Mutation of the Drosophila melanogaster serotonin transporter dSERT impacts sleep, courtship, and feeding behaviors

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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 serotonin transporter dSERT impacts sleep, courtship, and feeding behaviors. 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 that is mimicked by feeding the SSRI citalopram. Differences in daytime versus 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 and consistent with the pleomorphic influence of serotonin on the behavior of D. melanogaster. Starvation did not overcome the sleep drive in the mutants and in male 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 survival and is evolutionarily conserved from insects to mammals [1–5]. Both the amount and architecture of sleep can influence cognition and disruption of sleep in humans is linked to neurological and psychiatric disorders [6–8]. The neuromodulator serotonin (5-hydroxytryptamine, 5-HT) acts as a key regulator of sleep, and its involvement in sleep has been known for decades [9,10]. Previous studies in vertebrates have shown that serotonin signaling can promote wakefulness, while paradoxically, others demonstrate that serotonin is required for sleep induction and maintenance [11–15]. The circuits responsible for these effects and the cellular mechanisms by which serotonin regulates sleep remain unclear.

For over two decades, D. melanogaster has been utilized as a model system to study sleep [16,17] and serotonin [18–22] as well as other neuromodulators [23,24] have been shown to play significant roles in regulating sleep duration and quality. A role for serotonin in promoting sleep in D. melanogaster has been demonstrated in part by feeding the precursor 5-hydroxytryptophan (5-HTP) [18] or by utilizing mutants for the serotonin rate-limiting, synthetic enzyme tryptophan hydroxylase (TRH) [22]. Furthermore, of the five serotonin receptors expressed in D. melanogaster, mutations in three (d5-HT1A, d5-HT2B and d5-HT7) disrupt sleep behaviors, including total sleep amount, sleep rebound, and/or sleep architecture [18,20,22]. The powerful molecular genetic tools available in the fly have allowed some of the structures and cells required for sleep to be identified [18,22,25–32]. These tools have the potential to further dissect the mechanisms by which specific serotonergic circuits regulate sleep.

Serotonin modulates a variety of other behaviors in the fly including feeding [33–38] and sexual behavior [38–43]. It is possible that some of the circuitry underlyng serotonin’s effects on other behaviors is shared by pathways that regulate sleep. Alternatively, the serotonergic pathways for different behaviors may be hierarchical or otherwise conflict with one another. These relationships remain poorly understood.

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 [44]. 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) [45]. In addition to their therapeutic effects, SSRIs can dramatically influence a variety of other physiological functions and behaviors such as eating, libido, and sleep [46–49]. 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 [11,49,50]. The specific circuits and mechanisms by which changes in SERT activity influence these behaviors remain unclear.

A relatively weak hypomorph of dSERT has been previously described [51]. 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% [51]. 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 versus nighttime sleep may be modulated via distinct serotonergic circuits.
Results

Disruption of dSERT increases sleep in D. melanogaster

To generate new mutant alleles of dSERT, we used imprecise excision of a transposable element in the line XdPd04388 (BDSC #85438). To ensure a consistent genetic background, XdPd04388 was first outcrossed into w1118, and w1118 served as the primary control for our initial assays. The proximal end of the P element in XdPd04388 is 514 bp 5’ of the predicted transcriptional start site of dSERT (Fig 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 dSERT10 and dSERT16 respectively (Fig 1A). Both dSERT10 and dSERT16 delete the first non-coding exon and the first intron of dSERT. We identified two additional lines as controls; dSERT1 contains 41 additional bases that are remnants of the former P-element insertion but does not otherwise disrupt the dSERT gene and dSERT4 with no detectable genomic alterations at the former P-element insertion site.

To determine whether expression of dSERT was disrupted in dSERT10 and dSERT16, we first used qRT-PCR to quantify mRNA expression, using w1118 as a control. The dSERT transcript was not detectable in either of the mutant alleles (Fig 1B). By contrast, the previously published mutant allele generated by insertion of a MiMIC cassette was reported to retain ~50% expression relative to wild type [51]. To confirm these results and determine whether dSERT protein was similarly reduced, we performed western blots using a previously validated antibody to dSERT [52]. Compared to w1118 controls, both dSERT10 and dSERT16 show a decrease in dSERT protein expression (Fig 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 proceeded with our behavioral analyses.

We analyzed sleep in the dSERT mutants and found that both dSERT10 and dSERT16 mutants have dramatically increased sleep compared to w1118 controls (Fig 1D). 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 (dSERT10/dSERT16) were assayed and shown to exhibit a significant increase in total sleep compared to controls (S1A–S1B Fig). Consistent with these findings, dSERT16 homozygotes also demonstrated a significant increase in total sleep when compared to heterozygous dSERT16 flies (S1C–S1D Fig). To determine how genetic background may contribute to the behavior we observed, sleep in dSERT16 was also compared to the revertant dSERT4 control, which was obtained in the same screen as the imprecise excisions and shares the same genetic background. We also assayed sleep in the parental P-element line P[XP]d04388 (S1E–S1F Fig). We did not detect any difference in sleep between w1118 and dSERT4 and only a slight difference between w1118 and the parent, with the parent showing a slight decrease in sleep compared to w1118. Although it is impossible to completely rule out subtle effects caused by genetic background, these results demonstrate disruption of dSERT is primarily responsible for the increase in overall sleep and sleep drive we observe in the dSERT mutants.

