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Sensory Neurons in the *Drosophila* Genital Tract Regulate Female Reproductive Behavior

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SUMMARY

Females of many animal species behave very differently before and after mating. In *Drosophila melanogaster*, changes in female behavior upon mating are triggered by the sex peptide (SP), a small peptide present in the male's seminal fluid. SP activates a specific receptor, the sex peptide receptor (SPR), which is broadly expressed in the female reproductive tract and nervous system. Here, we pinpoint the action of SPR to a small subset of internal sensory neurons that innervate the female uterus and oviduct. These neurons express both *fruitless* (*fru*), a marker for neurons likely to have sex-specific functions, and *pickpocket* (*ppk*), a marker for proprioceptive neurons. We show that SPR expression in these *fru*$^+$ *ppk*$^+$ neurons is both necessary and sufficient for behavioral changes induced by mating. These neurons project to regions of the central nervous system that have been implicated in the control of reproductive behaviors in *Drosophila* and other insects.

INTRODUCTION

An animal's behavioral choices depend not only on external sensory input, but also on internal states that must be sensed and conveyed to the relevant neural circuits. The reproductive behaviors of *Drosophila melanogaster* females provide an ideal model system to explore the mechanisms by which internal states are sensed and to determine how they guide behavioral choices (Dickson, 2008). Virgin females are sexually receptive and lay only very few eggs, whereas females that have already mated are unreceptive and begin to lay eggs (Bloch Qazi et al., 2003; Kubli, 2003). To choose correctly between these distinct behaviors, the relevant central circuits must be informed of the female's mating status.

The cue that signals a female's mating status is the sex peptide, SP, a 36-amino acid peptide present in the male seminal fluid (Chen et al., 1988). Females do not choose postmating behaviors if they mate with males that lack SP (Chapman et al., 2003; Liu and Kubli, 2003), whereas injection of synthetic SP causes virgin females to behave as though they had mated (Chen et al., 1988). How does SP, present in the female reproductive tract, modulate the central circuits that control female mating behaviors?

The prevailing view is that SP is transported across the epithelium of the genital tract, enters the hemolymph, and acts directly on CNS targets (Kubli, 2003). Consistent with this view, SP, like many other male seminal fluid proteins (Lung and Wolfner, 1999; Monsma et al., 1990; Ravi Ram et al., 2005), can be detected in the hemolymph of mated but not virgin females (Pilpel et al., 2008). However, an alternative possibility is that the SP signal is conveyed to the CNS by a direct neural pathway from the reproductive tract. Such a route has been proposed for some species of moth, in which unidentified male substances elicit analogous postmating responses in females (Foster, 1993; Giebultowicz et al., 1990; Jurenka et al., 1993). An important first step toward distinguishing between these possibilities and ultimately understanding how SP modulates behavioral circuits in the CNS is to identify the cellular targets of SP.

We recently identified a molecular receptor for SP, called SPR, a member of the G protein-coupled receptor family (Yapici et al., 2008). SPR is broadly expressed in the female reproductive tract and nervous system, but the behavioral responses to mating can be entirely attributed to SPR function in the nervous system (Yapici et al., 2008). Moreover, expression of SPR in neurons that express the sex-specific transcripts of the *fruitless* (*fru*) gene is both necessary and sufficient for these behavioral responses (Yapici et al., 2008), supporting the notion that SP might act on some subset of the *fru* neurons (Kvitsiani and Dickson, 2006).

The *fru* gene labels ~2000 different neurons, including both sensory and central neurons (Billeter and Goodwin, 2004; Manoli et al., 2005; Stockinger et al., 2005). Which of these neurons are the specific targets of SP remains unknown. Here, we show that postmating behavioral responses are mediated by SPR function in a set of just 2–3 internal sensory neurons located on either side of the uterus. These sensory neurons have rich arborizations within the lumen of the reproductive tract and project to central targets in the abdominal and/or subesophageal ganglia. We propose that SP modulates signals that these neurons convey to the CNS, thereby regulating the central circuits that govern female reproductive behavior.

RESULTS

A GAL4 Screen Identifies Neurons that Require SPR Function

We initially identified SPR in a genome-wide pan-neuronal RNAi screen (Yapici et al., 2008). In this screen, we crossed the

panneuronal *elav-GAL4* driver to a genome-wide collection of RNAi transgenes (Dietzl et al., 2007) and scored female progeny for egg-laying defects. Mated *elav-GAL4 UAS-SPR-IR* females lay very few eggs and remain sexually receptive, and thus, like *SPR* null mutants, behave as though they were still virgins (Yapici et al., 2008). To define the cellular requirement for SPR function, we now inverted the logic of this screen, crossing the *UAS-SPR-IR* transgene to a collection of 998 GAL4 lines and scoring the female progeny for egg-laying defects in the same fashion (Figure 1A). In each of these lines, the GAL4 transcriptional activator is expressed in a random but stereotyped subset of cells, in which *SPR* function should now be inhibited by the *UAS-SPR-IR* transgene.

