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Mating reconciles fitness and fecundity by switching diet preference in flies

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Protein-rich diets shorten lifespan but increase fecundity in many organisms. Animals actively adjust their feeding behavior to meet their nutritional requirements. However, the neural mechanisms underlying the dynamic regulation of protein consumption remain unclear. Here we find that both sexes of fruit flies exhibit a preference for protein food before mating to prepare for reproduction. Mated female flies display an increased appetite for yeast to benefit their offspring, albeit at the cost of stress resistance and lifespan. In contrast, males show a momentarily reduced yeast appetite after mating likely to restore their fitness. This mating state-dependent switch between sexes is mediated by a sexually dimorphic neural circuit labeled with leucokinin in the anterior brain. Furthermore, intermittent yeast consumption benefits both the lifespan and fecundity of males, while maximizing female fecundity without compromising lifespan.

Food plays a significant role in shaping our behavior and physiology, and animals make dietary choices based on their internal states. Among various macronutrients, protein diets are known to be involved in muscle development[1], shaping the gut microbiome[2], and participating in numerous physiological activities. For instance, increased protein intake has been shown to reduce sleep arousal in flies and mice[3] and trigger a transition from feeding to courtship behavior[4]. Additionally, protein consumption has been found to impact brain inflammation and memory in mice[5].

The effects of protein intake extend to important aspects such as aging and fecundity in animals, attracting considerable attention in research. The protein exhibits contrasting effects on reproduction and lifespan[6]. Studies using the Geometric Framework in insects and rodents have revealed that low-protein diets are associated with longer lifespans[7]. However, excessive protein consumption promotes fertility in various species from mammals to insects[7], resulting in a protein-based trade-off between these traits. The mechanisms by which animals adjust their protein consumption to balance the trade-off between fecundity and fitness remain poorly understood.

The fruit flies access protein primarily from yeast. Female flies raised on high-yeast diets exhibit enhanced sexual attractiveness and greater fecundity[8]. To ensure an adequate protein supply for egg development, mating increases female flies' preference for protein-rich food[9–11]. Sex Peptide (SP) transferred with male seminal fluid acts through the SPSN-SAG circuitry which mediates enhanced yeast appetite[9]. However, excessive yeast consumption, exposure to yeast odorants, or the addition of amino acids to food reduce lifespan[12–14]. Therefore, animals must adapt protein choices to meet their needs.

Despite extensive studies in females, the impact of mating status on macronutrient-specific requirements in males remains unclear. Here, we find unmated males prefer yeast to maintain fertility while mating reduces males' yeast intake to prioritize fitness. Our study identifies the brain's anterior leucokinin (ALK) neurons that transmit mating states to yeast preference to apply the concept of allostasis in a sexually dimorphic manner. These conflicting interests between the sexes can give rise to sexual conflict and trigger an evolutionary arms race, where males strive to enhance female reproductive output while developing strategies to minimize their costs. Moreover, our findings demonstrate that intermittent yeast consumption, based on the regularity of mating, can have positive effects on both fitness and fecundity. These findings have implications for guiding mammals toward healthier dietary choices.

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Results

Yeast consumption enhances fecundity but reduces fitness

Feeding takes precedence over courtship in starved males, but this priority is reversed after consuming protein-rich food[4]. We first tested the effects of protein feeding on male fecundity and found that male flies fed on a yeast-rich diet displayed an increased courtship index (Fig. 1a). Furthermore, they also outcompeted the males fed on sugar-only food in a mating competing assay (Fig. 1b). Besides, males had increased numbers of germ stem cells (GSCs) after yeast consumption (Fig. 1c). To test the effects of yeast on competitive fertilization, we first paired virgin females with males fed on standard food[15]. Next, females were inseminated by a second male with sperm labeled with GFP, which allowed us to discriminate the replacement by sperm from the second mating. The sperm from the first male continued to be displaced by sperm from the second mating. By counting the sperm from the second male, we found that yeast consumption enhanced male sperm competitiveness (Fig. 1d). These results suggested that yeast intake increased both the sexuality and fertility of male flies.

However, we found that males fed on a yeast-containing diet had significantly shorter survival time during desiccation or starvation than those fed on a sucrose-only diet (Fig. 1e, f). Similar to males, females had decreased survival resistance when taking protein food (Fig. 1g, h). Therefore, the yeast in the diet can affect both fitness and fecundity but often function oppositely in both sexes.

Mating has opposite effects on yeast preference in male and female flies

Animals have the remarkable ability to adapt their behavior according to internal requirements. Our study hypothesized that flies might regulate the trade-offs between fitness and fecundity through the modulation of yeast appetite. Previous reports have indicated that mated females increase their yeast intake to maximize the fitness of their offspring[9], which aligns with our findings (Supplementary Fig. 1a). However, the impact of mating on the yeast preference of male flies remains poorly understood.

To test this, unmated males were deprived of yeast and paired with virgin females on the first day, then a CAFE assay was conducted on the second day[16]. Male flies were given a choice between 5% sucrose food and a mixture of 5% sucrose and 5% yeast food. In both wildtype *Canton S and* w^{1118} we found unmated males preferred yeast-rich food, whereas mating diminished the preference (Fig. 2a and Supplementary Fig. 1b). Furthermore, males mated once or sexually sated exhibited a similar behavioral change. Following the original CAFE assay protocol, we quantified the reduction in a capillary's content upon consumption by flies. Notably, unmated male flies exhibited a greater consumption of YS food compared to S food, a trend that reversed in mated male flies (Supplementary Fig. 1c). The results revealed a significant increase in the consumption of both YS and S food in mated males, suggesting that the shifts in appetite observed in mated males are not a result of decreases in the availability of YS food.

These findings were further validated with the two-choice feeding preference test (Fig. 2b). We also tested the yeast appetite of individual male flies using the flyPAD assay. Similarly, mated males exhibited decreased yeast appetite (Fig. 2c). Given the consistent results obtained from the three different methods, we utilized the CAFE assay for subsequent experiments. This assay is not only straightforward to operate but also well-suited for continuous monitoring.