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

The sleep phenotype we observed was stronger in dSERT16 compared to dSERT10, and we therefore focused on dSERT16 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 (e.g., near the food source) or to limited
Mutation of the *Drosophila* serotonin transporter increases sleep

**A**

Intronic position of *dSERT* and *XP* alleles

**B**

Relative *dSERT* expression

**C**

Western blot analysis

**D**

Sleep patterns for control, *dSERT*^{10}, and *dSERT*^{16} genotypes
movements that do not carry the flies past the tube’s midline, we repeated the assays with mon-
itors that use 4 separate beams to detect movement [53]. Since multiple sites within the tube
are simultaneously assessed for activity, multibeam monitors provide a more sensitive assay,
and a more conservative assessment of sleep. Our results show that in both single-beam and
multibeam monitors \textit{dSERT}\textsuperscript{16} mutants exhibit a significant increase in total sleep compared to
controls (Fig 2A).

Previous studies have shown that stimulation of aminergic pathways can increase grooming
and alter other sensorimotor behaviors that might affect sleep [54–56]. We therefore tested
whether grooming or negative geotaxis were altered in the \textit{dSERT}\textsuperscript{16} mutants. We did not detect
any differences from controls in either grooming rate (S2A Fig) or performance of negative
geotaxis in \textit{dSERT}\textsuperscript{16} flies (S2B Fig). Overall, these findings indicate that the behavioral change
we detect represents increased sleep in \textit{dSERT}\textsuperscript{16} 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 \textit{dSERT}\textsuperscript{16} flies
have increased sleep during the daytime (Fig 2B). This is further characterized by an increase in
daytime bout frequency compared to controls (Fig 2C). In addition, \textit{dSERT}\textsuperscript{16} mutants displayed
reduced latency to sleep at night (Fig 2D). The analysis of nighttime sleep behavior revealed that
\textit{dSERT}\textsuperscript{16} mutants similarly exhibit dramatically increased nighttime sleep (Fig 2E) but in contrast
to their daytime behavior, we observed a significant decrease in nighttime bout frequency (Fig
2F). The probability of transitioning from an awake to a sleep state, 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 \textit{dSERT} mutants [57]. Compared to controls, both \textit{dSERT}
mutants show a significant increase in P(Doze) (S1H Fig) and a reduction in P(Wake) (S1G Fig).
Together, these data indicate that disruption of \textit{dSERT} causes a significant increase in sleep and
alters sleep architecture in both the day and night periods.

To further confirm that increased sleep is a result of disrupted \textit{dSERT} function, we tested
whether feeding adult flies an SSRI to block \textit{dSERT} activity would impact sleep. \textit{w}\textsuperscript{1118} flies fed
the SSRI citalopram showed a dose dependent increase in total sleep (Fig 3A). Consistent with
our findings with the \textit{dSERT} mutants, both daytime sleep (Fig 3C) and nighttime sleep (Fig
3D) were significantly increased in wildtype flies fed citalopram. We do not detect any further
increase in sleep in \textit{dSERT}\textsuperscript{16} mutants fed citalopram (Fig 3B) supporting the idea that citalo-
pram’s effect on sleep is mediated by inhibition of \textit{dSERT} rather than a spurious, off-target
effect. In addition, these findings indicate that an increase in adult sleep can be caused by dis-
rupting \textit{dSERT} only in the adult and not during development. However, further experiments
will be needed to determine whether additional, and possibly more subtle developmental
defects may occur in the mutants.

\textit{dSERT}\textsuperscript{16} mutants exhibit rhythmic circadian behaviors

In addition to sleep, serotonergic pathways in mammals have been implicated in the regulation
of circadian rhythmicity [58]. To investigate a possible role for \textit{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 (Fig 4A and 4B) and their rhythmic behaviors persisted in free-running constant darkness (DD) cycles (Fig 4E and 4F). The average activity of \( dSERT^{16} \) flies while awake (activity counts/time awake) did not differ from control flies in LD (Fig 4C) nor DD (Fig 4G). In addition, \( dSERT^{16} \) mutants did not exhibit a significant decrease in either morning or evening anticipation behaviors for either LD (Fig 4D) or DD (Fig 4H) cycles. Periodogram analysis confirmed that \( dSERT^{16} \) mutants behave indistinguishably from wildtype controls (Fig 4I and 4J) and quantification of free-running circadian behaviors demonstrates that all of the \( dSERT^{16} \) flies (\( \tau = 23.6 \pm 0.04 \)) were rhythmic (Fig 4K) similar to control flies (\( \tau = 23.5\pm0.01 \)). In addition, the circadian periods of \( dSERT^{16} \) mutants did not detectably differ from controls (Fig 4L). In summary, we find that loss of \( dSERT \) does not detectably disrupt circadian rhythmicity. These data are consistent with previous work showing the feeding of either the serotonin precursor 5-HTP or the SSRI fluoxetine (Prozac), or overexpression of \( d5-HT1B \) did not detectably alter circadian rhythms in free-running conditions [19].
Sex dependent effects in dSERT mutants

All of our initial experiments analyzed sleep in mated females. Males and females exhibit different sleep patterns, with males exhibiting a more pronounced daytime siesta [59,60].

