Isoflurane Exposure Rescues Short-term Learning and Memory in Sleep-Disturbed *Drosophila melanogaster*.

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Sleep is known to play an important role in cognition, learning and memory. As *Drosophila melanogaster* have stable circadian rhythms and behavioral states similar to those of human sleep, they have been a useful model to investigate the effects of sleep on learning and memory. General anesthesia has been shown to cause cognitive impairments in humans. However, anesthesia also induces a behavioral state similar to sleep and may activate sleep pathways. This study examined learning and memory after an acute exposure of isoflurane in a *Drosophila* mutant model of restless leg syndrome. There were two possible outcomes: isoflurane (an anesthetic) could have impaired cognitive functioning or enhanced learning and memory by activating sleep pathways. Given the acute cognitive impairments often observed postoperatively, we believed the former outcome to be the most likely. Flies with fragmented sleep had impaired performance on an aversive phototaxic suppression learning and memory task compared to wildtype flies. This deficit was rescued with isoflurane exposure, as no differences in learning were observed between mutant and wildtype flies treated with anesthesia. This result suggests that anesthesia exposure can ameliorate impaired learning and memory due to sleep fragmentation. Further investigations are required to determine the type of memory impacted by anesthesia and the mechanisms by which anesthesia induces this effect.

Keywords: Anesthesia; Aversive Phototaxic Suppression; Isoflurane; Learning and Memory; Restless Leg Syndrome; Sleep Disorders;

Abbreviations: Electroencephalogram (EEG); Non-Rapid Eye Movement (NREM); Restless Leg Syndrome (RLS); Rapid Eye Movement (REM); Wanderlust mutants (WLST); Wildtype (WT)

Introduction

Sleep is beneficial for cognition and has been implicated in learning and memory (Brewster et al., 2015; Diekelmann et al., 2009; Schönauer et al., 2015; Stickgold, 2005). Studies have consistently shown that sleep, but not wakeful rest, after a learning task improves performance in subsequent trials (Backhaus et al., 2008; Wamsley et al., 2010). Furthermore, disruption or deprivation of sleep has been shown to impair cognition and memory in humans as well as in several animal models (de Bruin et al., 2017; Graves et al., 2003; Hagewoud et al., 2010, 2011; Kumar and Jha, 2012). Together, these findings demonstrate an integral role for sleep in memory consolidation that positively impacts cognition.

Mechanisms of sleep, learning, and memory have been extensively studied in *Drosophila melanogaster* (Dissel et al., 2015; Shaw et al., 2000). Like humans, *Drosophila* have stable circadian rhythms and activity patterns (Bushey and Cirelli, 2011). They are exceptionally active during the day with instances of short intermittent periods of rest. In darkness, they remain immobile as they sleep (van Alphen et al., 2013; Bushey and Cirelli, 2011).

There are several additional parallels between humans and fruit flies that make *Drosophila* useful models for the study of sleep, learning and memory. Importantly, the basic cellular and molecular mechanisms of sleep are highly conserved evolutionarily despite the gross anatomical discrepancies between the human and fruit fly brain (Hendricks et al., 2000; Kottler et al., 2013; Thimgan et al., 2013; Zimmerman et al., 2008). Several strains of *Drosophila* have been engineered to model various human sleep disorders, thus providing a tool to study the genetic and mechanistic underpinnings of these human disorders (Freeman et al., 2012). Furthermore, *Drosophila* exhibit cognitive decline following sleep deprivation, making them an especially useful model for studying the effects of sleep on learning and memory (Li et al., 2009).

