To Fight or Not to Fight

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http://dx.doi.org/10.1016/j.neuron.2017.08.029

In this issue of *Neuron*, Watanabe et al. (2017) uncover how octopamine, an invertebrate norepinephrine analog, modulates the neural pathways that bias *Drosophila* males toward aggression.

If you find yourself in a dark alley on a rainy night and a stranger approaches you, your heart might begin to race and you will be ready to fight or flee, depending on what the stranger does next. On the other hand, if you are walking through the park on a sunny day, you will likely smile and remain calm when a stranger approaches. Context is incredibly important, and our brains have evolved to allow us to flexibly alter our behavioral responses given the situation. In addition, internal drive can exert a powerful effect on behavioral choices (e.g., your reaction to the stranger on a sunny day might be quite different if you had only gotten an hour of sleep the night before). How do our nervous systems enable us to use both context and internal state to produce the right behavior at the right time? Numerous studies have pointed to an important role for neuromodulators (e.g., dopamine, serotonin, or norepinephrine). Studies in both worms and flies (with numerically simple nervous systems and powerful genetic tools that facilitate connecting neural function with behavior) reveal that such molecules, by increasing or decreasing the influence of particular synaptic connections, can flexibly alter how information is integrated or routed through neural circuits (Bargmann, 2012). But how neuromodulators exert their effects in a context-dependent manner remains less well understood.

Norepinephrine, and its invertebrate analog octopamine (OA), is a potent modulator of brain-wide states such as arousal (e.g., Suver et al., 2012), but can it also target specific circuits to bias behavioral choices? Addressing this question requires identifying the relevant receptor neurons (neurons that receive the neuromodulatory signal) and determining how they direct specific aspects of behavior given a certain context or internal state. This is precisely what Watanabe et al. achieve in this issue of *Neuron*, uncovering a previously uncharacterized node of integration between OA and a neural pathway involved in the choice between two mutually exclusive social behaviors: aggression and courtship (Figure 1).

A male fruit fly that has lived alone his entire adult life ("single-housed") will aggressively fight by lunging or producing wing threats when presented with another male and in the context of a resource worth fighting over (e.g., a fresh patch of fly food). In contrast, if presented with a female, he will instead chase her and sing a sonorous love song via wing vibration. Interestingly, if first housed with other males for a period of time ("group-housed"), he will act much less aggressively toward a new male—an effect shown to be modulated by OA (Zhou et al., 2008). To elucidate how OA is integrated into the known aggression/courtship circuitry, Watanabe et al. ask how OA-responsive neurons flexibly bias male flies toward or against aggression in the presence of another male.

To determine which subset of the roughly 100,000 neurons in the *Drosophila* brain are modulated by the OA signal to induce males to fight (Hoyer et al., 2008), the authors developed a new class of neural circuit tool generated by bashing up the cis-regulatory modules (CRMs) for all of the known OA receptors in the *Drosophila* genome. This produced a tractable number of enhancer lines (34) to be screened via neural silencing, testing for single-housed males that showed a decrease in lunging toward other males. Watanabe et al. identified line R47A04, which drives expression in just a few neuron types. When activated with NaChBac (a bacterial ion channel that constitutively depolarizes the cell membrane), it specifically increases the amount of aggression in group-housed males. However, the authors do not get the same result when driving the R47A04 neurons with phasic optogenetic or thermogenetic activation. They interpret this to mean that the neurons labeled in R47A04 are permissive, but not instructive, in driving male-male aggression. In their hands, depolarizing all OA neurons does not produce aggression either (in contrast with Zhou et al., 2008), also consistent with this hypothesis.

Line R47A04 was constructed using the CRM for the *Oamb* (octopamine α1) receptor, indicating that R47A04 neurons express this receptor. They confirm this with an elegant series of gene knockdown, rescue, and overexpression experiments—*Oamb* is specifically required in R47A04 neurons for the increased aggression phenotypes. But which specific neurons in the R47A04 line are involved in aggression? Here, Watanabe et al. make exquisite use of the *Drosophila* toolkit: using a recombination strategy to selectively remove expression of neurons within this line, they identify a crucial subset of male-specific neurons located in the superior medial protocerebrum (SMP), also known as aSP2 (Figure 1A). These neurons produce strong calcium responses to bath application of OA, although with a delay. The SMP is also home to the projections of another male-specific set of neurons (termed P1), known to induce both courtship and aggression behaviors (Hoopfer et al., 2015). Watanabe et al. find with functional imaging that R47A04^{aSP2} neurons produce

rapid calcium transients in response to P1 activation, and when R47A04^{aSP2} neurons are silenced, P1 neurons no longer induce aggression. These data indicate that R47A04^{aSP2} neurons are functionally downstream of P1 (although which specific subset of P1 neurons they respond to remains open); therefore, OA can bias males toward aggression by routing P1 activity through the R47A04^{aSP2} neurons (Figure 1B).