Fig 3. Wildtype flies fed SSRIs exhibit increased sleep. (A) Quantification of total sleep in w^{1118} control flies fed the indicated concentrations of citalopram (0-6 mM). (B) Total sleep in w^{1118} controls (grey) and dSERT^{16} mutants (red) fed vehicle (0 mM) or citalopram (3 mM). Analysis of daytime sleep (C) versus nighttime sleep (D) for the flies tested in panel A. Graphs show individual datapoints and group means ± SEM. A,C,D one-way ANOVA; B two-way ANOVA, with Tukey post-hoc test (p < 0.0332*, p < 0.0002***, p < 0.0001****).

https://doi.org/10.1371/journal.pgen.1010289.g003

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addition, the post-mating response in females includes a decrease in daytime sleep [61]. We therefore compared the effects of dSERT on males, mated females, and virgin females (S3 Fig).

Similar to mated females, total sleep (S3A Fig), daytime sleep (S3B Fig), and nighttime sleep (S3E Fig) were significantly increased in dSERT mutant males and virgin females compared to controls. For all three groups (mated females, male and virgin females) we also detect a reduction in latency in the dSERT mutant compared to controls, and a consistent change in nighttime sleep architecture, observed as a decrease in sleep bout frequency. Interestingly, in contrast to nighttime sleep architecture, the effects of dSERT on daytime architecture differed between the three groups; mated dSERT females showed an increase in daytime bout frequency compared to controls that was not observed in either males or virgin females (S3C Fig).

Fig 4. dSERTmutant s exhibit rhythmi c circadian behaviors. (A-B) 12h:12h LD and (E-F) 12h:12h DD locomotor activity group analysis for w1118 (A,E) and dSERT16 (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 with 0900 lights-on and 2100 lights-off and each column representing the mean, binned activity for 0.5 hr, n = 25–29 flies. Small dots above each column indicate the SEM. (C,G) The average activity while awake in dSERTmutant is no different than controls in both LD (C) and DD (G) cycles, individual datapoints and group means ± SEM. Student’s t-test, two-tailed unpaired. (D, H) Calculation of morning and evening anticipation indexes for dSERTmutant and control flies in LD (D) and DD (H) cycles. (I-J) Representative periodograms derived from activity records of individual w1118 (I) and dSERT16 (J) flies in constant darkness. (K) Percentage of flies with detectable rhythmicity was calculated for controls and dSERTmutant. (L) Circadian periods in DD were averaged from rhythmic flies per each genotype, error bars indicate SEM.

https://doi.org/10.1371/journal.pgen.1010289.g004
Loss of dSERT disrupts courtship and copulation behaviors

The difference between mated and virgin females in daytime sleep architecture suggested the possibility that dSERT could influence other sex-dependent behaviors. We therefore investigated whether dSERT<sup>16</sup> mutants might show defects in courtship and/or copulation and how this would interact with sleep. Previous work demonstrated that pairing male and female flies together overnight dramatically suppresses sleep due to increased courtship activity [62–64]. Consistent with these findings, co-housing pairs of wildtype male and female flies together resulted in a significant decrease in nighttime sleep compared to sleep recorded from isolated male or female flies, or female-female pairs (Fig 5A). In contrast to the controls, we did not detect a decrease in sleep when male and female dSERT<sup>16</sup> mutants were paired compared to single mutant flies or female-female pairs (Fig 5A). The failure of male-female pairing to decrease sleep in dSERT<sup>16</sup> mutants suggest that increased sleep drive outweighs copulation drive in the mutant. These data also suggested the possibility that loss of dSERT could decrease the drive for mating activity, but because the pairs were homotypically mutant or WT these data did not distinguish between a male and/or female phenotype for dSERT.

To determine whether reduced mating activity in dSERT<sup>16</sup> mutants was a male and/or female phenotype, we paired mutant males or females with wild type flies of the opposite sex. Pairing dSERT<sup>16</sup> mutant females with control males significantly reduces sleep compared to dSERT<sup>16</sup> male-female coupling. By contrast, we did not detect a statistically significant reduction in sleep when dSERT<sup>16</sup> males were coupled with wild type control females (Fig 5B). These data suggest that dSERT<sup>16</sup> males, but not females, show defects in sexual behavior.

To more directly test whether dSERT mutant males and/or females would show a sex-phenotype, we measured copulation rates (Fig 5C). We did not observe a significant difference in copulation success in dSERT<sup>16</sup> females compared to control females when paired with wildtype males. By contrast, when dSERT<sup>16</sup> males were paired with wildtype females, none of the pairs copulated within a standard period (one hour), in marked contrast to behavior of control males, of which 95% copulated with WT flies within the same time frame (Fig 5C). Since copulation and courtship might be differentially effected in dSERT males, we also assayed courtship behavior. dSERT<sup>16</sup> males exhibited significant defects in courtship behavior including increased latencies to initiate orientation (Fig 5D) and wing song (Fig 5E).