Similarities in the behavioral states characteristic of sleep and anesthesia have prompted study analyzing their underlying neural signatures and mechanisms (Brown et al, 2010). Like sleep, anesthesia induces an unconscious state associated with temporary paralysis, analgesia, and a lack of responsiveness to external stimuli, and as a result, is commonly used in clinical procedures (Brown et al., 2010, 2011; Mashour, 2011; Mashour and Pal, 2012; Tung et al., 2002). However, unlike sleep, it is not a readily reversible state of consciousness (Brown et al., 2010, 2011). Natural sleep induces a fluctuation between two states of brain activity: (1) Rapid Eye Movement (REM) with high frequency, low amplitude electroencephalogram (EEG) signatures, and (2) Non-REM (NREM) with three distinct EEG patterns throughout, most notably a period of high amplitude, low frequency activity (Brown et al, 2010). The importance of REM sleep in learning and cognition is noteworthy; interestingly, increases in REM sleep duration following a learning experience precede improvement on a trained cognitive task (Lucero, 1970). However, individuals under anesthesia appear to have similar brain activity to that of NREM sleep, as induction of general anesthesia also causes an increase in high amplitude, low frequency brain activity in EEG recordings (Brown et al., 2010). Additionally, the similar suppression of excitatory pathways and activation of inhibitory pathways observed at the onset of sleep occurs upon treatment with anesthesia (Mashour and Pal, 2012). Specifically, anesthesia-induced unconsciousness is facilitated by increasing the inhibitory effects of gamma-aminobutyric acid (GABA) and decreasing the excitatory effects of neurotransmitters in the cortex, thalamus and brainstem (Brown et al., 2010). Since many forms of anesthesia, including the GABA agonists propofol, sevoflurane, and isoflurane, target neurons in the arousal system of the brain stem, anesthesia induction can be likened to the activation of neural pathways associated with sleep onset (Brown et al., 2010, 2011; Kottler et al., 2013; Leung et al., 2014; van Swinderen and Kottler, 2014). As both sleep and anesthesia-induced unconsciousness are thought to share underlying neural pathways, anesthesia, like sleep, may influence learning and memory.

Few studies have compared the effects of anesthesia and sleep on learning and memory. Anesthesia is known to cause postoperative cognitive deficits in humans including memory disturbances, the risk and duration of which are increased in elderly patient populations (Qiao et al., 2015; Rörtgen et al., 2010; Rundshagen, 2014; Wang et al., 2014; Zhang et al., 2014, 2016). Interestingly, research suggests a connection between sleep and cognitive deficits following anesthesia. When treated with melatonin, a hormone that promotes sleep onset, mice experience notably reduced cognitive impairments on learning and memory tasks following administration of propofol (Xia et al., 2016; Yang et al., 2014). Individuals with sleep disorders are known to experience atypical postoperative effects following treatment with propofol and sevoflurane; such anomalies may include prolonged recovery time from general anesthesia and postoperative hypersomnia. However, the cognitive effects of anesthesia administration on individuals with sleep disorders have not yet been examined (Burrow et al., 2005; Mesa et al., 2000; Morimoto et al., 2011). While anesthesia induction is associated with cognitive induction, it is also thought to activate sleep pathways, and in this way may aid learning and memory processes in sleep-disturbed individuals. However, further research is necessary to explore such possible outcomes.

Here, we investigated the effects of the inhalation anesthetic isoflurane on learning and memory in a *Drosophila* model of restless leg syndrome in order to better understand the effects of anesthesia on cognition in a sleep-disturbed population (RLS; Freeman et al., 2012). Here, isoflurane was selected as the anesthetic of choice due to its common use in animal studies, potentially introducing additional behavioral variables in studies of memory or cognition, which warrants further investigation. Furthermore, as RLS mutant flies are thought to have sleep fragmentation due to increased motor activity, they are an ideal strain to assess the impacts of isoflurane on disordered sleep. This study examined whether acute exposure to isoflurane differentially impacts learning in flies with disordered sleep. This was accomplished using a learned aversive phototaxic suppression task, which has been shown to demonstrate a learned association while eliminating the potential confounding effects of habituation and sensitization (Seugnet et al., 2009; Ali et al., 2011). It is possible that exposure to isoflurane impairs short-term memory due to the cognitive impairments associated with anesthetization, and that this effect will be especially pronounced in mutant flies given their predisposition to fragmented sleep. However, it is alternatively possible that as isoflurane activates pathways associated with sleep onset, RLS mutants may have improved task performance following anesthetization given the cognitive benefits of sleep.

