Mutation of the *Drosophila* serotonin transporter dSERT disrupts courtship and feeding and increases both daytime and nighttime sleep

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Abstract

The Serotonin Transporter (SERT) regulates extracellular serotonin levels and is the target of most current drugs used to treat depression. The mechanisms by which inhibition of SERT activity influences behavior are poorly understood. To address this question in the model organism *Drosophila melanogaster*, we developed new loss of function mutations in *Drosophila SERT* (*dSERT).* Previous studies in both flies and mammals have implicated serotonin as an important neuromodulator of sleep, and our newly generated *dSERT* mutants show an increase in total sleep and altered sleep architecture. Differences in daytime vs. nighttime sleep architecture as well as genetic rescue experiments unexpectedly suggest that distinct serotonergic circuits may modulate daytime versus nighttime sleep. *dSERT* mutants also show defects in copulation and food intake, akin to the clinical side effects of SSRIs. Starvation did not overcome the sleep drive in the mutants. Additionally in males, but not female *dSERT* mutants, the drive to mate also failed to overcome sleep drive. *dSERT* may be used to further explore the mechanisms by which serotonin regulates sleep and its interplay with other complex behaviors.

Author Summary

Many medications used to treat depression and anxiety act by changing serotonin levels in the brain. Fruit flies also use serotonin and can be used as a model to study the brain. We have made a fly mutant for the serotonin transporter (SERT), which is the target of antidepressants in humans. The mutants sleep more, eat less, and have a decreased sex drive. These flies can be used to study the neuronal pathways by which serotonin regulates sleep, eating and sexual behaviors and may help us to understand the behavioral effects of antidepressants.

Introduction

Sleep is essential for life and is evolutionarily conserved from insects to mammals (1–3). Both the amount and architecture of sleep are critical for cognition and disruption of sleep in humans is linked to neurological and psychiatric disorders (4–6). The neuromodulator serotonin (5-hydroxytryptamine, 5-HT) acts as a key regulator of sleep, and its involvement in sleep has been known for decades (7,8). Previous studies in vertebrates have shown that serotonin signaling can promote wakefulness, while paradoxically, others demonstrate that serotonin is critical for sleep induction and maintenance (9–13). The circuits responsible for these effects and the cellular mechanisms by which serotonin regulates sleep remain unclear.

For over two decades, *Drosophila* has been utilized as a model system to study sleep (14,15) and both serotonin (16–20) and dopamine (21–25) have been shown to play significant roles in regulating sleep duration and quality. A role for serotonin in promoting sleep in *Drosophila* has been demonstrated in part by feeding the precursor 5-hydroxytryptophan (5-HTP) (16) or by utilizing mutants for the serotonin rate-limiting synthetic enzyme tryptophan hydroxylase (TRH) (20). Furthermore, of the five serotonin receptors expressed in *Drosophila*, mutations in three (*d5-HT1A*, *d5-HT2B* and *d5-HT7*) disrupt sleep behaviors, including total sleep amount, sleep rebound, and sleep architecture (16,18,20). The powerful molecular genetic tools available in the fly have allowed some of the specific structures and cells required for sleep to be identified (16,20,21,26–32). Similarly, these tools have the potential to uncover the mechanisms by which specific circuits regulate sleep.

The primary mechanism by which serotonin is cleared from the extracellular space in both mammals and flies is reuptake via the Serotonin Transporter (SERT) which localizes to the plasma membrane of serotonergic neurons (33). Blockade of SERT activity increases the amount of serotonin available for neurotransmission. It is the primary target for most current treatments

of depression and anxiety disorders, including the widely prescribed Selective Serotonergic Reuptake Inhibitors (SSRIs) (34). In addition to their therapeutic effects, SSRIs can dramatically influence a variety of other physiological functions and behaviors such as eating, libido, and sleep (35–38). Consistent with the complex relationship between serotonin and sleep, SSRIs often produce diverse and sometimes contradictory defects on sleep including insomnia, decreased REM sleep efficiency and daytime somnolence (9,38,39). It remains unclear how changes in SERT activity modifies sleep behavior in mammals.

A relatively weak hypomorph of *dSERT* has been previously described (40). The *dSERT* MiMIC insertion lies within the first intron just upstream of the first coding exon of the gene and reduces *dSERT* mRNA expression by ~50% (40). Its potential effects on sleep were not reported, and in general, it can be difficult to make firm conclusions about mutations that are not null or at least strong hypomorphs.

In this study, we have used P-element excision to generate new mutant alleles and find that *dSERT* is required for regulating both sleep amount and architecture. Our work further elucidates how the increased sleep drive exhibited in these *dSERT* mutants is affected in the context of other critical behaviors that are regulated by serotonin signaling including mating and feeding. Our data also suggest that *dSERT's* role in daytime vs. nighttime sleep may be modulated via distinct serotonergic circuits.

Results

Disruption of *dSERT* increases sleep drive in *Drosophila*

To generate new mutant alleles of *dSERT*, we used imprecise excision of a transposable element in the line XP^{d04388} (BDSC #85438). To ensure a consistent genetic background, XP^{d04388}

was first outcrossed into w^{1118}, and w^{1118} served as the primary control for our initial assays. The proximal end of the P element in XP^{d04388} is 514 bp 5' of the predicted transcriptional start site of *dSERT* (Figure 1A). We screened for loss of the *white* minigene in the P element and recovered two imprecise excision alleles of 1121bp and 1178bp which we designate as $dSERT^{10}$ and $dSERT^{16}$ respectively (Figure 1A). Both $dSERT^{10}$ and $dSERT^{16}$ delete the first non-coding exon and the first intron of *dSERT*. We identified two additional lines as controls; $dSERT^{1}$ contains 41 additional bases that are remnants of the former P-element insertion but does not otherwise disrupt the *dSERT* gene and $dSERT^{4}$ with no detectable genomic alterations at the former P-element insertion side.

To determine whether expression of *dSERT* was disrupted in $dSERT^{10}$ and $dSERT^{16}$, we first used qRT-PCR to quantify mRNA expression, using w^{1118} as a control. The *dSERT* transcript was not detectable in either of the mutant alleles (Figure 1B). By contrast, a previously published mutant allele generated by insertion of a MiMIC cassette was reported to retain ~50% expression relative to wild type (40). To confirm these results and determine whether dSERT protein was similarly reduced, we performed western blots using a previously validated antibody to dSERT (41). Compared to w^{1118} controls, both $dSERT^{10}$ and $dSERT^{16}$ show a decrease in dSERT protein expression (Figure 1C). It is unclear whether a faint band present in the western blots represents residual protein or non-specific background, and the intact coding sequence suggests that they may not be null alleles. Nonetheless, since they appeared to represent relatively strong hypomorphs with undetectable mRNA levels we preceded with our behavioral analyses.

We analyzed sleep in the *dSERT* mutants and found that both $dSERT^{10}$ and $dSERT^{16}$ mutants have drastically increased sleep compared to w^{1118} controls (Figure 1D). The probability

of transitioning from an awake to a sleep stake, P(Doze), and the probability of transition from a sleep to an awake state, P(Wake), were calculated to further analyze the changes in sleep drive and arousal in the *dSERT* mutants. Compared to controls, both *dSERT* mutants show a significant increase in P(Doze) (Figure 1E) and a reduction in P(Wake) (Figure 1F).

To confirm the increased sleep phenotypes are the result of *dSERT* disruption and not from other spurious changes to the genetic background, transheterozygous *dSERT* mutants ($dSERT^{10}/dSERT^{16}$) were assayed and shown to exhibit a significant increase in total sleep compared to controls (Sup Figure 1A-B). Consistent with these findings, $dSERT^{16}$ homozygotes also demonstrated a significant increase in total sleep when compared either to heterozygous $dSERT^{16}$ flies (Sup Figure 1C-D) or the revertant $dSERT^{4}$ control (Sup Figure 1E-F). Taken together, these results demonstrate disruption of *dSERT* causes a significant increase in overall sleep and sleep drive.

Loss of *dSERT* significantly increases sleep and alters sleep architecture in daytime and nighttime.

The sleep phenotype we observed was stronger in $dSERT^{16}$ compared to $dSERT^{10}$, and we therefore focused on $dSERT^{16}$ for further analysis. To verify that the phenotype was not due to a bias in the time spent on one end of the testing tube or to limited movements that do not carry the flies past the tube's midline, we assayed activity with both single-beam and multibeam monitors (42). Our results show that in both single-beam and multibeam monitors $dSERT^{16}$ mutants exhibit a significant increase in total sleep compared to controls (Figure 2A). These data eliminate the potential confound that can be caused by artefactual retention of the flies at one end of the assay tubes.

Previous studies have shown that stimulation of aminergic pathways can increase grooming and alter other sensorimotor behaviors that might effect sleep (43–45). We therefore tested whether grooming or negative geotaxis were altered in the *dSERT* mutants. We did not detect any differences from controls in either grooming rate (Sup Figure 2A) or performance of negative geotaxis in $dSERT^{16}$ flies (Sup Figure 2B). Overall, these findings indicate that the behavioral change we detect represents an increased sleep in *dSERT* mutants rather than an artifact caused by changes in other amine-associated behaviors.

More specific analysis of both daytime and nighttime sleep behavior shows that $dSERT^{16}$ flies have dramatically increased sleep during the daytime (Figure 2B). This is further characterized by an increase in daytime bout frequency compared to controls (Figure 2C). In addition, $dSERT^{16}$ mutants also displayed reduced latency to sleep at night (Figure 2D). The analysis of nighttime sleep behavior revealed that $dSERT^{16}$ mutants similarly exhibit dramatically increased nighttime sleep (Figure 2E) but in contrast to their daytime behavior, we observed significant decrease in nighttime bout frequency (Figure 2F). Together, these findings indicate that disruption of *dSERT* causes a significant increase in sleep and alters sleep architecture in both the day and evening periods.

$dSERT^{16}$ mutants exhibit rhythmic circadian behaviors.

In addition to sleep, serotonergic pathways in mammals have been implicated in the regulation of circadian rhythmicity (46). To investigate a possible role for dSERT activity in sustaining circadian rhythms, we analyzed circadian locomotor behaviors in $dSERT^{16}$ mutants. Both control and $dSERT^{16}$ mutants exhibited robust locomotor rhythms with bimodal activity peaks in 12h/12h light/dark (LD) cycles (Figure 3A-B) and their rhythmic behaviors persisted in

163 free-running constant darkness (DD) cycles (Figure 3E-F). Consistent with the increased sleep
164 behavior of *dSERT*^{16} mutants, the average activity of *dSERT*^{16} flies was significantly decreased
165 throughout both LD (Figure 3C) and DD (Figure 3G) conditions. Although the average activity
166 was dampened, *dSERT*^{16} mutants did not exhibit a significant decrease in either morning or
167 evening anticipation behaviors for either LD (Figure 3D) or DD (Figure 3H) cycles. Periodogram
168 analysis confirmed that *dSERT*^{16} mutants behave indistinguishably from wildtype controls
169 (Figure 3 I-J) and quantification of free-running circadian behaviors demonstrates that over 96%
170 of *dSERT*^{16} flies (tau=23.6 ±0.04) were rhythmic (Figure 3K) similar to control flies
171 (tau=23.5±0.01). In addition, the circadian periods of *dSERT*^{16} mutants did not appear
172 significantly changed (Figure 3L). In summary, we find that loss of *dSER*T does not appear to
173 disrupt circadian rhythmicity. These data are consistent with previous work showing the feeding
174 of either 5-HTP, the SSRI fluoxetine (Prozac), or overexpression of *d5-HT1B* in flies also did not
175 alter rhythms in free-running conditions (17).
176
177 **Loss of *dSERT* disrupts courtship and copulation behaviors.**
178 In clinical settings, SSRIs have number of side effects including sexual dysfunction,
179 raising the possibility that loss of *dSERT* could potentially influence these activities in the fly.
180 We first investigated whether *dSERT*^{16} mutants show defects in courtship and copulation and
181 how this would interact with sleep. Previous work demonstrated that pairing male and females
182 flies together overnight dramatically suppresses sleep as a result of increased courtship activity
183 (47–49). Consistent with these findings, co-housing pairs of wildtype male and female flies
184 together resulted in a significant decrease in nighttime sleep compared to sleep recorded from
185 isolated male or female flies (Figure 4A). However, pairing male and female *dSERT*^{16} mutants

together did not result in any significant sleep loss compared to isolated conditions (Figure 4A). These results indicate the increased sleep drive outweighs copulation drive in *dSERT*^{16} mutants and suggest that loss of dSERT might decrease sexual activity.