As a further test of male sexual behavior, we measured reproductive output in wildtype females paired for 2 days with dSERT<sup>16</sup> males. Wild type females paired with dSERT<sup>16</sup> males showed a reduction in the rate of egg laying (Fig 5F) as well as an increase in the proportion of unfertilized eggs (Fig 5G). These results indicate that while dSERT<sup>16</sup> males fail to copulate within one hour, over an increased time-period mating can occur, albeit with reduced fecundity compared to controls.

Loss of dSERT reduces food intake

Previous work has demonstrated that starvation as well as mating induces sleep loss in D. melanogaster presumably because starvation increases the drive to search for food during periods that are normally occupied by sleep [65]. We therefore tested whether starvation was sufficient to suppress sleep in dSERT<sup>16</sup> mutants. Consistent with previous studies, control flies dramatically suppressed their sleep when starved on agar medium compared to their sleep on baseline or recovery days when housed with standard food (Fig 6A). Surprisingly, starvation did not have a significant effect on sleep in the dSERT<sup>16</sup> mutants (Fig 6A). We directly tested feeding in the dSERT mutants and found that after 24 hours of starvation, food uptake in dSERT<sup>16</sup> was significantly decreased compared to controls (Fig 6B–6D). Together these results indicate that loss of dSERT not only enhances sleep but also reduces food intake.
The decreased effect of starvation on sleep in dSERT mutants could result from an increased resistance to starvation or perhaps dehydration. We assayed starvation resistance by housing flies on an agar substrate and found that dSERT mutants exhibit a significantly decreased probability of survival compared to controls (Fig 6E), with the dSERT mutants reaching a 50% mortality rate approximately 15% earlier than the controls. To test the effects of dehydration/desiccation we housed flies in empty vials (devoid of food and water). In contrast to the effects of starvation, we found that dSERT mutants exhibit a slight increase in resistance to desiccation and survived ~9% longer than controls (Fig 6F).

The lack of sleep reduction seen in dSERT mutants when starved (Fig 6A) or co-housed with the opposite sex (Fig 5A) could also be due to a higher threshold for arousal. We compared the arousability of sleeping flies for dSERT16 mutants and controls by exposing them to...
5 seconds of mechanical vibration every hour during both the daytime and nighttime. We did not detect any differences in arousability between control and dSER

T mutants when they were mechanically stimulated during the day using either stimulus (S4A Fig) or during the night using the stronger stimulus (1.0 g). By contrast, dSERT mutants exhibited a significant decrease in arousability compared to controls when exposed to a less intense vibrational stimulus (0.5 g) (S4B Fig). These data suggest that at night, dSERT mutants have a somewhat elevated arousal threshold, and an increased intensity of sleep. This decrease in arousability may contribute to the lack of wakefulness seen during starvation and co-housing conditions.

Transgenic expression of dSERT in serotonergic neurons is sufficient to rescue nighttime and/or daytime sleep defects

To further confirm that defects seen in dSERT16 mutants are due to a loss of dSERT we used “genetic rescue” and expressed a wild type dSERT transgene in the dSERT16 background, focusing primarily on defects in sleep amount. Although sleep in the fly has been generally approached as a single behavior, some genes and environmental factors can preferentially affect one phase [66–69]. Regulatory sequences from the gene that encodes the rate limiting
enzyme for serotonin biosynthesis *tryptophan hydroxylase* (TRH) have been utilized to generate multiple Gal4 lines that target broad populations of serotonergic neurons [22,70–73]. We first used the broad serotonergic driver designated as “TPH-Gal4” [71] to express UAS-dSERT. Although expression of UAS-dSERT using TPH-Gal4 did not fully reverse the increase in total sleep across the entire 24-LD cycle (Fig 7A) expression of dSERT using TPH-Gal4 was sufficient to restore the nighttime sleeping pattern to near control levels (Fig 7C). By contrast, day sleep was not altered in the dSERT16 mutants by TPH-Gal4>UAS-dSERT (Fig 7B). To extend our analysis we tested another broad serotonergic driver, TRH-Gal4 [70]. While both TRH-Gal4 and TPH-Gal4 include fragments of the Tryptophan Hydroxylase gene, they have been reported to exhibit differences in expression including relatively lower expression for TRH-Gal4 in processes that innervate the mushroom bodies [38] (see also S5 Fig). Similar to TPH-GAL4, total sleep levels across the entire 24-hour day were not fully rescued using TRH-Gal4 to express UAS-dSERT in the dSERT16 background (Fig 7D). However, in contrast to TPH-GAL4, TRH-Gal4 was sufficient to restore daytime sleep levels to that of controls (Fig 7E). Moreover, unlike TPH-Gal4, TRH-Gal4 did not rescue nighttime sleep levels (Fig 7F). We next tested whether combining the serotonergic drivers could fully restore sleep levels across the 24-hour period. Our results show that genetic rescue using both TPH-Gal4 and TRH-Gal4 to express UAS-dSERT in the dSERT16 mutant is sufficient to restore sleep levels to that of the controls in both the daytime and nighttime (Fig 7G–7I). Overall, these results demonstrate that the sleep defects in dSERT16 mutants can be genetically rescued, confirming that this phenotype is due to disruption of the dSERT gene. In addition, they suggest that distinct serotonergic circuits may regulate day and night sleep.

