Decision Making is Contextually Flexible

Behavior choices amongst grooming, feeding, and courting in *Drosophila* show contextual flexibility, not an absolute hierarchy of needs

Carla E Ladd[1] and Julie H Simpson[1,2]

[1]Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA, United States of America

[2]Corresponding author: jhsimpson@ucsb.edu

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Abstract

To determine the algorithmic rules and neural circuits controlling selection amongst competing behaviors, we established assays where adult *Drosophila melanogaster* choose between grooming and feeding, grooming and courting, or feeding and courting. We find that there is not an absolute hierarchy: while flies typically perform grooming first, they can choose to feed if sufficiently starved, or court if an appropriate female is available. Flies alternate between competing behaviors, performing short bouts of each action rather than completely satisfying one drive before transitioning to another. And we do not find evidence for a common genetic or neuronal locus that affects all decisions, suggesting that the fly may select the best course of action by discrete circuits regulating each pairwise comparison. Our results add to a growing body of work on decision-making in *Drosophila* and provide a foundation for future investigation of the exact neural circuits required to achieve appropriate choices.

Summary Statement

Flies alternate between competing behaviors. The choice amongst positive actions depends of context and relative drive levels. No central decision-making locus was identified.

Introduction

Flexibility in decision-making is a hallmark of a successful species (Devineni and Scaplen, 2021). The best choice can change with circumstances, and an animal that can adjust its actions according to risks and rewards, strengths of competing drives, and available resources thrives in a range of environments. Decision-making is impaired in human neurological conditions including Parkinson's Disease (Brand et al., 2004; Ryterska et al., 2014), Alcohol Use Disorder (Galandra et al., 2020), and schizophrenia (Barry et al., 2020). The behavioral rules, and genetic and neural bases of decision-making, have been studied in humans and in various other species (Bechara et al., 1994; Lakshminarayanan et al., 2010; Alabi et al., 2019; Rodríguez et al., 2001), but a full understanding of the control mechanisms and an assessment of common principles has been elusive.

Drosophila melanogaster has been an advantageous animal in which to study the genes, neurons, and circuits that contribute to perceptual decisions (DasGupta et al., 2014; Groschner et al., 2018), food selection (Münch et al., 2022), mate choice (Hindmarsh Sten et al., 2025; Dickson, 2008), mating duration (Gautham et al., 2024), oviposition site preference (Joseph et al., 2009; Yang et al., 2008), and escape strategies (von Reyn et al., 2014; von Reyn et al., 2017; Asinof and Card, 2024). Often these assays examine the trade-off between benefit and cost: will a male fly continue to mate in the presence of a threat (Cazale-Debat et al., 2024)? Do larvae risk hypoxia for food (Kim et al., 2017), or accept cold or bitter food, if they are very hungry (Wu et al., 2005)? Will they stay on a mediocre but available food source or seek richer resources (LeDue et al., 2016; de Belle et al., 1989)? Switching amongst individual and social behaviors driven by internal mechanisms (or at least uncontrolled stimuli) has also been assayed (Berman et al., 2016).

Flies make decisions about feeding based on how hungry they are, what specific nutrients they lack, what food is available, and prior experience (Nhuchhen et al., 2025; Marella et al., 2012). They can adjust meal size, select sugar or protein-rich foods, and will accept bitter or cold but nutritious substances if sufficiently motivated (LeDue et al., 2016; de Belle et al., 1989). And they can avoid food that previously made them sick (Stensmyr et al., 2012). Genes and neurons that affect feeding decisions include leucokinin (Yurgel et al., 2019), dopamine (Jin et al., 2023), diuretic hormone 31 (Lin et al., 2022), and tyramine (Cheriyamkunnel et al., 2021).

Flies also make decisions about courtship and mating actions. A male fly will evaluate whether a potential partner belongs to the same species, is male or female, and if she has previously mated (von Philipsborn et al., 2023). He will adjust his actions based on current sensory information and his past mating experience (Simon et al., 2011). Once he initiates courtship, he can escalate from orientation and chasing to singing with his wings depending on the cues from the female, and may proceed to tapping, licking, and attempted copulation (villella and Hall, 2008). The commitment to copulation and its duration can be influenced by perceived environmental threats: males adjust their choice between courtship and escape depending on external sensory cues, internal drives, and past experiences (Simon et al., 2011). Neural and genetic bases include leucokinin (Chen et al., 2025), tyramine (Cheriyamkunnel et al., 2021), and fruitless (Yamamoto and Koganezawa, 2013).

Our analysis of grooming behavior indicated that choice of which body part to clean is driven both by the acute distribution of debris and an intrinsic set of priorities: the eyes, antenna, and head are cleaned first (Seeds et al., 2014) – unless there is much more stimulation to mechanosensory bristles in posterior regions of the body (Zhang et al., 2020). An innate hierarchy organizes the grooming sequence from anterior to posterior body parts, with contextual flexibility to respond to the current sensory load. Neural circuits

that contribute to the grooming sequence include dopamine (Pitmon et al., 2016) and descending neurons (Guo et al., 2022).

Feeding, courting, and grooming are complex motor sequences made up of choices among smaller actions. We noticed, anecdotally, that grooming behavior superseded escape, courtship, and feeding in our standard grooming assays and we wondered if there was an absolute hierarchy amongst distinct behaviors, analogous to Maslow's hierarchy of needs (Maslow, 1943). How would a fly prioritize when given multiple options? Would this choice be fixed or flexible?

Much popular discussion on the disadvantages of multitasking and rapid task switching led us to examine whether flies finish one task before starting another or alternate between activities that satisfy competing drives.

We wondered if different decisions share common neural circuits – if there is a brain region involved in multiple decisions: a control center. We reasoned that if decision-making itself, rather than performance of a specific behavior, were impaired, then we would see differences in choices amongst several different behaviors. Altering neural activity in either a central, integration, or modulatory circuit might show normal sensory response thresholds for flies in individual behavioral assays but changes in the ratio or relative timing of behaviors when flies were given a choice. Previous research examined decisions between two behaviors such as courting and feeding showed roles for specific tyraminergic neurons (Cheriyamkunnel et al., 2021) and gut hormones (Lin et al., 2022), but whether these same neurons have a more universal effect across multiple decisions was not known.