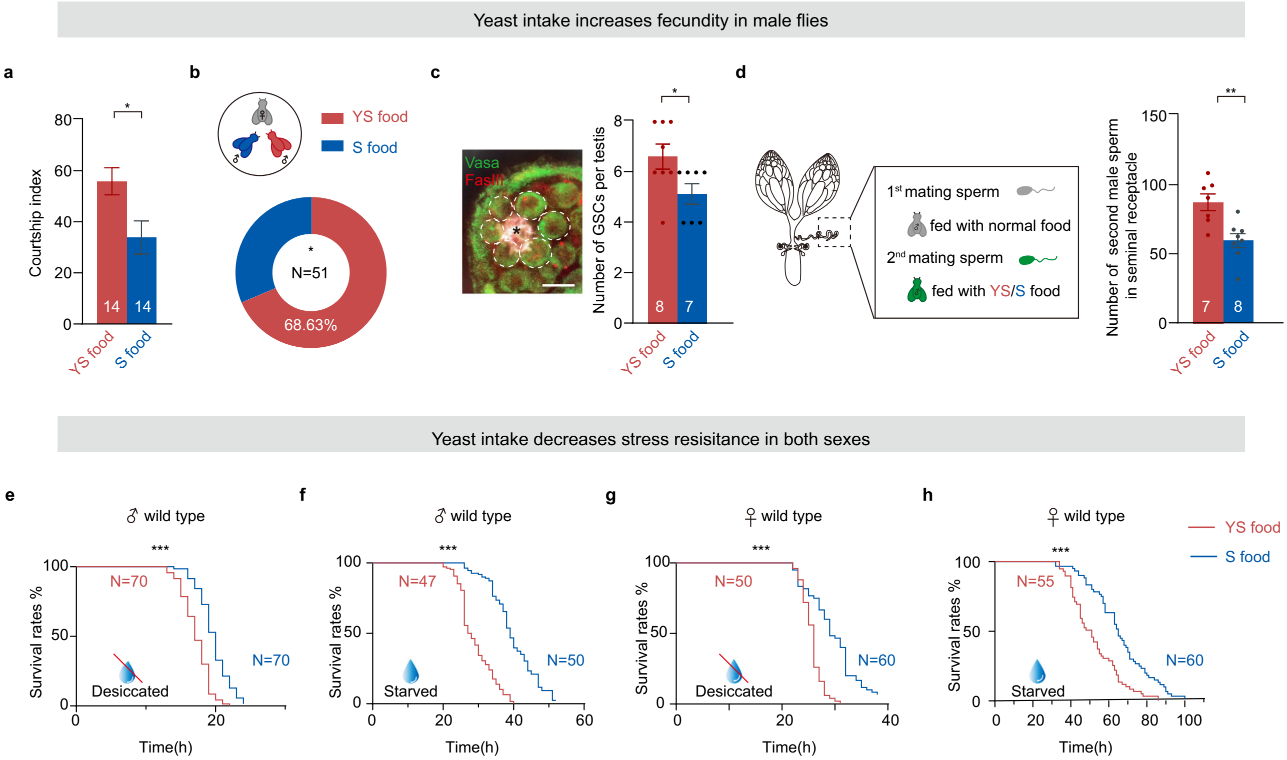


Fig. 1 | Yeast consumption increases fecundity but decreases stress resistance. **a** Courtship index of males fed on YS food or S food. Mean ± SEM. *$p < 0.05$, two-tailed unpaired *t*-tests. **b** Males' mating competitiveness when fed on YS food or S food. The top left panel represents a schematic diagram of the male mating competition assay. *$p < 0.05$, two-tailed Binomial test. **c** Left panel: The apical tip of a testis, where the FasIII-postive hub (asterix) is surrounded by Vasa-positive GSCs (dotted circle), the scale bar represented 10 μm. Right panel: Number of male GSCs when they were fed YS food or S food. Mean ± SEM. *$p < 0.05$, two-tailed Mann-Whitney nonparametric tests. **d** Male sperm competitiveness when fed on YS food or S food. Left panel: schematic diagram of male sperm competition assay. Right panel: number of GFP sperm remaining in the female seminal receptacle. Mean ± SEM. **$p < 0.01$, two-tailed unpaired *t* tests. Male resistance when fed YS food or S food. Survival rates of males under desiccation (**e**) and starvation (**f**) conditions. ***$p < 0.001$, Log-rank test. Female resistance when fed YS food or S food. Survival rates of males under desiccation (**g**) and starvation (**h**) conditions. ***$p < 0.001$, Log-rank test. Source data are provided as a Source Data file.

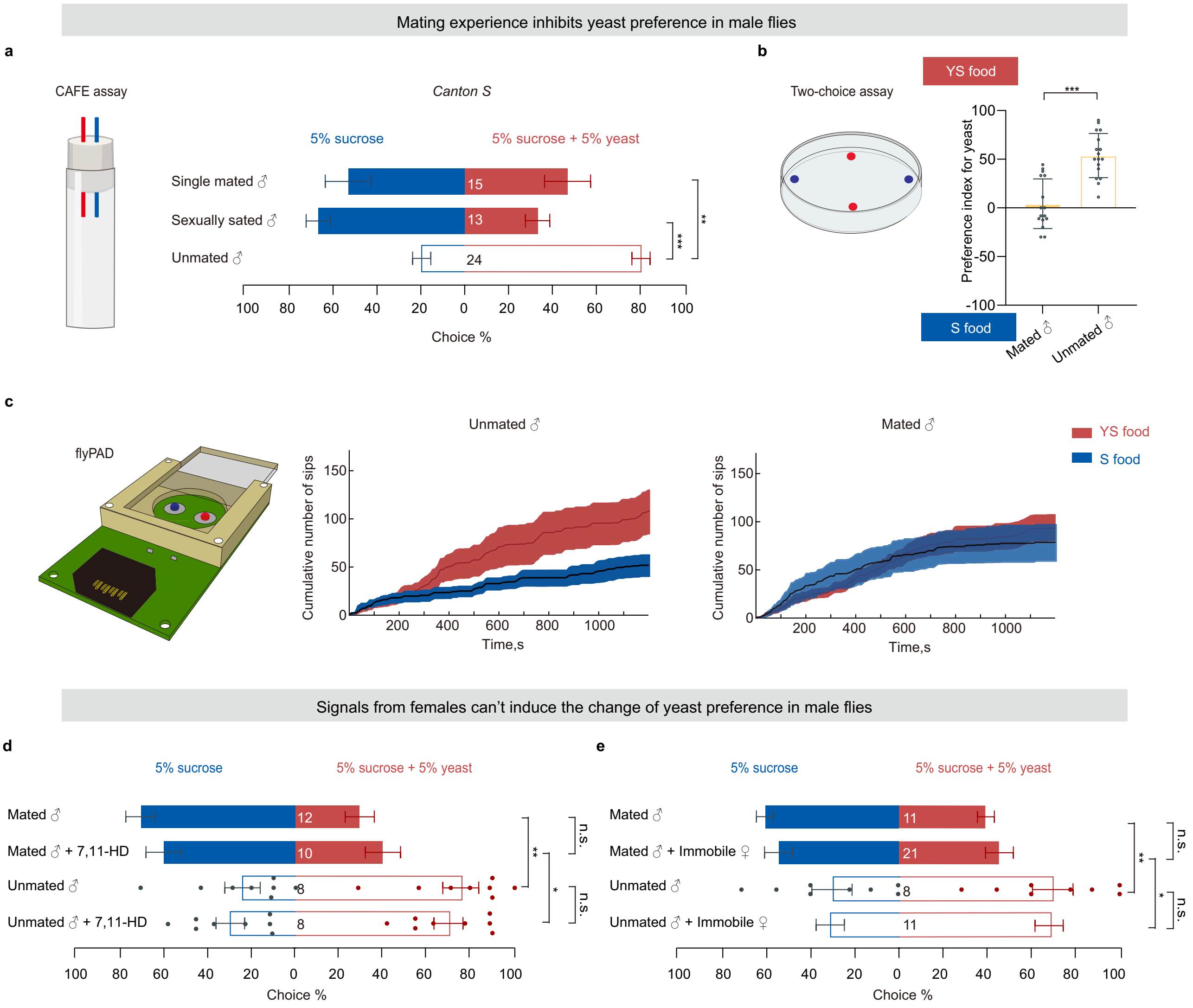


Fig. 2 | Mating decreases males' yeast preference. a Yeast preference of males in different mating statuses tested by CAFE assay. The left panel represents a schematic diagram of the CAFE assay. Mean ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$, two-tailed unpaired *t*-tests. **b** Yeast preference of males in different mating statuses tested by a two-choice assay. The left panel represents a schematic diagram of a two-choice assay. Mean ± SEM. $^{***}p < 0.001$, two-tailed unpaired *t*-tests. $n = 16, 17$. **c** Cumulative sips of YS food and S food in unmated and mated males recorded by flyPAD. The left panel represents a schematic diagram of the flyPAD assay. $n = 10, 10$. The line represents the mean, and the shading indicates the SEM. **d** Yeast preference of males when painted with 7,11-HD. The numbers on the columns represented replicate values. Mean ± SEM, $^{**}p < 0.01$,$^{*}p < 0.05$, n.s. not significant, two-tailed Mann–Whitney nonparametric tests. **e** Yeast preference of males when exposed to immobile females. Mean ± SEM, $^{**}p < 0.01$,$^{*}p < 0.05$, n.s. not significant, two-tailed unpaired *t*-tests. Source data are provided as a Source Data file.

Yeast mainly comprises sugar, protein, and salt. We also tested which components of yeast were responsible for the decreased yeast preference observed in mated males. CAFE assay revealed that mated males had decreased appetite for amino acids and salt (Supplementary Fig. 1d,e), suggesting that the post-mating change in yeast appetite is due to a reduced attractiveness to salt and protein.

We then asked whether female cues were sufficient to trigger the change in males' yeast preference. We painted unmated males with 7,11-HD, a female aphrodisiac pheromone[17]. Males' preference for yeast was not changed after the exposure to 7,11-HD (Fig. 2d). Moreover, the alteration in yeast preference was impeded when unmated males were grouped with females immobilized to prevent copulation (Fig. 2e). Similarly when males were exposed to feminized male[18], yeast appetite remained unchanged (Supplementary Fig. 1f). Collectively, we concluded that copulation is indispensable for the shift in male yeast preference.

Taken together, we supposed that the sexes employed divergent strategies to balance the trade-off between fitness and fecundity after mating. Unmated males preferred yeast consumption to maintain fecundity, while mated males may prioritize their survival by decreasing yeast appetite. Conversely, mated females increased their yeast intake to maximize their reproductive fitness, albeit at the cost of reduced stress resistance of their own (Supplementary Fig. 1g).

Intermittent yeast consumption increases fecundity and extends lifespan in males

To examine the duration of decreased yeast appetite in mated males, we conducted the CAFE assay on the second day after mating and found they restored the preference for yeast (Fig. 3a). This result suggested that the change in yeast preference lasted for two days and was subject to dynamic regulation by mating. In contrast, unmated males consistently displayed a preference for yeast (Supplementary Fig. 2a), implying that unmated males favor yeast, which is potentially associated with fecundity.