We identified 59 lines that resulted in a strong and reproducible egg-laying defect. Many of these lines were found to be broadly expressed, as revealed with a *UAS-mCD8-GFP* reporter. These lines were not examined further. More restricted neuronal expression was observed in seven lines, and for each of these we performed a series of secondary assays to confirm the egg-laying defect and to assess the receptivity of both virgin and mated females (Figure 1B). For all seven *GAL4* lines, SPR knockdown resulted in reduced egg laying and increased remating of mated females, but little if any change in the receptivity of virgin females (Figures 1C–1E). These defects were indistinguishable from those observed upon panneuronal SPR knockdown with the *elav-GAL4* driver (Figures 1C–1E), or in *SPR* null mutant females (Yapici et al., 2008). For the most restricted of our positive *GAL4* lines, *ppk-GAL4*, we confirmed that these defects can indeed be attributed to a diminished response to SP (Figure 1F).

SPR Is Required in *ppk*$^+$ Sensory Neurons in the Female Reproductive Tract

Increased remating is not an obligatory consequence of reduced egg laying (Barnes et al., 2007), yet for all 7 *GAL4* lines egg-laying defects were correlated with high remating frequencies. This suggests that the two postmating responses might be mediated by SPR function in a common set of cells, rather than the direct action of SP on distinct circuits for egg laying and receptivity. Accordingly, we sought to determine the sites of expression that are common to all seven *GAL4* lines.

Preliminary analyses identified *ppk-GAL4* as the line with the most restricted expression pattern. Using a nuclear targeted *UAS-lamin-EGFP* reporter, we found that *ppk-GAL4* drives expression almost exclusively in peripheral sensory neurons in the legs, wings, and body wall, as well as a small number of neurons associated with the female reproductive tract (Figure 1G). No cells are consistently labeled within the central nervous system (Figures 1H and 1I), although occasionally we detected one or two *ppk*$^+$ cell bodies near the base of the antennal nerve or in the lateral protocerebrum (Figure 1H). No *ppk*$^+$ cells could be detected within the ventral nerve cord (Figure 1I).

Like *ppk-GAL4*, the six other positive *GAL4* lines also labeled cells along the reproductive tract (see Figure S1 available online). In particular, all of these lines label the 2–3 sensory neurons located on either side of the uterus, whereas the sensory neurons in the legs, wings, and body wall were not consistently labeled by the other positive *GAL4* lines. Accordingly, we conclude that the behavioral changes induced by SP require *SPR* function in the *ppk*$^+$ reproductive tract sensory neurons.

ppk$^+$ *fru*$^+$ Sensory Neurons Innervate the Reproductive Tract

As visualized with a *UAS-mCD8-GFP* reporter, the *ppk*$^+$ reproductive tract neurons project fine processes between the muscle and epithelial cell layers to enter and arborize within the lumen of the uterus (Figures 2A–2E). An additional branch bifurcates close to the soma and innervates the lower regions of the common oviduct (Figure 2A). As judged by confocal microscopy, the arborizations in both the uterus and lower oviduct run along the inner surface of the epithelial cell layer.

We had previously mapped the requirement for SPR function to the set of ~2000 *fru*$^+$ neurons defined by expression of the *fru*^{GAL4} driver, but not of course the male-specific Fru^M protein (Yapici et al., 2008). We therefore suspected that some or all of the *ppk*$^+$ neurons might also be *fru*$^+$. Indeed, in *fru*^{GAL4} *UAS-mCD8-GFP* females we observed GFP$^+$ neurons near the uterus that appeared identical to the *ppk*$^+$ neurons (Figure 2F). Using a *ppk-EGFP* reporter (Grueber et al., 2003) and *fru*^{GAL4} *UAS-hist-RFP*, we confirmed that the *ppk*$^+$ uterus sensory neurons are indeed *fru*$^+$ (Figure 2G). The *fru*^{GAL4} driver is stronger than *ppk-GAL4* and with *UAS-mCD8-GFP* reveals additional fine processes extending into the lower uterus (Figure 2F). Like several of the other positive *GAL4* lines, *fru*^{GAL4} did not label the *ppk*$^+$ cells near the base of the ovary and the tip of the uterus.

SPR Expression in *ppk*$^+$ *fru*$^+$ Neurons Is Sufficient for the Mating Switch

Our RNAi knockdown experiments establish that *SPR* function is required in the *ppk*$^+$ *fru*$^+$ uterus sensory neurons, but they do not preclude an additional requirement for *SPR* function in other cells. To test this possibility, we used *ppk-GAL4* to drive a *UAS-SPR* transgene in *SPR* null mutant females. In these females, *SPR* function is present only in *ppk*$^+$ cells. In assays for virgin receptivity, egg laying, and remating frequency, these females behaved indistinguishably from the wild-type control females (Figures 2H–2J). In contrast, *SPR* mutants carrying only one of the two transgenes were not rescued. We also confirmed our previous finding (Yapici et al., 2008) that expression of *SPR* in *fru*$^+$ neurons alone is also sufficient to restore the post-mating switch in *SPR* null mutant females (Figures 2H–2J). The simplest interpretation of these data is that SP triggers the switch to post-mating behavior exclusively through its action on the *ppk*$^+$ *fru*$^+$ uterus sensory neurons.