Therefore, we established a set of behavioral assays where the fruit fly *Drosophila melanogaster* chooses amongst grooming, feeding, and courting (**Fig. 1**). All three of these behaviors are natural, productive actions, and we can adjust the drive to perform each one

115 by manipulating internal and external cues. Flies can clean themselves and have access to
116 food or mates; we measured behavior onset, bout durations, and relative time spent
117 grooming, feeding, or courting in pairwise assays.
118
119 **Methods and Materials**
120 ***Fly strains***
121 To study the behavior of wild type flies during decision making tasks, I used the CantonS
122 *Drosophila melanogaster* strain (**Table 1**). The flies were raised on common cornmeal in a
123 25°C incubator on a 12-hour light/dark cycle. Within 24 hours of becoming adults, the
124 males and females were separated into sex specific housing. Ice was used to anesthetize
125 flies during sorting. All lines crossed to *UAS-CsChrimson* were kept in a dark incubator and
126 raised on food containing 0.4 mM all-trans-retinal and starved on agar containing 0.4 mM
127 all-trans-retinal. All lines crossed to *UAS-Kir2.1; TubP-GAL80*^{ts} were kept at 30°C for 24
128 hours and then rested at room temperature (23ºC) for 1 hour prior to filming.

Table 1: Fly Strains

Strain	Nickname	Bloomington Stock ID	Obtained From
CantonS	CantonS		Simpson Lab
pBDPGal4U in attP2	Empty-GAL4	68384	Simpson Lab
w[*] TI{RFP[3xP3.cUa]=2A-GAL4}Tbh[2A-GAL4.KI]/FM7a	Oct-GAL4	86145	Bloomington
w[*]; P{w[+mC] = Tdc2-GAL4.C}2	Tdc2-GAL4	9313	Bloomington
w[1118]; TI{w[+mE.hs]=GAL4}TyrR[Gal4])	TyrR-GAL4	67129	Montell Lab
Th-GAL4	Th-GAL4		Simpson Lab
Trh-GAL4 (III)	Trh-GAL4		Simpson Lab
w[1118]; P{w[+mC]=Lk-GAL4.TH}2M	LK-GAL4	51993	Bloomington

w[1118]; P{y[+t7.7] w[+mC]=GMR65C07-GAL4}attP2	LKR-GAL4	39344	Bloomington
UAS-Kir2.1; Tub-GAL80ts	UAS-Kir2.1		Louis Lab
w[1118]; P{y[+t7.7] w[+mC]=20XUAS-IVS-CsChrimson.mVenus}attP40	UAS-CsChrimson	55135	Simpson Lab
w[*]; P{w[+mW.hs]=GawB}OK107 ey[OK107]/In(4)ci[D], ci[D] pan[ciD] sv[spa-pol]	OK107-GAL4	854	Bloomington
201Y-GAL4	201Y-GAL4		Simpson Lab
w[1118]; Pbac{WH}FoxPf03746	FoxP	85667	Bloomington

Behavioral experiments

For the competition assays, all filming was done at room temperature. Flies were filmed at 30 fps for 15 minutes. Each fly was only used once in one assay. Flies were anesthetized using ice for transferring to either dusting chambers or recording chambers, described in (Seeds et al., 2014). All flies recovered for 15 minutes prior to filming to ensure the flies were awake. The same recording chambers were used for every experiment; chambers were cleaned with water after each day of experimentation. Experimental and control flies were shaken in a consistent and reproducible manner before each filming to replicate the effects of dusting. All males and all virgin females were 3 days old at the onset of filming. All filming was done with a FLIR Blackfly S USB3 camera and recorded top down with an infrared backlight.

Grooming vs Feeding

Prior to filming, flies were starved on agar ("wet-starved": deprived of food but not water). CantonS flies were starved for 24 hours while OK107-GAL4>UAS-Kir2.1; TubPGAL80ts and

201Y>UAS-Kir2.1; TubPGAL80ts cross progeny were starved for 12 hours. Each fly was tested alone. Each assay chamber has four separate wells, so four flies can be assayed in each filming run. Four flies at a time were transferred to a transfer chamber to recover from being anesthetized for 15 minutes. From there they were transferred to a 24-well corning tissue culture plate filled with 5 mg Reactive Yellow 86 dust in each of the four center wells. Flies were shaken in a consistent and reproducible manner to evenly coat the flies in yellow dust. All dusting was performed under a Misonix WS-6 downflow hood. The flies were then transferred back into the transfer chamber and then finally into the recording chamber. For the feeding assay, a small plug of cornmeal food was added before the flies were introduced. The plug was made by inserting the end of a Pasteur pipette into a standard food vial and then slicing the tube into disks approximately 2 mm thick. Fresh food was used for each experiment. As soon as the flies were added, the chamber was placed under the camera and filming began.

Grooming vs Courting

Male flies were dusted according to the protocol outlined above and then transferred into recording chambers loaded with virgin females, resulting in four chambers each containing one dusted male and one clean virgin female. The virgin females were Canton S, aged 3 days, and allowed to recover for 15 minutes from anesthesia after transfer. The female flies were not dusted because we found that the male flies did not initiate courtship to dusted females. The flies were placed under the camera and filming began.

Courting vs Feeding

Only male flies were starved prior to filming. After being anesthetized, male flies rested in an empty recording chamber while females rested in a recording chamber containing a food plug as described above. Male flies were then shaken to simulate the shaking done in the grooming experiments. The chamber containing the males was then placed back-to-back with the chamber containing the females and the food to transfer the males into the

173 female and food chamber. As soon as the males were added, the chamber was placed
174 under the camera and filming began.
175
176 *Triple*
177 The protocol in this experiment was the same as described in the Grooming vs Feeding
178 protocol, except the dust-covered males were transferred to a recording chamber
179 containing a both a virgin female and food.
180
181 ***Screen***
182 To study what types of neurons were involved in decision-making, a targeted screen was
183 run using subsets found during a literature hunt (Pooryasin and Fiala, 2015;
184 Cheriyamkunnel et al., 2021; Huser et al., 2012; Certel et al., 2010; Münch et al., 2022;
185 Meschi et al., 2024). The GAL4/UAS system was used for both inactivation and activation of
186 subsets of neurons. GAL4 males (Oct, Tdc2, TyrR, Th, Trh, LK, LKR, and Empty {Control})
187 were crossed with *UAS-Kir2.1; TubPGAL80ts* (inactivation) and *UAS-CsChrimson*
188 (activation) virgin females. The resulting flies were then tested in all three decision-making
189 assays.
190
191 *Optogenetic activation*
192 Prior to the optogenetic activation experiments with UAS-Chrimson, flies were raised on
193 0.4 mM all-trans-retinal. Within 24 hours of becoming adults, males were transferred to
194 fresh tubes containing their regular cornmeal food mixed with retinol while virgin females
195 were transferred to a plain cornmeal food tube. During starvation experiments, male flies
196 were transferred to agar tubes containing 0.4 mM all-trans-retinal mixed into the agar.
197 During the experiment, an overhead FLDR-i132LA3 ring light (626 nm) producing
198 approximately 0.85 mW/cm^2 of red light was used for optogenetics activation.
199
200 ***Data analysis***
201 *Video annotation and ethograms*

All videos were annotated for behavior manually using the VCode system (http://social.cs.uiuc.edu/projects/vcode.html) for the first five minutes. Courting, feeding, and grooming behaviors were annotated separately (i.e., the video was watched three times). Courting behavior was annotated for orienting, chasing, wing singing, attempted copulation, and copulation. Feeding behavior was annotated for feeding and not feeding. Grooming behavior was annotated for anterior and posterior grooming; walking and standing behavior was also noted.