**Discussion**

We find that dSERT functions as a modulator of sleep, with loss of dSERT causing a robust increase in both daytime and nighttime sleep. Given that dSERT acts as the primary mechanism by which serotonin is cleared from the extracellular space, it is likely that dSERT mutants have an increase in the amount of serotonin available for neurotransmission. Their behavior is consistent with phenotypes caused by other changes in serotonergic signaling: feeding the serotonin precursor 5-HTP or overexpression of the rate-limiting enzyme TRH in dopaminergic and serotonergic cells [18] (using Ddc-Gal4) increases total sleep, similar to dSERT mutants. Conversely, a decrease in total sleep duration is exhibited by TRH mutants [22].

A role for serotonin has also been suggested to contribute to male courtship and mating based in part on studies of the gene *fruitless* (*fru*) [41,74]. Male specific FruM-positive abdominal-ganglionic neurons that innervate the reproductive organs have been shown to be serotonergic and fru mutants demonstrate defects in courting and mating events [41,74]. Furthermore, activation of serotonergic neurons causes defects in male copulation behavior [38]. Conversely, in females another study showed that knockout of TRH or silencing serotonergic neurons causes a reduction in virgin receptivity [43]. Our data are consistent with previous findings on the effects of serotonin on male sexual behavior [38]. In females, the decrease in receptivity was observed in flies with reduced serotonergic signaling [43], whereas loss of dSERT would predicted to increase extracellular serotonin and thereby increase serotonergic signaling. It is conceivable that dSERT females could more receptive than WT but we have not yet tested this possibility.

Previous studies have also addressed the complex relationship between increased serotonin signaling and feeding behavior in *D. melanogaster*. The use of either TPH-Gal4 or TRH-Gal4 to thermogenetically depolarize a broad population of serotonergic cells can significantly decrease food intake [33,38]. By contrast, the activation of a specific subset of serotonergic...
Fig 7. Transgenic expression of dSERT with both “TPH-Gal4” and TRH-Gal4 is sufficient to rescue increased sleep in dSERT mutant flies. (A-C) Quantification of total sleep (A), daytime sleep (B) and nighttime sleep (C) in control w^{1118};TPH-Gal4 (grey), w^{1118};TPH-Gal4, dSERT (red), and w^{1118};TPH-Gal4, dSERT; UAS-dSERT (green) flies. (D-F) Quantification of total sleep (D), daytime sleep (E), and nighttime sleep (F) in w^{1118};TRH-Gal4 (grey), w^{1118};dSERT; TRH-Gal4 (red), and w^{1118};dSERT; TRH-Gal4/UAS-dSERT (green) flies. (G-I) Quantification of total...
cells drives a contradictory behavior and induces feeding in sated flies [33]. The finding that broad activation of serotonergic cells reduces feeding is consistent with our data showing that globally expressed \textit{dSERT} mutants exhibit a significant decrease in feeding behavior. It will be interesting to test whether cell-specific knock-down of \textit{dSERT} could mimic the effects of activating a subset of serotonergic neurons [33,38].

Serotonin has also been implicated in the physiological functions of both the gut and the Malpighian tubules, the fly equivalent of mammalian kidneys [35,75–78]. For example, exogenous injection of serotonin into either the brain or the abdomen alters crop contractility [75]. These effects could potentially play a role in the differences in feeding behavior we observe in \textit{dSERT} mutants, their sensitivity to starvation and/or their modest resistance to desiccation. Similar to \textit{dSERT}, knockdown of either \textit{5-HT1A} [37] or \textit{5-HT1B} [35] can decrease resistance to starvation but increase resistance to desiccation. Further genetic experiments will be needed to determine whether these effects occur via independent pathways. We note that \textit{5-HT1A} and \textit{5-HT1B} are expressed in serotonergic neurons as well as post-synaptic neurons [79] and thus may function as autoreceptors. If so, knockdown of the receptors in pre- versus post-synaptic cells would be likely to have different effects and could appear similar or opposite to \textit{dSERT} phenotypes respectively.

Our work demonstrates that the increase in overall sleep in \textit{dSERT} \textit{16} mutants is mediated by at least two changes in sleep architecture that differ between daytime versus nighttime periods. During the nighttime, the increase in sleep in \textit{dSERT} \textit{16} flies is characterized by significantly decreased bout number (consolidated sleep) whereas during daytime, sleep bout number is increased. In addition, mated \textit{dSERT} females showed an increase in bout frequency compared to controls but only during the daytime and this effect was not observed in males or virgin females. Sex-peptide also induces changes in daytime sleep in mated females [61] and it is possible that sex-peptide and the post-mating response could modify the effects of \textit{dSERT}.