To elucidate the rationale behind the alteration in yeast appetite, we paired male flies with virgin females every three days for 3 h and

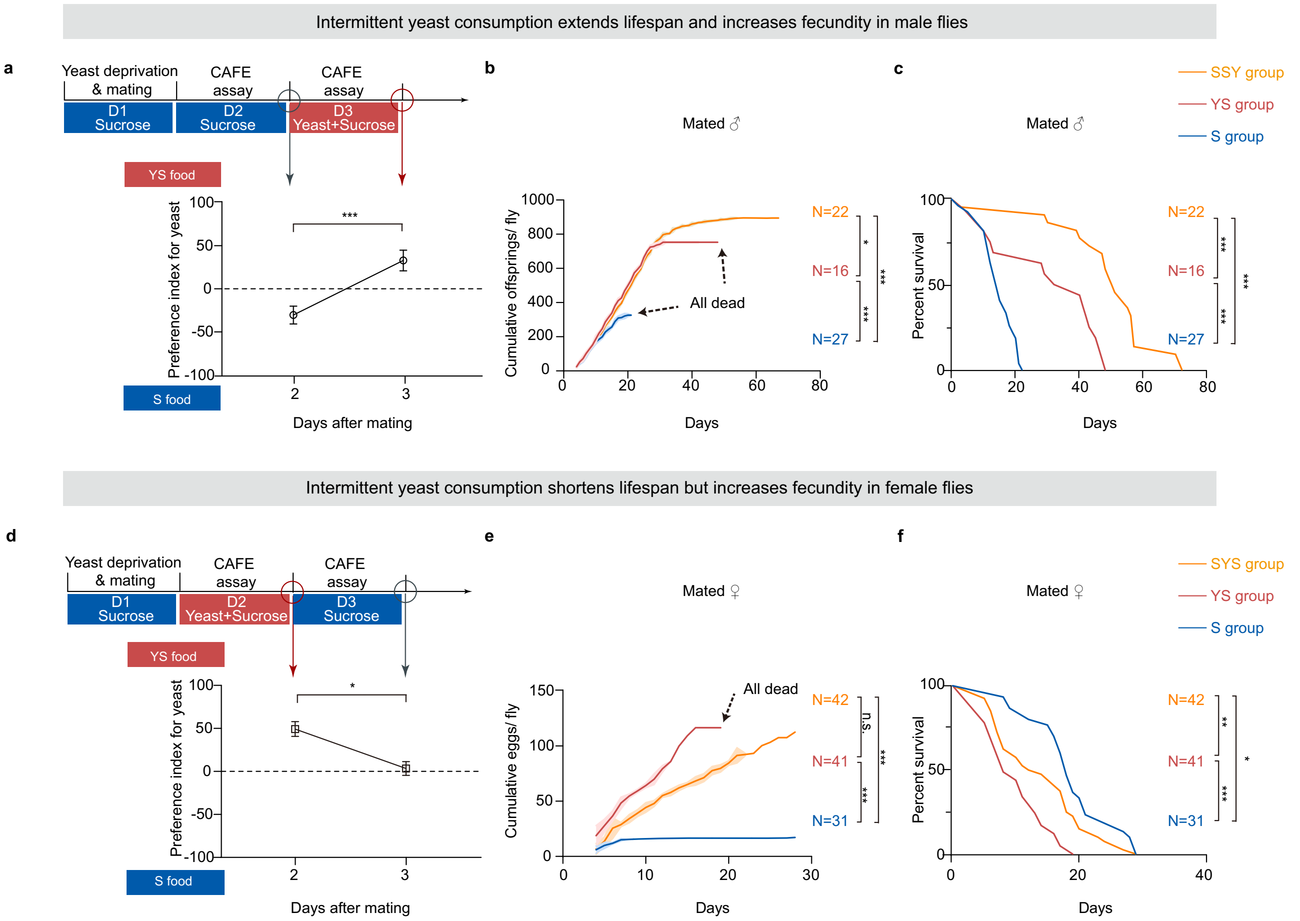


Fig. 3 | Intermittent yeast consumption regulates lifespan and fecundity in both sexes. a Duration of yeast preference in mated males. The upper panel is the schematic diagram showing the temporal pattern of the yeast preference test in males. Mean ± SEM. $^{***}p < 0.001$, two-tailed unpaired *t*-tests. $n = 15, 16$. **b** Cumulative number of offspring per male on different diets. Black arrows indicate the time point when all males died, as shown in Fig. 3c. Mean ± SDs. $^{*}p < 0.05$, $^{***}p < 0.001$, two-tailed ordinary ANOVA test, followed by Bonferroni's multiple comparisons tests. **c** Lifespan of mated males on different diets. $^{***}p < 0.001$, Log-rank test. **d** Temporal pattern of yeast preference in mated females. The upper panel is the schematic diagram showing the temporal pattern of the yeast preference test in females. Mean ± SEM. $^{*}p < 0.05$, two-tailed unpaired *t*-tests. $n = 20, 14$. **e** Cumulative number of eggs per female on different diets. Black arrows indicate the time point when females died, as shown in Fig. 3f. Mean ± SDs. $^{***}p < 0.001$, two-tailed ordinary ANOVA test, followed by Bonferroni's multiple comparisons tests. **f** Lifespan of mated females on different diets. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n.s. not significant, Log-rank test. Source data are provided as a Source Data file.

provided different feeding regimens to the mated males (as illustrated in Fig. 3a and Supplementary Fig. 2b). In comparison to a constant supply of sucrose or a combination of sucrose and yeast food, intermittent yeast consumption tailored to match their post-mating requirements (Fig. 3a) was advantageous for both lifespan and fecundity (Fig. 3b, c). These findings suggested that males adjust their feeding strategies to reconcile fecundity and overall fitness as a consequence of mating. (Supplementary Fig. 2c). Moreover, the consumption of yeast extended lifespan compared with those fed a sucrose-only diet in both unmated and mated males (Fig. 3c and Supplementary Fig. 2d), suggesting that tight restriction of protein and salt was harmful. In conclusion, our study underscored the significance of dynamic temporal patterns of yeast intake triggered by mating and provided insights into customizing diet patterns.

The effects of intermittent yeast consumption in female flies

We then wondered whether intermittent yeast consumption was also beneficial for both the lifespan and fecundity of female flies. We found that the appetite for yeast in female flies remained high for up to three days after mating (Fig. 3d). To simulate the nutritional requirement of mated females, we fed them a three-day cycle with one day of YS food sandwiched between two days of S food. Additionally, females were paired with males for 24 hours every three days (Supplementary Fig. 2e). Compared with constant consumption of YS food, intermittent yeast consumption extended the lifespan of female flies without reducing their fecundity (Fig. 3e, f), suggesting that intermittent yeast intake adequately supports their maximum fecundity while being less detrimental to their lifespan (Supplementary Fig. 2f).

The appetite for yeast in virgin females reversed in the second CAFE test (Supplementary Fig. 2g). Interestingly, yeast consumption extended their lifespan when compared to continuous sucrose consumption in virgin females (Supplementary Fig. 2h), a trend that was opposite to mated females. These findings indicate that persistent yeast intake may harm fitness by promoting fecundity in mated females (Fig. 3e, f).

The contrasting characteristics observed between mated males and females indicate that intermittent yeast intake potentially promotes lifespan extension in both sexes. However, it appears that mated females need to allocate additional energy toward their offspring, which could potentially compromise their health.

CRZ binds to anterior *LK* neurons to reduce yeast preference in mated males

We next aimed to decipher the molecular and circuit mechanisms underlying the mating-induced yeast appetite change in both sexes. Given the crucial role of copulation in male mating behavior, we asked