Ethograms showing the temporal distribution of behavior were made using MATLAB. Grooming, feeding, and courting are displayed on separate rows grouped by the individual fly to show instances of simultaneous behavior and to highlight the alternation pattern. Further analysis was done to look at both comparative amounts of each behavior as well as total behaviors. This analysis and the creation of dot plots, box plots, and line graphs were made using Excel. Significance was calculated using a T test with two-tailed distribution and equal variance.

Initiation was calculated as the first instance of a particular behavior. Wing singing was used as the marker for courtship initiation because it is more specific to courtship than chasing or orienting. Grooming initiation was not analyzed because eye cleaning always began before the recording chambers could be placed under the camera.

Bout lengths were calculated as time a fly continuously performed an action. Any standing or walking breaks in behavior for less than a second were disregarded. Copulation was not included in bout length analysis.

Results

Characterization of pairwise behavioral choices

We examined how flies choose between grooming and feeding when both drives are present (**Fig. 2**). When flies are covered in dust or debris, they groom. The mechanosensory

stimulus provides strong external drive and the behavioral response is robust. Most flies spend 60-70% of their time grooming in a 5-minute assay. When flies are deprived of food for at least 8 and up to 36 hours and then presented with a food source, they spend up to 80% of their time feeding (**Fig. S1**). Hunger is a strong internal drive and most flies spend time feeding to satisfy it. There is a large amount of variability in percent time feeding: we hypothesize that this is caused by differences in fat storage or exact time of last meal before experimental food deprivation began. When flies are starved for 24 hours, covered in dust, and presented with food, they alternate between feeding and grooming. The total time spent grooming and the total time spent feeding are both reduced when compared to performance without competition. We also noted that starvation without food does not reduce time spent grooming, and that access to food does not reduce grooming when the flies are not starved.

We started analysis of the decision-making behavior during competition between grooming and feeding by first creating *ethograms*, records of the actions the flies perform over the assayed time (**Fig. 2A**). We observe that the bout lengths of grooming shorten as feeding competition increases (**Fig. S2**). We also observe that the fly alternates between bouts of grooming and feeding, supporting the Alternation Model proposed in **Figure 1E**.

Quantifying total grooming time and total feeding time for each fly for the first five minutes of observation shows individual variability but clear trends (**Fig. 2B**). Flies perform less of each behavior when they have a choice and there is a trade-off: more feeding means less grooming and vice versa. This is shown with data points for each individual fly in **Figure 2B** and quantified as a population average for 10 flies in each competition condition in **Figure 2C.**

We conclude that the presence of two competing drives causes the flies to alternate, dividing their time between behaviors to reduce both motivating drives.

Similar results occur when grooming and courting drives compete (**Fig. 3**). Clean male flies spend on average 90% of their time courting when they share a small chamber with a conspecific virgin female, but when the male is covered with dust, courting is reduced, and grooming is increased. In the competitive condition, males alternate between grooming and courting.

Starved male flies will alternate between courtship and feeding when given access to both virgin females and food (**Fig. 4**). Starvation itself reduces courtship, but the total amount of time spent courting is more significantly reduced when food is available. Most males alternate between courting and feeding with bouts of shorter duration, rather than feeding for a sustained period before switching to courtship.

We observe rare attempts to multitask: occasionally a fly will perform posterior grooming or courtship singing during feeding and occasionally grooming a wing while pursuing a female. These combinations are physically possible (**Fig. S3**) but infrequent. We also note that when both male and female flies are covered in dust, courtship is reduced to near zero; apparently a dusty female does not elicit male mating response. Whether this is due to masked olfactory or pheromonal cues, altered visual appearance, or other factors is unclear.

Behavior initiation is delayed by competing drives

When flies are presented with three competing behavior choices, they initiate grooming first, followed by feeding, and then courting. This order could reflect priority or opportunity: the flies are covered in dust, so they receive the mechanosensory stimulation that drives the grooming response immediately. They usually encounter the virgin female fly moving around in the assay chamber before they locate the food patch, but while the female is free roaming and requires pursuit, the food is stationary and readily available. Therefore, the order of initiation could be a consequence of which opportunity to satisfy a drive they reach first, rather than relative drive strength or an intrinsic hierarchy of importance. The

chambers are small (15.6 mm in diameter), and the subject male fly usually encounters the food and/or female quickly, so we believe the allocation of time spent is primarily due to drive strength rather than just proximity.

The average time of courtship initiation is delayed by the presence of food and/or dust, while the initiation time of feeding is delayed by dust and/or virgin females (**Fig. 5**). Thus, measurements of change in behavior initiation time support the analysis of total time spent in each behavior to indicate that flies faced with competing drives alternate between the actions required to satisfy them.

As the fly's initiation of grooming, feeding, and courting actions are delayed, we also see a reduction in bout length (**Fig. S2**). As the competing drive strength increases, the duration of feeding and courting bouts become shorter compared to the bout lengths when no competition is present. The feeding bout length reduced from an average of 13.56 seconds (s) when no competition was present, to 10.36 s when a female was present and 6.30 s when food was present. The courtship bout length (not including actual mating) reduces from an average of 4.82 s with no competition to 1.19 s when dust was present and 1.74 s when food was present. The grooming behavior bouts are also reduced when competition is present, but less so than the feeding and courting bouts: when no competition is present, the fly averages 12.45 s grooming bouts, but the presence of a female drops the duration to 9.05 s and the presence of food drops it to 7.27 s. Together this data supports the Alternation Model proposed in **Figure 1E** as the way flies organize decision-making when faced with competing drives.

During the triple competition assay, feeding bout lengths dropped to 3.55 s and courting bouts dropped to 0.59 s, but the average grooming bout length of 9.54 s had no significant change. This suggests that grooming is less affected by competition than feeding or courtship and may support a soft hierarchy of grooming, then feeding, then courting as proposed in **Figure 1C**.