Silencing *ppk*$^+$ *fru*$^+$ Neurons Induces Postmating Behaviors in Virgin Females

We used *ppk-GAL4* and a *UAS-shi*^{ts} transgene (Kitamoto, 2001) to acutely block synaptic transmission from *ppk*$^+$ neurons. When we silenced *ppk*$^+$ neurons by culturing flies for 90 min at the restrictive temperature of 30°C, virgin females were significantly less receptive to mating than control females that lacked one of the two transgenes, as well as those that carried both but were maintained at 22°C (Figure 3A). Indeed, upon silencing the *ppk*$^+$ neurons, virgins were as unreceptive as normal mated females (Figures 1E, 2I, and 3A). Virgin *ppk-GAL4 UAS-shi*^{ts}

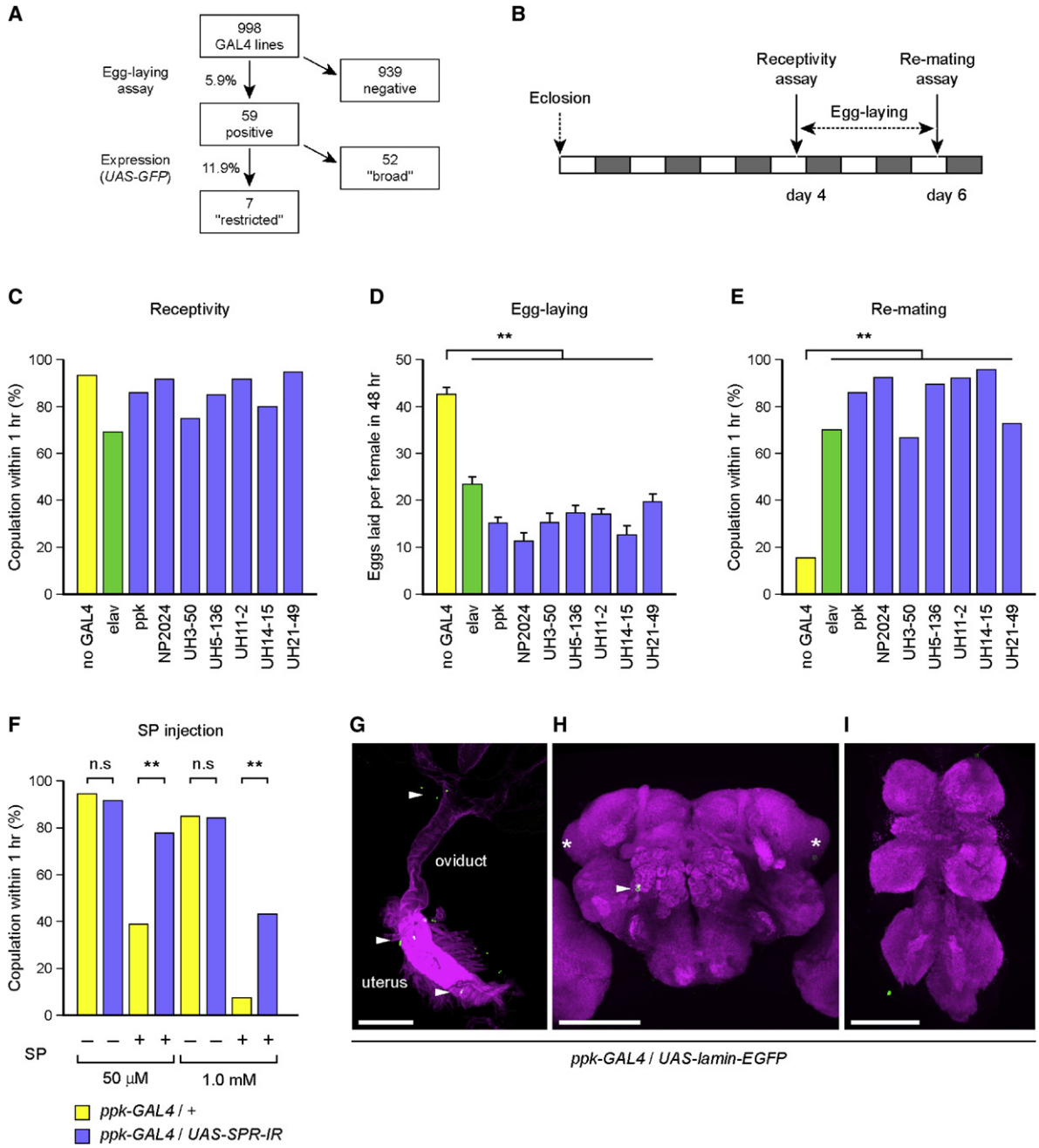

Figure 1. Identification of GAL4 Lines in an *SPR* RNAi Screen

(A) Overview of the primary screen.

(B) Protocol for secondary assays in (C)–(E).

(C) Receptivity of virgin females carrying the indicated GAL4 line and *UAS-SPR-IR*, n = 59–120.

(D) Number of eggs laid per female during the 48 hr period after mating, n = 43–112. Data are shown as mean ± SEM. **p < 0.0001, Student's t test.

(E) Remating frequencies, n = 42–110. **p < 0.0001, exact binomial test.

(F) Receptivity of virgin females of the indicated genotype upon injection with either 50 μM SP, 1.0 mM SP, or buffer alone (–), n = 36–40. n. s., p > 0.05; ** p < 0.0001; exact binomial test.

(G) Reproductive tract of *ppk-GAL4 UAS-lamin-EGFP* female stained with anti-GFP (green) and phalloidin (magenta). Arrowheads indicate locations of ppk^+ neurons. Scale bar, 200 μm.

