the endothelial barrier, suggesting that OxPAPC might have different effects in the alveolar and intravascular compartments (Nonas et al., 2006). These seemingly discrepant findings could be reconciled in part if systemic challenge with OxPAPC directly (via TLR4) or indirectly desensitizes the activation of circulating leukocytes.

Given that the MyD88 pathway is critical to the host response to bacterial infections (Skerrett et al., 2007), the results of Imai and colleagues suggest that new strategies to modulate the TRIF-TRAF6 pathway, while leaving the MyD88 pathway largely intact, might be beneficial in some forms of ALI. Although the proximal event that creates the initial oxidative environment in the lungs remains unclear, neutrophil recruitment and activation are likely to be important because of the neutrophil's potent respiratory burst and because of the protection noted in Ncf1-deficient mice. Likewise, the key molecular "switch" that controls whether TRIF or MyD88 is activated by TLR4 remains a key unanswered question.

Almost 41 years after the clinical description of ALI, we have only one treatment that definitely improves survival, and this involves reducing the volume of air applied to the lungs during mechanical ventilation (Acute Respiratory Distress Syndrome Network, 2000). The work of Imai and colleagues points to potential molecular approaches that could further improve outcomes for this clinically important syndrome.

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# Fly Courtship Song: Triggering the Light Fantastic

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**In a study in this issue, Clyne and Miesenböck (2008) apply an ingenious optogenetic technology to activate neurons that generate male-specific courtship song in flies. This work sheds new light on the neural circuitry underlying sexually dimorphic behaviors in *Drosophila*.**

Courtship in the fruit fly *Drosophila melanogaster* is largely the domain of the male and consists of a series of intricate behaviors designed to achieve successful copulation. These behaviors include following, tapping and licking the female, and the extension of the male wing that is closest to the female and its vibration to generate male courtship song (reviewed by Billeter et al., 2006a). These behaviors depend on complex sensory and motor neural circuitry acting on specific effector tissues such as the limbs, wings, proboscis, and abdominal muscles of the male. The action of the neurons involved in these circuits can be related directly to the behavior they modulate such as courtship song production, which is critical for copulatory success. This behavioral output is robust and easily quantified, and so lends itself to structure/function analyses.

The ability to perform these sex-specific behaviors is dependent on the existence of a sexually dimorphic nervous system. Differences, both in neuronal numbers and projection patterns,

exist between male and female nervous systems and, although subtle, could result in profound dimorphic behavioral outputs (Stockinger et al., 2005; Kimura et al., 2005; Billeter et al., 2006b; Rideout et al., 2007; Datta et al., 2008). In *Drosophila*, the sex determination genes *fruitless* (*fru*) and *doublesex* (*dsx*) orchestrate the developmental events necessary for most aspects of "maleness" and "femaleness" (Billeter et al., 2006a). Insights into how these genes function in specifying sexual behavior can be inferred from their temporal and spatial expression patterns. In particular, distribution of the male-specific *fruitless* proteins ($Fru^M$) encoded by *fru* is highly suggestive, especially with respect to modulation of underlying behavioral circuitry, as it is expressed in subsets of neurons implicated in male courtship behaviors (Billeter et al., 2006a).

But why is courtship song exclusive to males? Studies of *Drosophila* sexual mosaic mutants show that both the protocerebrum region of the brain and the thoracic ganglia of the ventral nerve cord are required for male courtship song to occur (von Schilcher and Hall, 1979). In addition, expression of male-specific isoforms of both *dsx* ($Dsx^M$) and *fru* ($Fru^M$) are required for the production of complete wild-type courtship song (Rideout et al., 2007). So is this male-specific behavior an inherent consequence of differences in gene expression that affect excitability or connectivity within the song neural circuit? Or do both males and females possess the song circuitry but only males receive the appropriate input from higher-order "command neurons" to activate the circuit? These two models are not mutually exclusive; indeed, there is evidence for their coexistence (Kimura et al., 2005; Kvitsiani and Dickson 2006; Billeter et al., 2006b; Rideout et al., 2007; Datta et al., 2008). The study in this issue by Clyne and Miesenböck (2008) sets out to answer these questions. The authors use artificial photostimulation of a local *fru*-expressing neural circuit in flies to demonstrate that although wing extension and courtship song can be generated in both males and females, this song requires modulation by both local $Fru^M$ expression and by higher-order command neurons.