The differential rescue of nighttime or daytime sleep via \textit{TPH-Gal4} or \textit{TRH-Gal4} respectively, supports the idea that different serotonergic circuits may regulate nighttime versus daytime sleep. Previous work has shown that knockdown of \textit{TRH} in the dorsal paired median (DPM) neurons that innervate the MB causes a decrease in nighttime sleep [21]. While the effects on daytime sleep were not clear, the \textit{d5-HT1A} receptor mutant exhibited a clear decrease in nighttime sleep, nighttime bout length, and an increase in nighttime bout number, and all of these behaviors can be genetically rescued via expression of a \textit{d5-HT1A} transgene in the MBs [18]. \textit{TPH-Gal4} but not \textit{TRH-Gal4} driven expression of \textit{UAS-dSERT} rescued the nighttime sleep phenotype of \textit{dSERT} \textit{16}, and \textit{TPH-GAL4} may show higher expression in the MBs than the \textit{TRH-Gal4} driver used in our study [20](S5A–S5B Fig). We speculate that the increased and consolidated nighttime sleep seen in \textit{dSERT} \textit{16} may be caused by an increase in extracellular serotonin in the MBs and a resultant increase in the activation of \textit{5-HT1A} in the MBs; however, further experiments will be needed to directly test this hypothesis.

We also speculate that some aspects of the daytime \textit{dSERT} sleep phenotype, such as the increased frequency of daytime sleep bouts, may be mediated by the ellipsoid body and the \textit{d5-HT7} receptor. Activation of EB neurons leads to an increase in the number of sleep episodes specifically during the daytime [20]. \textit{d5-HT7} mutants and treatment with a \textit{5-HT7} antagonist similarly causes a reduction in sleep episode frequency [20]. However, it is likely that the
daytime dSERT sleep phenotype is also dependent on other circuits and/or receptors, since dSERT16 exhibits an increase in sleep amount that was not detected in the d5-HT7 mutant [20].

Serotonin has been previously shown to affect development in both mammals (80,81) and D. melanogaster [82–91]. Interestingly, patients with mutations in the autism risk gene CHD8 show defects in sleep initiation and maintenance, and mutation of the D. melanogaster ortholog kismet disrupts sleep architecture and increases serotonin labeling of the gut during development [92]. The hyper serotonergic state observed during development appeared to decrease rather than increase sleep [92] in contrast to most of the reported effects of serotonin in the adult fly. It is possible that developmental effects of dSERT influence the adult sleep phenotype, and in future experiments we will use genetic rescue during development versus adulthood to explore this idea. For now, we emphasize that we observe an increase in sleep comparable to the phenotype seen in dSERT16 following administration of the SSRI citalopram to wildtype adults. Occlusion of the drug’s effects on sleep by the dSERT16 mutant confirm that they were caused by inhibition of dSERT rather than a non-specific target (Fig 3B). These data indicate that inhibition of dSERT in the adult is sufficient to cause at least some aspects of the sleep phenotype, but do not rule out the possibility of additional, or even contrary, developmental effects.

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 [93–95], while an increase in serotonin levels caused by a SERT KO mouse has the opposite effects and increases REM [96,97]. Knockouts of either 5-HT1B or 5-HT1A also exhibit more REM [98,99], but the increase in REM caused by the SERT KO can be reduced by developmental blockade of 5-HT1A [97].

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 [100] that is linked to the efficacy of SSRIs [101] and sleep deprivation [102]. 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 [103–106], and sexual dysfunction is a well-documented side effect of SSRI treatment [46,47]. Our work begins to address the competition between the pathways that regulate these effects in the context of D. melanogaster sleep, as we demonstrate that loss of dSERT induces an increased sleep drive that appears to outweigh the drive for both normal feeding and mating behaviors (Figs 5–6). We speculate that further studies of dSERT mutants may help to uncover the relationship between other serotonergic pathways that regulate 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 [70,107–111].

**Materials & methods**

**Fly strains**

Fly stocks were reared on standard cornmeal media (per 1 L H2O: 12 g agar, 29 g Red Star yeast, 71 g cornmeal, 92 g molasses, 16 mL methyl paraben 10% in EtOH, 10 mL propionic acid 50% in H2O) at 25°C with 60% relative humidity and entrained to a daily 12 h light,12 h dark schedule (12hr:12hr LD). w1118 flies were obtained from Dr. Henrike Scholz. UAS-dSERT (BL24464) and UAS-MCD8::GFP (BL32185) were obtained from the Bloomington Drosophila Stock Center. TPH-Gal4 was a gift from Dr. Jongkyeong Chung [71] and TRH-Gal4 was a gift from Dr. Edward Kravitz [70].
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 generations to the w1118 line of the Scholz lab to isogenize the genetic background. Second, females of the P[XP]d04388 line were crossed to w1118; CyO/+; {Δ2–3; Ki}/TM2 [Δ2–3; Ki]/+ males. Next, w1118; P[XP]d04388/CyO females 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 chromosome. 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 male flies was isolated using Trizol 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 to the manufacturer’s instructions. For qRT-PCR analysis, the MESA BLUE qPCR MasterMix Plus for SYBR Assay (Eurogentec) with 100 ng cDNA as template was used. Detection was performed using the iCycler iQ5 Multicolour Real-Time PCR Detection System. The data were analyzed using the ΔΔCt method [112]. The NormFinder software [113] was used for control primer 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’-GGTGCCTCACGATCTGGAAG-3’ and 5’-CAGCCGATATCGTGTTGTA-3’. Data are shown as fold changes in dSERT relative to the controls.