(H and I) Brain (H) and ventral nerve cord (I) of *ppk-GAL4 UAS-lamin-EGFP* female stained with anti-GFP (green) and the synaptic marker mAb nc82 (magenta). Arrowhead indicates a ppk^+ neuron near the antennal nerve, asterisks indicate the positions of weakly stained cells in the lateral protocerebrum. Scale bar, 100 μm.

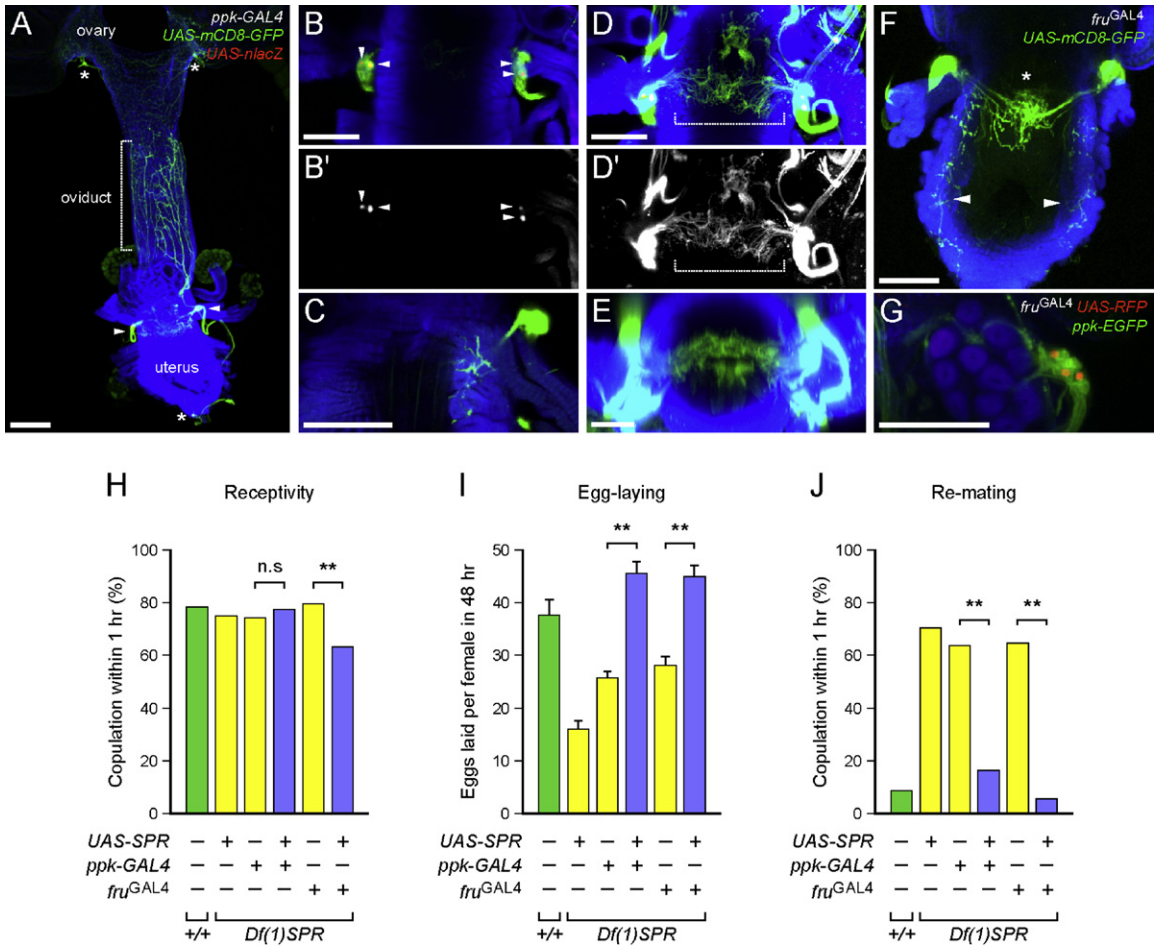

Figure 2. SPR Acts in *ppk*$^+$ *fru*$^+$ Sensory Neurons Innervating the Female Reproductive Tract

(A–E) Reproductive tract of *ppk-GAL4 UAS-mCD8-GFP UAS-nlacZ* females, stained with anti-GFP (green), anti-β-galactosidase (red), and phalloidin (blue). (A) Oviduct and uterus. Arrowheads, *ppk*$^+$ cell bodies flanking the uterus; dashed line, projections along the oviduct; asterisks, additional *ppk*$^+$ neurons near the base of the ovaries and the tip of the uterus. (B and B′) Higher magnification views showing two *ppk*$^+$ neurons on each side of the uterus (arrowheads). (B′) shows the anti-β-galactosidase staining alone. (C) Confocal section showing processes of *ppk*$^+$ neurons that penetrate between the muscle and epithelial cells to enter the lumen of the uterus. (D and D′) Processes of *ppk*$^+$ neurons in the lumen of the uterus (dashed line). (E) View along the central axis of the uterus, which is surrounded by a ring of muscle fibers (blue).

(F) Reproductive tract of *fru*^{GAL4} *UAS-mCD8-GFP* female stained with phalloidin (blue), with GFP fluorescence in green. Asterisk indicates arborizations in the uterus, which includes fine processes extending to the lower uterus that are less obvious with *ppk-GAL4* (arrowhead).