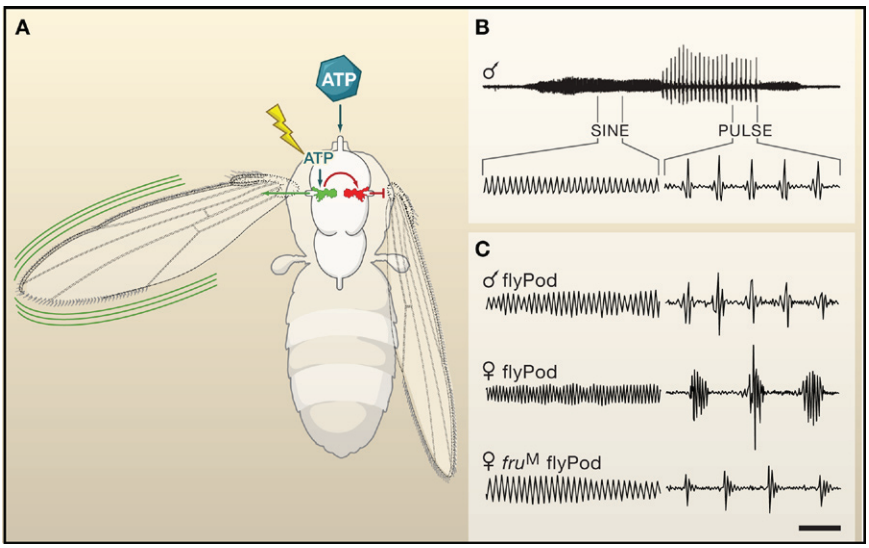


**Figure 1. Photoactivation of Male Courtship Song**

(A) In an optogenetic system used to generate unilateral wing extension and courtship song in male flies, a caged ATP molecule (DMNPE-ATP; blue hexagon) is injected into the cervical stalk of males. The ATP is converted into a free agonist through photolysis of the cage using 100 ms pulses of ultraviolet light. The free ATP then selectively depolarizes neurons expressing the *fruitless* (*fru*) gene that also express the ATP receptor P2X2 (green cluster) in one hemisegment of the thoracic ganglia. This results in transmission of a positive motor signal to the adjacent wing with consequent extension of that wing and the generation of courtship song (green arrow and curved green lines). These cells may send a negative signal (curved red arrow) to adjacent contralateral *fruitless* neuronal clusters also expressing P2X2 (red cluster) resulting in reciprocal inhibition and prevention of bilateral wing extension (red inhibitory bar).

(B) Voltage-time plot of wild-type *Drosophila* male courtship song. Sine and pulse components of courtship song may communicate species-specific information as well as increase receptivity of females to copulation (Billeter et al., 2006a). Sine and pulse segments, each ~200 ms in duration, are shown at an expanded timescale (black lines). In this example, the sine song frequency is 157 Hz and the interpulse interval average is 38 ms (Clyne and Miesenböck, 2008).

(C) Voltage-time plots of sine and pulse segments of songs elicited from decapitated flies (flyPods). Male flyPods most closely mimic the sine and pulse pattern of wild-type male courtship song. Female song is less clean and requires a 4-fold higher level of photostimulation. Females expressing the male isoform of the *fruitless* gene $Fru^M$ (she-males) more closely mimic the courtship song of wild-type males (Clyne and Miesenböck, 2008). Scale bar, 50 ms.

Clyne and Miesenböck expressed a light-activated ion channel (Lima and Miesenböck, 2005) (Figure 1A) in all *fru*-expressing neurons, enabling them to photoactivate *fru* expression at will (Stockinger et al., 2005). Initial behavioral outputs after photostimulation were observed in <2% of whole adult flies. The authors reasoned that this was due to potential conflicting signals when all *fru* neurons are stimulated simultaneously. To circumvent this problem, they performed experiments on decapitated adult flies (which the authors call "flyPods") lacking a brain but retaining the ventral nerve cord. Stimulation of these male flyPods resulted in recognizable wing extension 46% of the time. In addition, they demonstrated that photostimulation of ~20 *fru*-expressing neurons connecting the brain and the thoracic ganglia did not initiate wing extension, confirming that the circuitry necessary to generate this behavior resides solely within the thoracic ganglia.