Candidate genes, neurons and brain regions may affect aspects of decision-making

Previous experiments implicated mutations in the FoxP transcription factor in the time course of perceptual decisions when sensory stimuli were ambiguous (Palazzo et al., 2020). We tested whether available *FoxP* mutations behaved differently in our pairwise competition assays. The genetic background of the mutants affected the starvation conditions required to balance the choice between feeding and grooming or courting, but the primary effect we observed was an increase in the average bout duration of anterior grooming, perhaps indicating that transitions between anterior and posterior grooming actions was impaired (**Fig. S4**). This observation encouraged us to investigate bout duration as another manifestation of decision-making, potentially acting over a shorter timescale and within an overarching behavioral context. *FoxP* mutants do not show differences in action selection within courtship. Our initial grooming bout duration results were observed with a hypomorphic *FoxP* allele, f03746 (Mendoza et al., 2014), and when we were unable to confirm them with the additional knock-out allele 5-SZ-3955 (DasGupta et al., 2014), we did not pursue this gene candidate further.

A fortuitous observation in our undergraduate lab class showed that activation of the neurons targeted by *leucokinin-GAL4* caused reduced grooming in response to dust – and an increase in proboscis extension. This led us to test whether these neurons affected the choice between feeding and grooming. Activation of *leucokinin-GAL4* neurons in single behavior control assays showed a small increase in time spent feeding and a large decrease in grooming. In our two-choice competition assays, grooming time was reduced but neither feeding nor courtship increased (**Fig. 6**). Silencing the leucokinin-GAL4 neurons reduced feeding in both single-behavior control assays and in competition without impairing locomotion, grooming, or courtship. *Leucokinin-GAL4* drives expression in more than 20 neurons in the brain and ventral nerve cord, and the behavioral effects are more consistent with roles in individual behavior drives (Nässel, 2021) than defects in decision-making generally.

Aminergic neurons were previously implicated in various decision tasks (Gautham et al., 2024; Cheriyamkunnel et al., 2021), but in our competition assays, evaluated by the ratio of time spent in each behavior, we did not observe strong or specific decision-making phenotypes (**Fig. S5**). Further higher-resolution behavioral quantification will be needed to determine if they have more subtle contributions to choice and alternation timing.

Finally, the mushroom bodies are a brain area involved in associating positive and negative valence to olfactory cues in associative learning paradigms and metabolic contexts (Zhang et al., 2007; Parnas et al., 2024; Suárez-Grimalt et al., 2024). We tested whether silencing mushroom body neurons (using broad drivers *201Y-* and *OK107-GAL4*) would alter grooming, feeding, or courtship in individual behavior assays or competitive choice. We saw a strong reduction in percent time spent courting in both the individual and choice assays (**Fig. 7**). There was an increase in time spent grooming in the grooming vs courting choice assay, but not quite to the level of grooming that occurs when there is no competing drive. These phenotypes are more consistent with a defect in courtship than decision-making more generally.

Discussion

We observe variability in behavior choices among individuals. The representative ethograms shown in **Figures 2, 3,** and **4** illustrate that onset timing, bout durations, and alternation patterns differ, even in genetically homogeneous flies subjected to the same experimental conditions. The trends in organization of behavior choice are statistically significant when comparing groups of flies (histograms), but differences in drive strength and flexibility in action selection are apparent. Difficulty achieving uniform starvation conditions, dust distributions, and mate access may explain some of the variability, but stochasticity in the neural mechanisms of action selection is another possible explanation for the observed difference in individual behavioral responses. This variability in the choice outcomes makes screening for neurons or genes with subtle effects challenging – we

would be measuring a change in the relative distribution of time spent in competing actions rather than a change from a baseline/set point. This would require higher throughput analysis, potentially possible with automatic behavior recognition methods.

Future experiments could extend our multiple-choice assay to include aggression or escape, in order to search for small numbers of neurons or genes affecting multiple behavioral choices and thus perhaps decision-making in general. While we have not yet found a locus that influenced multiple decisions, we have yet to test the central complex, another plausible candidate brain region with roles in learning and memory (Solanki et al., 2015). We did see differences in behavioral priorities when comparing CantonS and transgenic lines in the w1118 background. The w1118 flies were less robust to starvation, with a higher fraction dying at 24 hrs starved, and they showed a higher frequency of multitasking behaviors. w1118 also prioritized grooming more strongly in all competitive choice assays, indicating that genetic background can indeed influence aspects of decision-making.

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Competing interests

No competing interests declared.

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Data and resource availability

Data and resource availability: All relevant data and details of resources can be found within the article and its supplementary information. Reagents available upon request.

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Figure Legends

Figure 1: Conceptual overview and choice assay measuring how flies select among competing positive actions.

A) A graphical abstract of the decision-making assay. B) An outline of the assay and analysis pipeline. C) Two models hypothesizing the presence or absence of a behavioral hierarchy. D) Two models hypothesizing the decision-making process at an anatomical level. E) Two models hypothesizing temporal distribution of behaviors resultant of competing drives.

Figure 2: Grooming and feeding behaviors alternate when drives compete.

A) Ethograms depicting the first five minutes of the assay. B) A dot plot comparing the total percent time spent grooming to the total percent time spent feeding for each fly. C) A box plot comparing total percent time spent on each behavior.

Figure 3: Grooming and courting behaviors alternate when drives compete.
A) Ethograms depicting the first five minutes of the assay. B) A dot plot comparing the total percent time spent grooming to the total percent time spent courting for each fly. C) A box plot comparing total percent time spent on each behavior.