(G) High-magnification confocal image of the uterus of a *ppk-EGFP fru*^{GAL4} *UAS-hist-RFP* female, stained with phalloidin (blue) and showing GFP fluorescence in green and RFP fluorescence in red.

Scale bars: (A), 100 μm; (B–G), 50 μm.

(H–J) Rescue of *SPR* function. Females were assayed according to the protocol in Figure 1B. n = 60 for the first two genotypes and 112–120 for all others. *Df(1)SPR* is the cantonized *Df(1)Exel6234* strain (Yapici et al., 2008). n.s., $p > 0.05$; **$p < 0.0001$; exact binomial tests in (H) and (J), Student's t test in (I). Data in (I) are mean ± SEM. Note that, in slight contrast to (Yapici et al., 2008), a small but significant reduction in virgin receptivity upon expression of *UAS-SPR* with *fru*^{GAL4} (A), possibly as a result of *SPR* overexpression.

females also laid many eggs when they were maintained for 2 days at 30°C (Figure 3B), and in this respect too they resembled normal mated females (Figures 1D and 2J). Indeed, egg-laying rates of females with silenced *ppk*$^+$ neurons were equally high regardless of whether or not they had mated (Figures 3B and 3C). By comparison, control females laid very few eggs as virgins (Figure 3B) but large numbers after mating (Figure 3C). Silencing the *ppk*$^+$ neurons thus induces postmating behaviors in virgin females, as has previously been observed upon silencing of all *fru*$^+$ neurons (Kvitsiani and Dickson, 2006). Silencing the *ppk*$^+$ *fru*$^+$ uterus sensory neurons evidently mimics exposure to SP.

Central Projections of *ppk*$^+$ *fru*$^+$ Sensory Neurons

We sought to determine the central projections of the *ppk*$^+$ neurons by combining *ppk-GAL4* with either the membrane marker *UAS-mCD8-GFP* (Figures S2A–S2C) or the presynaptic marker *UAS-syt-GFP* (Figures 4A–4D). Because very few CNS cells are *ppk*$^+$ (Figures 1H and 1I), any GFP$^+$ processes in the CNS derive from

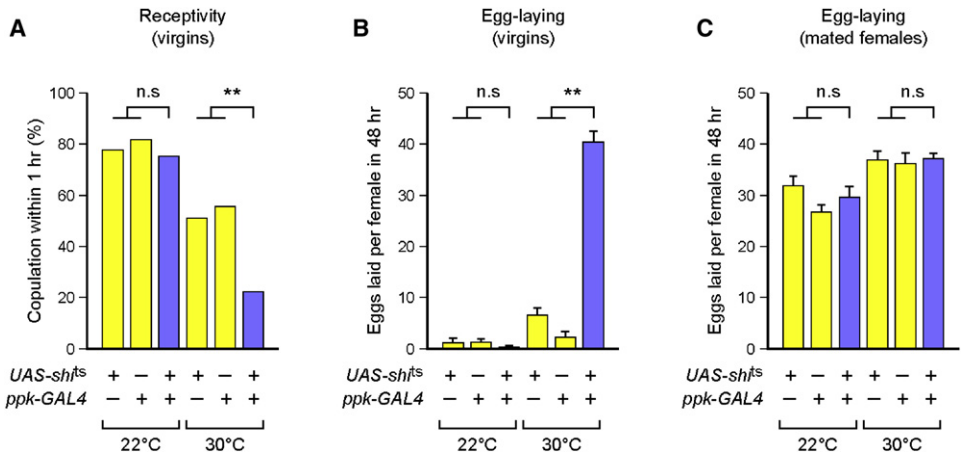

Figure 3. Silencing the *ppk*$^+$ Neurons

(A) Receptivity of virgin females raised at 22°C and kept at the indicated temperature for 90 min before and 60 min during the mating assay. n = 137–190 for assays at 22°C, n = 90 for all genotypes at 30°C. n.s., $p > 0.05$; **$p < 0.0001$; exact binomial test.

(B) Number of eggs laid by virgin females raised at 22°C and then kept at the indicated temperature for 2 days. n = 50–55. Data are shown as mean ± SEM. n.s., $p > 0.05$; **$p < 0.0001$; Student's t test.

(C) Number of eggs laid by females raised at 22°C, mated to wild-type males, and then kept at the indicated temperature for 2 days. n = 39–66. Data are shown as mean ± SEM. n.s., $p > 0.05$, Student's t test.

peripheral *ppk*$^+$ neurons. This includes, but is not limited to, the *ppk*$^+$ *fru*$^+$ sensory neurons on the uterus. These neurons contribute afferents to the abdominal trunk nerve. Most abdominal trunk afferents terminate in the abdominal ganglion, but at least some of these GFP$^+$ fibers extend further anteriorly. They are difficult to trace any further, however, due to the many additional GFP$^+$ processes that enter through the leg and wing nerves.

