Mechanosensitive Neurons on the Internal Reproductive Tract Contribute to Egg-Laying-Induced Acetic Acid Attraction in Drosophila

Highlights

Drosophila females activate acetic acid attraction prior to egg laying

Distention of the internal reproductive tract can trigger AA attraction

Tract distention is monitored by mechanosensitive tract ppk1 neurons

Function of mechanosensitive tract ppk1 neurons is essential for AA attraction

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In Brief

Pregnancy can change the behavior of females significantly. Gou et al. show that for female fruit flies, the need to lay eggs temporarily increases their attraction for acetic acid and that mechanical stretch of their internal reproductive tract—induced by egg delivery in the tract—is one source of such need. This study identifies the neural basis of an important reproductive need for female flies and provides a model for studying how such need modifies behavior and sensory processing of females.
Mechanosensitive Neurons on the Internal Reproductive Tract Contribute to Egg-Laying-Induced Acetic Acid Attraction in *Drosophila*

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**SUMMARY**

Selecting a suitable site to deposit their eggs is an important reproductive need of *Drosophila* females. Although their choosiness toward egg-laying sites is well documented, the specific neural mechanism that activates females’ search for attractive egg-laying sites is not known. Here, we show that distension and contraction of females’ internal reproductive tract triggered by egg delivery through the tract plays a critical role in activating such search. We found that females start to exhibit acetic acid (AA) attraction prior to depositing each egg but no attraction when they are not laying eggs. Artificially distending the reproductive tract triggers AA attraction in non-egg-laying females, whereas silencing the mechanosensitive neurons we identified that can sense the contractile status of the tract eliminates such attraction. Our work uncovers the circuit basis of an important reproductive need of *Drosophila* females and provides a simple model for dissecting the neural mechanism that underlies a reproductive need-induced behavioral modification.

**INTRODUCTION**

The need to care for offspring can alter behaviors of animal mothers significantly. Many commonly described maternal behaviors such as feeding and aggression against intruders serve the purpose of nurturing and protecting the newborns. But in some species, pregnancy alone is sufficient to induce changes in sensory processing and behaviors (Rosenblatt and Lehrman, 1963). Pregnancy-induced hormonal changes are thought to play a role in activating “prenatal care behaviors” (Kristal, 2009), but the exact circuit mechanism by which the presence of a fetus in utero modifies the behaviors of expectant mothers remains not well understood.

The fruit fly *Drosophila melanogaster* has emerged as a suitable model to study the genetic and circuit basis of female reproductive behaviors. Similar to higher animals, virgin and mated/pregnant flies show significant differences in their behaviors and physiologies. For example, virgins are receptive to male courtship and lay very few eggs, whereas mated females are unresponsive to courtship, lay eggs frequently, and preferentially consume proteins over sugars (Carvalho et al., 2006; Kubli, 2003; Ribeiro and Dickson, 2010). Much progress has been made in recent years in elucidating the molecular and circuit basis by which the experience of mating modifies physiologies and behaviors of female flies (Bussell et al., 2014; Feng et al., 2014; Häsemeyer et al., 2009; Rezával et al., 2012; Yang et al., 2009; Zhou et al., 2014). In contrast, whether and how egg-laying need influences how female flies interpret the valence of external stimuli—so as to guide their decision of whether to move toward or away from specific stimuli—remains little explored, despite the fact that female flies are known to be highly selective about where to lay eggs (Azanchi et al., 2013; Dweck et al., 2013; Joseph et al., 2009; Joseph and Heberlein, 2012; Rockwell and Grossfield, 1978; Schwartz et al., 2012; Yang et al., 2009).

Here, we show that egg-laying need increases female flies’ attraction for acetic acid (AA) significantly. Behavioral analysis reveals that signs of AA attraction emerge prior to physical egg deposition. Manipulating the internal egg-delivery process (that precedes physical egg deposition) reveals that artificial distention of the internal reproductive tract is sufficient to activate AA attraction and that mechanical stretch of the reproductive tract is sensed and relayed to the CNS by a set of piezo-expressing sensory neurons. Our results suggest a model in which *Drosophila* females modify their AA attraction by assessing, via mechanosensitive neurons on the tract, whether eggs are being pushed through their reproductive tract. We propose such activation of AA attraction in anticipation of impending physical egg laying may be considered a rudimentary form of maternal care and provide a suitable model to study the circuit mechanism by which reproductive needs modify female behaviors.