Material and Methods

Flies

Experiments were conducted with wild type (WT) and genetically matched Wanderlust (WLST, dBTBD7) mutant *Drosophila melanogaster* strains provided by Dr. A. Freeman (Emory University). Wanderlust mutants lack the *D. melanogaster* gene CG1826, analogous to the human gene dBTBD9. Importantly, the dBTBD9 genotype designates loss of the fly gene CG1826 (Freeman et al., 2012). In humans, a polymorphism in this gene on chromosome 6 is correlated with an increased risk for RLS (Freeman et al., 2012). Flies were cultured in vials with Formula 4-24 Instant Drosophila Medium (Carolina, Burlington NC) on a 12/12-hour light/dark cycle. Like humans, flies predominantly exhibit activity during the day and rest during the night; therefore, a 12/12 light/dark cycle is commonly used in *Drosophila* research to reflect their natural circadian rhythm (Shaw et al., 2000). Temperature and humidity were maintained at 24˚C and 20% relative humidity. Flies were transferred to fresh vials every three days.

Drosophila Sleep Activity Monitoring

Activity of WT and WLST flies (N = 30 per group, age 4-7 days old) were recorded using Drosophila Activity Monitor and DAMSystem3038 software (Trikinetics, Waltham, MA). A separate cohort of flies was used for sleep activity monitoring, which did not undergo anesthesia. To observe circadian rhythm and sleep/wake cycles, activity was recorded for one full day with a 12 hour light/dark schedule with light on at 6PM. Flies were placed in activity tubes with agar/sucrose food and were allowed 30 minutes of habituation prior to the start of monitoring (Chiu et al., 2010). An infrared beam passing through the center of each tube would detect each time the fly moved across it. Each time the fly crossed through the beam indicated an instance of fly activity (Pfeiffenberger et al., 2010).

Sleep Data Analysis

Activity recorded from a 24-hour period was analyzed in one-minute bins (giving a total of 1440 data points per fly). The number of sleep bouts was counted during both light and dark phases. A sleep bout was defined as a period of inactivity lasting five minutes or longer (van Alphen et al., 2013). The total sleep time per phase was calculated using the free running period tool by measuring the length of each sleep bout. The average length of sleep bout was then calculated from these two metrics. All sleep monitoring analysis was conducted using ActogramJ (Schmid et al., 2011).

Isoflurane Exposure

Awake flies were transferred from home culture vials to a 150mL empty house-made anesthesia chamber. A 1.5% isoflurane/oxygen anesthetic gas mixture (isoflurane provided by Patterson Veterinary Supply) was delivered to the chamber through plastic tubing at a flow rate of 0.4 L/min. All flies fell to the bottom of the chamber and were immobile within 6 minutes. Anesthesia was delivered continuously for two hours after the flies fell to the bottom of the chamber. Flies were then immediately transferred to individual ventilated 15mL centrifuge tubes. A recovery period of 1.24 hours (± 0.04 SEM) preceded introduction to the aversive phototaxic suppression task.

Aversive Phototaxic Suppression Task

Female WLST mutants and WT (N=20 per group, age 4-6 days old) completed an aversive phototaxic suppression paradigm testing short-term memory acquisition and recall (Ali et al., 2011; Le Bourg, 2004; Le Bourg and Buecher, 2002). Only females were used because the genetic mutation in WLST flies lies on the X chromosome, thus making the mutant phenotype stronger in females (A. Freeman, personal communication).

The task apparatus consisted of two 15mL centrifuge tubes, one dark and one lit with a fiber optic light, which were connected through a custom-made passageway (Figure 1). Flies were housed in the dark chamber, and a barricade in the passageway was intermittently removed to give flies access to the lit chamber (Ali et al., 2011). Only photopositive flies that entered the lit chamber when initially given access to it were used for the task, and all other flies were discarded.

Filter paper soaked with a 1μM quinine solution was employed as an aversive stimulus in the lit chamber to encourage suppression of phototaxic tendencies. The learning phase of the task consisted of ten one-minute-long trials, allowing flies to associate the quinine solution with light. Learning trials were immediately followed by five ten-second-long test trials in which the chamber containing quinine was replaced with a clean chamber. The goal of this task was for the fly to learn to associate the light with the quinine, an aversive odor frequently used for such conditioning paradigms (Ali et al., 2011). If this were accomplished, the fly would then avoid the lit chamber during the test trials even though the unpleasant scent was removed. An entrance into the lit chamber within the ten-second test period was considered a failed trial. Error rates for each fly were calculated based on the proportion of failed trials out of total test trials (i.e. an error rate of 0.6 corresponds to three failed trials out of five total test trials).