We found that a proximal 1.0 kb promoter fragment from the *ppk* gene drives expression in most of the *ppk*$^+$ leg neurons, but not in the uterus neurons (Figures S2D–S2G), and so used this *ppk1.0* promoter to drive expression of GAL80, a repressor of GAL4 (Figures 4E–4N). In *ppk-GAL4 ppk1.0-GAL80 UAS-syt-GFP* animals, the uterus neurons were still strongly labeled (Figure 4H), but reporter expression within the ventral nerve cord was largely restricted to the two sets of fibers that enter through the abdominal trunk and the mesothoracic nerves. (Figure 4G). In 3D confocal images from these animals, we could now trace a medial pair of bilateral GFP$^+$ fibers that emerge from the dense network in the abdominal ganglion, traverse the entire length of the nerve cord along its ventral side (Figures 4K and 4L) and extend through the cervical connective (Figures 4J, 4M, and 4N) into the brain.

These ascending fibers terminate in the posterior region of the suboesphageal ganglion (SOG; Figure 4I). *ppk1.0-GAL80* suppresses marker expression in almost all other *ppk*$^+$ inputs to the brain, with the exception of processes that enter near the antennal nerve and a few cells near the prothoracic ganglion that send fibers into the lateral SOG (Figures 4I and 4J). None of these other processes come into proximity of the medial posterior SOG, and so we conclude that the GFP$^+$ termini in this region derive exclusively from the ascending fibers from the abdominal nerve. Whether this are indeed the *ppk*$^+$ uterus sensory neurons or some other unidentified *ppk*$^+$ neurons that also contribute to the abdominal nerve cannot be resolved with certainty. Nonetheless, these data indicate that the neural signal generated or modulated by SP is conveyed to targets in the abdominal ganglion of the nerve cord and most likely also to targets in the posterior subesophageal ganglion in the brain (Figure 4O).

DISCUSSION

We have described here a set of internal *ppk*$^+$ *fru*$^+$ sensory neurons in the female reproductive tract and provided evidence that SPR functions in these neurons to trigger the behavioral changes induced by SP upon mating. This conclusion rests on two complementary sets of observations. First, SPR is required in both *ppk*$^+$ and *fru*$^+$ cells, because postmating responses are eliminated upon knockdown of SPR in either cell population. Second, SPR is sufficient in either *ppk*$^+$ or *fru*$^+$ cells alone, as expression in either restores the postmating response in *SPR* null mutant females. This forces the conclusion that SPR acts exclusively in cells that are both *ppk*$^+$ and *fru*$^+$. The sensory neurons innervating the uterus are the only cells we have been able to identify that express both of these markers. There are typically four to six such cells, and we do not yet know if they are functionally equivalent, or if egg laying and receptivity are regulated by two distinct cell subtypes.

Silencing synaptic transmission of *ppk*$^+$ *fru*$^+$ neurons mimics the activity of SP, in that they both cause virgin females to become unreceptive and initiate egg laying. Thus, an attractive hypothesis is that activation of SPR by SP reduces the synaptic output of these neurons. Like other *ppk*$^+$ neurons (Adams et al., 1998; Grueber et al., 2003), the *ppk*$^+$ *fru*$^+$ uterus neurons are probably mechanosensory. They may therefore have an important function as uterus stretch receptors in the coordination of sperm transfer, fertilization, and egg release. They may have two distinct functional states, depending on the presence or absence of SP. Because receptivity can be genetically