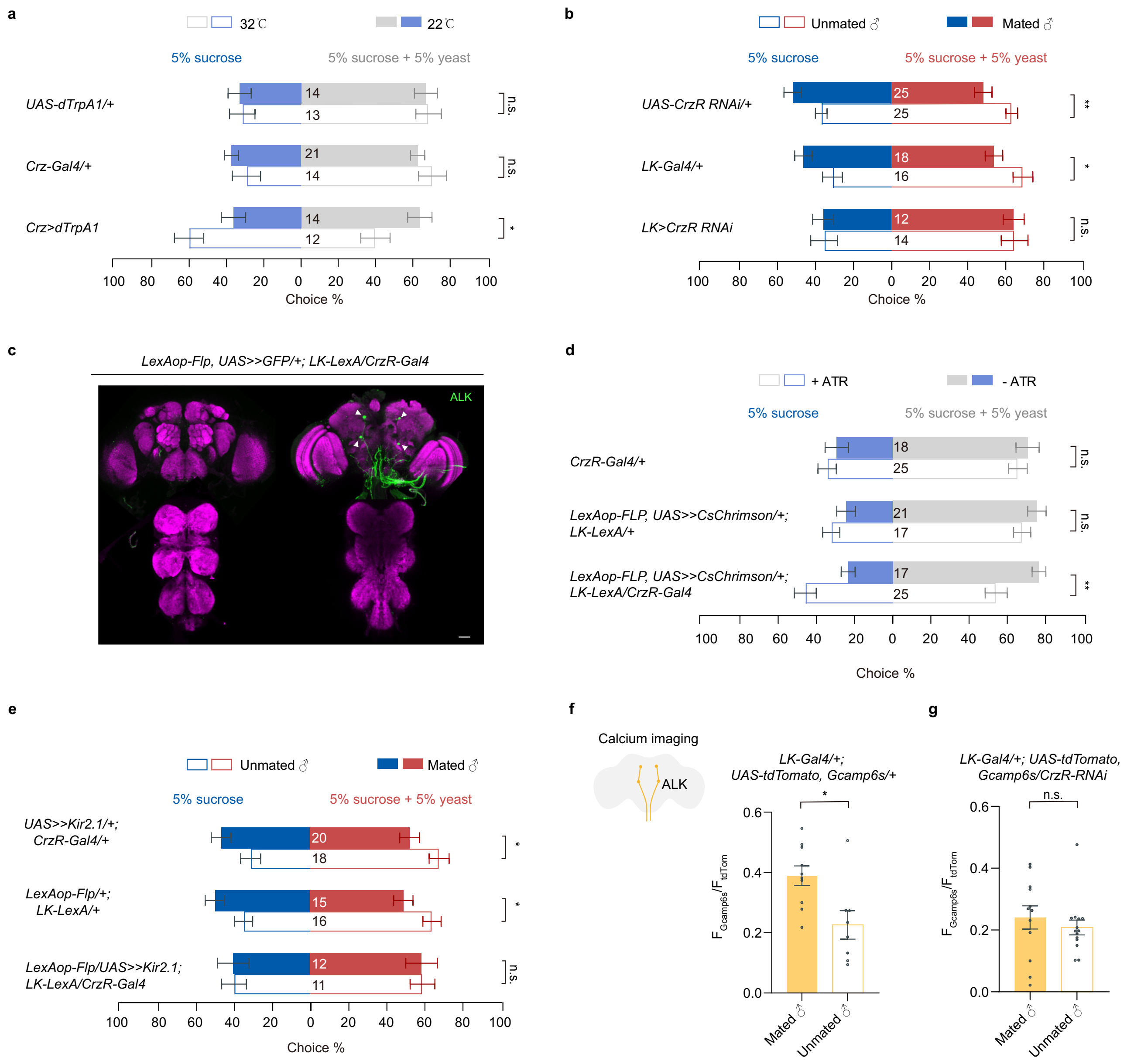


Fig. 4 | Crz bound to ALK neurons to modulate post-mating yeast preference in males. a Yeast preference of unmated males when *Crz* neurons were activated. Mean ± SEM. $^{*}p < 0.05$, n.s., not significant, two-tailed unpaired *t*-tests. **b** Yeast preference of males when knocking down CrzR in *LK* neurons. The numbers on the columns represented replicate values. Mean ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$, n.s. not significant, two-tailed unpaired *t*-tests. **c** Intersection between *CrzR* neurons and *LK* neurons showed expression pattern of ALK neurons in the male central nervous system. White arrows indicated the cell bodies of ALK neurons. Green: GFP. Magenta: nc82. The scale bar represented 50 µm. **d** Yeast preference of unmated males when ALK neurons were activated. The hollow column represents flies fed with all-trans-retinal (ATR). The solid column represents flies fed without ATR. Mean ± SEM. $^{**}p < 0.01$, n.s. not significant, two-tailed unpaired *t*-tests. **e** Yeast preference of male when silencing ALK neurons. The hollow column represents unmated males. The solid column represents mated males. The numbers on the columns represented replicate values. Mean ± SEM; $^{*}p < 0.05$, n.s., not significant, two-tailed unpaired *t*-tests. **f** Ca^{2+} signal of male ALK neurons in different mating states. The right panel indicated the relative fluorescence ($F_{Gcamp6s}/F_{tdTom}$) of male ALK neurons. Mean ± SEM. $^{*}p < 0.05$, two-tailed Mann–Whitney nonparametric tests. $n = 10, 8$. **g** Ca^{2+} signal of male ALK neurons in different mating states when CrzR was knocked down. Mean ± SEM. n.s. not significant, two-tailed Mann–Whitney nonparametric tests. n = 12, 14. Source data are provided as a Source Data file.

whether ejaculation alone induced the change of yeast appetite of males. Previous studies reported that ejaculation was triggered by the activation of *Crz* neurons[19]. We found that activating *Crz* neurons with the warm-activated cation channel dTrpA1 at 32 °C led to a significant decrease in yeast preference in unmated males (Fig. 4a), suggesting that *Crz* neurons and ejaculation were sufficient to induce the yeast appetite change in males. Mated males of *Crz-Gal4/+*, *Uas-dTrpA1*, and *Crz>dTrpA1* exhibited a reduced yeast appetite under both 32 °C and 22 °C conditions (Supplementary Fig. 3a).

To further understand how the CRZ peptide modulated yeast preference, we examined several neuronal populations known to mediate the preference for dietary protein or amino acids in flies, including WED neurons[20], FB-LAL neurons, and peptidergic neurons expressing diuretic hormone-44 (DH44)[21], insulin-like peptide-2 (Ilp2)[22], or leucokinin (LK)[23,24]. We hypothesized that CRZ might target some of these neurons to induce yeast preference. Among them, specifically knocking down CrzR in *LK* neurons eliminated the mating-induced decrease in yeast preference (Supplementary Fig. 3b), while genetic control groups exhibited a decreased yeast preference after mating (Fig. 4b). To confirm this, we employed another CrzR RNAi line marked as *CrzR RNAi*[26017], and the results were consistent (Supplementary Fig. 3c). Importantly, knocking down CrzR in *LK* neurons did not impact mating behaviors (Supplementary Fig. 3d,e). The results of flyPAD revealed that knocking down CrzR in *LK* neurons reduced the number of sips taken on yeast-rich food (Supplementary Fig. 3f-i). These results suggested that CRZ modulated yeast preference in the fly through the CRZ receptor on *LK* neurons.

We then examined the expression patterns of *LK* neurons and *CrzR* neurons respectively. Two to three neurons per brain were labeled in the lateral protocerebrum by the *LK-Gal4* (Supplementary Fig. 3j), while *CrzR* neurons were distributed broadly across the brain (Supplementary Fig. 3k). By intersection strategy, we were able to label the overlapping neurons between *LK-LexA* and *CrzR-Gal4*. Two pairs of neurons in the anterior brain were identified (Fig. 4c), termed anterior *LK* neurons (ALK) for short[25]. Using the same intersection strategy, we expressed CsChrimson in ALK neurons and activated them with red light. Unmated males with their ALK neurons activated exhibited a significant decrease in yeast preference compared to control males (Fig. 4d). Moreover, silencing ALK neurons with Kir2.1 impeded the mating-induced reduction of yeast preference in males (Fig. 4e).

Furthermore, the calcium level of ALK neurons in mated male flies was higher than that in unmated groups (Fig. 4f). The activation was dependent on the CRZ signal, as the calcium response of ALK neurons in newly mated males did not show a significant increase when we knocked down CrzR in *LK* neurons (Fig. 4f). Thus, we concluded that CRZ released during ejaculation bound to CrzR on ALK neurons to reduce yeast appetite in mated males.

FB-LAL neurons are activated by released sNPF from ALK neurons

To explore the downstream circuit of ALK neurons in yeast preference regulation, we analyzed the transcriptional level of different neuropeptides in male ALK neurons using the Fly Cell Atlas (FCA) database and found that short neuropeptide F (sNPF) had the highest expression level[26] (Supplementary Fig. 4a). The functions of sNPF resembled those suggested for NPY in mammals, including feeding regulation[27]. Therefore, we hypothesized that sNPF in ALK neurons may reduce yeast preference after mating. The intersection between *LK-LexA* and *sNPF-Gal4* suggested that ALK neurons were sNPF positive (Fig. 5a). Furthermore, we performed an intersection of *sNPF-LexA* and *CrzR-Gal4*, which was simultaneously stained with an LK antibody. The results confirmed that LK neurons expressing sNPF are also positive for CrzR (Supplementary Fig. 4b). However, the observed additional co-expression may be attributed to the specific characteristics of the *sNPF-LexA* line.

Knocking down sNPF in ALK neurons blocked the switch of yeast preference after male mating (Fig. 5b), suggesting that sNPF in ALK neurons was responsible for the decreased yeast appetite in mated males. In contrast, knocking down LK in ALK neurons did not impede the change in yeast appetite after mating (Supplementary Fig. 4c), indicating that LK in ALK neurons was dispensable for yeast preference.

We then investigated how the released sNPF acted on downstream neural circuits. It has been reported that FB-LAL neurons were activated to promote prolonged protein intake under protein deprivation conditions[20]. We hypothesized that sNPF may target FB-LAL neurons to inhibit yeast consumption after mating. To test this, we knocked down sNPFR in FB-LAL neurons labeled with *R75B10-GAL4* or *R70G12-GAL4* respectively. Remarkably, both manipulations effectively blocked the mating-induced decrease in protein appetite (Fig. 5f). As previously reported, the intersection between *R70G12-Gal4* and *R75B10-LexA* specifically revealed two pairs of FB-LAL neurons[20].