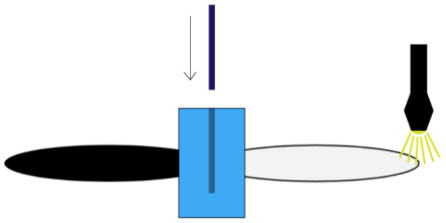

Figure 1: Aversive phototaxic suppression task apparatus. Two 15mL centrifuge tubes, one lit with a fiberoptic cable and one dark, are connected by a custom-made passageway (light blue). Flies were housed in the dark chamber (left) and intermittently allowed access to the lit chamber (right) by inserting or removing a barrier (dark blue). Learning trials were conducted with photopositive flies, as they were given access to the lit chamber in which filter paper soaked in an aversive quinine solution was placed. Flies underwent ten one-minute learning trials, followed by five ten-second test trials in the absence of the aversive stimulus to test their memory (Ali et al., 2011).

Statistics

Statistical analysis was conducted using a two-way ANOVA and a Student's t-test. Outliers were removed from data sets using Grubbs' tests. In all cases, $\alpha = 0.05$ was considered statistically significant. Statistical analysis was conducted using PRISM GraphPad statistical software.

Results

Sleep

Activity of WT and WLST flies was monitored over 24 hours in order to confirm WLST sleep disturbances (Figure 2A). The total time spent sleeping during each phase of the light cycle was measured, and a main effect of light phase was found (two-way ANOVA, F (1,

114) = 152.4, $p \leq .0001$, Figure 2B) such that both WT and WLST flies spent more time sleeping during the dark phase than during the light phase. However, no main effect of fly type and no interaction between the two factors were observed. Upon further investigation, no differences were identified between the total sleep of WT and WLST flies in either dark or light phases.

The number of sleep bouts during both phases of the light cycle was examined, and a main effect of light phase (two-way ANOVA, F (1, 116) = 8.56, p = .004), as well as an interaction between fly type and light phase (two-way ANOVA, F (1, 116) = 7.85, p = .006), was found (Figure 2C). Further examination revealed a difference between WT and WLST in number of sleep bouts such that WLST flies had fewer periods of sleep during the light phase (Student's t-test, t = 2.739, df = 58, p = .008), thus confirming disturbances in WLST sleep patterns.

To determine whether WT and WLST have different lengths of sleep bouts, the average length of sleep bout during the light and dark phase was calculated for each fly (Figure 2D). A main effect of light phase (two-way ANOVA, F (1, 112) = 32.12, $p \leq .001$), but not of fly type, was identified. Additionally, an interaction between fly type and light phase was found (two-way ANOVA, F (1, 112)= 6.34, p = .01) such that WT flies were found to have a longer average sleep length than WLST during the dark phase (Student's t-test, t = 2.095, df = 57, $p < .001$). These results suggest that mutant flies sleep for shorter periods during the dark period, which is indicative of fragmented sleep.

Learning and Memory

An aversive phototaxic suppression paradigm was employed in order to examine the effects of isoflurane treatment on cognition in flies with fragmented sleep (Figure 3). Female WT and WLST flies, either treated with isoflurane or untreated (control), were evaluated on their short-term memory performance. While no main effect was found for isoflurane