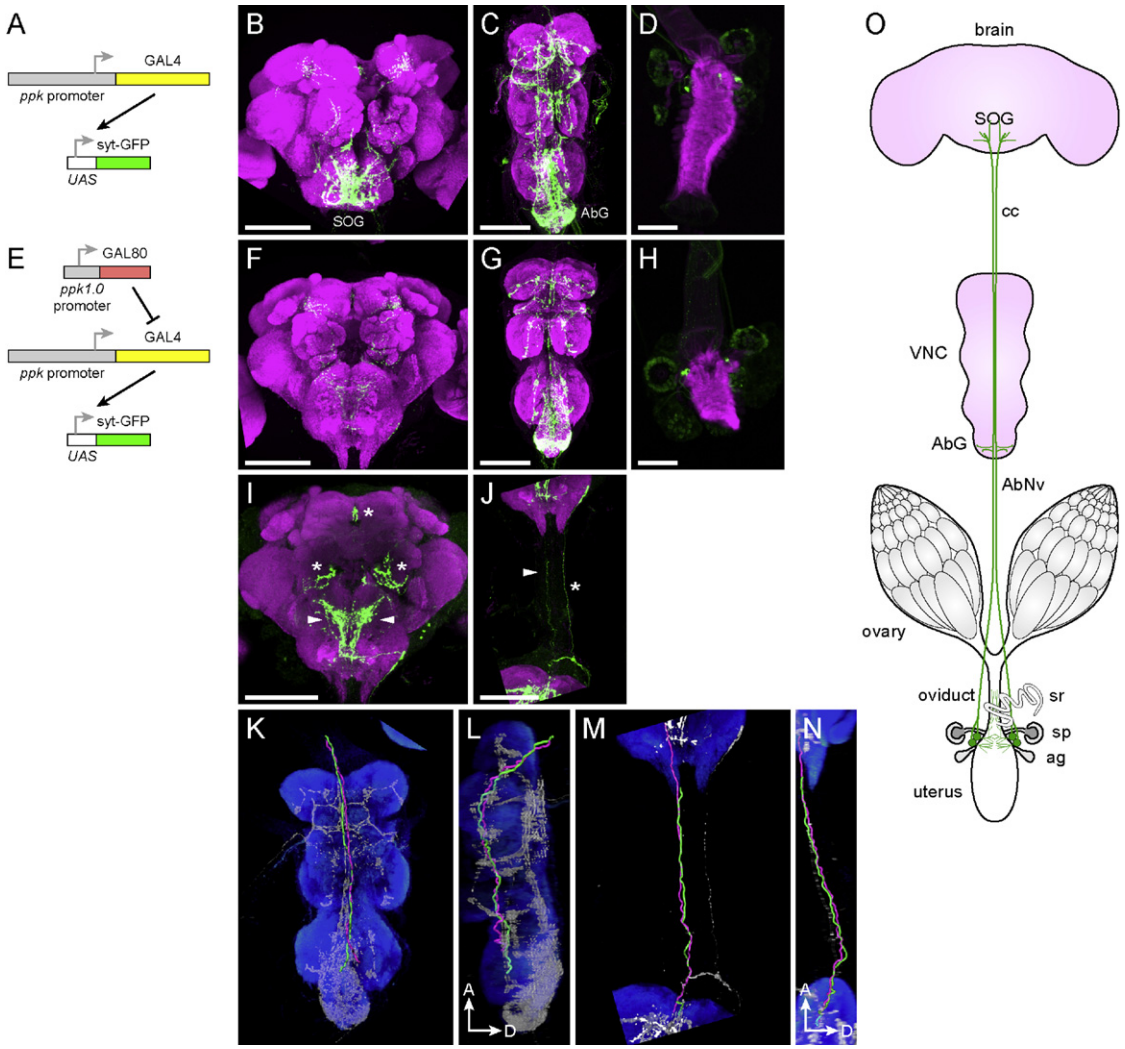

Figure 4. Central Projections of *ppk*$^+$ Neurons

(A–D) GFP expression in *ppk-GAL4 UAS-syt-GFP* (A) female. Brain (B) and ventral nerve cord (C) stained with anti-GFP (green) and mAb nc82 (magenta). Reproductive tract (D) stained with anti-GFP (green) and phalloidin (magenta). SOG, subesophageal ganglion; AbG, abdominal ganglion.

(E–H) GFP expression in *ppk-GAL4 ppk1.0-GAL80 UAS-syt-GFP* (E) female, showing brain (F), ventral nerve cord (G) and reproductive tract (H). Samples were stained and imaged under identical conditions to those shown in (B)–(D).

(I and J) Single confocal section of the posterior brain of a *ppk-GAL4 ppk1.0-GAL80 UAS-syt-GFP* female (I) imaged at higher gain than in (F). *ppk*$^+$ fibers in the medial posterior SOG (arrowheads in [I]) can be traced to a medial pair of ascending *ppk*$^+$ fibers visible in the maximum intensity projection of cervical connective (J, arrowhead). Lateral fibers (asterisk in [J]) originate from a cell loosely associated with the prothoracic ganglion that is frequently lost during dissection. Other *ppk*$^+$ fibers appear to enter the brain through the antennal nerve (asterisk in [I]).

(K–N) Tracings of *ppk*$^+$ fibers along the ventral aspect of the nerve cord (K and L) and the cervical connective (M and N) of a *ppk-GAL4 ppk1.0-GAL80 UAS-syt-GFP* female, stained with mAb nc82 (blue) and anti-GFP (gray). The two medial GFP-positive pathways are traced in green and magenta. (K and M) ventral views, (L and N) lateral views.

(O) Schematic of central projections of *ppk*$^+$ uterus neurons. AbNv, abdominal nerve; cc, cervical connective; sr, seminal receptacle; sp, spermathecae; ag, accessory gland (parovaria).

Scale bars: 100 μm.

uncoupled from egg production and egg laying (Barnes et al., 2007), we infer that SP can also act independently of any stretch signal in the uterus. Modulation of receptivity and egg laying might be mediated through either distinct *ppk*$^+$ *fru*$^+$ subtypes or distinct central synapses.

How might SP regulate these sensory neurons? We can envision two possibilities. First, the *ppk*$^+$ *fru*$^+$ neurons may detect SP in the reproductive tract and alter their firing rate accordingly. In this model, passage of SP into the hemolymph would not be required to induce the postmating response. A second possibility is that SP enters the circulatory system and acts presynaptically to modulate the release of these neurons at their central targets. The fact that SP can indeed be detected in the hemolymph of mated females (Pilpel et al., 2008) does not in itself

exclude the former possibility. At least some effects of SP, such as stimulating juvenile hormone synthesis in the corpus allatum (Moshitzky et al., 1996), probably do require SP to enter the hemolymph. Similarly, the fact that SP triggers a postmating response even when injected directly into the hemolymph (Chen et al., 1988) is also consistent with either model. The somata and some processes of the *ppk*$^+$ *fru*$^+$ neurons lie outside the uterus and would be readily accessible to factors in the hemolymph. A neural rather than a circulatory route has been proposed to mediate postmating responses in several species of moths (Foster, 1993; Giebultowicz et al., 1990; Jurenka et al., 1993). However, this conclusion is based upon the loss of this response upon nerve cord transection, a result predicted by both of these models. Thus, both models are consistent with currently available evidence from studies in *Drosophila* and other species, and distinguishing between them will require detailed studies of the physiological properties of the *ppk*$^+$ *fru*$^+$ neurons in response to SP.