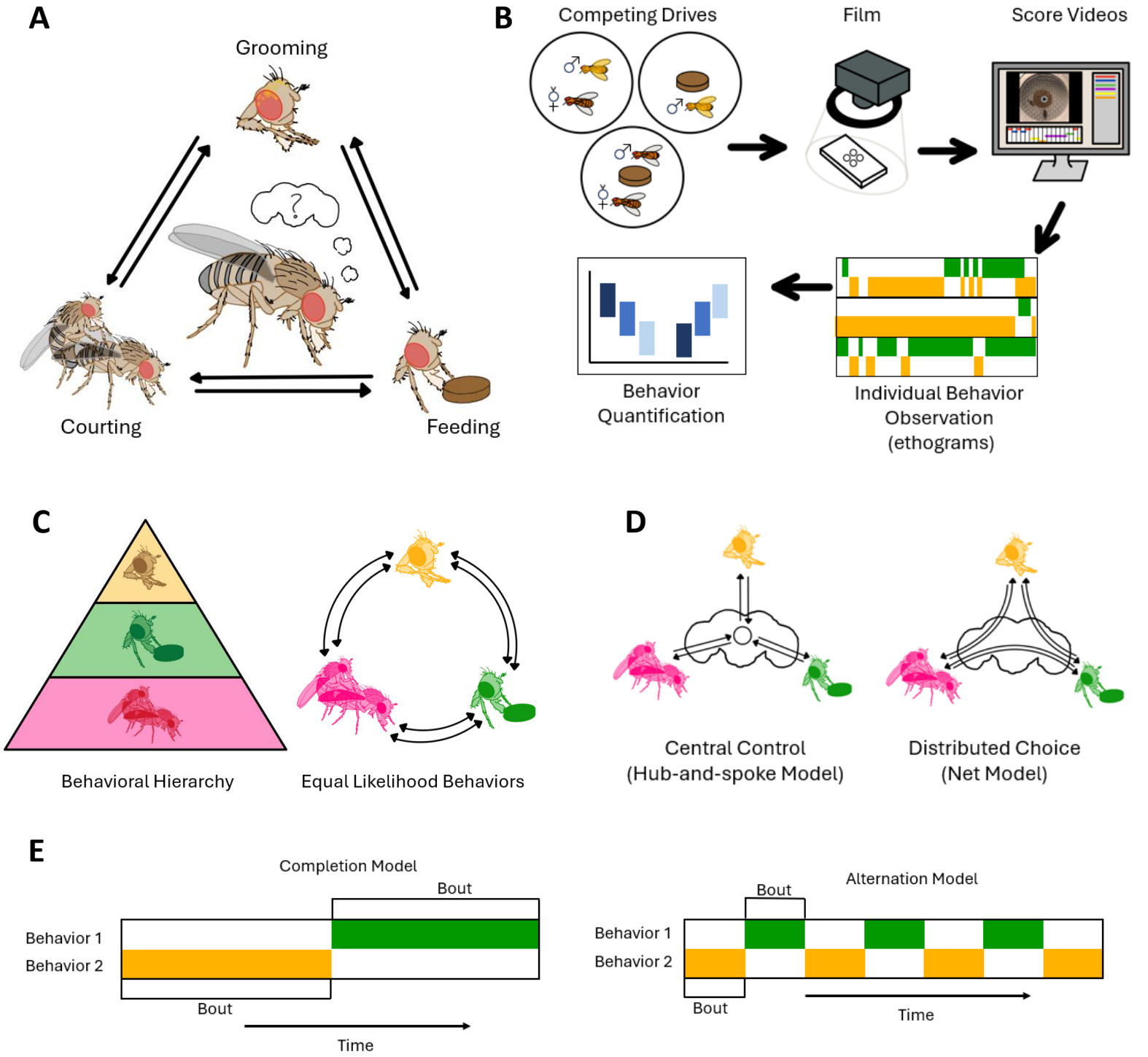
Figure 2: Courting and feeding behaviors alternate when drives compete.
A) Ethograms depicting the first five minutes of the assay. B) A dot plot comparing the total percent time spent courting to the total percent time spent feeding for each fly. C) A box plot comparing total percent time spent on each behavior.

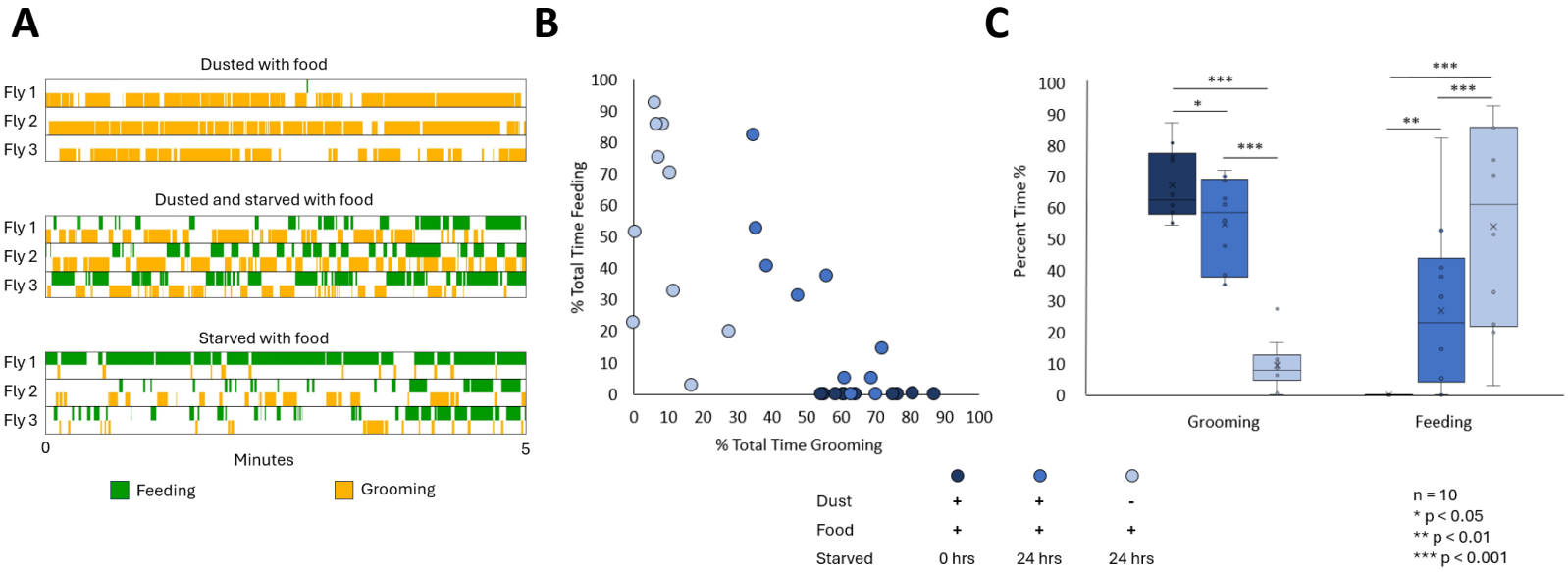
Figure 5: Competing drives result in delayed behavior initiation.
A) A line graph tracking initiation of wing singing courtship behavior. The dotted line represents the 50% mark. B) A line graph tracking initiation of feeding behavior. The dotted line represents the 50% mark.