The investigators showed that unilateral wing extension in male flyPods is a behavioral output related to song circuitry, because photostimulation of the neurons innervating flight muscles resulted in bilateral rather than unilateral wing extension. Furthermore, the authors noticed that the initial asymmetric wing choice, although apparently random, showed marked repetition, perhaps due to physiological or experimental constraints. Clyne and Miesenböck speculated that a *fru*-expressing neuronal cluster stimulated unilateral wing extension and also caused reciprocal inhibition of the contralateral cluster within the localized circuit of the pattern generator resulting in the blocking of bilateral wing extension (see Figure 1A).

The investigators then asked whether recognizable courtship song was generated during wing extension in males, females, and in females expressing $Fru^M$ (she-males) (Stockinger et al., 2005). Strikingly, they observed recognizable song patterns consisting of both sine and pulse song components not only in male but also in female flyPods (Figure 1C). Female flyPod song, however, required a 4-fold higher level of photostimulation and was "less clean," lacking the stereotypical pulse form and stable sine and pulse frequencies (Figure 1C). This difference between male and female flyPods appears to be due to variations within the underlying circuitry rather than a consequence of physiology, as $fru^M$ she-males (which have a masculinized neuronal circuit but a female morphology) were able to produce a song more akin to males at lower levels of photostimulation (Figure 1C). This result is notable given that, while the underlying circuitry necessary to generate song resides in the thoracic ganglia of both sexes, females and $fru^M$ she-males have ~20 fewer $Dsx^M$-dependent *fru*-expressing neurons per hemisegment than males (Rideout et al., 2007). Clyne and Miesenböck speculate that these male-specific *fru* neurons are critical for connecting and modulating the song circuit with descending interneurons. This speculation is reinforced by the fact that intact $fru^M$ she-males, although capable of wing extension comparable to males (Demir and Dickson 2005), are not able to generate recognizable courtship song (Rideout et al., 2007).

Can the flyPod-generated courtship song be recognized by wild-type females? The authors set out to determine the authenticity of song production in male, female, and $fru^M$ she-male flyPods by assaying the effectiveness of songs to induce copulation between virgin wild-type females and "dewinged" (and hence mute) males. Mute males are normally unsuccessful in achieving copulation due to their inability to produce song. Playing the song produced by the female flyPod had no effect on alleviating the courtship defect of mute males. However, when the courtship song from a male or $fru^M$ she-male flyPod was played, mute males were able to successfully copulate with virgin wild-type females. The effects of the $fru^M$ she-male song are particularly notable given that live, intact she-males rarely sing and what they do sing is largely incoherent (Demir and Dickson 2005; Rideout et al., 2007). The authors are careful to note that the male and she-male flyPod songs, although possessing the attributes of normal courtship song and capable of inducing copulatory behavior, still lack the metronomic precision and higher-pulse repetition rates of wild-type male courtship song.

With this study, Clyne and Miesenböck demonstrate the existence of an underlying localized neural circuit capable of generating wing extension and courtship song in flies. This circuit, although present in both males and females, must be modulated by $Fru^M$ expression to allow generation of an appropriate sex-specific courtship song and requires descending inputs from higher-order command neurons for song initiation and coordination. This new work clearly advances our functional understanding of a localized behavioral circuit. Importantly, in their attempt to identify what comprises the command structure for this circuit, the authors have taken us closer to elucidating how central circuits that regulate motor outputs are formed. Furthermore, this method of manipulating a defined neural circuit through artificial photostimulation complements other new optogenetic techniques, such as those for fine anatomical mapping of neurons and their projections (Datta et al., 2008). In future studies, this powerful tool kit will allow the marrying of circuit architecture and underlying cellular and synaptic properties to elucidate further how neural pathways control behaviors.

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