To determine whether the reduced mating activity was a result of either decreased male courting or female receptivity in *dSERT*^{16} mutants, we paired the mutants with wild type flies. Pairing *dSERT*^{16} mutant females with control males significantly reduces sleep compared to *dSERT*^{16} male-female coupling. By contrast, we did not detect a statistically significant reduction in sleep when *dSERT*^{16} males were coupled with wild type control females (Figure 4B). These behavioral analyses suggested that *dSERT*^{16} males, but not females, exhibit defects in sexual behavior.

To more directly test whether *dSERT* mutants would show defects in sexual activity, we measured copulation rates. We did not observe a significant difference in copulation success in *dSERT*^{16} females compared to control females when paired with wildtype males. However, when *dSERT*^{16} males were paired with wildtype females none were found to copulate within one hour in marked contrast to the majority of control males (Figure 4C).

In flies, copulation and courtship are regulated in part by distinct pathways. We therefore assayed courtship in the *dSERT* mutant. *dSERT*^{16} males exhibited significant defects in courtship behavior including increased latencies to initiate orientation (Figure 4D) and wing song (Figure 4E).

As a further test of male sexual behavior, we measured reproductive output in wildtype females paired for 2 days with *dSERT*^{16} males. Wild type females paired with *dSERT*^{16} males showed a reduction in egg laying rate (Figure 4F) as well as an increase in the proportion of unfertilized eggs (Figure 4G). These results indicate that while *dSERT*^{16} males fail to copulate

209 within one hour, over an increased time-period mating can occur, albeit with reduced fecundity
210 compared to controls.
211
212 **Loss of *dSERT* reduces food intake.**
213 In addition to alterations in sexual activity, common clinical side effects of SSRIs include
214 changes in food intake, weight gain and gastrointestinal distress (37,50). Previous work has
215 demonstrated that starvation induces sleep loss in *Drosophila* and increases activity to search for
216 food (51). We therefore tested whether starvation was sufficient to suppress sleep in $dSERT^{16}$
217 mutants. Consistent with previous studies, control flies dramatically suppressed their sleep when
218 starved on agar medium compared to their sleep on baseline or recovery days (Figure 5A). By
219 contrast, starvation did not have a significant effect on sleep in the $dSERT^{16}$ mutants (Figure 5A).
220 Furthermore, we directly tested the feeding in these *dSERT* mutants and found that after 24 hours
221 of starvation food uptake in $dSERT^{16}$ mutants was significantly decreased compared to controls
222 (Figure 5B). Taken together these results indicate that loss of *dSERT* not only enhances sleep but
223 reduces food intake.
224
225 **Transgenic expression of *dSERT* in serotonergic neurons is sufficient to rescue nighttime or**
226 **daytime sleep defects.**
227 To further confirm that defects seen in $dSERT^{16}$ mutants are due to a loss of *dSERT* we
228 used "genetic rescue" and expressed a wild type *dSERT* transgene in the $dSERT^{16}$
229 background, focusing primarily on defects in sleep. We first used the broad serotonergic
230 driver *TRH-Gal4* (52) to express *UAS-dSERT*. Although, sleep in the fly has been generally
231 approached as a single behavior, some genes and environmental factors can preferentially affect

one phase (53–56). Expression of *UAS-dSERT* using *TRH-Gal4* did not fully reverse the increase in total sleep across the 24-LD cycle in $dSERT^{16}$ mutants (Figure 6A-B). However, separately analyzing day versus night sleep shows that expression of *dSERT* using *TRH-Gal4* is sufficient to restore the daytime sleeping pattern to control levels (Figure 6C). By contrast, night sleep was not altered in the $dSERT^{16}$ mutants by *TRH-Gal4>UAS-dSERT* (Sup Figure 3A). We further analyzed whether sleep architecture could be rescued. Expression of *UAS-dSERT* by *TRH-Gal4* in $dSERT^{16}$ mutant was also able to restore bout frequency to the level of control flies for daytime sleep (Figure 6D). Conversely, we did not observe rescue of the nighttime sleep consolidation phenotype (Sup Figure 3B).

To extend our analysis we tested another broad serotonergic driver, *designated as "TPH-Gal4"* (57). While both *TRH-Gal4* and *TPH-Gal4* include fragments of the *Tryptophan Hydroxylase* gene*,* they have been reported to exhibit differences in expression (58) including a relatively lower of expression for *TRH-Gal4* in processes that innervate the mushroom bodies for. Similar to *TRH-GAL4*, total sleep levels were not fully rescued using *TPH-Gal4 expressing UAS-dSERT in the* $dSERT^{16}$ *background* (Figure 6E, F). However, in contrast to *TRH-GAL4*, analysis of nighttime sleep demonstrates that rescue via *TPH-Gal4* is sufficient to restore nighttime sleep levels to that of controls (Figure 6G). Moreover, unlike *TRH-Gal4*, *TPH-Gal4* did not rescue daytime levels (Sup Figure 3D). Further analysis of sleep architecture revealed that expression of *UAS-dSERT* with *TPH-Gal4* in $dSERT^{16}$ mutants was able to rescue the nighttime consolidation phenotype (Figure 6H). while daytime architecture did not significantly differ from the $dSERT^{16}$ mutant (Sup Figure 3E).

To further extend our rescue experiments to sexual and feeding in the context of sleep, we repeated our experiments using either starvation or co-housing, focusing on nighttime sleep

and rescue with *TPH-Gal4.* Consistent with the results shown in Figure 4, control flies reduced nighttime sleep when male and females were paired and $dSERT^{16}$ mutants did not show any significant decrease in sleep in co-housing conditions (Figure 6I). However, flies expressing *UAS-dSERT* under the control of the *TPH-Gal4 driver*, significantly decrease their nighttime sleep in co-housing conditions compared to sleep levels of individual male or females (Figure 6I). While we cannot yet clearly parse the specific effects of rescue on sexual behavior versus sleep, these data suggest that expression of *UAS-dSERT* with *TPH-Gal4* restored the relative balance of sexual behavior vs. sleep and most likely rescued both behaviors.

We next tested if genetic rescue using *TPH-Gal4* could also restore sleep to wild type levels under starvation conditions. When starved, nighttime sleep was again decreased in control flies but was not significantly reduced in $dSERT^{16}$ mutants (Figure 6J). However, under starvation conditions, expression of *dSERT* under the control of *TPH-Gal4* in $dSERT^{16}$ mutants did not significantly decrease nighttime sleep when starved (Figure 6J). Overall, these results demonstrate that the sleep defects in $dSERT^{16}$ mutants can genetically rescued, confirming that this phenotype is due to disruption of the *dSERT* gene. In addition, they strongly suggest for the first time that distinct serotonergic circuits may regulate day and night sleep separately. Moreover, they suggest that while the serotonergic circuits that control nighttime sleep and sexual behavior may share at least some of the same pathways, the serotonergic pathways responsible for regulating feeding and nighttime sleep are likely to be independent.

Discussion

We find that dSERT functions as a critical modulator of sleep, with loss of *dSERT* causing a severe increase in both daytime and nighttime sleep. Given that dSERT acts as the

278 primary mechanism by which serotonin is cleared from the extracellular space, it is likely that
279 *dSERT* mutants have an increase in the amount of serotonin available for neurotransmission.
280 Their behavior is consistent with phenotypes caused by other changes in serotonergic signaling:
281 feeding the serotonin precursor 5-HTP or overexpression of the rate-limiting enzyme *TRH* in
282 dopaminergic and serotonergic cells (16) (*Ddc-Gal4*) increases in total sleep, similar to *dSERT*
283 mutants. Conversely, a decrease in total sleep duration is exhibited by *TRH* mutants (20).
284 A role for serotonin has also been suggested to contribute to male courtship and mating
285 on based in part on studies of the gene *fruitless* (*fru*) (59,60). Male specific Fru^M positive
286 abdominal-ganglionic neurons that innervate the reproductive organs have been shown to be
287 serotonergic and *fru* mutants demonstrate defects in courting and mating events (59,60).
288 Furthermore, consistent with our findings, activation of serotonergic neurons causes defects in
289 male copulation behavior (58).
290 Previous studies have also addressed the complex relationship between increased
291 serotonin signaling and feeding behavior in *Drosophila*. The use of either *TPH-Gal4* or *TRH-*
292 *Gal4* to thermogenetically depolarize a broad population of serotonergic cells can significantly
293 decrease food intake (58,61), whereas the activation of a specific subset of serotonergic cells
294 drives a contradictory behavior and induces feeding in sated flies (61).
295 Our work demonstrates that the increase in overall sleep in $dSERT^{16}$ mutants is mediated
296 by two different changes in sleep architecture that differ during daytime versus nighttime
297 periods. During the nighttime the increase in sleep in $dSERT^{16}$ flies is characterized by
298 significantly decreased bout number (consolidated sleep) whereas during daytime sleep bout
299 number is increased. The differential rescue nighttime or daytime sleep via *TPH-Gal4* or *TRH-*
300 *Gal4* respectively, support the idea that different serotonergic circuits may regulate nighttime vs.

daytime sleep. Previous work has shown that knockdown of *TRH* in the dorsal paired median (DPM) neurons that innervate the MB causes a decrease in nighttime sleep (19). We therefore speculate that changes in nighttime sleep caused by dSERT may be mediated primarily through an increase in serotonergic signaling to the mushroom body (MB). Consistent with our findings that *TRH-Gal4* could rescue only daytime sleep, previous work has reported that the expression pattern of the *TRH-Gal4* shows relatively little labeling of processes that innervate the MBs (18). We have confirmed that *TRH-Gal4* shows less labeling to the of the MB compared to *TPH-Gal4* (Sup Figure 3C, F). Further evidence for the possibility that the MBs mediate nighttime sleep include the observation the *d5-HT1A* receptor mutant exhibits a decrease in nighttime sleep, nighttime bout length, and an increase in nighttime bout number, and that all of these behaviors can be genetically rescued via expression of a *d5-HT1A* transgene in the MBs (16). Previous work has demonstrated the MBs are dispensable for sleep-feeding interactions (51), consistent with our results showing that even though transgenic expression of *dSERT* with *TPH-Gal4* could restore nighttime sleep levels, it did not rescue the a decrease in sleep via starvation seen in wt flies. Together these data suggest that the increased and consolidated nighttime sleep seen in $dSERT^{16}$ mutants is the result of an increase in signaling through the d5-HT1A receptor caused by an increase in extracellular serotonin in the MBs.

We speculate that some aspects of the daytime dSERT sleep phenotype, such as the increased frequency of daytime sleep bouts may be mediated by the ellipsoid body and the d5-HT7 receptor. Activation of EB neurons leads to an increase in the number of sleep episodes specifically during the daytime (18). *d5-HT7* mutants and treatment with a 5-HT7 antagonist similarly causes a reduction in sleep episode frequency (18). However, it is likely that the daytime *dSERT* sleep phenotype is dependent on other circuits and/receptors, since *dSERT*

exhibit an increase in sleep amount that was not detected by disruption of *d5-HT7* (18). We plan to use the *dSERT* mutants to further map serotonergic circuits that may differentially daytime vs nighttime sleep.

Studies in mice underscore the complexity of serotonin's effects on sleep. For example, an increase in serotonergic signaling caused by increased activity of raphe serotonergic cells reduces REM sleep (62)(63)(64) while an increase in serotonin levels caused by a *SERT KO* mouse has the opposite effects and increases REM (65,66). Knockouts of either 5-HT1B or 5-HT1A also exhibit more REM (67,68), but the increase in REM caused by the SERT KO can be reduced by developmental blockade of 5-HT1A (66).