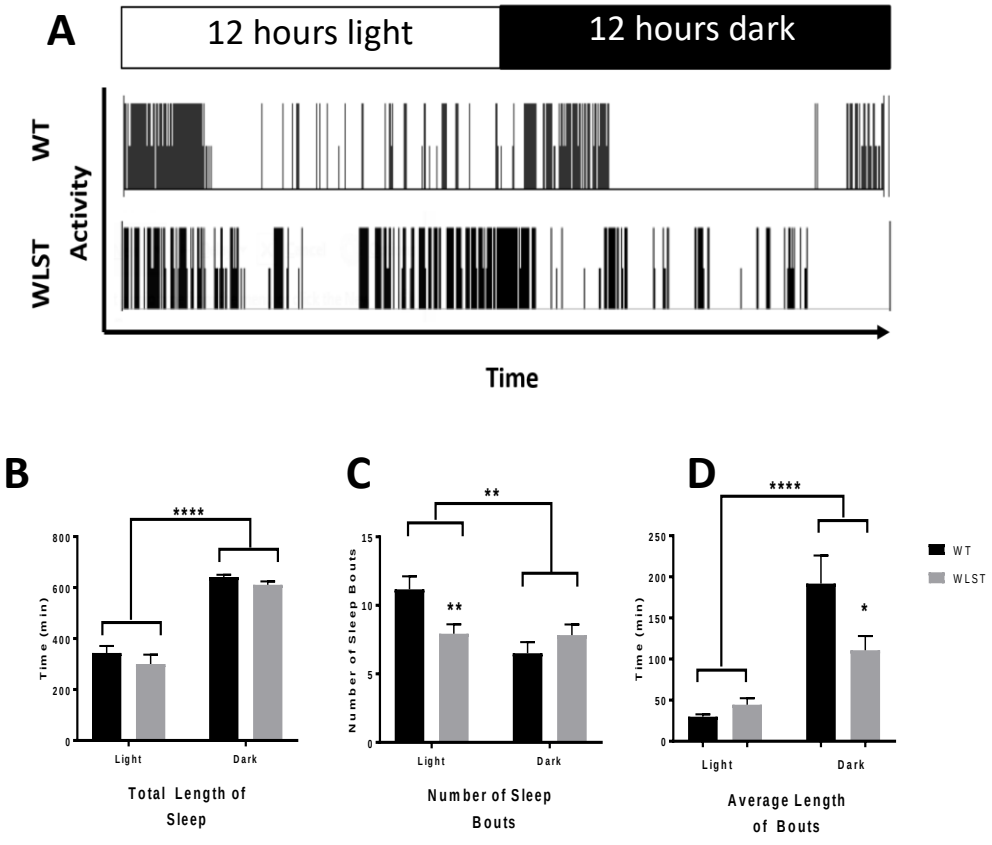

Figure 2: WLST mutant *Drosophila melanogaster* show sleep disturbances.
A. Representative actograms of activity for one WT and one WLST fly over the course of 24 hours under a 12-hour light/dark schedule. B. No difference was found between WT and WLST flies in total sleep time. A main effect of light phase was found, such that both WT and WLST flies slept less during the light cycle (two-way ANOVA, F (1, 114) = 152.4, $p \leq .0001$). C. Both WT and WLST flies had more bouts of sleep during the light cycle (two-way ANOVA, F (1, 116) = 8.56, p = .004). WLST flies had fewer sleep bouts during the light phase compares to WTs (independent Student's t-test, t = 2.739, df = 58, p = .008). D. A main effect of light phase was observed (two-way ANOVA, F (1, 112) = 32.68, $p \leq .001$), such that WT and WLST flies had shorter sleep bouts during the light phase. Additionally, WLST flies had shorter sleep bouts during the dark phase compared to WTs (independent Student's t-test, t = 2.095, df = 57, p = .04), suggesting sleep disturbances. N = 30 per group. Mean ± SEM shown. * $p \leq .05$, ** $p \leq .01$, **** $p \leq .0001$.

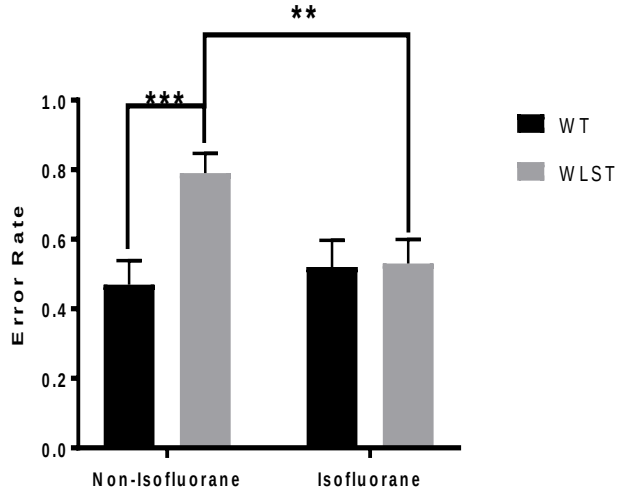

Figure 3: Isoflurane exposure rescues learning and memory functionality in sleep-deprived WLST. Female WLST and WT flies, either treated with isoflurane or untreated, were subjected to an aversive phototaxic suppression task, and their error rates were tabulation as the proportion of trials failed. A main effect was found for fly type (two-way ANOVA, $F(1, 76) = 5.784$, $p = .019$). An interaction was found between fly type and anesthesia treatment (two-way ANOVA, $F(1, 76) = 5.015$, $p = .026$). WLST flies not treated with isoflurane had a higher error rate than their WT counterparts (independent Student's t-test, $t = 3.59$, $df = 38$, $p = .0065$) and equally as well as the WTs of either anesthetic treatment. $N = 20$ per group. Mean ± SEM shown. ** $p \leq .01$, *** $p \leq .001$.