We examined the expression patterns of neurons labeled with *sNPFR* and *R75B10-GAL4* neurons respectively. These neurons contain cell bodies located in the posterior medial protocerebrum and sent projections to the fan-shaped body (FB), extending more anteriorly to the lateral accessory lobe (LAL) area (Supplementary Fig. 4d,e). By intersecting the *sNPFR-Gal4* and *R75B10-LexA* lines, we identified one of the labeled neuron groups as putative FB-LAL neurons (Fig. 5c).

We then monitored the neuronal activity of FB-LAL neurons with Arclight, a genetically encoded fluorescent voltage indicator in unmated and mated males[28]. The fluorescence signal of FB-LAL neurons increased by mating, suggesting that FB-LAL neurons were inhibited (Fig. 5d, e). In contrast, when we specifically knocked down sNPFR in FB-LAL neurons, we observed a complete reversal of the changes in the signal of Arclight following mating (Fig. 5g, h). This indicated that sNPFR in FB-LAL neurons was crucial for mediating the alterations in neural activity associated with mating-induced changes in yeast appetite.

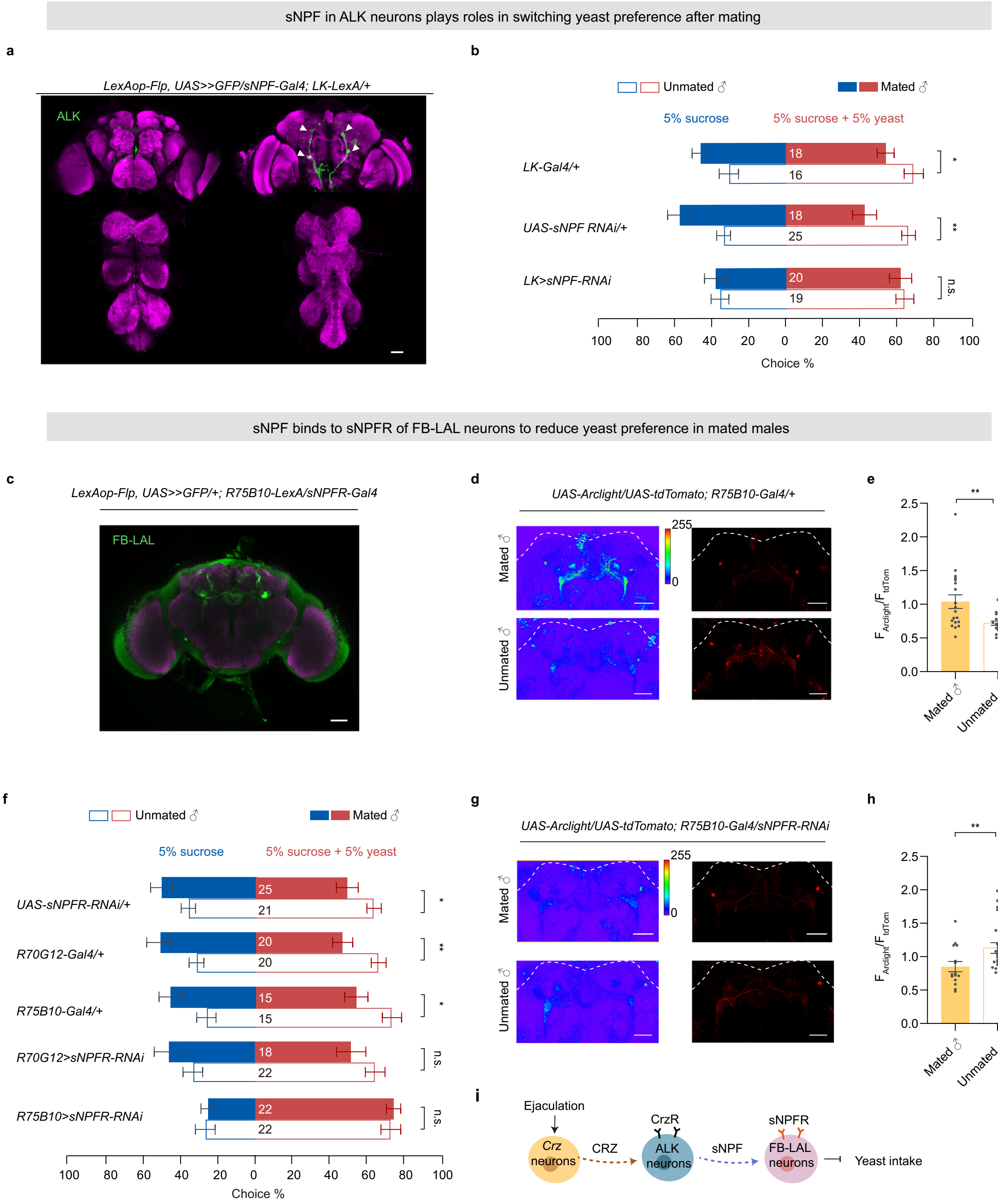
In summary, we found that the switch in yeast preference caused by mating was likely induced by CRZ released upon copulation which acted directly on ALK neurons in the brain. The ALK neurons released sNPF upon activation which bound to the sNPFR of FB-LAL neurons to reduce yeast preference in mated males (Fig. 5i).

ALK neurons mediate the post-mating switch of yeast preference in female flies

We then asked whether ALK neurons also regulated female yeast preference. Firstly, we examined the projection pattern of *LK* neurons and *CrzR* neurons in females and found that these neurons were not sexually dimorphic in the brain (Supplementary Fig. 5a,b). Using an intersection strategy, we specifically labeled ALK neurons in females, whose cell bodies were located in the anterior brain (Fig. 6a). Silencing ALK neurons with Kir2.1 hindered the mating-induced elevation of yeast preference in females (Fig. 6b). We also discovered that the calcium level of ALK neurons in mated females was higher than that in virgins (Fig. 6c), indicating that ALK neurons were activated by mating.

ALK neurons played opposite roles in regulating yeast appetite between the two sexes, despite the lack of a sexually dimorphic pattern. To further investigate the mechanism, we analyzed the transcriptional levels of different neuropeptides in female ALK neurons using the FCA database and found that the expression pattern of neuropeptides in female ALK neurons was different from that in males (Supplementary Fig. 5c)[26]. We conducted targeted knockdown to interfere with the expression of LK, TK, and sNPF in female ALK neurons. Unlike in males, knocking down LK specifically in ALK neurons effectively blocked the increase in yeast preference of mated females (Fig. 6d), suggesting that LK in ALK played an important role in mediating yeast preference in female flies.

Our findings demonstrated that distinct neuropeptides functioned in male and female ALK neurons to modulate the changes in yeast appetite induced by mating. Next, we wanted to determine whether the released LK acted on female FB-LAL neurons to increase yeast consumption. The projection pattern of female FB-LAL neurons was identical to males' (Supplementary Fig. 4f). We monitored the neuronal activity of FB-LAL neurons in both virgin and mated females using Arclight as an indicator, and we observed no significant differences in the signal between the two states (Fig. 6e). To further corroborate this result, we recorded the level of Ca^{2+} in ALK neurons using GCaMP6m and found no changes in the Ca^{2+} level after mating (Supplementary Fig. 5d). These findings suggested that female FB-LAL neurons were dispensable for the post-mating switch in yeast preference, and implicated that different peptides and their downstream signaling pathways play sex-specific roles in regulating the post-mating modulation of protein preference (Fig. 6g). This mechanism likely enables distinct strategies to balance the trade-offs between fecundity and fitness in males and females.



Discussion

To ensure survival and reproduction, animals must balance their nutrient intake to match their current needs. Our study reveals that flies budget their investment between fitness and fecundity by switching their yeast appetite in a sex-specific manner. Mated males have decreased yeast preference to prioritize fitness compared with unmated males. On the contrary, mated female have an increased yeast appetite to ensure enough supply for the development of their offspring.