In humans, the complex relationship between sleep, depression, and the effects of SSRIs underscores the importance of understanding the role of SERT in sleep. Variation in the 5' flanking region of the human *SERT* gene have been associated with a change in SERT expression levels *in vitro* (69), that is linked to the efficacy of SSRIs (70) and sleep deprivation (71). In addition to sleep, SSRIs impact other critical behaviors such as feeding and mating. SSRIs can induce hypophagic effects and interfere with normal feeding behavior (72–75), and sexual dysfunction is a well-documented side effect of SSRI treatment (35,36). Our work begins to address the competition between the pathways that regulate these effects in the context of *Drosophila* sleep, as we demonstrated that loss of *dSERT* induces an increased sleep drive that outweighs the propensity for both normal feeding and mating behaviors (Figure 5). We speculate that further studies of *dSERT* mutants may help to uncover the relationship between other serotonergic pathways that regulate other complex behaviors common to mammals and flies such as aggression and may be used further as a genetic model for the behavioral effects of SSRIs (52,76–80).

Materials & Methods

Fly Strains

Fly stocks were reared on standard cornmeal media (per 1L H20: 12 g agar, 29 g Red Star yeast, 71 g cornmeal, 92 g molasses, 16mL methyl paraben 10% in EtOH, 10mL propionic acid 50% in H20) at 25 C with 60% relative humidity and entrained to a daily 12 h light,12 h dark schedule (12hr:12hr LD). w^{1118} flies were obtained from Dr. Seymour Benzer. *UAS-dSERT* (BL24464) and *UAS-MCD8::GFP* (BL32185) were obtained from the Bloomington *Drosophila* Stock Center. *TPH-Gal4* was from Dr. Jongkyeong Chung(57).

P-element mutagenesis

The *dSERT* alleles were generated by imprecise excision of the *P{XP}d04388* transposon that is inserted 514 bases upstream of the *dSERT* gene on the second chromosome. First, the *P{XP}d04388* line was backcrossed for five generation to the w^{1118} line of the Scholz lab to isogenize the genetic background. Second, females of the *P{XP}d04388* were crossed to w^{1118}; *CYO/+; [Δ2-3; Ki]/TM2* males. Next, w^{1118}*; P{XP}d04388/CYO*; [Δ 2-3; Ki]/+ males were mated with *P{XP}d04388* females. In the next generation, 200 single male flies carrying possible deletion of the *P{XP}d04388* over *P{XP}d04388* were crossed to *Df(2R) PX2/CYO* females that carry a genomic lesion including the complete *dSERT* gene. The new dSERT alleles complemented the lethality associated with the deficiency. The males of the F1 generation were screened for loss of the P-element insertion, e.g. white-eyed male flies carrying the *CYO* allele. 89 lines were established as stocks. The new alleles were analyzed for genomic lesions using

PCR. The following primers were used to confirm lesion and amplify DNA for sequencing: 5'-TGACCCACTAAATGCCATGA-3' and 5' CCAGAAAAAGCGAAATCTGC-3'.

Quantitative Real Time PCR (qRT-PCR)

Total RNA of 30 flies was isolated using Trizol™ method followed by a DNAse digest for 30 min at 37° C. To synthesize cDNA, the SuperScript™ II Reverse Transcriptase (Invitrogen™) and oligo^{dT} primers were used according manufacturer instruction. For qRT-PCR analysis the MESA BLUE qPCR MasterMix Plus for SYBR® Assay (Eurogentec) with 100 ng cDNA as template was used. The detection was performed using the iCycler iQ5 Multicolour Real-Time PCR Detection System. The data were analyzed using the ΔΔCt method(81). The NormFinder software(82) was used for control primers selection. The following control primers recognizing *RpLPO* were selected: 5'-CAG CGT GGA AGG CTC AGT A-3' and 5'-CAG GCT GGT ACG GAT GTT CT-3'. For *dSERT* the following primers recognizing sequences in the third and fourth exon were used: 5'- GTTGCCTCAGCATCTGGAAG-3' and 5'-CAGCCGATAATCGTGTTGTA-3'. Data are shown as fold changes in *dSERT* relative to the controls.

Western blot analysis

To isolate proteins of fly heads, 500-1000 flies were frozen in liquid nitrogen and heads were separated with a sieve. The heads were pulverized with a sterile pestle and resuspended in buffer containing 10mM NaCL, 25 mM Hepes, pH 7.5, 2mM EDTA and 1x cOmplete™ Mini protease inhibitors (Merck). The suspension was kept on ice for 10 min with gentle mixing before

spinning it at 18300 x g at 4°C for 15 min. The remaining pellet was resuspended in a buffer containing 10mM NaCL, 25 mM Hepes, pH 7.5, 1x cOmplete™ Mini protease inhibitors (Merck) and 0.5% CHAPS and incubated at 4°C for 10 minutes with gentle mixing. The solution was centrifuged at 3000 x g for 5 min to recover membrane extract. 20 µg protein of each sample were used for western blotting according standard procedures. The membrane was blocked in 5% milk and the primary rabbit anti-dSERT antibody (Giang et al., 2011) were used at a dilution of 1: 20 000 and mouse anti-β-Actin (mAbcam 8224) at 1:10 000.

Behavioral Analysis

Sleep : Sleep was measured as previously described(83). In brief, 3-5 day old female flies (co-housing experiments also utilized male flies) were individually loaded into 65mm long glass tubes and inserted into *Drosophila* activity monitors to measure locomotor activity (Trikinetics Inc; Waltham MA, USA) and periods of inactivity for at least 5 minutes were classified as sleep. Custom Visual Basic scripts(83) in Microsoft Excel were used to analyze Trikinetics activity records for sleep behaviors. MB5 monitors (Trikinetics Inc; Waltham MA, USA) were used for multibeam monitoring experiments.

Starvation: For starvation and sleep experiments 3-5 day old, mated females were loaded into DAMs tubes with standard food for acclimation. After 1 day of acclimation, baseline sleep was measured for 24 hours (Day 1). On the experimental day (Day 2) flies were transferred to tubes containing 1% agar at ZT7 to be starved for 17 hours. Flies were then transferred back to fresh tubes containing standard food at ZT0 (Day 3) and activity was recorded for 24-hour recovery period.

Co-housing: For co-housing sleep experiments males and females were loaded individually into DAMs tubes and baseline sleep was recorded for 2 days. On experimental day at ZT0 flies were combined into male-female pairs (either same genotype or mixed as specified in the text) in new DAMS tubes and sleep was recorded for 24 hours. The following day at ZT0 flies were separated back into individual tubes and activity was recorded for 24-hour recovery period.

Grooming: For grooming experiments, 3–5-day old, mated females were observed in individual chambers using a Dinolite USB video camera AM7023CT. A single grooming event was scored when a fly rubbed its legs over its head or abdomen or rubbed legs together. For each fly, grooming was analyzed over three separate 2 min periods and quantified as an average number of grooming events per minute. Each scoring period was started by the observation of an initial single grooming event.

Negative Geotaxis: For negative geotaxis experiments, 3 groups of 10 flies were placed in two empty 28.5 x 95 mm polystyrene vials that are vertically joined by tape facing each other. After gently tapping the vials, the flies were allowed to climb for 10s. Each group was scored for the number of flies that climbed above the 8cm mark compared to those remaining below the 8cm mark. Each group was tested 5 times (with one minute recovery between each trial), and the average for those five trials was quantified.

Locomotor Activity and Circadian Rhythmicity: Experiments were performed with 3-5-day-old females in *Drosophila* activity monitors (Trikinetics Inc; Waltham MA, USA) that were first

entrained for 2-3 days in 12hr:12hr LD. For DD analysis, data were analyzed for 3-6 days from the second day in DD. Data analysis was done with Faas software 1.1 (Brandeis Rhythm Package, Michel Boudinot). Rhythmic flies were defined by χ^2 periodogram analysis with the following criteria: power ≥20, width ≥2h with selection of 24hr ± 8hr upon period value. Power and width are the height and width of the periodogram peak, respectively, and give the significance of the calculated period. Mean daily activity (number of events per 0.5hr ± SEM) is calculated over 3-4-day period in LD or DD conditions. Morning anticipation index was calculated as(84) (activity for 3hrs before lights-on)/(activity for 6hrs before lights-on) and evening anticipation index as (activity for 3hrs before lights-off)/(activity for 6hrs before lights-off).

Courtship & Copulation Assays: Virgin females were aged 3-5 days in groups ~10 and naïve males 4 days individually. Single pairs of female and male flies were gently aspirated into a chamber of a mating wheel and video recording was started. The percent of males achieving copulation within 1 hour was measured as well as the time elapsed between the introduction into the chamber and the male display of courtship steps such as orientation and wing extension.

Egg Laying and Hatchability: Five-day old virgin females fed with wet yeast for one day were placed with males (5 females to 10 males) in one bottle for egg laying on molasses plates over 2 days (with removal and replacement of plates every 24 hours). Once molasses plates were removed, they were kept in sealed Tupperware and after 24 hours in 25°C the number of unhatched eggs were manually counted under a microscope.

Feeding Assays: Groups of 10 flies were starved for 24 hours on 1% agar medium as a water source. After starvation, flies were transferred to blue food (I will add food recipe) and allowed to feed for 15 mins. The amount of blue food dye ingested was measured using visual inspecting feeding scores as previously described(61). From 0 being no dye visible in the abdomen to 3, the abdomen is swollen and filled with dye. The average feeding score was quantified by averaging the feeding scores of each fly in the vial.

Immunohistochemistry

Immunostaining was performed following a standard procedure comprising brain dissection, fixation in 3% glyoxal for 25 min, blocking in PBTG (PBS plus 0.2% Triton, 0.5% BSA and 2% normal goat serum), and primary and secondary antibody staining diluted in PBTG. The following primary antibodies were used: rabbit anti-GFP (1:500, Invitrogen) and mouse anti-DLG (1:20, 4F3 -Developmental Study Hybridoma Bank). Alexa Fluor 488 and Alexa Flour 555 goat secondary antibody (1:500; Invitrogen) were used as secondary antibodies. Confocal images were obtained using a Zeiss LSM 880 confocal microscope with Zen software. Images were processed using Fiji/Image J software.

Statistics

Data were analyzed in Prism 9 (GraphPad; San Diego CA, USA). Group means were compared using two-tailed t tests or one- or two-way ANOVAs, with repeated-measures where appropriate, followed by planned pairwise comparisons with Holm-Sidak multiple comparisons tests. Sample sizes for each experiment are depicted in each figure panel or in the appropriate figure legend. All group averages shown in data panels depict mean ± SEM.

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Competing interests statement

The authors declare that they have no competing financial interests.

Figure Legends

Figure 1. *dSERT* imprecise excision alleles. (A) Schematic of *dSERT* gene: grey and blue boxes indicate non-coding exons and coding exons respectively. Deletions of the $dSERT^{10}$ and $dSERT^{16}$ flies are indicated with red dotted lines. (B) qRT-PCR was performed on cDNA

synthesized from whole male flies with *RplP0* for reference. Gene expression of *dSERT* in the mutant was normalized to controls with +1 representing expression in w^{1118}. *dSERT* transcript levels were significantly down-regulated in $dSERT^{10}$ (0.01±0.02) and $dSERT^{16}$ (0.001±0.003). Error bars represent SD. P***≤0.001 (Student's t-test). (C) Western blot analysis shows a band representing dSERT at ~65kD that is reduced in intensity in both mutants. Actin was used as a loading control and shows no difference across genotypes. (D) Hourly sleep traces in wild-type w^{1118} (black) $dSERT^{10}$ (orange) and $dSERT^{16}$ (red) flies. (E-F) Quantification of P(Doze) (E) and P(Wake) (F) during the light period (LP) and dark period (DP) in control w^{1118} (grey), $dSERT^{10}$ mutants (orange), and $dSERT^{16}$ mutants (red). Sleep trace shows mean ± SEM and graphs show individual datapoints and group means ± SEM. P****≤0.0001 (one-way ANOVA with Holm-Sidak multiple comparisons tests).