treatment, a main effect was observed for fly type (two-way ANOVA, $F(1, 76) = 5.784$, $p = .019$). Control WLST flies had a higher error rate than WTs (Student's t-test, $t = 3.59$, $df = 38$, $p = .009$). An interaction between fly type and isoflurane treatment was identified (two-way ANOVA, $F(1, 76) = 5.015$, $p = .026$), such that the error rate of WLST flies decreased upon treatment with isoflurane (Student's t-test, $t = 2.879$, $df = 38$, $p = .0065$) to match that of WTs under either anesthetic treatment, but no change was observed in WTs error rates across treatments. These results demonstrate the differential cognitive effects of isoflurane treatment in flies with fragmented sleep patterns.

Discussion

Here, we showed that the WLST flies exhibited fragmented sleep patterns and had impaired short-term learning and memory capabilities based on their performance on an aversive phototaxic suppression task. WLST flies showed sleep disturbances, including fewer sleep bouts during the light phase, shorter average sleep bout length during the dark phase (Figure 2C, $p = .004$, Figure 2D, $p = .04$), and deficits in short-term memory when untreated with isoflurane compared to WTs (Figure 3, $p = .009$). Interestingly, treatment with isoflurane improved WLST performance on the task whereas it had no effect on WT performance (Figure 3; WLST: $p = .0065$, WT: $p = .95$). These results suggest that WLST flies with fragmented sleep have impaired learning and memory abilities, which may be rescued upon treatment with isoflurane. Isoflurane exposure may act as a proxy for sleep in WLST flies to enhance their performance on the task. If exposure to this anesthetic is indeed acting as a proxy for sleep, these results lend support to the hypothesis that isoflurane activates pathways associated with sleep onset and, as a result, may similarly affect learning and memory processes.

A previous study supports the present finding that WLST flies have fragmented sleep, providing evidence that they are a strong and robust model for RLS (Freeman et al. 2012). Freeman et al. show that WLST flies display shorter average length of sleep bouts and an increased number of sleep bouts during the dark phase (2012). In addition, results from the present study identified a decreased number of sleep bouts during the light phase (Figure 2C, $p = .004$) and shorter length of sleep bouts during the dark phase (Figure 2D, $p = .04$) for WLST flies compared to WTs, further supporting that this model experiences sleep fragmentation.

The present study additionally demonstrated impaired cognitive function in WLST flies, as they had a higher average error rate on the aversive phototaxic suppression task compared to WTs (Figure 3, $p = .0065$). Previous studies have similarly identified cognitive impairments in patients suffering from RLS compared to age-matched controls (Fulda et al.,

2011; Pearson et al., 2006). Furthermore, patients with neurodegenerative diseases frequently develop sleep disorders such as RLS and, as a result, demonstrate cognitive impairments including impaired learning (Raggi and Ferri, 2010; Sonni et al., 2014). Therefore, it is important to further study the impact of sleep on cognition in order to improve the quality of life for these patients. Additionally, the results from the present study suggest the need for a more individualized and comprehensive postoperative monitoring system, particularly for patients with documented sleep disorders.

While previous studies have strongly associated the mechanism of isoflurane action with the activation of pathways implicated in the onset of sleep, few have assessed the effects of anesthesia on sleep-mediated processes, such as cognition (Gardner et al., 2016; Kottler et al., 2013; van Swinderen and Kottler, 2014). One study has demonstrated that sleep pressure and physiological effects caused by sleep deprivation are not alleviated in WT *Drosophila* upon treatment with propofol (Gardner et al., 2016). Unlike the results of the present study, which suggest that isoflurane treatment may mitigate learning and memory deficits in flies with fragmented sleep, potentially by activating sleep pathways, the Gardner et al. 2016 study suggests that treatment with propofol does not provide the same physiological benefits or restorative effects as sleep. However, this discrepancy may be due to the use of different *Drosophila* strains in these studies, as the present experiment assessed a fragmented sleep mutant model, while others have examined sleep-deprived WTs. WT flies show no change in performance on the aversive phototaxic suppression task following isoflurane treatment, which may support the proposed hypothesis regarding the discrepancy between this study and previous findings: while isoflurane may amend effects of sleep fragmentation and, as a result, rescue cognitive deficits as seen in WLST flies, it may not ameliorate sleep deprivation and sleep pressure as observed in WTs. However, this discrepancy illustrates the need for a wider array of model sleep disorder populations to elucidate the biological mechanism behind this phenomenon.