Western blot analysis

To isolate proteins of fly heads (male and female), 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 followed by centrifugation at 18300 x g at 4˚C for 15 min. The pellet, containing larger membranous particles and the bulk of dSERT 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 the membrane extract. 20 μg protein of each sample were used for western blotting according to standard procedures. The membrane was blocked in 5% milk and the primary rabbit anti-dSERT antibody (Giang et al., 2011) was used at a dilution of 1: 20 000 with a mouse anti-β-Actin (mAbcam 8224) at 1:10 000 used as a loading control.

Behavioral analysis

Sleep: Sleep was measured as previously described [114]. In brief, 3–5-day old mated female flies (unless otherwise stated) were individually loaded into 65 mm 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. The
sleep duration and architecture were quantified for both daytime (lights on) and nighttime (lights off), Zeitgeber hours 0 to 12, and 13 to 24, respectively. DAM2s were used for single beam monitors and DAM5Ms were used for multibeam monitors (4 independent beams per tube with 9.1 mm adjacent beam spacing). Custom Visual Basic scripts \[114\] in Microsoft Excel or SCAMP analysis scripts in MATLAB were used to analyze Trikinetics activity records for sleep behaviors.

Arousal thresholds were assayed by fastening Trikinetics activity monitors to microplate adapters on vortexers (VWR). 0.5 g or 1 g of force was used to stimulate flies for 5 s every hour for 24 hours. The unit g is used to express vibration amplitude with 1 g equal to the gravitational constant. Experiments were performed twice, and arousal rates were averaged together.

**SSRI feeding and sleep**

For sleep experiments with SSRIs, flies were loaded into tubes that either contained vehicle or varying concentrations of citalopram hydrobromide (Sigma PHR1640) in vehicle (5% sucrose, 1% agar, and 1% blue food dye in dH\(_2\)O) and allowed to feed for 20 hours before sleep was analyzed. Blue food dye in vehicle was used to ensure that each fly fed during the initial 20 hours.

**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 flies were loaded individually into DAMs tubes and baseline sleep was recorded for 2 days. On the experimental day at ZT0 flies were combined into male-female or female-female pairs (either same genotype or mixed as specified in the text) in new DAMS tubes and sleep was recorded for 24 hours.

**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 2 min scoring period was initiated by the observation of a grooming event.

**Negative geotaxis**

For negative geotaxis experiments, 3 groups of 10 flies 3-5-days old were placed in two empty 28.5 x 95 mm polystyrene vials fastened together with their open ends facing each other to form a continuous 190 mm space. The long axis of the joined tubes was oriented vertically above a lab bench. After gently tapping the vials on the bench, the flies were allowed to climb for 10 s. Each group was scored for the number of flies that climbed above the 8 cm mark (measured from the bottom of the tube facing the lab bench) compared to those remaining
below the 8 cm mark. Each group was tested 5 times (with one minute recovery between each trial), and the average for the five trials was quantified and used for analysis.

**Locomotor activity and circadian rhythmicity**

Experiments were performed with 3-5-day old females in *D. melanogaster* 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 $\chi^2$ periodogram analysis with the following criteria: power $\geq 20$, width $\geq 2$ h with selection of 24 hr $\pm$ 8 hr. Power and width are the height and width of the periodogram peak, respectively, and give the significance of the calculated period. The average activity while awake (number of activity counts/time awake $\pm$ SEM) was calculated over 3-day period in LD or DD conditions. Morning anticipation index was calculated as (activity for 3 hrs before lights-on)/ (activity for 6 hrs before lights-on) and evening anticipation index as (activity for 3 hrs before lights-off)/(activity for 6 hrs before lights-off).

**Courtship & copulation assays**

Virgin females were aged 3-5-days in groups of ~10 and naïve males were aged individually for 4 days. Single pairs of female and male flies were gently aspirated into a chamber of a custom acrylic device 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 including orientation and wing extension. Analysis of courtship behavior was scored in blind.