Supplemental Figure 1. Genetic controls. Hourly sleep traces (A) and quantification of total sleep (B) in control w^{1118} (grey), transheterozygous $dSERT^{10}/dSERT^{16}$ mutants (yellow), $dSERT^{10}$ mutants (orange), and $dSERT^{16}$ mutants (red). Hourly sleep traces (C) and quantification of total sleep (D) in control w^{1118} (grey), $dSERT^{16}$ heterozygotes (purple), and $dSERT^{16}$ homozygous mutants (red). Hourly sleep traces (E) and quantification of total sleep (F) in control w^{1118} (grey), $dSERT^{4}$ revertants (blue), and $dSERT^{16}$ mutants (red). Sleep traces show mean ± SEM and graphs show individual datapoints and group means ± SEM, p≤0.0001****, one-way ANOVA, Holm-Sidak multiple comparisons tests.

Figure 2. *dSERT*^{16} mutants exhibit increased sleep behavior and changes in sleep architecture. Sleep was recorded with both single-beam and multibeam monitors. (A)

Quantification of total sleep in wild-type w^{1118} (grey) and $dSERT^{16}$ (red) flies. Analysis of daytime sleep (B) and daytime bout frequency (C). (D) Latency after light-off was significantly decreased in $dSERT^{16}$ mutants. Quantification of nighttime sleep (E) and nighttime bout frequency (F). Graphs show individual datapoints and group means ± SEM. Student's t-test, two-way, unpaired, p≤0.0001****

Supplemental Figure 2. *dSERT*^{16} mutant sleep phenotype is not an artifact of additional amine-linked behaviors. (A) $dSERT^{16}$ mutants (purple) show no change in grooming behavior compared to wild-type w^{1118} flies (grey). Mean± SEM, unpaired Student's t-test. (B) Male and female $dSERT^{16}$ mutants behave indistinguishably from control flies in negative geotaxis assays. Mean ± SEM, one-way ANOVA.

Figure 3. *dSERT*^{16} mutants exhibit rhythmic circadian behaviors. (A-B) 12h:12h LD and (E-F) 12h:12h DD locomotor activity group analysis for w^{1118} (A,E) and $dSERT^{16}$ (B,F) flies. Histograms represent the distribution of activity through 24 h, averaged over three days, number of flies indicated in C, D, G, H. Lighter and darker bars indicate day and night phases, respectively. Dots indicate the SEM of the activity for each 0.5 hr (0900 is lights-on and 2100 is lights-off). (C,G) Mean daily activity for $dSERT^{16}$ mutants is reduced during both LD (C) and DD (G) cycles, individual datapoints and group means ± SEM. Student's t-test, two-way, unpaired, p****≤0.0001.Calculation of morning and evening anticipation indexes for $dSERT^{16}$ and control flies in LD (D) and DD (H) cycles. (I-J) Representative periodograms derived from activity records of individual w^{1118} (I) and $dSERT^{16}$ (J) flies in constant darkness. (K) Percentage

552 of flies with detectable rhythmicity was calculated for control and *dSERT*^{16}. (L) Circadian
553 periods in DD were averaged from rhythmic flies per each genotype, error bars indicate SEM.
554
555 **Figure 4. *dSERT*^{16} mutants exhibit defects in courtship and copulation.** (A) Quantification of
556 nighttime sleep for males (blue) and females (red) individually housed in DAMs tubes for both
557 control *w*^{1118} (open bars/circles) and *dSERT*^{16} (shaded bars/circles). Following 2 days of
558 individual housing male and females were paired together in DAMs tubes (purple) and nighttime
559 sleep was quantified for both *w*^{1118} (open bar/circles) and *dSERT*^{16} (shaded bar/circles). (B)
560 Quantification of nighttime sleep for *dSERT*^{16} males (shaded blue) and females (shaded red)
561 individually housed or in male-female pairs (shaded purple). Nighttime sleep averages are also
562 shown for mixed co-housing of control males with *dSERT*^{16} females (open bars with red circles)
563 or *dSERT*^{16} males with control females (open bars with blue circles). (C-G) Mating pairs made
564 up of either wildtype (WT) males (red) or WT females (blue) with controls (open bars) or
565 *dSERT*^{16} mutants (shaded bars). (C) Percentage of pairs that copulated within 1 hr.
566 Quantification of average latency to orientation (D), and wing song (E). Quantification of egg
567 laying (F) and percentage of eggs that failed to hatch 24 hours after being laid (G). All graphs
568 (except C) show means ± SEM. P****≤0.0001 (A-C, one-way ANOVA); (D-G Two-way
569 unpaired t test).
570
571 **Figure 5. *dSERT*^{16} mutants exhibit defects in feeding.**
572 (A) Quantification of nighttime sleep for wild-type *w*^{1118} (grey) and *dSERT*^{16} mutants (red) over
573 a 3-day period that began 1 day after initial loading into DAMs tubes. Day 1 (Baseline): flies
574 kept on standard food. Day 2 (Starvation): flies transferred to agar for food deprivation. Day 3

(Recovery): flies transferred to fresh food. (B) Food uptake is significantly reduced in starved *dSERT*^{16} mutants (red) compared to controls (grey). (C) Representative images depict visual feeding score used to assay food uptake. All graphs show individual datapoints and group means ± SEM. P****≤0.0001 (two-way ANOVA (A), two-way unpaired t test (B)).

Figure 6. Transgenic expression of *dSERT* with *TRH-Gal4* or *TPH-Gal4* is sufficient to rescue daytime defects or nighttime defects respectively in *dSERT*^{16} mutants. (A) Hourly sleep traces in wild-type *w*^{1118}*;TRH-Gal4* (grey), *dSERT*^{16}*; TRH-Gal4* (red), *TRH-Gal4/UAS-dSERT* (blue), and *dSERT*^{16} *; TRH-Gal4/UAS-dSERT* (green) flies. Quantifications of total sleep (B), daytime sleep (C), and daytime bout number (D). (E) Hourly sleep traces in wild-type *w*^{1118}*;TPH-Gal4* (grey), *TPH-Gal4*, *dSERT*^{16} (red), *TPH-Gal4;UAS-dSERT* (blue), and *TPH-Gal4*, *dSERT*^{16} *; UAS-dSERT* (green) flies. Quantifications of total sleep (F), nighttime sleep (G) and nighttime bout number (H). (I) Quantification of nighttime sleep for males and females individually housed in DAMs tubes for control wild-type *w*^{1118}*;TPH-Gal4* (grey), *TPH-Gal4*, *dSERT*^{16} (red), and *dSERT*^{16} *; TRH-Gal4/UAS-dSERT* (green) flies. Following 2 days of individual housing male and females were paired together in DAMs tubes and nighttime sleep was quantified. (J) Quantification of nighttime sleep for wild-type *w*^{1118}*;TPH-Gal4* (grey), *TPH-Gal4*, *dSERT*^{16} (red), and *dSERT*^{16} *; TRH-Gal4/UAS-dSERT* (green) flies over a 3-day period that began 1 day after initial loading into DAMs tubes. Day 1 (Baseline): flies kept on standard food. Day 2 (Starvation): flies transferred to agar for food deprivation. Day 3 (Recovery): flies transferred to fresh food. Sleep traces show mean ± SEM and graphs show individual datapoints and group means ± SEM. P****≤0.0001 (one-way ANOVA (B-D, F-H) and two-way ANOVA (I-J)).

598

599 **Supplemental Figure 3**. Quantification of nighttime sleep (A) and nighttime bout frequency (B)
600 in wild-type *w*^{1118}*;TRH-Gal4* (grey), *dSERT*^{16}*; TRH-Gal4* (red), *TRH-Gal4/UAS-dSERT* (blue),
601 and *dSERT*^{16} *; TRH-Gal4/UAS-dSERT* (green) flies. (C,F) Representative pictures show
602 expression of UAS-MCD8::GFP (green) driven by *TRH-Gal4* (C) or *TPH-Gal4* (F) and DLG
603 (magenta) in mushroom bodies. The different lobes of the mushroom body are labeled in white.
604 Quantification of daytime sleep (D) and daytime bout frequency (E) in wild-type *w*^{1118}*;TPH-*
605 *Gal4* (grey), *TPH-Gal4*, *dSERT*^{16} (red), *TPH-Gal4;UAS-dSERT* (blue), and *TPH-Gal4*, *dSERT*^{16}
606 *; UAS-dSERT* (green) flies. All graphs show individual datapoints and group means ± SEM.
607 P****≤0.0001 (one-way ANOVA).

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References

1. Crocker A, Sehgal A. Genetic analysis of sleep. Genes Dev. 2010 Jun 15;24(12):1220–35.

2. Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW. CONTROL OF SLEEP AND WAKEFULNESS. Physiol Rev. 2012 Jul;92(3):1087–187.

3. Keene AC, Duboue ER. The origins and evolution of sleep. J Exp Biol. 2018 Jun 1;221(11):jeb159533.

4. Spira AP, Chen-Edinboro LP, Wu MN, Yaffe K. Impact of Sleep on the Risk of Cognitive Decline and Dementia. Curr Opin Psychiatry. 2014 Nov;27(6):478–83.

5. Goel N, Rao H, Durmer JS, Dinges DF. Neurocognitive Consequences of Sleep Deprivation. Semin Neurol. 2009 Sep;29(4):320–39.

6. Bonnet MH, Arand DL. Clinical effects of sleep fragmentation versus sleep deprivation. Sleep Med Rev. 2003 Aug 1;7(4):297–310.

7. Jouvet M. Biogenic Amines and the States of Sleep. Science. 1969 Jan 3;163(3862):32–41.

8. Monti JM. Serotonin control of sleep-wake behavior. Sleep Med Rev. 2011 Aug 1;15(4):269–81.

9. Ursin R. Serotonin and sleep. Sleep Med Rev. 2002 Feb 1;6(1):55–67.

10. Jouvet M. Sleep and Serotonin: An Unfinished Story. Neuropsychopharmacology. 1999 Aug;21(1):24–7.

11. Portas CM, Bjorvatn B, Ursin R. Serotonin and the sleep/wake cycle: special emphasis on microdialysis studies. Prog Neurobiol. 2000 Jan 1;60(1):13–35.

12. Solarewicz JZ, Angoa-Perez M, Kuhn DM, Mateika JH. The sleep-wake cycle and motor activity, but not temperature, are disrupted over the light-dark cycle in mice genetically depleted of serotonin. Am J Physiol-Regul Integr Comp Physiol. 2014 Nov 12;308(1):R10–7.

13. Oikonomou G, Altermatt M, Zhang R wei, Coughlin GM, Montz C, Gradinaru V, et al. The Serotonergic Raphe Promote Sleep in Zebrafish and Mice. Neuron. 2019 Aug 21;103(4):686-701.e8.

14. Shaw PJ, Cirelli C, Greenspan RJ, Tononi G. Correlates of Sleep and Waking in Drosophila melanogaster. Science. 2000 Mar 10;287(5459):1834–7.

15. Hendricks JC, Finn SM, Panckeri KA, Chavkin J, Williams JA, Sehgal A, et al. Rest in Drosophila Is a Sleep-like State. Neuron. 2000 Jan 1;25(1):129–38.

16. Yuan Q, Joiner WJ, Sehgal A. A Sleep-Promoting Role for the Drosophila Serotonin Receptor 1A. Curr Biol. 2006 Jun 6;16(11):1051–62.

17. Yuan Q, Lin F, Zheng X, Sehgal A. Serotonin modulates circadian entrainment in Drosophila. Neuron. 2005 Jul 7;47(1):115–27.

18. Liu C, Meng Z, Wiggin TD, Yu J, Reed ML, Guo F, et al. A Serotonin-Modulated Circuit Controls Sleep Architecture to Regulate Cognitive Function Independent of Total Sleep in Drosophila. Curr Biol CB. 2019 04;29(21):3635-3646.e5.

19. Haynes PR, Christmann BL, Griffith LC. A single pair of neurons links sleep to memory consolidation in Drosophila melanogaster. Davis GW, editor. eLife. 2015 Jan 7;4:e03868.

20. Qian Y, Cao Y, Deng B, Yang G, Li J, Xu R, et al. Sleep homeostasis regulated by 5HT2b receptor in a small subset of neurons in the dorsal fan-shaped body of drosophila. Sehgal A, editor. eLife. 2017 Oct 6;6:e26519.