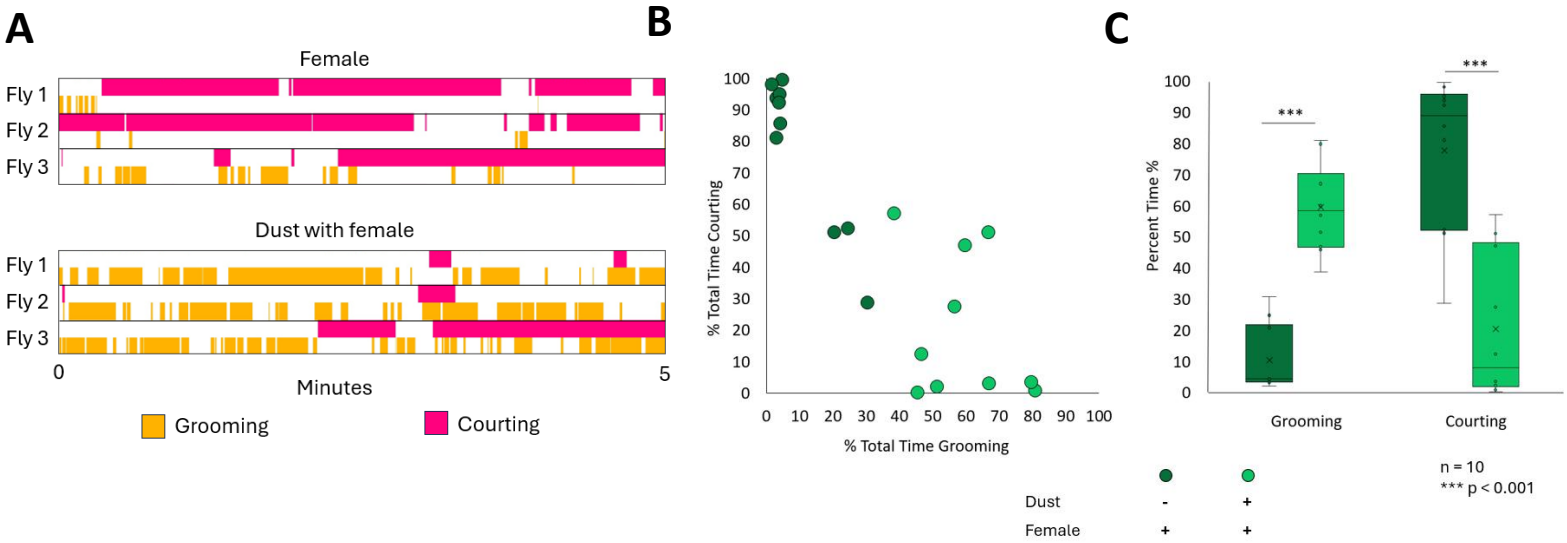
Figure 6: Inactivation and activation of leukokinin producing neurons in the three choice assays.
A) Single drive analysis of inactivated leukokinin producing neurons. B) Single drive analysis of activated leukokinin producing neurons. C) Grooming vs Feeding inactivation ethogram. D) Grooming vs Feeding activation ethogram. E) Box plot corresponding to C. F) Box plot corresponding to D. G) Grooming vs Courting inactivation ethogram. H) Grooming vs Courting activation ethogram. I) Box plot corresponding to G. J) Box plot corresponding to H. K) Courting vs Feeding inactivation ethogram. L) Courting vs Feeding activation ethogram. M) Box plot corresponding to K. N) Box plot corresponding to L.

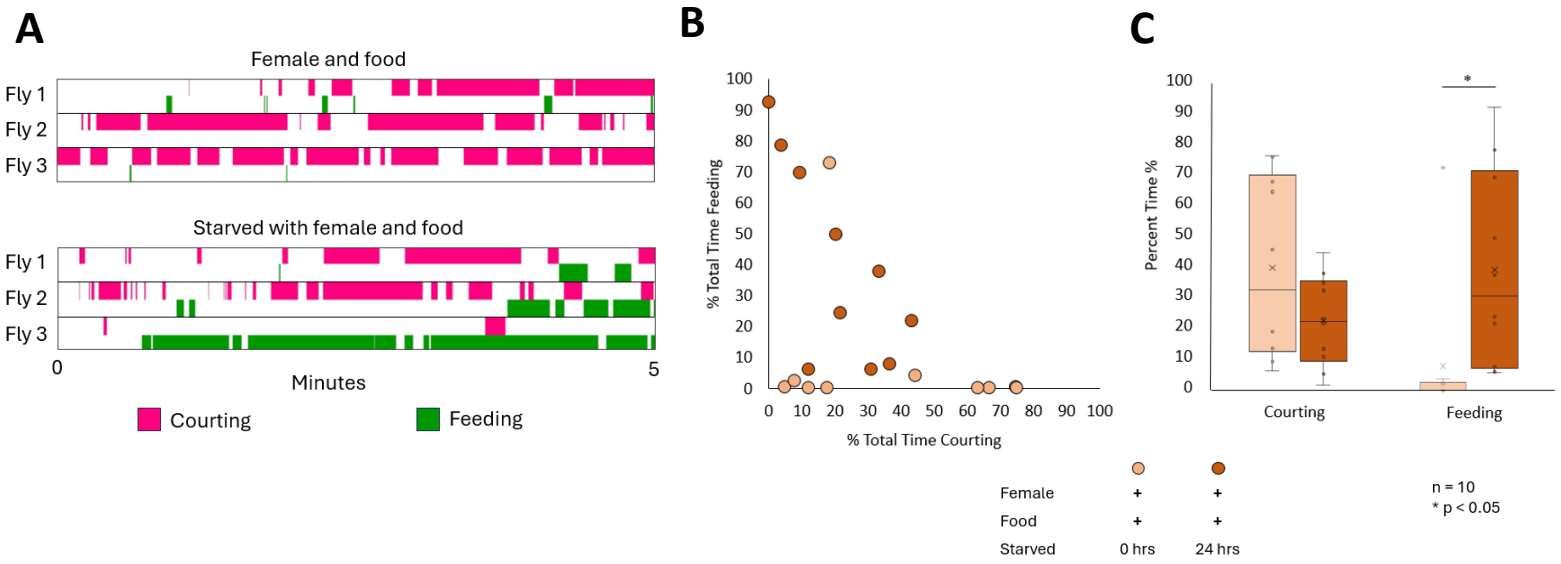
Figure 7: Inactivation of mushroom bodies in the three choice assays.
A) Single drive analysis of inactivated mushroom bodies. B) Grooming vs Feeding inactivation ethogram. C) Grooming vs Courting inactivation ethogram. D) Courting vs

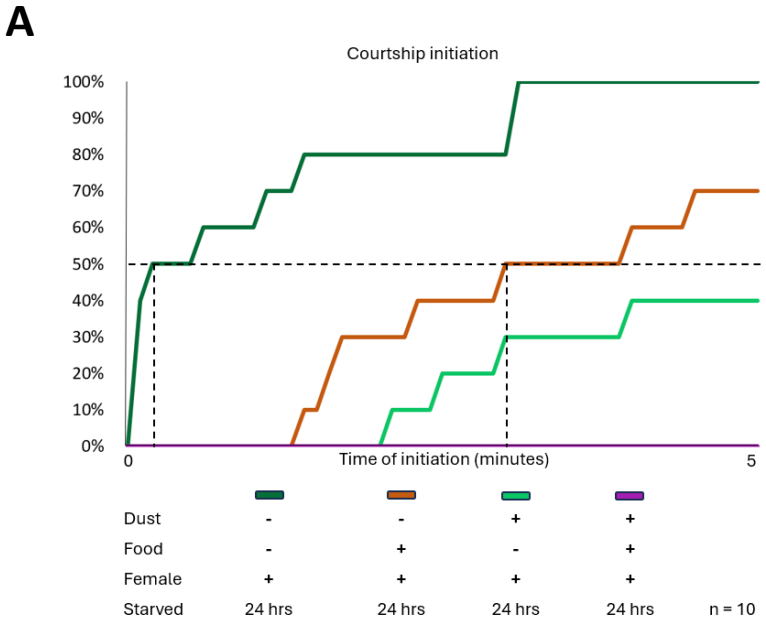
Feeding inactivation ethogram. E) Box plot corresponding to B. F) Box plot corresponding to C. G) Box plot corresponding to D.