The ecological and evolutionary interpretation of nutrition's impact on physiology, life history, and fitness has long been a topic under debate. It is commonly assumed that adaptive reallocation of nutrients from reproduction to somatic maintenance enhances survival during food shortages in nature[29–31]. However, if this were true, it would imply that longevity under dietary restriction and high fecundity under abundant protein resources are mutually exclusive due to competition for limited nutrients. Another theory explaining the trade-off between fitness and fecundity is the disposable soma theory, which posits that organisms allocate more resources toward reproduction than to body maintenance[32,33]. Here, we present an additional perspective on how the rapid reconfiguration of the neuronal circuits that control animal food intake based on internal needs

Fig. 5 | FB-LAL neurons were activated by sNPF from ALK to reduce yeast preference in mated males. a The intersection between *sNPF* neurons and *LK* neurons in the male central nervous system. White arrows indicated the cell bodies of ALK neurons. Green: GFP. Magenta: nc82. The scale bar represented 50 µm. **b** Yeast preference of males when blocking down sNPF in ALK neurons. Mean ± SEM. $^*p < 0.05$, $^{**}p < 0.01$, n.s., not significant, two-tailed unpaired *t*-tests. **c** The intersection between the male brain's sNPFR and FB-LAL neurons. Green: GFP. Magenta: nc82. The scale bar represented 50 µm. **d** The voltage signal of male FB-LAL neurons in different mating states. The left panels represent the signals of Arclight, and the right panels represent the signals of tdTomato. White dotted lines outline the brain. The calibration bar ranges from 0–255. Scale bar, 10 µm. **e** The relative fluorescence intensity ($F_{\mathrm{Arclight}}/F_{\mathrm{tdTom}}$) of male FB-LAL neurons in different mating statuses. Mean ± SEM. $^{**}p < 0.01$, two-tailed unpaired *t*-tests. $n = 19, 20$. **f** Yeast preference of males with sNPFR knocked down in FB-LAL neurons. Mean ± SEM. $^{**}p < 0.01$, $^*p < 0.05$, n.s. not significant, two-tailed Mann–Whitney nonparametric tests. **g** The voltage signal of male FB-LAL neurons in different mating states when sNPFR was knocked down. The left panels represent the signals of Arclight, and the right panels represent the signals of tdTomato. White dotted lines outline the brain. The calibration bar ranges from 0–255. Scale bar, 10 µm. **h** The relative fluorescence intensity ($F_{\mathrm{Arclight}}/F_{\mathrm{tdTom}}$) of male FB-LAL neurons with sNPFR knockdown in different mating statuses. Mean ± SEM. $^*p < 0.05$, two-tailed Mann–Whitney nonparametric tests. $n = 15, 22$. **i** Graphic model showed the neural circuits behind reduced yeast preference after mating in males. Source data are provided as a Source Data file.

reconciles the trade-offs between fitness and fecundity. Mating-induced intermittent yeast consumption led to longer lifespans and increased fecundity in males and reduced the cost of mating in females, indicating that animals actively make food choices based on the priority of optimizing fecundity and overall fitness.

Lifespan and reproductive capability are commonly thought to antagonize one another, which is found to be influenced by the protein-to-carbohydrate ratio in food in various organisms7. In flies, the Geometric Framework analysis has demonstrated that the highest longevity is observed at a protein-to-carbohydrate ratio of 1:16, while maximum egg-laying occurs at 1:2. Furthermore, an intermediate P: C ratio of 1:4 results in optimal allostatic performance[34,35]. Based on our results, the P: C ratio of the recipe, which extends lifespan and increases fecundity, is approximately 1:3. This finding indicates that animals can attain an optimal P: C ratio through the regulation of their yeast appetite, which in turn maintains both fitness and fecundity. However, it would be important to measure daily intake to accurately verify the precise P:C ratio in both sexes at different reproductive states. Noticeably, salt intake may also contribute to the post-mating change in yeast appetite in both sexes. Increased salt consumption has been shown to enhance egg production in female *Drosophila*[10]. However, it warrants further investigation whether and how salt intake regulate the post-mating physiology in male animals.

It is well known that pregnancy and postpartum in female animals are associated with physiological and behavioral adaptations. For example, female post-mating behavior in fruit flies is characterized by reduced sexual receptivity, increased egg laying, and changes in food appetite, sleep, and memory[9,36–39]. While males and females exhibit significant differences in their reproductive behaviors[40], studies have revealed that mating can also trigger behavioral changes in males. For instance, multiple copulations reduce male fertility and propensity to engage in mating[41,42], and mating inhibits sex-related male-male aggression[43] Additionally, a recent finding suggested that mating experience decreased ethanol preference[44]. In addition to the modulation of male behavior, mating also alters gene expression patterns, lifespan, metabolism, and antioxidant systems[45,46]. Our study uncovers a shift in protein preference in males after mating, adding insights into mating-induced behavioral adaptation. These findings together suggest that male post-mating behaviors were often overlooked, and highlight the importance of comparative studies between female and male post-mating behaviors.

Protein intake acutely activates *Diuretic hormone 31* (*Dh31*) enteroendocrine cells in the male gut, and gut-derived Dh31 propels a switch to courtship[4]. In agreement with this finding, we found that the inhibition of yeast consumption may reduce the propensity of mated males to engage in mating. Furthermore, we found that CRZ released after mating binds to ALK neurons to reduce yeast appetite in males, while ALK neurons are suggested to function to enhance yeast appetite in females. There are two possible reasons that ALK neurons exhibit sexually dimorphic functions. Firstly, ALK neurons may receive different inputs from males and females. The male-derived SP in seminal fluid is defined as a classical modulator to induce female post-mating behavioral switch, and its modulatory effect is transmitted to pC1 neurons in the brain via the SPSNs-SAG-PC1 circuits[37,47–49]. Female ALK may receive input from this circuit, although further studies are needed to test this hypothesis. Secondly, activation of ALK neurons may release different neuropeptides to target distinct downstream circuits that control feeding in two sexes. For instance, FB-LAL works downstream of male ALK neurons but does not participate in the modulation of yeast appetite in females.

In addition to mating status, male yeast appetite is also modulated by nutritional states to support protein homeostasis[50,51]. Flies shift their preference towards protein-rich food sources after protein deprivation. Multiple mechanisms have been proposed to regulate protein intake. Gustatory receptor neurons in the proboscis expressing Ir76b and Ir25a mediate yeast feeding, and their sensitivity to amino acids is increased after protein deprivation[50]. A functional atlas of the ventral fly brain revealed that the metabolic state of protein shapes sensorimotor processing across large sections of the neuropil[51]. Furthermore, the neuropeptide CNMamide (CNMa) is highly induced in enterocytes of the anterior midgut during protein deprivation, and the EAA-deprived condition is communicated to the brain through CNMa to promote yeast-rich food intake[52]. Moreover, severe protein deprivation activated FB-LAL neurons to promote prolonged protein intake[20]. The shift in yeast appetite induced by mating in protein-deprived flies was mediated by ALK neurons, suggesting that yeast appetite modulation is influenced by distinct internal states independently. The neural circuit that integrates all the internal and external information to direct the protein intake warrants future investigation.

Methods

Fly stock

Fly stocks were maintained in a 25 °C incubator with a 12 h/12 h light/dark cycle and humidity control. The fly lines used in this study were summarized in detail in the Supplementary Information.

The flies utilized in our study were between 5 and 7 days old, except those subjected to gene knockdown using RNAi, which were aged between 7 and 10 days. The genotype of virgin females was w^{1118}, and wild-type males were *Canton S*, except for the males used in Supplementary Fig. 1b. Flies were reared on a standard medium, and all behavior tests were done at 25 °C and 40% humidity unless otherwise stated.

Food preparation

Flies were reared on standard food (45 L water, 1102.5 g yeast, 2250 g brown sugar, 787.5 ml antiseptic, 450 g agar, 15 g yeast, 2250 g cornmeal). Yeast and sucrose food (YS food) was prepared by mixing 5% yeast extract (OXOID, 1357060) and 5% sucrose (BIOBYING, CAS:57-50-1). Sucrose food (S food) was prepared by adding 5% sucrose. Yeast deprivation was achieved by maintaining the flies on S food supplemented with 1% agar (LABLEAD, CAS:9002-18-0).

For amino acid preference tests, a liquid medium consisting of a 100 mM AA mixture and 5% sucrose or 5% sucrose alone was used[53]. The AA mixture comprised 20 amino acids, which were obtained from

Sigma. To prepare the 20 amino-acid mixture solution, L-alanine (CAS: 56-41-7), L-arginine (CAS: 74-79-3), L-asparagine (CAS: 70-47-3), L-aspartic acid (CAS: 56-84-8), L-cysteine (CAS: 52-90-4), L-histidine (CAS: 71-00-1), L-isoleucine (CAS: 73-32-5), L-leucine (CAS: 61-90-5), L-lysine (CAS: 56-87-1), L-glutamine (CAS: 56-85-9), L-glutamic acid (CAS: 56-86-0), Glycine (CAS: 56-40-6), L-methionine (CAS:63-68-3), L-