21. Tomita J, Ban G, Kato YS, Kume K. Protocerebral Bridge Neurons That Regulate Sleep in Drosophila melanogaster. Front Neurosci. 2021;15:647117.

22. Driscoll M, Buchert SN, Coleman V, McLaughlin M, Nguyen A, Sitaraman D. Compartment specific regulation of sleep by mushroom body requires GABA and dopaminergic signaling. Sci Rep. 2021 Oct 8;11(1):20067.

23. Karam CS, Williams BL, Jones SK, Javitch JA. The Role of the Dopamine Transporter in the Effects of Amphetamine on Sleep and Sleep Architecture in Drosophila. Neurochem Res. 2021 Feb 25;

24. Kasture A, El-Kasaby A, Szöllősi D, Asjad HMM, Grimm A, Stockner T, et al. Functional Rescue of a Misfolded Drosophila melanogaster Dopamine Transporter Mutant Associated with a Sleepless Phenotype by Pharmacological Chaperones. J Biol Chem. 2016 Sep 30;291(40):20876–90.

25. Kume K, Kume S, Park SK, Hirsh J, Jackson FR. Dopamine is a regulator of arousal in the fruit fly. J Neurosci Off J Soc Neurosci. 2005 Aug 10;25(32):7377–84.

26. Lee J, Lim C, Han TH, Andreani T, Moye M, Curran J, et al. The E3 ubiquitin ligase adaptor Tango10 links the core circadian clock to neuropeptide and behavioral rhythms. Proc Natl Acad Sci U S A. 2021 Nov 23;118(47):e2110767118.

27. Chatterjee A, Lamaze A, De J, Mena W, Chélot E, Martin B, et al. Reconfiguration of a Multi-oscillator Network by Light in the Drosophila Circadian Clock. Curr Biol. 2018 Jul 9;28(13):2007-2017.e4.

28. Jackson FR, Ng FS, Sengupta S, You S, Huang Y. Glial cell regulation of rhythmic behavior. Methods Enzymol. 2015;552:45–73.

679 29. Flores CC, Loschky SS, Marshall W, Spano GM, Massaro Cenere M, Tononi G, et
680 al. Identification of Ultrastructural Signatures of Sleep and Wake in the Fly Brain.
681 Sleep. 2021 Sep 18;zsab235.

682 30. Shafer OT, Keene AC. The Regulation of Drosophila Sleep. Curr Biol CB. 2021 Jan
683 11;31(1):R38–49.

684 31. Li Q, Jang H, Lim KY, Lessing A, Stavropoulos N. insomniac links the development
685 and function of a sleep regulatory circuit. eLife. 2021 Dec 15;10:e65437.

686 32. Driscoll ME, Hyland C, Sitaraman D. Measurement of Sleep and Arousal in
687 Drosophila. Bio-Protoc. 2019;9(12):e3268.

688 33. Rudnick G, Krämer R, Blakely RD, Murphy DL, Verrey F. The SLC6 transporters:
689 perspectives on structure, functions, regulation, and models for transporter
690 dysfunction. Pflugers Arch. 2014 Jan;466(1):25–42.

691 34. Blakely RD, El Mestikawy S, Robinson MB. The brain in flux: Genetic, physiologic,
692 and therapeutic perspectives on transporters in the CNS. Neurochem Int. 2019
693 Feb;123:1–6.

694 35. Kennedy SH, Rizvi S. Sexual Dysfunction, Depression, and the Impact of
695 Antidepressants. J Clin Psychopharmacol. 2009 Apr;29(2):157–64.

696 36. Schweitzer I, Maguire K, Ng C. Sexual Side-Effects of Contemporary
697 Antidepressants: Review. Aust N Z J Psychiatry. 2009 Sep 1;43(9):795–808.

698 37. Serotonin and Norepinephrine Reuptake Inhibition and Eating Behavior - HAINER -
699 2006 - Annals of the New York Academy of Sciences - Wiley Online Library
700 [Internet]. [cited 2022 Apr 28]. Available from:
701 https://nyaspubs.onlinelibrary.wiley.com/doi/full/10.1196/annals.1367.017

702 38. Wichniak A, Wierzbicka A, Walęcka M, Jernajczyk W. Effects of Antidepressants
703 on Sleep. Curr Psychiatry Rep [Internet]. 2017 [cited 2020 Sep 9];19(9). Available
704 from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5548844/

705 39. Biard K, Douglass AB, Koninck JD. The effects of galantamine and buspirone on
706 sleep structure: Implications for understanding sleep abnormalities in major
707 depression: J Psychopharmacol (Oxf) [Internet]. 2015 Aug 10 [cited 2020 Sep 9];
708 Available from: https://journals.sagepub.com/doi/10.1177/0269881115598413

709 40. Hidalgo S, Molina-Mateo D, Escobedo P, Zárate RV, Fritz E, Fierro A, et al.
710 Characterization of a Novel Drosophila SERT Mutant: Insights on the Contribution
711 of the Serotonin Neural System to Behaviors. ACS Chem Neurosci. 2017 Oct
712 18;8(10):2168–79.

713 41. Giang T, Rauchfuss S, Ogueta M, Scholz H. The Serotonin Transporter Expression
714 in Drosophila melanogaster. J Neurogenet. 2011 Mar 1;25(1–2):17–26.

42. Garbe DS, Bollinger WL, Vigderman A, Masek P, Gertowski J, Sehgal A, et al. Context-specific comparison of sleep acquisition systems in Drosophila. Biol Open. 2015 Oct 30;4(11):1558–68.

43. Yellman C, Tao H, He B, Hirsh J. Conserved and sexually dimorphic behavioral responses to biogenic amines in decapitated Drosophila. Proc Natl Acad Sci. 1997 Apr 15;94(8):4131–6.

44. McClung C, Hirsh J. Stereotypic behavioral responses to free-base cocaine and the development of behavioral sensitization in Drosophila. Curr Biol. 1998 Jan 15;8(2):109–12.

45. Chang HY, Grygoruk A, Brooks ES, Ackerson LC, Maidment NT, Bainton RJ, et al. Overexpression of the Drosophila vesicular monoamine transporter increases motor activity and courtship but decreases the behavioral response to cocaine. Mol Psychiatry. 2006 Jan;11(1):99–113.

46. Morin LP. Serotonin and the regulation of mammalian circadian rhythmicity. Ann Med. 1999 Feb;31(1):12–33.

47. Fujii S, Krishnan P, Hardin P, Amrein H. Nocturnal Male Sex Drive in Drosophila. Curr Biol CB. 2007 Feb 6;17(3):244–51.

48. Machado DR, Afonso DJ, Kenny AR, Öztürk-Çolak A, Moscato EH, Mainwaring B, et al. Identification of octopaminergic neurons that modulate sleep suppression by male sex drive. Griffith LC, editor. eLife. 2017 May 16;6:e23130.

49. Beckwith EJ, Geissmann Q, French AS, Gilestro GF. Regulation of sleep homeostasis by sexual arousal. Griffith LC, editor. eLife. 2017 Sep 12;6:e27445.

50. Fava M. Weight gain and antidepressants. J Clin Psychiatry. 2000;61 Suppl 11:37–41.

51. Keene AC, Duboué ER, McDonald DM, Dus M, Suh GSB, Waddell S, et al. Clock and cycle Limit Starvation-Induced Sleep Loss in Drosophila. Curr Biol. 2010 Jul 13;20(13):1209–15.

52. Alekseyenko OV, Lee C, Kravitz EA. Targeted manipulation of serotonergic neurotransmission affects the escalation of aggression in adult male Drosophila melanogaster. PloS One. 2010 May 24;5(5):e10806.

53. Yang Y, Edery I. Daywake, an Anti-siesta Gene Linked to a Splicing-Based Thermostat from an Adjoining Clock Gene. Curr Biol CB. 2019 May 20;29(10):1728-1734.e4.

54. Wang J, Fan JY, Zhao Z, Dissel S, Price J. DBT affects sleep in both circadian and non-circadian neurons. PLoS Genet. 2022 Feb;18(2):e1010035.

55. Lone SR, Potdar S, Venkataraman A, Sharma N, Kulkarni R, Rao S, et al. Mechanosensory Stimulation via Nanchung Expressing Neurons Can Induce Daytime Sleep in Drosophila. J Neurosci Off J Soc Neurosci. 2021 Nov 10;41(45):9403–18.

56. Ishimoto H, Lark A, Kitamoto T. Factors that Differentially Affect Daytime and Nighttime Sleep in Drosophila melanogaster. Front Neurol. 2012 Feb 27;3:24.

57. Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, et al. Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. Nature. 2006 Jun;441(7097):1157–61.

58. Pooryasin A, Fiala A. Identified Serotonin-Releasing Neurons Induce Behavioral Quiescence and Suppress Mating in Drosophila. J Neurosci. 2015 Sep 16;35(37):12792–812.

59. Lee G, Hall JC. Abnormalities of Male-Specific FRU Protein and Serotonin Expression in the CNS of fruitless Mutants inDrosophila. J Neurosci. 2001 Jan 15;21(2):513–26.

60. Lee G, Villella A, Taylor BJ, Hall JC. New reproductive anomalies in fruitless-mutant Drosophila males: Extreme lengthening of mating durations and infertility correlated with defective serotonergic innervation of reproductive organs. J Neurobiol. 2001;47(2):121–49.

61. Albin SD, Kaun KR, Knapp JM, Chung P, Heberlein U, Simpson JH. A Subset of Serotonergic Neurons Evokes Hunger in Adult Drosophila. Curr Biol CB. 2015 Sep 21;25(18):2435–40.

62. Kiianmaa K, Fuxe K. The effects of 5,7-dihydroxytryptamine-induced lesions of the ascending 5-hydroxytryptamine pathways on the sleep wakefulness cycle. Brain Res. 1977 Aug 12;131(2):287–301.

63. Trulson ME, Jacobs BL. Raphe unit activity in freely moving cats: correlation with level of behavioral arousal. Brain Res. 1979 Mar 9;163(1):135–50.

64. Benington JH, Kodali SK, Heller HC. Scoring transitions to REM sleep in rats based on the EEG phenomena of pre-REM sleep: an improved analysis of sleep structure. Sleep. 1994 Feb;17(1):28–36.

65. Wisor JP, Wurts SW, Hall FS, Lesch KP, Murphy DL, Uhl GR, et al. Altered rapid eye movement sleep timing in serotonin transporter knockout mice. Neuroreport. 2003 Feb 10;14(2):233–8.

66. Alexandre C, Popa D, Fabre V, Bouali S, Venault P, Lesch KP, et al. Early Life Blockade of 5-Hydroxytryptamine 1A Receptors Normalizes Sleep and Depression-Like Behavior in Adult Knock-Out Mice Lacking the Serotonin Transporter. J Neurosci. 2006 May 17;26(20):5554–64.

787 67. Boutrel B, Franc B, Hen R, Hamon M, Adrien J. Key role of 5-HT1B receptors in the
788 regulation of paradoxical sleep as evidenced in 5-HT1B knock-out mice. J Neurosci
789 Off J Soc Neurosci. 1999 Apr 15;19(8):3204–12.

790 68. Boutrel B, Monaca C, Hen R, Hamon M, Adrien J. Involvement of 5-HT1A
791 receptors in homeostatic and stress-induced adaptive regulations of paradoxical
792 sleep: studies in 5-HT1A knock-out mice. J Neurosci Off J Soc Neurosci. 2002 Jun
793 1;22(11):4686–92.

794 69. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, et al. Association
795 of Anxiety-Related Traits with a Polymorphism in the Serotonin Transporter Gene
796 Regulatory Region. Science. 1996;274(5292):1527–31.

797 70. Smeraldi E, Zanardi R, Benedetti F, Di Bella D, Perez J, Catalano M.
798 Polymorphism within the promoter of the serotonin transporter gene and
799 antidepressant efficacy of fluvoxamine. Mol Psychiatry. 1998 Nov;3(6):508–11.

800 71. Benedetti F, Serretti A, Colombo C, Campori E, Barbini B, di Bella D, et al.
801 Influence of a functional polymorphism within the promoter of the serotonin
802 transporter gene on the effects of total sleep deprivation in bipolar depression. Am
803 J Psychiatry. 1999 Sep;156(9):1450–2.