While sleep is known to be important for cognition, it may also amend anesthesia-induced cognitive impairments (Stickgold, 2005; Walker, 2009). Mice that underwent long-term treatment with isoflurane experienced disrupted circadian rhythm and impaired spatial learning and memory (Xia et al., 2016). However, treatment with melatonin prior to long-term isoflurane exposure prevented abnormal circadian rhythm and impaired spatial memory performance. This finding further demonstrates the benefits of sleep for cognition, as well as the complex relationship between the mechanisms underlying sleep and the molecular action of anesthetics associated with the activation of sleep pathways. Previous studies suggest that anesthesia causes circadian dysregulation in the days following treatment, but do not postulate why (Krenk et al., 2012, 2014; Xia et al., 2016; Zhang et al., 2014). Melatonin levels in patients who were anesthetized with isoflurane for surgical purposes decreased at night following surgery (Kärkelä et al., 2002). Additionally, patients were found to have circadian disturbances following treatment with anesthesia, including shorter sleep period, lower quality of sleep, and fragmented sleep caused by increased awakenings at night (Kärkelä et al., 2002). Together, these findings suggest that the chemical promotion of sleep onset is delayed and sleep is not well maintained after anesthetization. Similar findings in rodents demonstrate that propofol causes changes in the melatonin cycle, resulting in sleep fragmentation (Dispersyn et al., 2009, 2010). It is important to note that since we only investigated the effects of isoflurane, this study does not have implications for all classes of anesthetics.

The improved cognition seen in WLST flies after treatment with isoflurane may suggest that isoflurane could serve as a proxy for sleep, and may therefore cause shifts in circadian rhythm. Such a circadian shift may compensate for fragmented sleep in these flies, allowing them to perform equally well as WT controls on memory tasks. However, this same circadian dysregulation may disrupt the intact circadian rhythm in WT flies, as suggested by the previously discussed studies regarding altered

melatonin levels after administration of anesthesia (Dispersyn et al., 2009, 2010; Kärkelä et al., 2002). We suggest that it is this circadian rhythm disruption that resulted in no cognitive benefits on the learning task for the WT flies. However, as the circadian rhythms of flies in the present study were not examined following exposure to anesthesia, no conclusions may be drawn as to whether isoflurane induces circadian dysfunction in WT flies. Future experiments examining sleep patterns of WT and WLST flies after long-term isoflurane exposure must be conducted in order to determine whether the anesthetic does indeed cause circadian shifts that disrupt WT sleep, but compensate for fragmented WLST sleep. Furthermore, experiments must assess whether such circadian shifts underlie the changes in cognition observed in the present study upon treatment with isoflurane. Such experiments may elucidate whether anesthesia has a positive role in sleep regulation in dysregulated models. Alternatively, modification in synaptic transmission is another candidate mechanism underlying the changes in cognition given that isoflurane targets the GABAergic system to mediate its effects (Brown et al., 2010).

While the present study assessed learning and short-term memory in WLST flies exposed to isoflurane, further studies must be conducted in order to determine the effect of isoflurane treatment on long-term memory in flies with fragmented sleep patterns. Such experiments were attempted in the present study by assessing the error rates of flies on the aversive phototaxic suppression task six hours after the start of initial learning trials. However, due to experimental confounds such as testing short-term memory of flies during their light phase but testing long-term memory during their dark phase, when flies are lethargic and less motile, no conclusion could be drawn from the collected data. Additionally, insufficient exclusion criteria were defined for assessing mobility and lethargy of flies prior to testing long-term memory. Therefore, future experiments must extend the present results to elucidate the long-term effects of isoflurane on cognition, learning, and memory in flies with fragmented sleep patterns.

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