**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 using a dissecting microscope.

**Feeding assays**

Groups of 10 flies 3-5-days old were starved for 24 hours on 1% agar medium as a water source. After starvation, flies were transferred to blue food (10% sucrose, 5% active yeast, 1% agar, and 4% blue food dye (McCormick) and allowed to feed for 15 mins. The amount of blue food dye ingested was measured using visual inspecting feeding scores or spectrophotometry of blue dye as previously described [33]. For visual inspection, each fly was scored with 0 defined as no dye visible in the abdomen, 1 as dye is just visible as a small spot in the crop, 2 as crop with dye extends 1/3-2/3 the length of the abdomen and 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. For spectrophotometric quantification of blue dye, 10 flies were homogenized in 400 µl PBS and centrifuged (14,000 rpm for 3 min at ambient temperature). 250 µl of the supernatant was aspirated into a fresh tube, avoiding the pelleted debris and re-centrifuged (speed, 3 min, temperature). 200 µl of the supernatant was loaded into a 96 well microplate for absorbance readings (Biotek Synergy 2 Multi-Mode Plate Reader). The corrected absorbance of the dye was calculated by subtracting the absorbance at 750 nm (outside of blue dye spectrum) from the absorbance at 630 nm (peak of blue dye) as previously described [33].
Desiccation and starvation assay
3–day old females were isolated and groups of ten were placed in empty vials (for desiccation) or vials containing 1% agar substrate in dH₂O (for starvation). Flies were visually inspected every 6 hours and surviving flies were manually counted. The assay was designated to be finished when the last fly of each genotype was dead. Data is shown as percentage of live flies over time. For both experiments 10 groups of 10 flies were assayed for each genotype.

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 X-100, 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, and followed by Tukey’s post-hoc 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.

Supporting information
S1 Fig. Genetic controls, P(doze) and P(wake). Hourly sleep traces (A) and quantification of total sleep (B) in w¹¹¹⁸ controls (grey), transheterozygous dSERT¹⁰/dSERT¹⁶ mutants (yellow), homozygous dSERT¹⁶ mutants (orange), and homozygous dSERT¹⁶ mutants (red). Hourly sleep traces (C) and quantification of total sleep (D) in w¹¹¹⁸ controls (grey), dSERT¹⁶ heterozygotes (purple), and dSERT¹⁶ homozygous mutants (red). Hourly sleep traces (E) and quantification of total sleep (F) in w¹¹¹⁸ controls (grey), dSERT¹⁶ revertant (light blue), d04388 parental, P element line (teal), and dSERT¹⁶ mutants (red). (G-H) Quantification of P(Wake) (G) and P(Doze) (H) during the light period (LP, Zeitgeber hours 1–12) and dark period (DP, Zeitgeber hours 13–24) in w¹¹¹⁸ controls (grey), dSERT¹⁰ mutants (orange), and dSERT¹⁶ mutants (red). For all panels, sleep traces show mean ± SEM and histograms show both individual datapoints and group means ± SEM. One way ANOVA with Tukey post-hoc test (p ≤ 0.0332*, p ≤ 0.0021**, p ≤ 0.0002***, p ≤ 0.0001****). LP and DP were analyzed separately in G, H.

(PDF)

S2 Fig. dSERT¹⁶ mutant sleep phenotype is not an artifact of additional amine-linked behaviors. (A) dSERT¹⁶ mutants (purple) show no change in grooming behavior compared to w¹¹¹⁸ controls (grey). The average number of grooming events per minute for three separate 2 min periods with three experimental replicates is shown. In each experimental replicate n = 5 flies for each genotype. Mean± SEM, unpaired Student’s t-test. (B) Male and female dSERT¹⁶ mutants behave indistinguishably from control flies in negative geotaxis assays. Mean ± SEM,
one-way ANOVA.

S3 Fig. Sleep in dSERT mutants of different genders and mating status. (A) Quantification of total sleep in w[1118] and dSERT[16] males (blue), virgin females (pink), or mated females (red). Analysis of daytime sleep (B) and daytime bout frequency (C) and latency (D). Quantification of nighttime sleep (E) and nighttime bout number (F). Graphs show individual datapoints and group means ± SEM. Two-way ANOVA, with Tukey post-hoc test (p < 0.0332, p < 0.0002, p < 0.0001).

S4 Fig. Arousal threshold of dSERT[16] flies. Proportion of w[1118] (black) and dSERT[16] (red) flies awakened after 5 seconds of either 0.5 g or 1 g vibrational stimulation during the daytime (A) or nighttime (B) (n = 24 trials per groups, n = 30–32 flies per group). Mean ± SEM, Two-way ANOVA with Tukey post-hoc test (≤ 0.0021).

S5 Fig. (A-B) Representative pictures show expression of UAS-MCD8::GFP (green) driven by TRH-Gal4 (A) or “TPH”-Gal4 (B) and labeled with an antibody to DLG (magenta) in mushroom bodies. Mushroom body lobes are labeled with white text.

S1 Data. Raw data.

Acknowledgments
We thank Drs Jongkyeong Chung and Edward Kravitz for sharing fly lines. We thank Dr. Cezar Nave and Prabhjit Singh in J.D.’s laboratory for technical support and discussion. We thank Dr. Tim Lebestky and Mikhayla Armstrong for their early contributions to the analysis of the dSERT mutants.

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References


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


55. PLoS Genetics | https://doi.org/10.1371/journal.pgen.1010289 November 21, 2022 23 / 26


111. Dierick HA, Greenspan RJ. Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. Nat Genet. 2007 May; 39(5):678–82. https://doi.org/10.1038/ng2029 PMID: 17450142