**RESULTS**

Egg Laying, but Not Mating, Correlates with Mated Females’ Positional Preference for AA

It has been shown that, as a population, mated females show stronger positional preference for AA than virgins do, and they also prefer to lay eggs on an AA-containing versus an AA-free
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substrate (Joseph et al., 2009). Because mating induces several behavioral and physiological changes, we first ascertained that mated females' increased AA preference is triggered by egg-laying need as opposed to other mating-induced changes. We custom-built an apparatus that contains several chambers, each of which can house one AA+ and one AA− substrate (Figure S1B). We loaded females into this apparatus (one per chamber), recorded their behaviors for 4 hr (Figure S1A; Movie S1), and tracked their positions using Ctrax (Branson et al., 2009). Similar to what was reported previously (Joseph et al., 2009), we found that mated, egg-laying females show a clear preference to lay eggs and to spend time on an AA site (Figure 1A; Table S1).

To disentangle the impact of mating versus egg laying on positional preference for AA, we compared AA preference of mated females that lay very few eggs versus virgins that lay many. (We have discovered that mated females reduce their egg-laying rate significantly if their diet is deprived of yeast paste, a protein source that boosts egg production. Conversely, virgins that have been continuously fed yeast paste after eclosion can sometimes lay many eggs.) We found that mated females that lay very few eggs show no positional preference for AA, but virgins that lay many do (Figure 1A). Importantly, neither yeast-fed males nor yeast-fed virgins that lay few eggs show any AA preference (Figure S1F). Together, these results suggest egg laying, not mating or yeast feeding, is responsible for mated females' positional preference for AA.

**Egg-Laying Females Show “Active Attraction” for AA**

We next determined whether egg-laying need triggers “active AA attraction” in addition to positional preference for AA. We noticed that females move constantly between AA+ and AA− free substrates but occasionally reverse their running direction in the middle of the chamber. We labeled a reversal where females would switch from moving away to moving toward AA substrate an “attractive return” and proposed that it indicates active AA attraction (Figure 1B, right panel). A recent report uses a similar criterion to define behavioral attraction (Gao et al., 2013). Similarly, we proposed a reversal from moving toward to moving away from AA substrate signals active AA aversion and labeled it an “aversive return” (Figure 1B, left panel). We can then determine net AA attraction by calculating an “AA attraction index” (Figure 1B). Indeed, regardless of their mating status, females that lay many eggs show a significantly stronger AA attraction than ones that lay very few eggs (Figure 1B; Figure S1G), suggesting increased egg laying is sufficient to induce active AA attraction.

We have so far compared AA attraction of two types of females: one that lays many eggs and one that does not. But if egg-laying need is indeed the trigger for AA attraction, the same female should exhibit different levels of AA attraction depending on her egg-laying rate in a given time period. So we sought to correlate egg-laying rate and AA attraction in the same animals and found that temporal distribution of egg-laying events is nonuniform: there are clearly periods when a female lays eggs frequently and ones when she does not (Figure 1C, blue and green traces). Moreover, females consistently move faster during “no egg-laying” periods than “high egg-laying” periods (Figure 1C, blue and green traces), allowing us to use locomotion speed to separate their trajectories into high versus low egg-laying states (Figures 1C, S1C, and S1D). We found that individuals show stronger AA attraction when they are actively laying eggs than when they are not (Figure 1D). An examination of the “return point” (Figure 1B) further revealed that females in a high egg-laying state tend to execute their attractive returns at positions closer to AA (Figures 1E and 1E′), suggesting that when laying eggs actively, females are less tolerant about staying away from AA.

Finally, if egg-laying need is a trigger for AA attraction, then signs of attraction might emerge prior to each egg laying. We examined the trajectory in the 1 min window immediately before and after 151 egg-laying events and found that, indeed,
attractive returns tend to occur before, but not after, egg laying (Figures 1F and 1F). Moreover, when we segmented the trajectory before each egg laying into four consecutive 1 min periods, we found that the number of attractive returns increases as females are nearing to laying an egg (Figures 1F and 1F). In contrast, aversive returns occur very rarely in these windows (Figure S1E). Taken together, our analysis shows that Drosophila females are more attracted to AA when they are actively laying eggs and that such attraction emerges prior to physical egg laying.