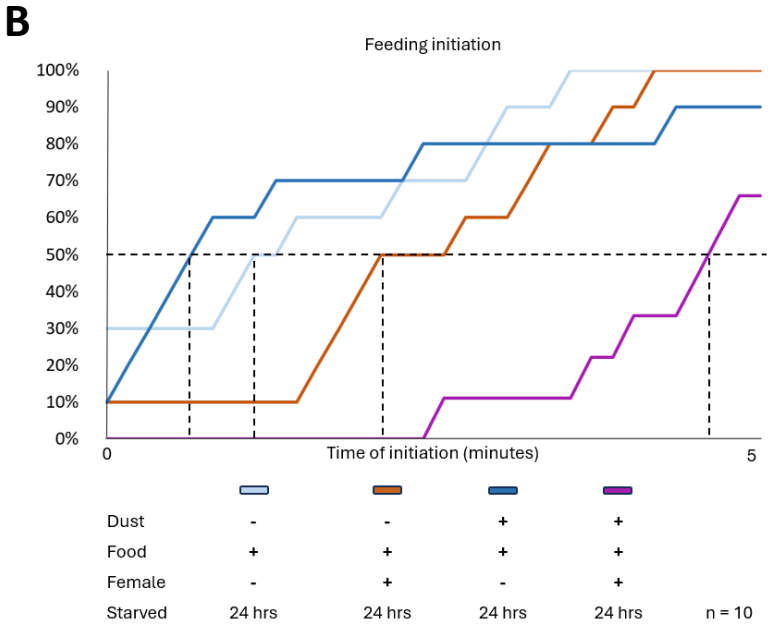

A
Grooming
Courting
Feeding
B
Competing Drives
Film
Score Videos
Behavior Quantification
Individual Behavior Observation (ethograms)
C
Behavioral Hierarchy
Equal Likelihood Behaviors
D
Central Control (Hub-and-spoke Model)
Distributed Choice (Net Model)
E
Completion Model
Bout
Behavior 1
Behavior 2
Bout
Time
Alternation Model
Bout
Behavior 1
Behavior 2
Bout
Time

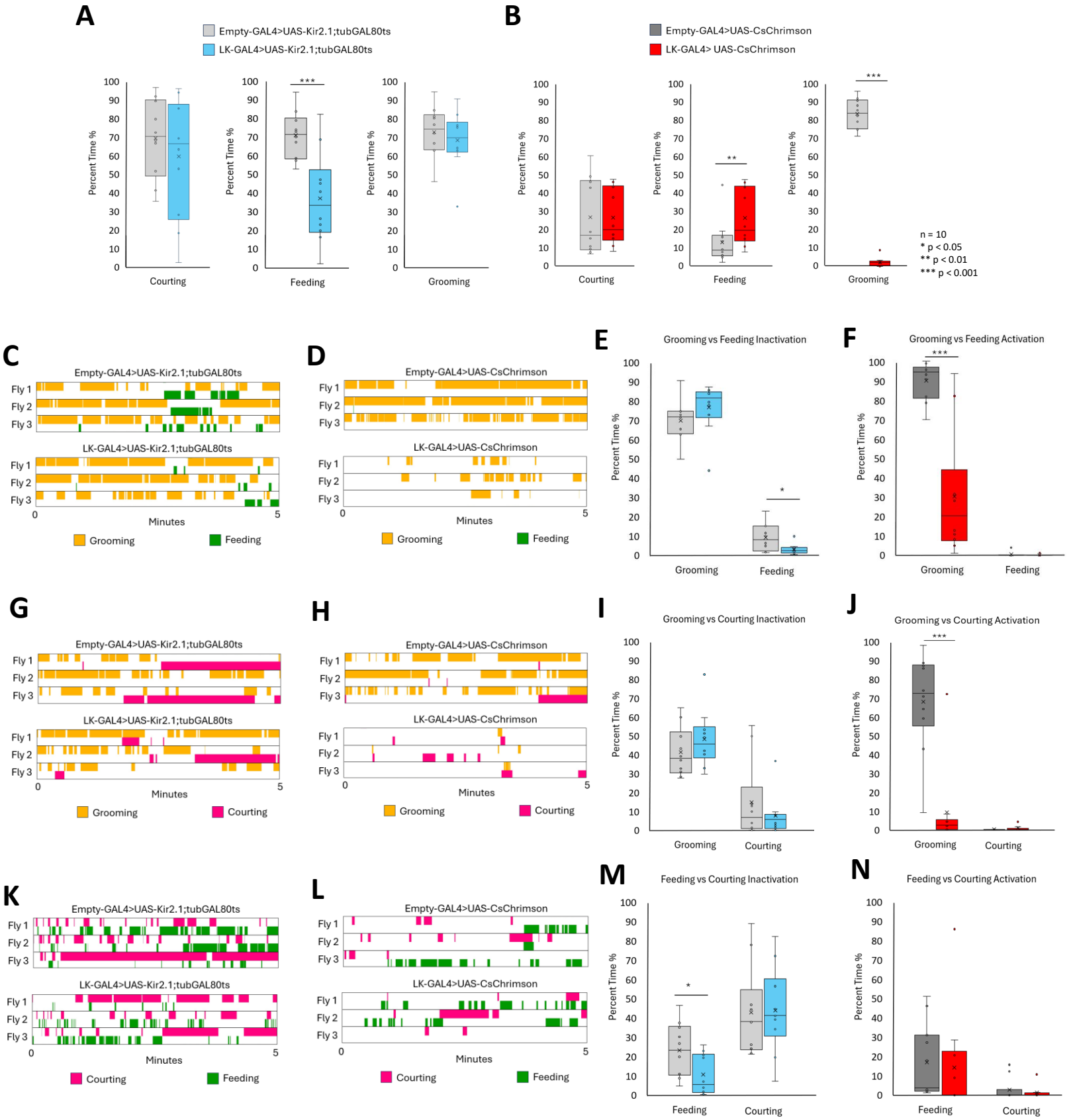

A
Dusted with food
Fly 1
Fly 2
Fly 3
Dusted and starved with food
Fly 1
Fly 2
Fly 3
Starved with food
Fly 1
Fly 2
Fly 3
0
5
Minutes
Feeding
Grooming
B
% Total Time Feeding
% Total Time Grooming
C
Percent Time %
Grooming
Feeding
Dust + + -
Food + + +
Starved 0 hrs 24 hrs 24 hrs
n = 10
* p < 0.05
** p < 0.01
*** p < 0.001