The central targets of the *ppk*$^+$ *fru*$^+$ sensory neurons include the abdominal and/or subesophageal ganglia—regions of the CNS likely to contain circuits that mediate behavioral responses to mating. The abdominal ganglion houses the octopaminergic neurons that are believed to regulate the release and passage of mature eggs from the ovary to the uterus (Cole et al., 2005; Middleton et al., 2006; Monastirioti, 2003; Monastirioti et al., 1996; Rodriguez-Valentin et al., 2006). We suspect that these neurons are direct or indirect targets of the *ppk*$^+$ *fru*$^+$ sensory neurons and that these circuits serve to ensure that ovulation and oviposition are coordinated with the presence of sperm.

Some *ppk*$^+$ fibers project from the abdominal trunk nerve right through to the SOG, potentially forming a direct neural connection from the reproductive tract to the brain. We suspect that these projections may feed into circuits that regulate female receptivity and other postmating behaviors. Virgin females are enticed to mate by the male's courtship song. Most auditory sensory neurons project to the mechanosensory neuropil in the lateral SOG (Kamikouchi et al., 2006), close to the terminal arborizations of the *ppk*$^+$ neurons. The proximity of the auditory processing centers and the ascending *ppk*$^+$ projections raises the attractive possibility that mating modulates an early step in song processing. The SOG also contains processes of the Ilp7 neurons, which function in egg-laying site selection after mating (Yang et al., 2008). Direct evidence for mating-induced changes in SOG circuit function is lacking in flies but has been obtained in other insects. In some species of moth, mating induces a long-term inhibition of the SOG neurosecretory cells that regulate female pheromone biosynthesis, making mated females less attractive to other males (Ichikawa, 1998).

Having identified sensory neurons that detect SP in the reproductive tract, it will now be important to characterize the central pathways that process these signals to regulate female behavior. In the olfactory system, sensory neurons that detect pheromones are *fru*$^+$ (Kurtovic et al., 2007; Root et al., 2008), as are their postsynaptic partners in the brain (Datta et al., 2008; Stockinger et al., 2005). Given that the sensory neurons that detect SP are also *fru*$^+$, and many *fru*$^+$ neurons are also located in both the abdominal and subesophageal ganglia (Billeter and Goodwin, 2004; Manoli et al., 2005; Stockinger et al., 2005), it is enticing to think that a similar logic may apply in these pathways too. Elucidating the operation of these circuits should reveal how the female CNS integrates both external and internal information to switch between two very different behavioral patterns.

EXPERIMENTAL PROCEDURES

GAL4 Screen

Virgin females homozygous for *UAS-SPR-IR1* on the third chromosome (Yapici et al., 2008) and *UAS-Dcr2* on the first chromosome (Dietzl et al., 2007) were obtained from the appropriate Y, *hs-hid* stocks and crossed to males from the various GAL4 lines. Five to six *UAS-SPR-IR* females were crossed to three to five *GAL4* males. Progeny were raised on semidefined medium at 25°C on a 12:12 hr dark: light cycle. Parents were removed from the vial after 3 days, and adult progeny left in the vial for 3–4 days posteclosion to allow mating. Twenty to thirty adult females and three to five males were then removed and transferred to a fresh food vial and again transferred to a fresh vial after 24 hr and 48 hr. After 72 hr, the adult flies were discarded. The number of eggs in each of the three vials was estimated and scored on a 1–5 scale as follows: 1, ~100 or more eggs (normal); 2, ~50–100 eggs; 3, ~20–50 eggs; 4, ~5–20 eggs; 5, ~0–5 eggs. A 3 day average score of 3 or more was regarded as positive. For a quick assessment of GAL4 expression patterns, lines were crossed to *UAS-GFP* on the second chromosome and the brains, ventral nerve cords, and reproductive tract were dissected from adult female progeny and examined live under wide field fluorescent microscopy. For further details of all fly stocks used, see Supplemental Data.

Behavioral Assays

Quantitative assays for detailed phenotypic characterization were performed as described (Yapici et al., 2008). For the *UAS-shi*^{ts} experiments, flies were raised, collected, and maintained at 22°C and if appropriate shifted to 30°C 90 min before the assay. Assays for receptivity or egg laying were then performed in parallel at 22°C and 30°C.

Immunohistochemistry and Tracing of *ppk*$^+$ Fibers

Staining of the CNS and reproductive tract were performed using rabbit anti-GFP (Torrey Pines Biolabs, 1:6000), mouse anti-GFP (Promega, 1:1000), mAb nc82 (DSHB, 1:20 [Wagh et al., 2006]), and/or rhodamine-phalloidin or Alexa 647-phalloidin (both Molecular Probes/Invitrogen, 1:100). For axon tracing, the stained ventral nerve cord and brain was imaged at maximum optical resolution and high gain on a Zeiss LSM 510 confocal microscope. The image stack was deconvolved using Huygens Essential (Scientific Volume Imaging) and a custom point spread function obtained from the confocal setup. Axons were traced in 3D using a custom module in Amira (Evers et al., 2005; Schmitt et al., 2004).

SUPPLEMENTAL DATA

The Supplemental Data include two figures and Supplemental Experimental Procedures and can be found with this article online at http://www.neuron.org/supplemental/S0896-6273(09)00076-2.

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