How do R47A04^{aSP2} neurons shape the balance between courtship and aggression (for example, in natural environments, when males are likely to encounter both males and females)? Male-specific P1 neurons are known to be activated by a courtship-promoting cuticular hydrocarbon, 7,11-heptacosadiene, produced by females (Clowney et al., 2015), whereas the OA neurons are downstream of taste receptors that detect 7-tricosene, a male-specific molecule known to suppress courtship and promote aggression (Andrews et al., 2014). R47A04^{aSP2} neurons may thus constitute a neural correlate for "jealousy," biasing males toward aggression, but only in the presence of a female. This provides a clear example of how contextual cues can shape behavioral decisions via neuromodulation (Anderson, 2016). OA neurons are known to encode internal states, such as wakefulness (Crocker et al., 2010), and might also encode other contextual cues. For example, visual feedback plays a prominent role in shaping both aggressive lunging (Hoyer et al., 2008) and courtship song patterning (Coen et al., 2016)—OA neurons are known to modulate the gain of *Drosophila* visual pathways (Suver et al., 2012), and thereby may also affect the choice between aggression and courtship. Finally, neuromodulation can also occur at the level of the P1 neurons, where dopaminergic modulation scales with social history (Zhang et al., 2016).

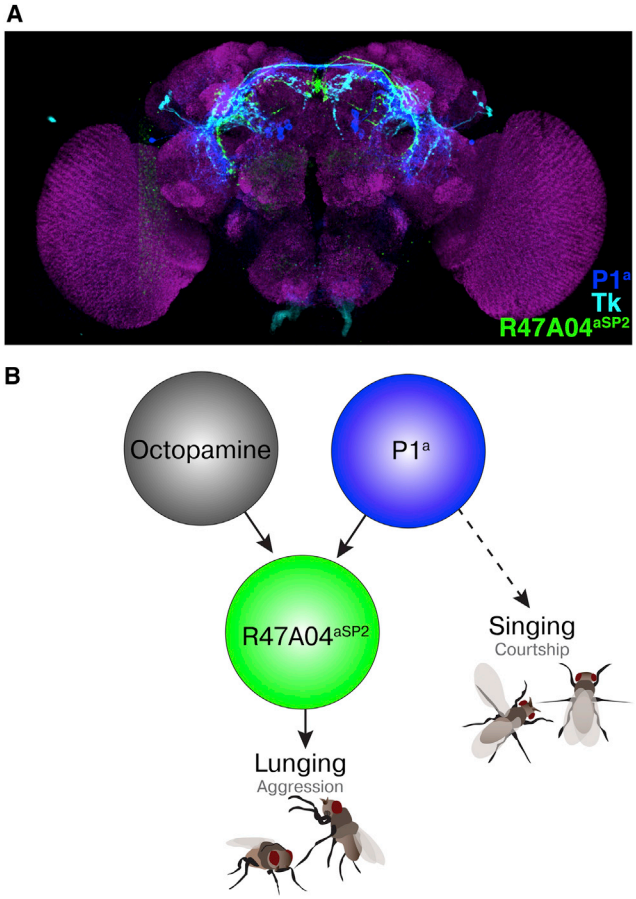

Figure 1. A Circuit Module Linking Neuromodulation with the Control of Aggression Behaviors in *Drosophila*

(A) Aggression-promoting neurons P1 (Hoopfer et al., 2015; blue), Tk (Asahina et al., 2014; cyan), and R47A04^{aSP2} (Watanabe et al., 2017; green) all overlap in the superior medial protocerebrum (SMP) of the *Drosophila* brain. P1^{a} are a subset of P1 (Hoopfer et al., 2015). Images courtesy of Eric Hoopfer, Margot Wohl, Kenta Asahina, and Kiichi Watanabe. Registration performed by Diego Pacheco (Murthy lab).

(B) R47A04^{aSP2} neurons bias males toward aggression in response to octopaminergic modulation. Schematic reproduced from Watanabe et al. (2017) and images reproduced with permission from Hoopfer (2016).