804 72. Blundell JE, Hill AJ. Dexfenfluramine and appetite in humans. Int J Obes Relat
805 Metab Disord J Int Assoc Study Obes. 1992 Dec;16 Suppl 3:S51-59.

806 73. Goudie AJ, Thornton EW, Wheeler TJ. Effects of Lilly 110140, a specific inhibitor of
807 5-hydroxytryptamine uptake, on food intake and on 5-hydroxytryptophan-induced
808 anorexia. Evidence for serotoninergic inhibition of feeding. J Pharm Pharmacol.
809 1976 Apr;28(4):318–20.

810 74. Clifton PG, Barnfield AMC, Philcox L. A behavioural profile of fluoxetine-induced
811 anorexia. Psychopharmacology (Berl). 1989 Jan 1;97(1):89–95.

812 75. Simansky KJ, Vaidya AH. Behavioral mechanisms for the anorectic action of the
813 serotonin (5-HT) uptake inhibitor sertraline in rats: comparison with directly acting
814 5-HT agonists. Brain Res Bull. 1990 Dec;25(6):953–60.

815 76. Alekseyenko OV, Chan YB, de la Paz Fernandez M, Bülow T, Pankratz MJ, Kravitz
816 EA. Single serotonergic neurons that modulate aggression in Drosophila. Curr Biol
817 CB. 2014 Nov 17;24(22):2700–7.

818 77. Falkner AL, Dollar P, Perona P, Anderson DJ, Lin D. Decoding Ventromedial
819 Hypothalamic Neural Activity during Male Mouse Aggression. J Neurosci. 2014 Apr
820 23;34(17):5971–84.

821 78. Lin D, Boyle MP, Dollar P, Lee H, Perona P, Lein ES, et al. Functional identification
822 of an aggression locus in the mouse hypothalamus. Nature. 2011 Feb
823 10;470(7333):221–6.

824 79. Dierick HA, Greenspan RJ. Molecular analysis of flies selected for aggressive
825 behavior. Nat Genet. 2006 Sep;38(9):1023–31.

826 80. Dierick HA, Greenspan RJ. Serotonin and neuropeptide F have opposite
827 modulatory effects on fly aggression. Nat Genet. 2007 May;39(5):678–82.

828 81. Pfaffl MW. A new mathematical model for relative quantification in real-time RT–
829 PCR. Nucleic Acids Res. 2001 May 1;29(9):e45.

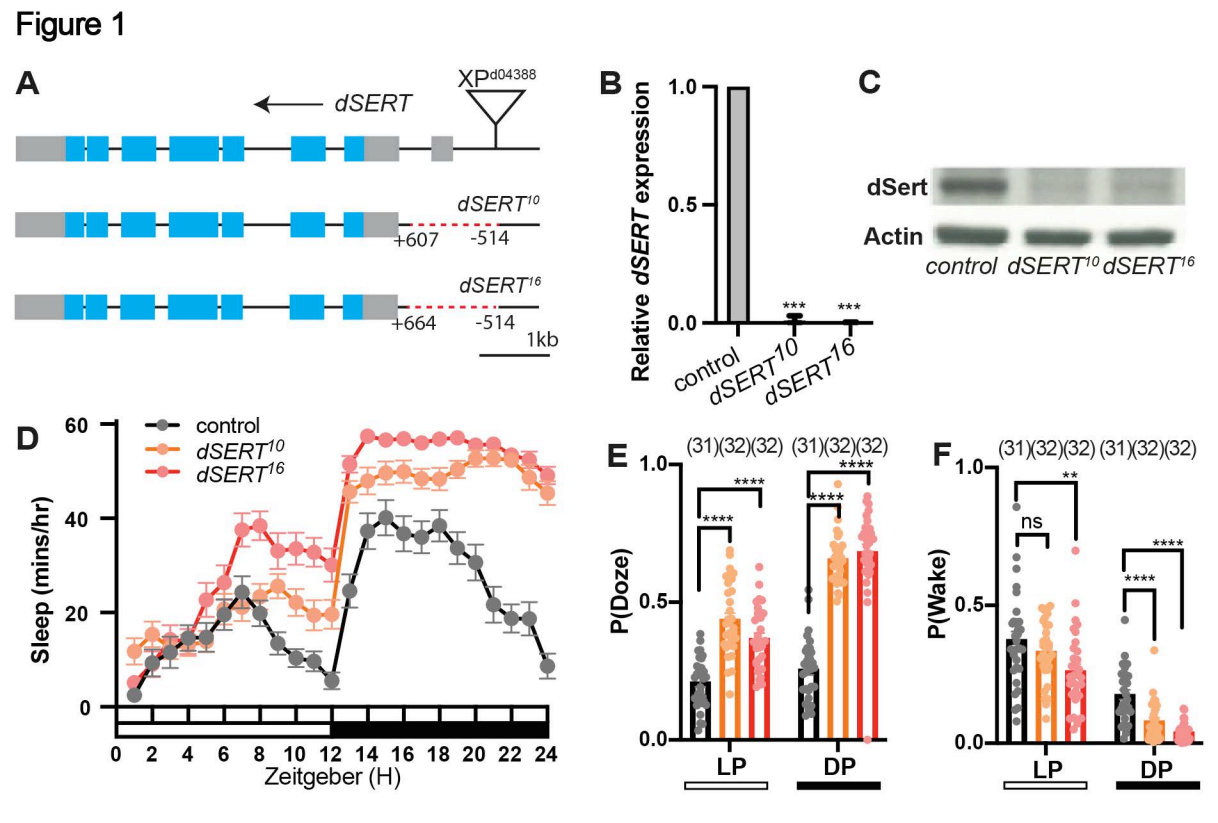
830 82. Andersen CL, Jensen JL, Ørntoft TF. Normalization of real-time quantitative
831 reverse transcription-PCR data: a model-based variance estimation approach to
832 identify genes suited for normalization, applied to bladder and colon cancer data
833 sets. Cancer Res. 2004 Aug 1;64(15):5245–50.

834 83. Shaw PJ, Tononi G, Greenspan RJ, Robinson DF. Stress response genes protect
835 against lethal effects of sleep deprivation in Drosophila. Nature. 2002 May
836 16;417(6886):287–91.

837 84. Im SH, Taghert PH. PDF Receptor Expression Reveals Direct Interactions between
838 Circadian Oscillators in Drosophila. J Comp Neurol. 2010 Jun 1;518(11):1925–45.

839

Figure 1


A
dSERT
XP d04388
dSERT10
+607
-514
dSERT16
+664
-514
1kb
B
Relative dSERT expression
1.0
0.5
0.0
***
***
control
dSERT10
dSERT16
C
dSert
Actin
control
dSERT10
dSERT16
D
control
dSERT10
dSERT16
Sleep (mins/hr)
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Zeitgeber (H)
E
(31)(32)(32)
(31)(32)(32)
P(Doze)
1.0
0.5
0.0
****
****
****
****
LP
DP
F
(31)(32)(32)
(31)(32)(32)
P(Wake)
1.0
0.5
0.0
**
ns
****
****
LP
DP

Supplemental Figure 1

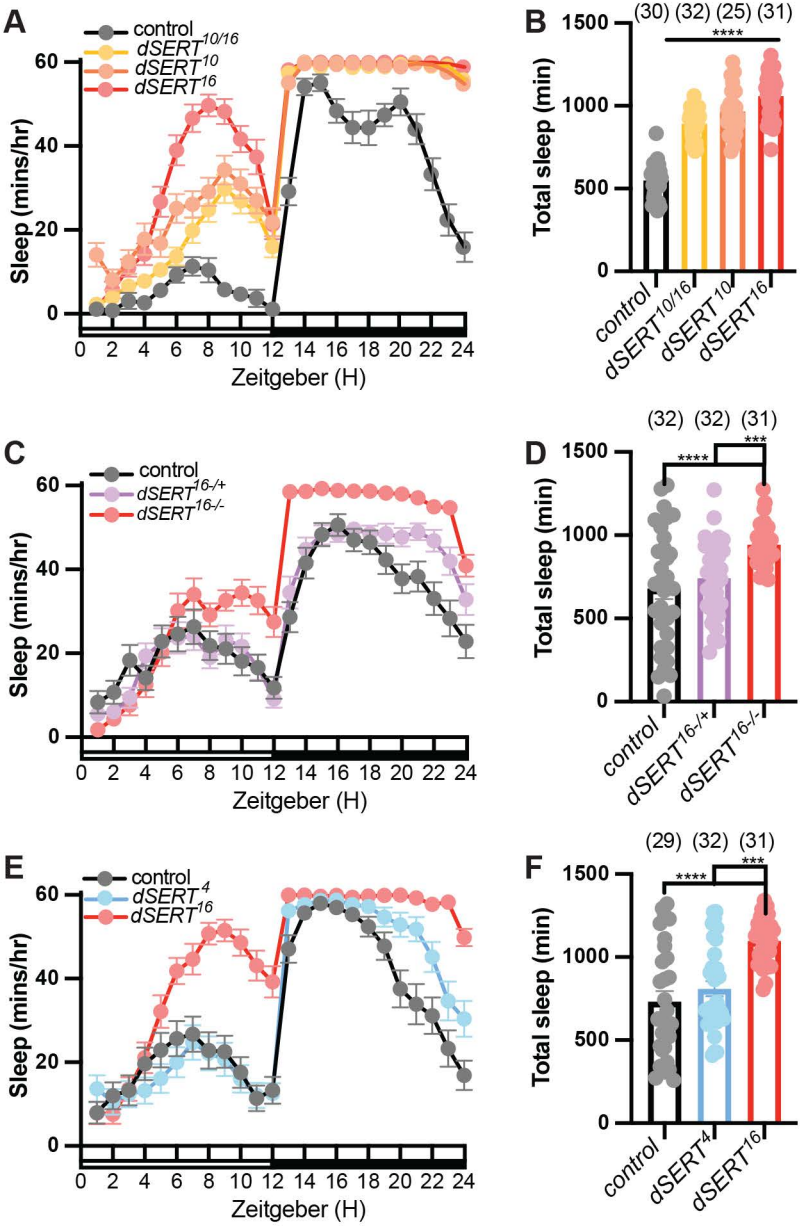
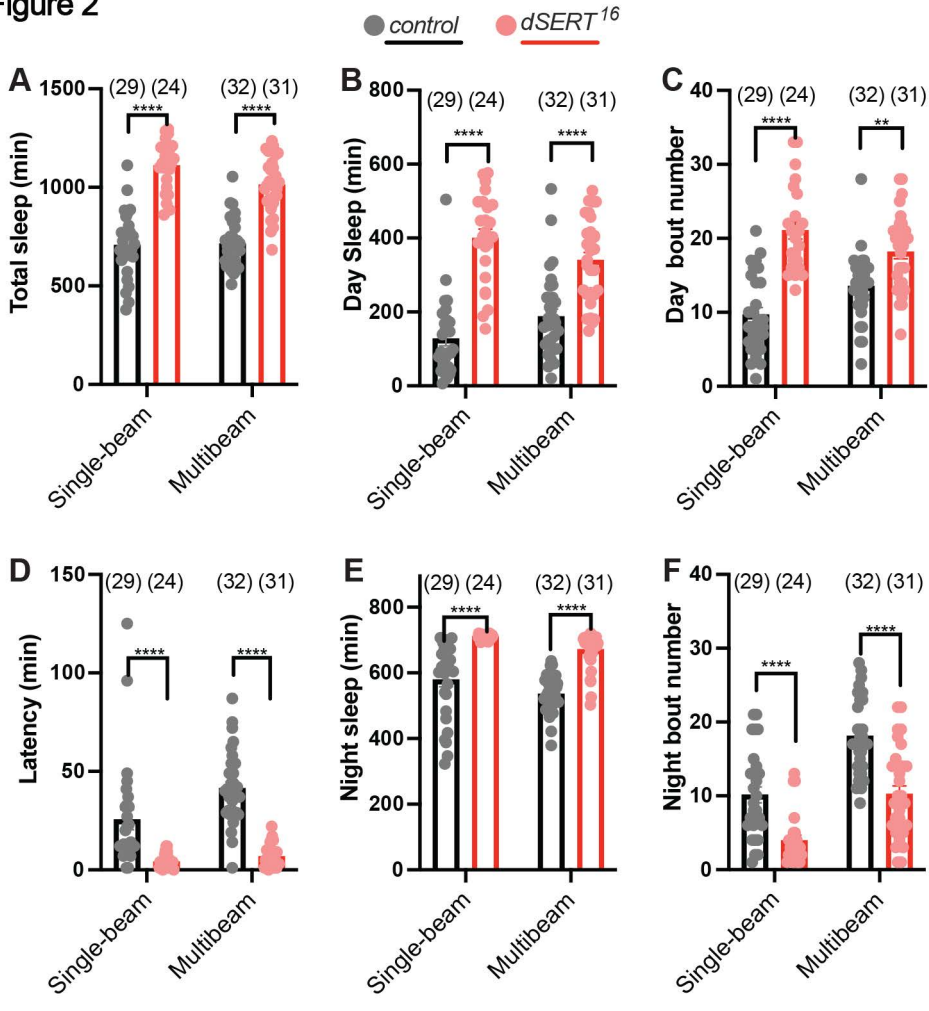