Persistent Presence of Egg(s) in the Internal Reproductive Tract Is Sufficient to Trigger AA Attraction

Next, we wanted to uncover the neural basis by which egg-laying need activates AA attraction prior to physical egg laying. We looked closer at the egg-delivery process that occurs in females’ internal reproductive tract. While egg-laying becomes obvious to observers only when females begin to display the ovipositor motor program (Yang et al., 2008), it has begun once an egg starts to descend from the ovaries into the internal reproductive tract—an epithelial tube that connects the two ovaries to the uterus, where eggs are fertilized (Figure 2A). The reproductive tract has a small diameter and is encased by muscles that are innervated by sensory and motor neurons (Castellanos et al., 2013; Hässemeyer et al., 2009; Yang et al., 2009). Because egg delivery in the tract precedes physical egg deposition, we hypothesize that perhaps females start to increase AA attraction once they sense eggs are being pushed through the tract.

To test this idea, we first assessed AA attraction of females who have eggs “persistently trapped in the tract.” It has been shown recently, and we confirmed, that some of the ILP7-Gal4-expressing neurons in the ventral nerve cord (VNC) are motor neurons that innervate the tract (Figure 2B) (Castellanos et al., 2013). Importantly, inhibiting ILP7 neurons with Kir2.1 (Baines et al., 2001) consistently causes one or more eggs to be “jammed” in the tract (Castellanos et al., 2013; Yang et al., 2008) (Figure 2C), providing us with the desired phenotype to test our hypothesis. Indeed, females with inhibited ILP7 neurons
showed clear AA attraction, despite that they cannot physically lay any eggs (because their tract is jammed) (Figure 2E). Importantly, females of the same genotype but deprived of yeast paste showed much reduced egg jamming (Figure 2D) as well as much reduced AA attraction (Figure 2E), suggesting that the persistent presence of eggs in the tract, not other changes induced by silencing ILP7 neurons, is what triggers AA attraction. Thus, we propose that during regular egg laying, AA attraction is activated each time an egg is being squeezed through the tract.

**A Group of ppk1-Expressing Sensory Neurons on the Reproductive Tract Can Sense Tract Contraction**

How do females sense that an egg is being squeezed through their reproductive tract? We speculate some sensory neurons on the tract are mechanosensors. We have previously shown that the ppk-Gal4-labeled sensory neurons on the tract can be divided into at least two groups (Häsemeyer et al., 2009; Yang et al., 2009) (Figure 3A). The first group expresses the fruitless transcripts, senses sex peptide (SP), and has relatively short dendrites (Häsemeyer et al., 2009; Rezával et al., 2012; Yang et al., 2009).

**Figure 3. A Subgroup of ppk1-Expressing Sensory Neurons that Innervate the Reproductive Tract Can Sense Tract Contraction**

(A) A diagram showing a subset of ppk1 neurons that extends dendrites on the tract and project axons to the VNC. There is one ppk1 neuron on each lateral duct (red arrows) and two to three on each side of the base of the common duct (green arrows). Black arrow points to the anterior/posterior (A/P) divide demarcated by the ppk1 dendrites. Note that we use ppk1 and ppk1-Gal4 interchangeably.

(B) Dendrites (red arrows) of ppk1 neurons partition the tract into distinct domains. Note that cell bodies of ppk1 neurons on the lateral ducts are suspended outside of the tract and are easily torn off during dissection, but one cell body remains attached to the tract in this picture (blue arrow).

(C) piezo-Gal4 labels the same “tract-tiling” neurons as ppk1-Gal4. Blue arrows, somas of the sensory neurons on the lateral ducts; black arrow, A/P divide.

(D and E) Dendrites and axons of the tract ppk1 neurons labeled by ppk1-Gal4 driving GFP in the presence of ppk1-Gal80. (D) The “subtracted” animals still show labeling of tract-tiling sensory neurons. (E) The tract-tiling ppk1 neurons target their axons to the posterior tip of the VNC. Scale bar for (E) is 25 μm. See Figure S2 for a comparison of the axonal projection labeled by ppk1-Gal4 before and after ppk1-Gal80-mediated “subtraction.”

(F) Our preparation. To activate ILP7 neurons, we used ILP7-LexA to express LexAop2-P2X2 and bath-applied ATP. To assess activity changes of ppk1 neurons, we used ppk1-Gal4 to express UAS-GCaMP3 and imaged their axonal termini in the VNC. The connectives between VNC and reproductive tract in were usually carefully preserved, but we severed them when assessing whether the GCaMP3 increase in ppk1 axons might be due to local activation of ppk1 axons by ILP7 neurons (see also Figure S3).