This study raises a number of new questions. First, what circuits downstream of the R47A04^{aSP2} neurons control and pattern aggression behaviors? The R47A04^{aSP2} neurons do not appear to be downstream of the Tachykinin (Tk) aggression- promoting neurons (Asahina et al., 2014), so they may represent a separate pathway for modulating aggression. Second, what can be made of the timescale of responses in R47A04^{aSP2} neurons to OA application (delayed) versus P1 activation (immediate)? Activation of P1 neurons in the presence of males induces wing extensions (and likely also singing) while the activating stimulus is on and lunging only after the stimulus has been turned off (Hoopfer et al., 2015): P1 activation may therefore have a "priming" effect on aggression behaviors via R47A04^{aSP2} neurons. R47A04^{aSP2} neurons do not, in contrast with P1 neurons, induce wing extensions when activated; these neurons therefore may bring the system above threshold for aggression, but not for courtship. Interestingly, males with R47A04^{aSP2} neurons silenced show an increase in wing extensions, indicating that turning down aggression-related behaviors is coordinated with turning up courtship-related behaviors. It will be interesting to determine if there exists a similar neuromodulatory pathway that biases the circuit instead toward courtship behaviors, and how this modulatory pathway interacts with the R47A04^{aSP2} neurons. However, it is important to keep in mind that all of the existing data are still consistent with the existence of two separate populations of P1 neurons that control courtship versus aggression; new reagents that specifically label subpopulations of P1 should help to resolve this issue. Third, the neurons studied here are all male-specific; however, OA has been shown to be important for both male and female aggression (Zhou et al., 2008). Does OA have a conserved role in modulating the choice between mating and aggression behaviors, but via distinct circuits, in females? Finally, aggression and courtship are high-level terms used to describe groupings of actions flies produce. Beyond lunging, what other actions do the R47A04^{aSP2} neurons modulate? The use of largely "unsupervised" methods to segment *Drosophila* behavior (Berman

et al., 2014) may provide important insights into how precisely OA and R47A04^{aSP2} neurons influence the dynamic movements and sensorimotor transformations that comprise fly social interactions.

Previous studies of OA and aggression in *Drosophila* were all consistent with OA (like its norepinephrine analog) broadly regulating arousal, and thereby having an indirect effect on aggression. By combining careful behavioral analysis with sophisticated genetic and neural circuit manipulations, the study from Watanabe et al. now reveals that OA can modulate specific networks that control social behaviors, biasing the output of the network in favor of driving aggression. It is tempting to speculate that similar mechanisms might underlie the effects of norepinephrine on social behaviors in larger brains, including those of humans.

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Synapse-Specific Encoding of Fear Memory in the Amygdala

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http://dx.doi.org/10.1016/j.neuron.2017.08.020

Input specificity is a fundamental property of long-term potentiation (LTP), but it is not known if learning is mediated by synapse-specific plasticity. Kim and Cho (2017) now show that fear conditioning is mediated by synapse-specific LTP in the amygdala, allowing animals to discriminate stimuli that predict threat from those that do not.

In order to survive, animals must be able to discriminate dangerous stimuli from those that are safe. That is, when confronted with an aversive and potentially lethal event, animals must learn the specific stimuli in the environment that predict danger so that they can mobilize adaptive defensive responses to those stimuli in the future. Without a mechanism for learning specific stimulus-outcome relationships, fear and defensive behavior broadly generalize to many stimuli and settings. This is a maladaptive state of affairs that may underlie fear and anxiety disorders.

Pavlovian conditioning is a fundamental form of learning that permits animals to encode specific stimulus-outcome associations and produce adaptive behavior in anticipation of those outcomes. For example, during fear conditioning, an innocuous stimulus, such as an acoustic tone (i.e., the conditioned stimulus [CS]), that has come to predict an aversive outcome, such as an electric shock (i.e., the unconditioned stimulus [US]), produces a host of defensive responses, including freezing behavior. Importantly, animals will readily learn to discriminate a CS (e.g., a CS+) that predicts the US from one that does not (e.g., a CS−).

Decades of work have now revealed the neural circuits underlying Pavlovian fear conditioning (Herry and Johansen, 2014). Sensory information from many brain areas, particularly the thalamus, hippocampus, and cortex, converge in the basolateral complex of the amygdala (BLA; including the lateral, basolateral, and basomedial nuclei). Considerable work indicates that long-term potentiation (LTP) at synapses transmitting CS information to the BLA underlies fear conditioning. That is, the ability of the once neutral CS to generate defensive behavior is mediated by an LTP-mediated increase in synaptic transmission onto BLA principal neurons (Bocchio et al., 2017).