Figure 2


control
dSERT16
A
Total sleep (min)
1500
1000
500
0
(29) (24)
(32) (31)
****
****
Single-beam
Multibeam
B
Day Sleep (min)
800
600
400
200
0
(29) (24)
(32) (31)
****
****
Single-beam
Multibeam
C
Day bout number
40
30
20
10
0
(29) (24)
(32) (31)
****
**
Single-beam
Multibeam
D
Latency (min)
150
100
50
0
(29) (24)
(32) (31)
****
****
Single-beam
Multibeam
E
Night sleep (min)
800
600
400
200
0
(29) (24)
(32) (31)
****
****
Single-beam
Multibeam
F
Night bout number
40
30
20
10
0
(29) (24)
(32) (31)
****
****
Single-beam
Multibeam

Supplementary Figure 2

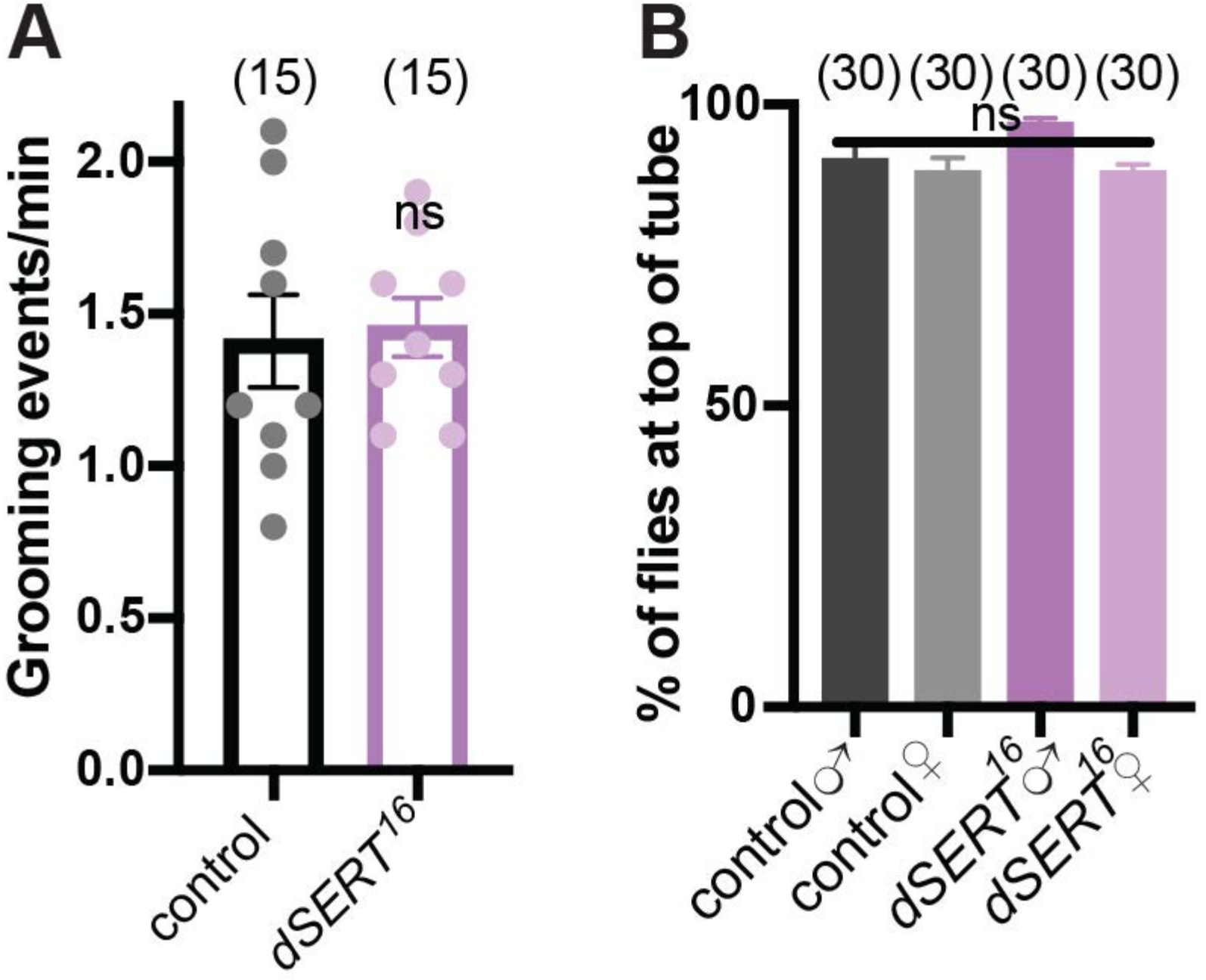


Figure 3

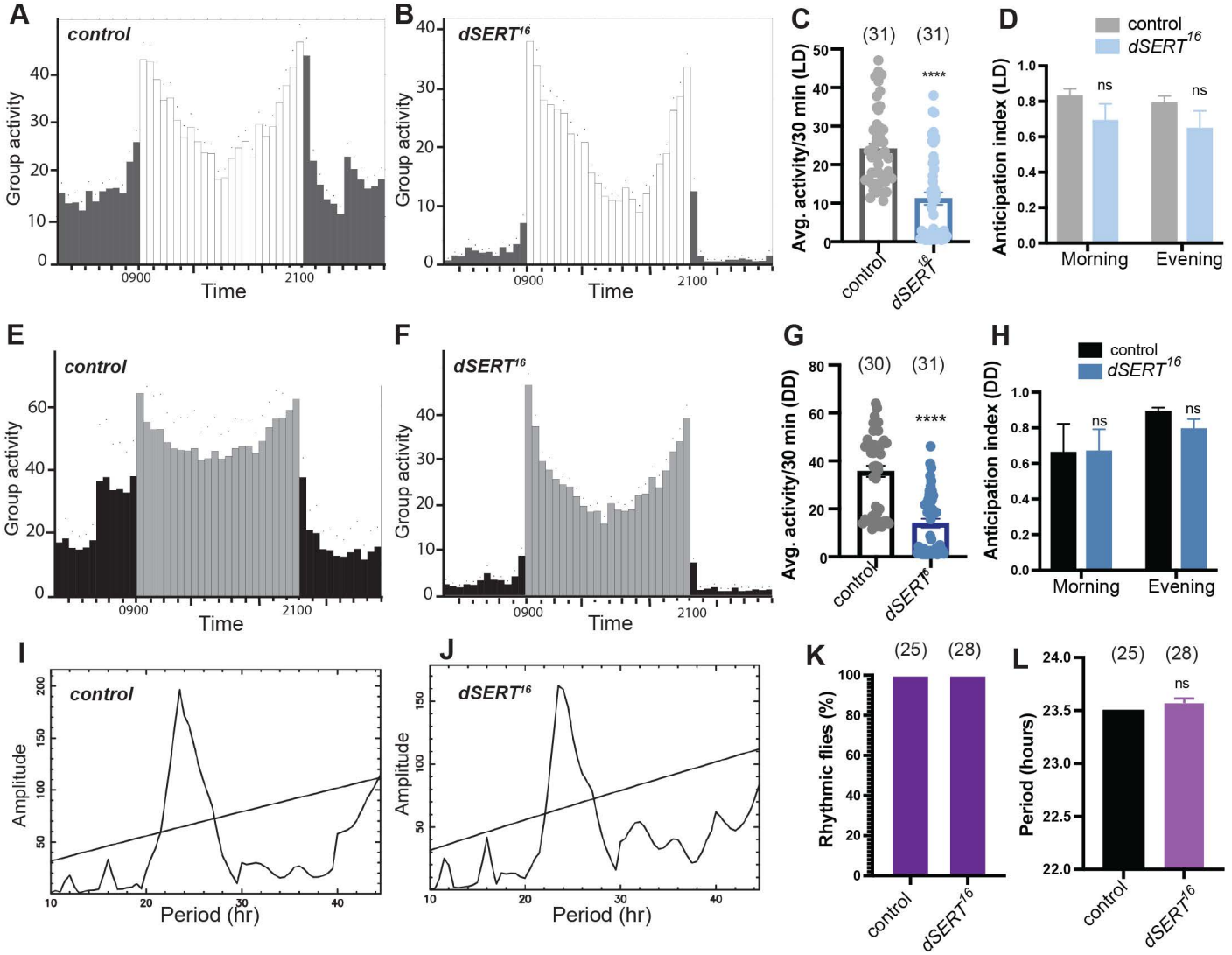

A
control
Group activity
Time
0900
2100
B
dSERT16
C
(31) (31)
****
Avg. activity/30 min (LD)
control
dSERT16
D
control
dSERT16
Anticipation index (LD)
ns
Morning
Evening
E
F
G
(30) (31)
Avg. activity/30 min (DD)
H
Anticipation index (DD)
I
Amplitude
Period (hr)
J
K
(25) (28)
Rhythmic flies (%)
L
Period (hours)