(G and H) Stimulating ILP7 neurons induces a clear contraction of the reproductive tract. (G) Tract before stimulation. ILP7 axons are visible because they coexpress GCaMP3 and P2X2. (H) ATP causes a clear contraction of the tract and a significant GCaMP3 increase (n = 10 animals). Scale bar for (G) and (H) is 25 μm. See also Movie S2.

(I–K) Stimulating tract contraction (via stimulating ILP7 neurons) induces a clear GCaMP3 increase in tract ppk1 neurons with (9/20) and without (8/8) the presence of ppk1-Gal80. (I) GCaMP3 response of ppk1 axons when perfused with buffer. (J) GCaMP3 response of the same axons when perfused with ATP. (K) Quantification of the ATP-induced GCaMP3 change. ***p < 0.001. Mann-Whitney test. Note that the GCaMP3 increase is more pronounced in the area where tract ppk1 axons terminate (pink arrow) than where other axons terminate (white arrow). See also Movie S3.
Laying-Induced AA Attraction

We suspect ppk neurons of the second group, two on lateral ducts and four to six at the base of the common duct (Figures 3B, S2H and S2I), are mechanosensors. They have large dendrites that “tile” the entire surface of tract (Figure 3B) and appear to express the mechanosensitive channel Piezo (Coste et al., 2010; Coste et al., 2012; Kim et al., 2012)(Figures 3C and S2E). However, there is no direct evidence showing that these “tract-tiling” ppk1 neurons are indeed capable of sensing tract contraction/distention.

Next, we devised a three-step approach to test whether these tract-tiling ppk1 neurons are mechanosensors (Figure 3F). First, we used the calcium sensor GCaMP3 (Tian et al., 2009) to monitor activities of axonal termini of tract ppk1 neurons. The (GCaMP3 signal in ppk axons in the VNC is less likely to move out of the focal plane when tract contraction occurs.) To define the specific VNC region targeted by tract ppk1 neurons, we made use of a ppk1.0-Gal80 (Häsemeyer et al., 2009). This ppk1.0-Gal80 suppresses Gal4-dependent expression in nearly all ppk1-Gal4 neurons except ones that reside on the tract, revealing that axons of tract ppk1 neurons consistently target to the posterior tip of the VNC (Figures 3D, 3E,S2A, and S2B).

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To determine if the mechanosensitive tract ppk1 neurons play a role in promoting AA attraction, we next targeted them for inhibition. In our first “subtraction-based” approach, we used the ppk1-Gal4 to express Kir2.1 in the presence of ppk1-Gal80. This manipulation caused females to have eggs jammed in their tract (Figure 4A), but unlike ILP7-inhibited females, they showed no AA attraction (Figure 4A). In our second “intersection-based” approach, we used the 21-7-Gal4 that labels tract ppk1 neurons, but not many of the none tract ppk1 neurons (Song et al., 2007), to express an UAS-FRT-stop-FRT-Kir2.1 (Yang et al., 2009) and introduced a ppk1-LexA and a source of fip into the same animals. Because 21-7-Gal4 and ppk1-LexA are coactive mostly only in the tract ppk1 neurons, animals bearing...
all four transgenes (ppk1-LexA, LexAop2-flo, 21-7-Gal4, and UAS-FRT-stop-FRT-Kir2.1) should mostly have only their tract ppk1 neurons inhibited (Figures S2C and S2D). Again, these “intersected” animals show an “egg-jamming” phenotype but no AA attraction (Figure 4C). Lastly, restricting Kir2.1 expression to only the adult stage still reduced AA attraction (Figure 4B), ruling out potential developmental problems due to chronic silencing of ppk1 neurons as the cause for the lack of AA attraction we observed. Thus, active tract ppk1 sensory neurons are required to activate AA attraction induced by egg presence in the tract.

To test whether the mechanosensitivity of the tract ppk1 neurons is important for AA attraction, we reduced their piezo expression. We first used 21-7-Gal4 to express a piezo-RNAi (Kim et al., 2012). In addition, we also used two copies of ppk1-Gal4—in the presence of ppk1-Gal80—to express the piezo-RNAi. Neither set of animals showed AA attraction, suggesting that piezo expression in tract ppk1 neurons is important for AA attraction (Figure 4C).