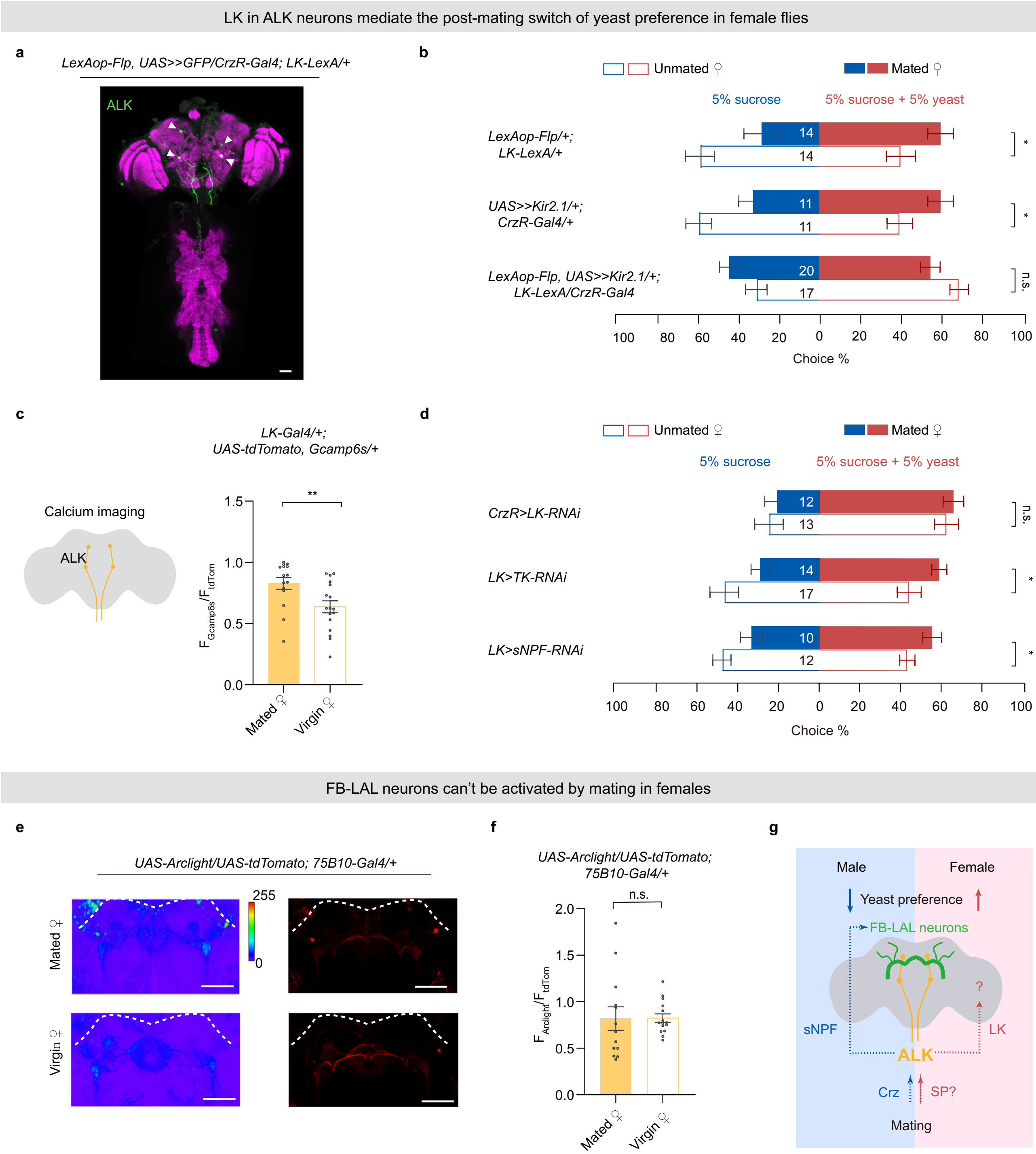


Fig. 6 | ALK neurons functioned in modulating female post-mating yeast preference. a The intersection between *CrzR* neurons and *LK* neurons showed the expression pattern of ALK neurons in the female central nervous system. The white arrow indicated the cell bodies of ALK neurons. Green: GFP. Magenta: nc82. The scale bar represented 50 µm. **b** Yeast preference of females when ALK neurons were silenced with Kir2.1. Mean ± SEM. $^{*}p < 0.05$, n.s. not significant, two-tailed unpaired *t*-tests. **c** Ca^{2+} signal of female ALK neurons in different mating states. The right panel showed the relative fluorescence intensity ($F_{Gcamp6s}/F_{tdTom}$) of female ALK neurons in different mating states. Mean ± SEM. $^{**}p < 0.01$, n.s. not significant, two-tailed unpaired *t*-tests. $n = 15, 17$. **d** Yeast preference of females when knocking down different neuropeptides in female *LK* neurons. Mean ± SEM. $^{*}p < 0.05$, n.s. not significant, two-tailed unpaired *t*-tests. **e** The voltage signal of female FB-LAL neurons in different mating statuses. The left panels represent the signals of Arclight, and the right panels represent the signals of tdTomato. White dotted lines outline the brain. The calibration bar ranges from 0–255. Scale bar, 10 µm. **f** The relative fluorescence intensity ($F_{Arclight}/F_{tdTom}$) of female FB-LAL neurons in different mating statuses. Mean ± SEM. n.s. not significant, two-tailed unpaired *t*-tests. $n = 13, 15$. **g** Graphic model of a sex-specific mechanism where different peptides and their downstream signaling pathways play crucial roles in regulating the post-mating modulation of yeast preference. Source data are provided as a Source Data file.

phenylalanine (CAS: 63-91-2), L-proline (CAS: 147-85-3), L-serine (CAS:56-45-1), L-threonine (CAS: 72-19-5), L-tryptophan (CAS: 73-22-3) and L-valine (CAS: 72-18-4) were dissolved in distilled water at a concentration of 5 mM for each amino acid. The 19 amino-acid mixture stock solution plus 5 mM L-tyrosine (CAS: 60-18-4) was used as an AA mixture. L-tyrosine was added separately because of its low solubility[53].

To test salt preference, the liquid medium consisted of 0.015% sodium chloride (GENERAL-REAGENT, CAS:7647-14-5) and 5% sucrose, or 5% sucrose only.

CAFE (CApillary FEeder) assay

As described in a previous study[16], males were subjected to a 24-h yeast deprivation period by providing them with a 5% sucrose solution before the CAFE assay. To test yeast preferences, a liquid medium containing either YS food or S food was used, with the addition of 0.01% dye to each food. The red dye is Cochineal (TCI, CAS:3520-42-1), and the blue dye is Brilliant Blue (TCI, CAS:3844-45-9). The assignment of food color was randomized for each experiment. The CAFE assay utilized a plastic food vial with a porous cap, enabling the ingress of air and water vapor. Plastic food vials were equipped with a Titan filter paper (15 cm) soaked in 500 µl of water to guarantee a stable water supply. Capillaries filled with the liquid food were inserted through the cap using truncated 10 µl pipette tips. During the experiments, the food vials were maintained inside a light-proof plastic box. Each CAFE food vial consisted of 10 flies.

Following the 24-h CAFE tests, flies were frozen, and the amount of ingested dye was determined by visual inspection of the abdomen. The number of flies consuming different foods was counted. The choices preference was calculated as $N_{(\text{YS food or S food})}/(N_{(\text{YS food})} + N_{(\text{S food})}) \times 100\%$. A capillary measuring 100 mm in length and containing 5 µL of food solution was utilized to quantify the reduction in food content, thereby enabling the calculation of food intake.

Once-mated males were paired with virgin females in a 1:1 ratio for 24 h while simultaneously being deprived of yeast. As for sexually sated males, they were paired with females in a 1:15 ratio during the last 3 h of yeast deprivation. In Fig. 3, the food in the capillaries was changed daily to assess the duration of yeast preference. S food was provided for yeast deprivation on the first day. Subsequently, during the CAFE assays conducted on the second and third days, yeast was supplied for flies to choose freely.

The recipe shown in Supplementary Fig. 2b, e was tailored based on the yeast preference exhibited by the flies. During the CAFE assay conducted on the second day, mated females displayed a preference for YS food. By the third day, the yeast appetite of mated females was restored. Consequently, a SYS food mixture was established. Conversely, mated males showed an appetite for S food on the second day, with their yeast appetite being restored by the third day. Based on this observation, an SSY food mixture was established.

Two-choice assay

Before the two-choice assay, males were yeast-deprived for 24 h. The assay utilized a 90 mm diameter petri dish divided into four quadrants, with each quadrant containing a filter paper soaked in 30 µl distilled water to maintain humidity[54]. Additionally, 5 µl of YS or S food in 1% agar was placed next to each filter paper. Following 24 h of feeding, flies were anesthetized, and the amount of ingested dye was determined by visually inspecting the abdomen. The preference index for YS food was calculated using the formula: $(N_{(\text{YS food})} - N_{(\text{S food})})/(N_{(\text{YS food})} + N_{(\text{S food})} + N_{(\text{food both})}) \times 100\%$.

flyPAD feeding assay

After a 24-h yeast deprivation, male flies were placed in the recording arena of flyPAD containing two food types with 0.25% agar, and their feeding behavior was recorded for 20 min. The sips' signal was analyzed using MATLAB and calculated as described in a previous study[55].

Mating behavior assay

Male flies underwent a 24-h yeast deprivation and were subsequently fed either YS or S food supplemented with 1% agar on the following day. Virgin females were aspirated into courtship chambers 4 mm in height and 10 mm in diameter, where they were paired with the males. The male courtship index was defined as the proportion of time that the male spends on courtship behaviors toward the female until copulation. Courtship mainly comprises following, wing extension, and attempted copulation, as tapping and licking are subtle behaviors and are more difficult to quantify. Courtship behavior was recorded til copulation or for up to 5 min[56].