Figure 4

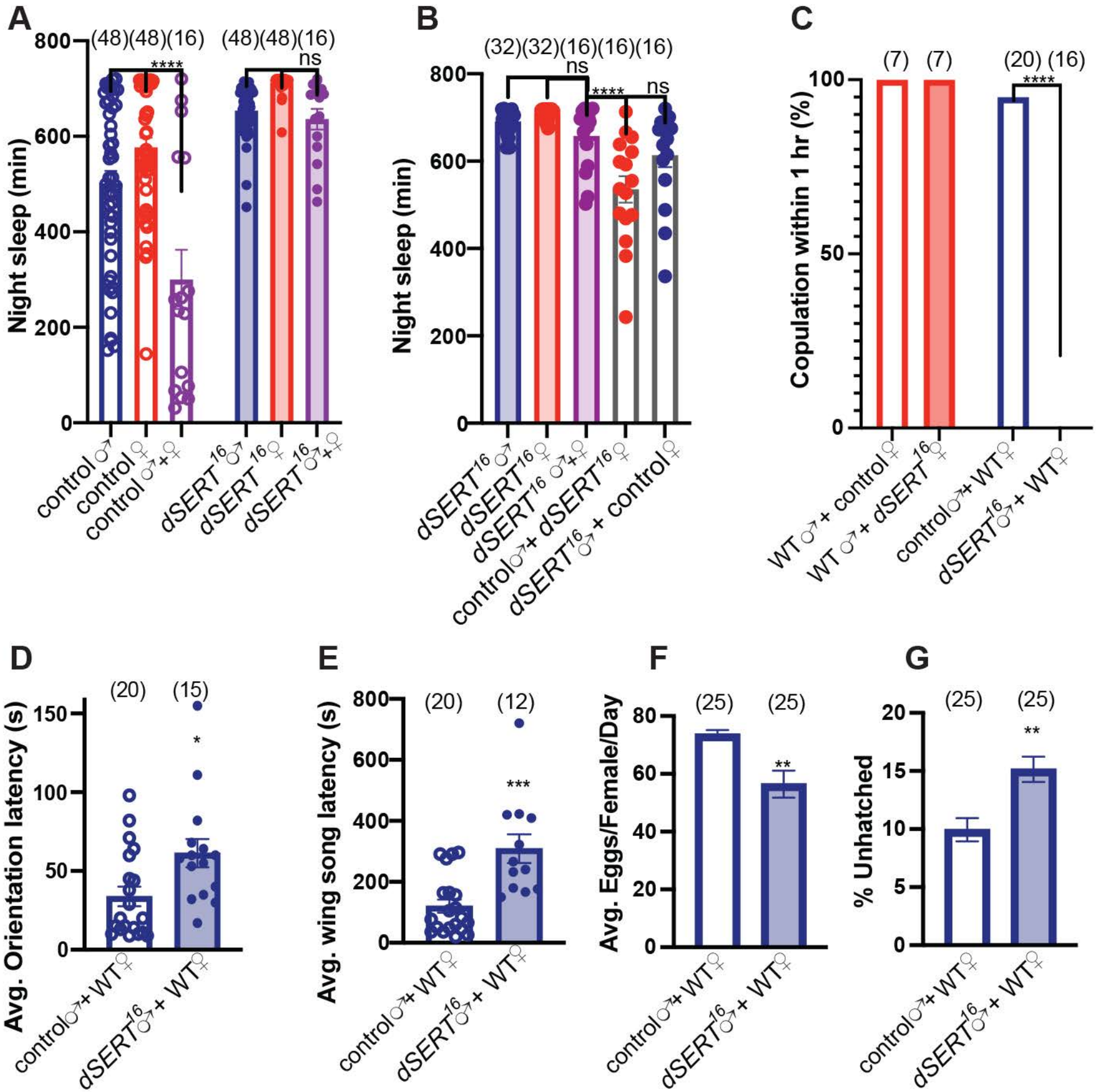

A
(48)(48)(16)
(48)(48)(16)
****
ns
Night sleep (min)
800
600
400
200
0
control♂
control♀
control♂+♀
dSERT16♂
dSERT16♀
dSERT16♂+♀
B
(32)(32)(16)(16)(16)
ns
****
ns
Night sleep (min)
dSERT16♂
dSERT16♀
dSERT16♂+♀
control♂+ dSERT16♀
dSERT16♂+ control♀
C
(7) (7) (20) (16)
****
Copulation within 1 hr (%)
100
50
0
WT♂+ control♀
WT♂+ dSERT16♀
control♂+WT♀
dSERT16♂+ WT♀
D
(20) (15)
*
Avg. Orientation latency (s)
150
100
50
0
control♂+WT♀
dSERT16♂+ WT♀
E
(20) (12)
***
Avg. wing song latency (s)
800
600
400
200
0
control♂+WT♀
dSERT16♂+ WT♀
F
(25) (25)
**
Avg. Eggs/Female/Day
80
60
40
20
0
control♂+WT♀
dSERT16♂+ WT♀
G
(25) (25)
**
% Unhatched
20
15
10
5
0
control♂+WT♀
dSERT16♂+ WT♀

Figure 5

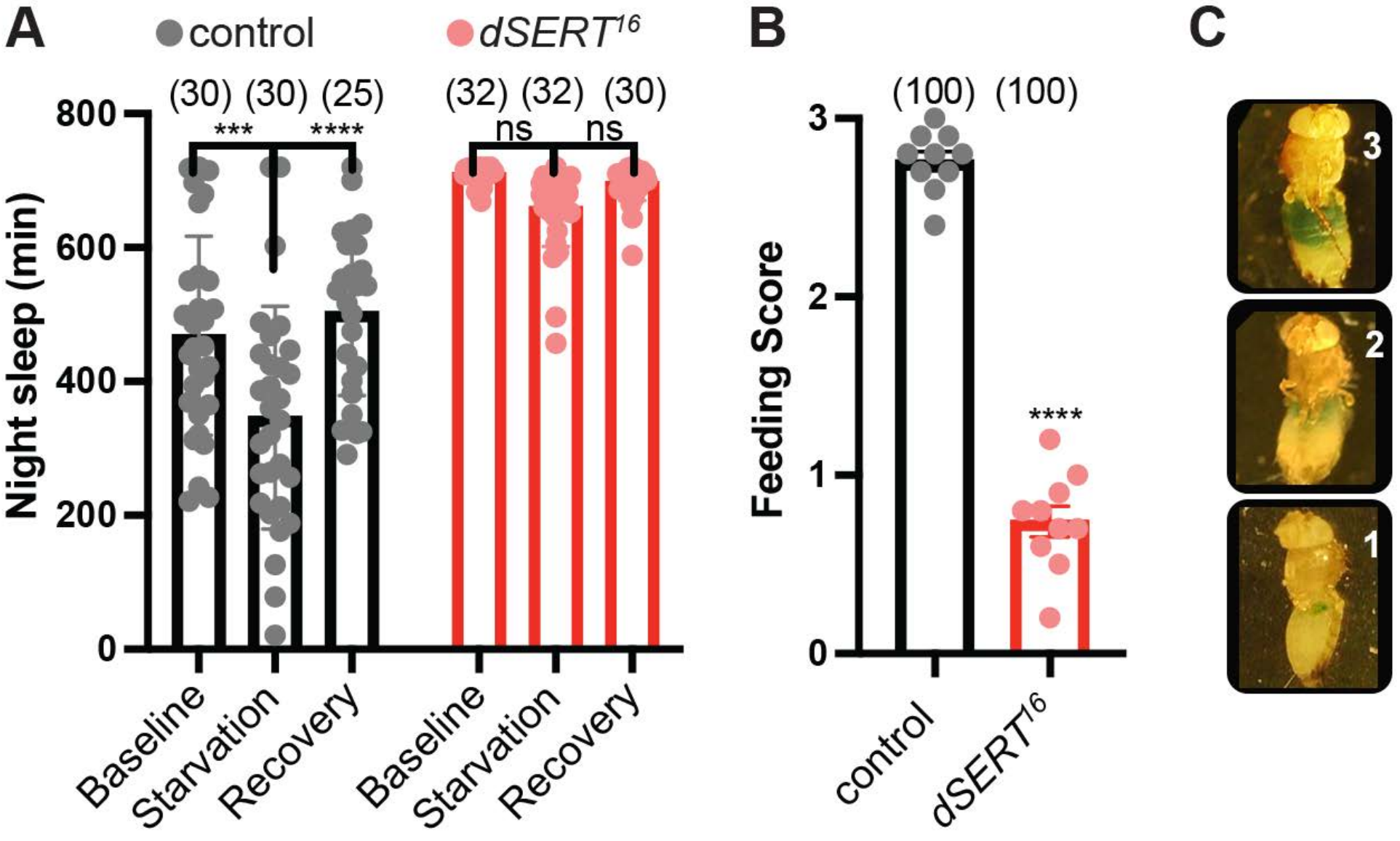
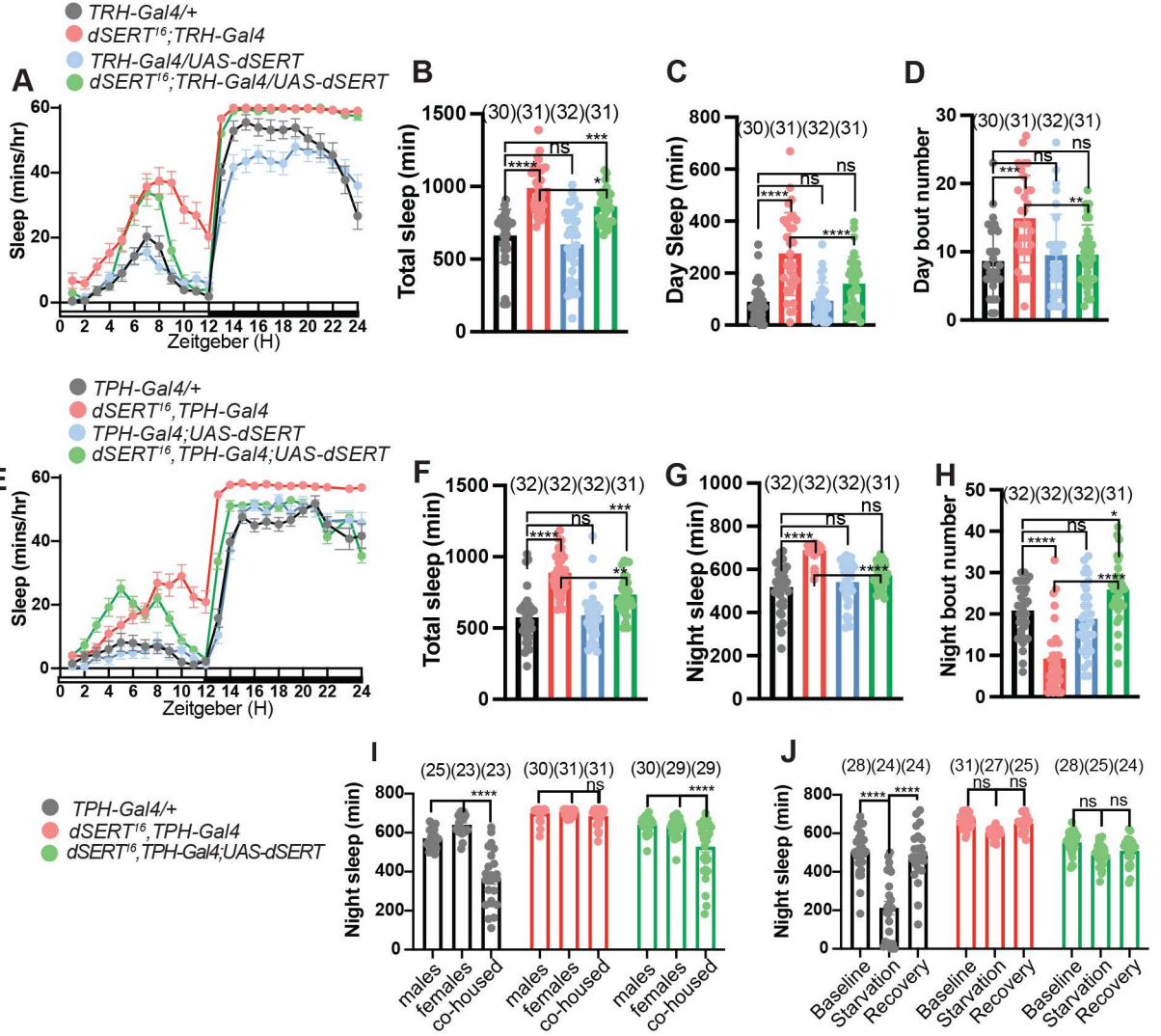

A
control
dSERT16
(30) (30) (25)
(32) (32) (30)
***
****
ns
ns
Night sleep (min)
800
600
400
200
0
Baseline
Starvation
Recovery
Baseline
Starvation
Recovery
B
(100) (100)
Feeding Score
3
2
1
0
****
control
dSERT16
C
3
2
1

Figure 6


TRH-Gal4/+
dSERT16;TRH-Gal4
TRH-Gal4/UAS-dSERT
dSERT16;TRH-Gal4/UAS-dSERT
A
Sleep (mins/hr)
Zeitgeber (H)
B
Total sleep (min)
(30)(31)(32)(31)
C
Day Sleep (min)
(30)(31)(32)(31)
D
Day bout number
(30)(31)(32)(31)
TPH-Gal4/+
dSERT16,TPH-Gal4
TPH-Gal4;UAS-dSERT
dSERT16,TPH-Gal4;UAS-dSERT
E
Sleep (mins/hr)
Zeitgeber (H)
F
Total sleep (min)
(32)(32)(32)(31)
G
Night sleep (min)
(32)(32)(32)(31)
H
Night bout number
(32)(32)(32)(31)
TPH-Gal4/+
dSERT16,TPH-Gal4
dSERT16,TPH-Gal4;UAS-dSERT
I
Night sleep (min)
(25)(23)(23)
(30)(31)(31)
(30)(29)(29)
males
females
co-housed
J
Night sleep (min)
(28)(24)(24)
(31)(27)(25)
(28)(25)(24)
Baseline
Starvation
Recovery

Supplemental Figure 3