminergic neurons, as detected by staining for TβH, the key enzyme in octopamine synthesis. Right, the octopamine biosynthetic pathway. (**b**) *TβH* mutant flies are not aggressive. Aggressive behavior is rescued by returning TβH expression to the antennal lobe (AL) and SOG octopaminergic neurons. (**c**) Aggression is not rescued when TβH function is restored in only a subset of the SOG octopaminergic neurons. (**d**) Expression of TβH in only 2–5 octopaminergic neurons in ventral SOG is sufficient to fully rescue aggressive behavior. These neurons therefore represent the octopaminergic fighting center in the fly brain.

neurons. This was not sufficient to reinstate wild-type levels of aggression (**Fig. 1c**), thus ruling out the antennal lobe and most (but not all) of the SOG octopaminergic neurons as the neurons responsible for aggression.

Would TβH activity in those other SOG neurons rescue the aggressive behaviors? To answer this, the authors first rescued TβH expression in the antennal lobe and SOG octopaminergic neurons using the TDC2-GAL4 line as before. They then turned off expression of transgenic TβH specifically in the cha-Gal4–positive neurons in which they already knew TβH was not required, by expressing GAL80, the GAL4 inhibitor, under the same 'cha' promoter that drove cha-GAL4. As a result, TβH expression was restored only in a small group of 2–5 octopaminergic neurons in the SOG (there was slight variation between individual animals). Notably, the aggressive behavior of TβH mutant flies was fully rescued (**Fig. 1d**). Thus, these few octopaminergic neurons are a major source of aggression in the fly brain.

This study opens up a whole avenue of exciting challenges and questions. If these 2–5 octopaminergic neurons are specifically silenced, is aggression abolished? What are the neuronal targets for these specific octopaminergic neurons and how do these target neurons regulate aggression? How are octopamine levels regulated in these few neurons? What neurons target these particular octopaminergic neurons and how do they regulate aggression? How are the activities of these neurons correlated with and modified by fighting? Can social rearing affect the amount of octopamine in these few neurons? Are these neurons also positive for *fruitless* (as intriguingly suggested by a recent study[9]) and can *fruitless* (or other *fruitless*-positive neurons) affect octopamine levels in these neurons? As a genetic model system, *Drosophila* has the tools to answer most of these questions[13]. Furthermore, what we learn from fighting flies might also be applicable to mammals. The mammalian counterpart of octopamine is norepinephrine. Mice that lack norepinephrine show essentially no aggressive behaviors, whereas increases in norepinephrine signaling lead to enhanced aggression[14,15]. Genetic dissection of neural circuits[13] using similar strategies in mice might identify aggression centers in the mammalian brain.

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