Finally, we asked whether artificial activation of tract ppk1 neurons in the absence of active egg delivery is sufficient to trigger AA attraction. We found that expressing in tract ppk1 neurons the sodium channel NaChBac (Luan et al., 2006), a commonly used effector for increasing membrane potential, failed to trigger AA attraction in yeast-deprived females (Figure 4D). Because NaChBac overexpression may not be effective in stimulating ppk1 neurons, we also used the heat-gated dTRPA1 to stimulate ppk1 neurons. However, increasing temperature alone causes a significant alteration in AA attraction, making data interpretation difficult. Thus, we are unable to conclude whether stimulating ppk1 neurons is sufficient to induce AA attraction.

**DISCUSSION**

In this report, we discovered that egg-laying need can activate AA attraction in Drosophila females and that mechanical stretch of the tract—induced by egg delivery through their internal reproductive tract—is one origin of the “egg-laying need.” We demonstrate that egg delivery in the internal reproductive tract is an important physiological signal that modulates how Drosophila females interpret the valence of sensory stimuli. The flexibility in egg-laying need-induced modification of sensory processing contrasts significantly to that triggered by SP: once SP gains control of the female CNS, it keeps the mated females unreceptive to AA. Thus, there is no need for females to remate until the stored sperm is passed of eggs through the tract may trigger release of hormones from the tract that then act together with ppk1 neurons to modulate behavior. Nevertheless, it is interesting to note that Drosophila is not the only species that relies on reproductive tract-generated mechanical stimuli to activate “mating behaviors.”

Mechanical stimulation of uterine wall has been shown to contribute to maternal behavior activation in sheep, dogs, and rats (Hayes and De Vries, 2007; Kendrick et al., 1991; Keverne et al., 1983; Lévy et al., 2010; Poindront et al., 1989; Yeo and Keverne, 1986), raising the possibility that this feature of behavior control may be evolutionarily conserved.

Which sensory system is responsible for egg-laying-induced AA attraction? Several reports suggest AA can promote attraction through the olfactory system (Ai et al., 2010; Root et al., 2011; Semmelhack and Wang, 2009), but Joseph et al. showed that egg-laying preference for AA depends on taste, but not olfaction (Joseph et al., 2009). Indeed, we have found that Ir64a and Or83b, two olfaction mutants with defective AA sensing (Ai et al., 2010; Semmelhack and Wang, 2009), both show strong egg-laying-induced AA attraction (Figures S4E and S4F). But if egg-laying-induced AA attraction is indeed taste driven, then it may be partly driven by taste memory. This is because “attractive returns” occur in the middle of the chamber where females’ taste neurons are not in contact with AA. Mushroom body (MB) has been shown to regulate egg-laying-induced positional preference for AA (Joseph et al., 2009), but we did not observe a significant change in AA attraction when we ablated it (Figures S4A–S4D), suggesting the taste memory that guides attractive returns in our paradigm is likely stored elsewhere. Determining the identity of the AA-sensing taste neurons that promote AA attraction will be an important next step.

Lastly, we also do not know which brain center(s) responds to tract ppk1 neurons to modify valence of AA signal. Pars intercerebralis, the neuroendocrine center, may be one potential (indirect) target, given that it was proposed recently to be a potential target of tract neurons that signal mating status change (Feng et al., 2014) and that hypothalamus, its vertebrate counterpart, is important for modulating reproductive behaviors also.

**EXPERIMENTAL PROCEDURES**

**Stocks**

The following stocks were used in this work: ILP7-Gal4 (Yang et al., 2008), piezo-Gal4 (Kim et al., 2012), 21-7-Gal4 (Song et al., 2007), hs-FLP (Gordon and Scott, 2009), UAS-Kir2.1eGFP (Baines et al., 2001), ppk1-Gal4, UASmCD8-GFP, UAS-mCD8-RFP, UAS-NaChBac (Bloomington Drosophila Stock Center), UAS-FRT-CD2-stop-FRT-Kir2.1eGFP (Yang et al., 2009), UAS-P2X2 (Lima and Miesenböck, 2005), UAS-GCaMP3 (Tian et al., 2009), UAS-piezo-RNAi (Kim et al., 2012), ppk1-LexA, ppk1.0-Gal80 (Häsemyer et al., 2009), ILP7-LexA, LexAop2-FLP (Pan et al., 2012), LexAop2-P2X2, ppk1-GS-Gal4, LexAop2-GCaMP3, and w1118.