In Supplementary Fig. 3, latency refers to the time elapsed between courtship initiation and the start of copulation, while duration represents the length of time the copulation. The courtship index was calculated as the time spent on courtship behaviors divided by the latency or 5 minutes.

Mating competing assay

A virgin female was paired with two *Canton S* males. Male flies were deprived of yeast for 24 h and subsequently fed either YS food or S food supplemented with 1% agar on the following day. To facilitate identification, markings were randomly made on the notum of one of the males. The results in Fig. 1b indicated the percentage of flies that successfully copulate within 30 min.

7-11 HD painting

Five micrograms 7-11 HD (500 ng/fly) dissolved in 10% ethanol was applied to filter paper and left to evaporate for 2 h before the CAFE assay[57]. Unmated males were exposed to the filter paper in the CAFE tube, while control males were exposed to filter paper with 10% ethanol alone.

Immobilizing females

10 immobile 5-7 day-old females or feminized males were affixed to a cover slide using light-curing adhesive. Subsequently, the slides were introduced into the CAFE tube.

Optogenetics and thermogenetics

Newly eclosed flies were collected and transferred into food containing 500 µl of sugar-retinal solution (500 µM all-trans-retinal diluted in 100 mM sucrose solution)[58]. Flies were kept in the dark and used for optogenetics experiments after being fed with all-trans-retinal for 3–5 days. The experiments were conducted in a dark room, where the flies were subjected to 590 nm light stimulation every 10 min for 1 h before the CAFE assay.

For heat activation experiments, flies were raised in an incubator of 22 °C. Before the CAFE assay, flies were heated for 30 min.

Survival assay

Flies were deprived of yeast for a 24-h and then provided with either YS or S food supplemented with 1% agar on the following day. To assess their resistance to starvation, the flies were transferred to empty vials with filter paper soaked with 500 µl of water. To evaluate the resistance to desiccation, the flies were transferred to empty food tubes, and the number of surviving flies was recorded at hourly intervals.

Lifespan and fecundity assay

Newly eclosed flies were collected and provided with a diet as outlined in Supplementary Fig. 2a,d. The food was replaced daily to ensure optimal nutrition. For lifespan assay, daily records of deceased flies were recorded until the demise of all flies in the experiment.

The fecundity assay was initiated on the fourth day after eclosion. For males, fed individuals were paired with virgin females at a ratio of 1:3. This mating process occurred in an empty tube for 3 hours every 3 days, ensuring that female fertility remained unaffected by nutritional factors. The mated females were then collected and raised on standard food for egg-laying. The fresh food was replaced daily for 3 days. After 9 days, the number of offspring was manually counted upon their emergence.

Regarding female fecundity, fed females were paired with males in a 1:1 ratio for 24 h every 3 days. Following mating, the males were discarded, and the number of eggs laid by the females was counted daily.

Sperm competition test

3-day-old virgin females were initially mated with *Canton S* males. After 3 days, the mated females were randomly assigned to mate with second males for 6 h, which were given a one-day diet consisting of either YS food or S food after 24 h of yeast deprivation. The sperm of the second males were labeled by protamineB-eGFP, and the female reproductive organs were dissected 45 min after the end of the second mating to count the number of sperm present in the female seminal receptacle[15].

Tissue dissection, staining, and imaging

Brains and ventral nerve cords (VNC) of 5–7-day-old flies were dissected in dissection buffer (PBST: 0.015% Triton X-100 in 1X PBS) and fixed in 4% paraformaldehyde (PFA) at room temperature on a shaker for 20 minutes. Subsequently, the tissues were washed for 4 × 20 min in wash buffer (0.3% Triton X-100 in 1X PBS) and blocked in block buffer (1X heat-inactivated normal goat serum with 0.3% Triton X-100 in 1X PBS) for 30 minutes at room temperature. The samples were then incubated with primary antibodies at 4 °C overnight. The primary antibodies used were Rabbit-GFP (Invitrogen, A11122, 1:500 dilution), Chicken-GFP (Invitrogen, A10262, 1:500 dilution), Mouse-LK (a gift from Yufeng Pan, Institute of Life Sciences, Southeast University, 1:500 dilution), and Mouse-NC82 (Hybridoma Bank DSHB, Brunchpilot, 1:50 dilution). Chicken-GFP was only used in Supplementary Fig. 4b. On the second day, samples were washed for 4 × 20 min and then incubated in secondary antibodies. The secondary antibodies were all from Invitrogen and used at 1:200 dilution: Alexa Fluor 555 anti-Rabbit (A-21428), Alexa Fluor 647 anti-Mouse (A-2123511031) and Alexa Fluor 488 anti-Chicken (A-11039). After incubation, tissues were washed for 4× 10 minutes. VNC and brains were mounted on a slide for imaging. Confocal imaging was performed using an Olympus FV1000 microscope with a 20× air lens.

Testes were stained with antibodies against Vasa to mark GSCs and Fasiclin III (FasIII) to mark the hub cells. The number of marked cysts arising from the marked GSCs was counted[59].

Calcium imaging of ALK neurons

The whole brain was dissected in a recording chamber containing external solution (103 mM NaCl, 3 mM KCl, 5 mM TES, 10 mM trehalose, 10 mM glucose, 26 mM $NaHCO_3$, and 1 mM NaH_2PO_4, pH 7.25, 310 mm). 4 mM $MgCl_2$ and 2 mM $CaCl_2$ were added to the saline before use.

Calcium imaging was performed using an Olympus FV1000 microscope with a 20X air lens. The Ca^{2+} indicator GCaMP6s was used to measure the Ca^{2+} signal. td-Tomato was used as an internal reference. GCaMP6s was excited with 488 nm light and td-Tomato was excited with 556 nm light. Regions of interest (ROIs) were manually selected from the cell body area using ImageJ. The value of fluorescent intensity was calculated as $F_{\mathrm{Gcamp6s}}/F_{\mathrm{td\text{-}tomato}}$.

To image the activity of male ALK neurons after mating, 5–7-day-old *LK-Gal4/+; UAS-tdTomato, Gcamp6s/+* males were paired with virgin females in a 1:1 ratio. They were dissected immediately after mating. The intensity of the excitation light remained consistent when recording the signals of the state before and after mating.

Imaging of the activity of ALK neurons in female flies after mating was conducted by pairing a 5–7 day-old *LK-Gal4/+; UAS-tdTomato, Gcamp6s/+* virgin female with a male in the courtship chamber until mating was completed. The females were dissected 24 h after mating, and imaging was performed. Notably, the intensity of the excitation light remained consistent when recording the signals of the state before and after mating.

Analyzing transcriptional profiles of ALK neurons

We downloaded the raw count data of 10X stringent head datasets from the Fly Cell Atlas (https://flycellatlas.org/). Within these datasets, we detected six cells co-expressing *LK* and *CrzR*, consisting of two males and four females. To identify and compare sex-specific expression patterns of neuropeptides in this cell type, we first scaled the counts using the scale function in the R package and then calculated the average counts. The codes we used have been uploaded to GitHub[60].

Voltage imaging of FB-LAL neurons

The entire brain was dissected in an external solution described above. Arclight imaging was performed using an Olympus FV1000 microscope with a 20× air lens, and voltage changes were measured while td-Tomato was used as a reference. Arclight was excited with 488 nm light and td-Tomato was excited with 556 nm light. ROIs were manually selected from the cell body area using ImageJ, and the fluorescent intensity was calculated as $F_{\mathrm{Arclight}}/F_{\mathrm{td\text{-}tomato}}$.

In male flies, the activity of FB-LAL neurons after mating was carried out by pairing a 5–7 day-old male with a w^{1118} virgin female in the courtship chamber. The male flies were dissected immediately upon completion of copulation. For female flies, 5–7 day-old females were paired with *Canton S* males, and brain imaging was performed after a 24-h post-mating. The intensity of the excitation light remained consistent when recording the signals of the state before and after mating.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism Software. All data presented in bar and line graphs were means ± SEMs. In Fig. 3b,e, means ± SDs are shown in error bands. To evaluate the statistical significance between the two datasets, two-tailed unpaired *t-tests*, ordinary ANOVA test, and two-tailed Mann–Whitney nonparametric test were used. The Log-rank test was used to analyze survival rates, whereas the *Binomial* test was employed to analyze mating competitiveness results.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

We declare that all data supporting the findings are provided in the Source Data file. Source data are provided with this paper.

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Author contributions

C.L. and N.T. performed the experiments and analyzed the data. P.C. analyzed transcriptional profiles of ALK neurons and uploaded the codes to GitHub. C.L. and W.Z. wrote the manuscript. All authors discussed and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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