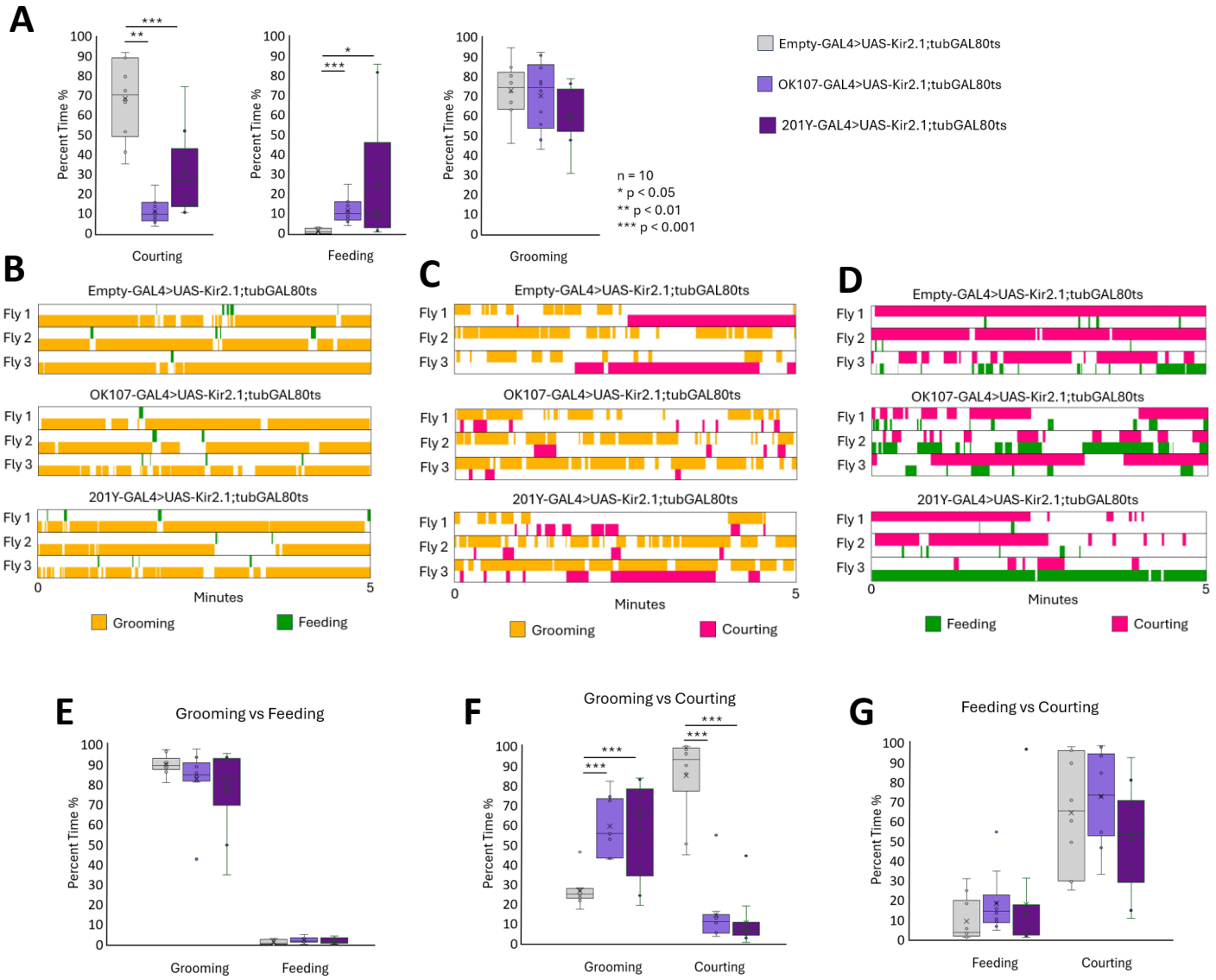

A
Female
Fly 1
Fly 2
Fly 3
Dust with female
Fly 1
Fly 2
Fly 3
0
5
Minutes
Grooming
Courting
B
% Total Time Courting
% Total Time Grooming
C
Percent Time %
***
***
Grooming
Courting
Dust - +
Female + +
n = 10
*** p < 0.001


A
Female and food
Fly 1
Fly 2
Fly 3
Starved with female and food
Fly 1
Fly 2
Fly 3
0
5
Minutes
Courting
Feeding
B
% Total Time Feeding
% Total Time Courting
C
Percent Time %
Courting
Feeding
*
Female
Food
Starved
0 hrs
24 hrs
n = 10
* p < 0.05


A
Courtship initiation
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
Time of initiation (minutes)
5
Dust - - + +
Food - + - +
Female + + + +
Starved 24 hrs 24 hrs 24 hrs 24 hrs n = 10


B
Feeding initiation
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
Time of initiation (minutes)
5
Dust - - + +
Food + + + +
Female - + - +
Starved 24 hrs 24 hrs 24 hrs 24 hrs n = 10


A
Empty-GAL4>UAS-Kir2.1;tubGAL80ts
LK-GAL4>UAS-Kir2.1;tubGAL80ts
Percent Time %
Courting
Feeding
Grooming
***
B
Empty-GAL4>UAS-CsChrimson
LK-GAL4> UAS-CsChrimson
**
***
n = 10
* p < 0.05
** p < 0.01
*** p < 0.001
C
Empty-GAL4>UAS-Kir2.1;tubGAL80ts
LK-GAL4>UAS-Kir2.1;tubGAL80ts
Fly 1
Fly 2
Fly 3
Minutes
Grooming
Feeding
D
Empty-GAL4>UAS-CsChrimson
LK-GAL4>UAS-CsChrimson
E
Grooming vs Feeding Inactivation
*
F
Grooming vs Feeding Activation
G
H
Courting
I
Grooming vs Courting Inactivation
J
Grooming vs Courting Activation
K
L
M
Feeding vs Courting Inactivation
N
Feeding vs Courting Activation


A
Percent Time %
Courting
Feeding
Grooming
Empty-GAL4>UAS-Kir2.1;tubGAL80ts
OK107-GAL4>UAS-Kir2.1;tubGAL80ts
201Y-GAL4>UAS-Kir2.1;tubGAL80ts
n = 10
* p < 0.05
** p < 0.01
*** p < 0.001
B
C
D
Fly 1
Fly 2
Fly 3
Minutes
Grooming
Feeding
Courting
E
Grooming vs Feeding
F
Grooming vs Courting
G
Feeding vs Courting