**Transgenic Animals**

ILP7-LexA, ppk1-LexA constructs were produced by cloning the 1 kb promoter upstream of ILP7 and ppk1 genes, respectively, into the recently modified LexA construct (Pfeiffer et al., 2010). LexAop2-GCaMP3 and LexAop2-P2X2 were constructed by cloning the GCaMP5 (Tian et al., 2009) and P2X2 (Lima and Miesenböck, 2005) genes into LexAop2 vectors (Pfeiffer et al., 2010), respectively.
et al., 2010). These constructs were then injected into attp-carrying animals following standard protocol.

**Immunohistochemistry**

Tissues were processed following the same protocol as previously described (Yang et al., 2008). Images were acquired using a Zeiss LSM 700 laser scanning confocal microscope. The following primary antibodies were used: mouse anti-Dig (1:10, DHSB), rat anti-mcD8 (1:100, Invitrogen), mouse anti-GFP (1:100, Sigma-Aldrich), and rabbit anti-GFP (1:1,000, Invitrogen). The following secondary antibodies were used: goat anti-mouse Alexa 488, goat anti-mouse Cy3, goat anti-rat Alexa 488, goat anti-rabbit Alexa 488, and goat anti-rabbit Cy3.

**Hydroxyurea Treatment to Ablate the Mushroom Body**

To ablate the mushroom body, 0- to 1-hr-old larvae were collected and fed hydroxyurea (HU) (H8627, Sigma) dissolved in inactive yeast paste (50 mg/ml) for 5 hr and then transferred into fresh HU-free food.

**Egg-Laying Preference Assay**

Females were collected into groups of 20–30 with 15–20 males at the first day of eclosion and kept in fly incubator (25°C and 65% humidity) for 4–5 days. The vials where females were kept were supplemented with active yeast paste unless we wanted to reduce their egg production. Prior to egg-laying/behavior assays, individual female flies were loaded into chambers in a custom-designed two-choice egg-laying apparatus (Figures S1A and S1B). The 3% AA (v/v) substrate and plain substrate were made from 1% agarose that has been preheated in the 55°C water bath. The substrates were allowed to set for 30 min before experiments commence.

**Behavior Analysis**

Files were raised and placed into chambers as described above. A camera holder with four Microsoft LifeCam Cinema cameras was then attached to the top of the chamber-containing apparatus (Figure S1A). Each camera was positioned above two egg-laying chambers to record two flies. Videos were acquired by CamUniversal and then converted to allow faster tracking using Avidemux. Egg-laying events were annotated manually by visually inspecting the videos. Citrax (Branson et al., 2009) was used to track the fly positions. Custom MATLAB code was used to detect parameters we used to calculate positional preference index and attraction index and to separate the trajectories into high and low egg-laying states based on the flies’ locomotion speed (Figures S1C and S1D). The speed is calculated first by determining the path length every 2 s (at 7.5 frames/s). We imposed a 1 min threshold when separating the low versus high locomotion states. This is because flies sometimes increase their running speed suddenly and briefly—even though they have been consistently in a state of low locomotion—and they then immediately slow down and lay an egg (Figure 1C). Thus, if flies do not maintain their increased speed for at least 1 min, we consider such changes too transient to be labeled as “high locomotion state.”

**Tract Contraction Assay**

To determine whether stimulating ILP7 can cause tract contraction (Figures 3G and 3H), we isolated the tract from the rest of the body and pinned it onto a custom-made perfusion chamber that contains insect physiology buffer (Xiang et al., 2010). We then either perfused the chamber with buffer alone or with buffer with ATP (400 μM).

**GCaMP3 Recording**

Intact VNC (with their connection to the tract carefully preserved) was dissected and mounted in a custom-built perfusion chamber and imaged through a water-immersion 40× lens on our Zeiss LSM 700 confocal scope. To image neuronal activity, we recorded GCaMP3 fluorescence at one frame per second using the time-series function software. We normally waited for the baseline to stabilize before perfusing buffer alone for 3 min and then buffer with ATP to stimulate specific neurons and recorded changes in GCaMP3 signal. The acquired images were then analyzed using a custom MATLAB code. Change in fluorescence (ΔF/F) in a given ROI was averaged from signals from all focal planes.

**REFERENCES**


**SUPPLEMENTAL INFORMATION**

Supplemental Information includes four figures, one table, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.09.033.

**AUTHOR CONTRIBUTIONS**

C.-H.Y. conceived the project with help from B.G. and Y.L. B.G. and Y.L. performed the experiments and analyzed the results with help from A.G., U.S., and C.-H.Y. U.S. designed the behavior setup. B.G., Y.L., and C.-H.Y. wrote the paper.

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