Relating Major Depressive Disorder (MDD) to circadian signaling in *Drosophila melanogaster*

Research Paper

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Abstract

Today, over 3.2 million adolescents aged 12-17 experience Major Depressive Disorder (MDD), and 73% of adolescents who experience MDD do not get enough sleep each night. Depression, which is characterized by lower-than-normal levels of serotonin, can be modeled in *Drosophila* via chronic vibrational stress (VS). The purpose of this experiment was to elucidate the relationship between circadian oscillator modification and the severity of symptoms of MDD in the model *Drosophila melanogaster*. The Drosophila Activity Monitor and custom Python code were used to measure sleep. VS caused arrhythmic sleep architecture in w1118 controls. During both the daytime and nighttime, *fumin* (insomnia-like)mutants had higher numbers of sleep bouts than the w1118 flies, indicating that the *fumin* mutants experienced less sleep consolidation because their arousal is characterized by enhanced alertness, due to the fact that they have a higher amount of extracellular dopamine and a decreased arousal threshold. Flies with elevated tryptophan hydroxylase (Trh) (generated by crossing UAS-Trh;Elav-Gal4) alleviated arrythmicity induced by VS. Trh is rate-limiting in serotonin synthesis, so elevating it was posited to increase brain serotonin, relieving the effects of VS. VS exposure increased the number of sleep bouts because it caused more fragmented sleep, reducing sleep consolidation in the flies via the inhibition of 5-HT release to the 5-HT-1A receptors in the α-lobes of the MBs. Thus, VS-induced sleep irregularity was enhanced with insomnia-like predisposition and alleviated with increased brain serotonin.

I. Introduction

According to the National Institute of Mental Health (2019), 17.3 million American adults and 3.2 million adolescents aged 12-17 (7.1% and 13.3% of respective U.S. populations) experience Major Depressive Disorder (MDD). 63.8% of adults and 70.8% of adolescents who experienced MDD exhibited severe impairment of quality of life. Currently, there are no cures for MDD, and treatment plans consisting of medication and therapy can cost anywhere from hundreds of dollars to several thousand dollars per month (Winerman 2017). A 2017 National Sleep Foundation poll revealed that 73% of adolescents who experienced MDD did not get enough sleep per night.

Depression in humans as well as *Drosophila melanogaster* has been characterized by lower-than-normal levels of serotonin, or 5-hydroxytryptamine (5-HT). Mushroom body 5-HT1A receptors control 5-HT synthesis/activity, while 5-HT2B receptors in the mushroom body lobes, while the 5-HT1A receptors control behavioral quiescence. Depression-like behaviors, such as the lack of resistance to stimuli such as uncomfortable heat or electric shocks, can be induced via chronic stress, such as heat punishment (Ries et al. 2017).

Serotonin, or 5-HT, is a neurotransmitter ubiquitously present in mammals and insects. It is also implicated in regulating sleep-wake cycles (Yuan et al. 2006). Yuan et al. (2006) implicated 5-HT in sleep promotion (ablating serotonergic cells led to insomnia) as well as wakefulness (the timing of serotonin secretion is correlated with neuronal activity). d5-HT1A, d5-HT1B, d5-HT2, and d5-HT7 serotonin receptors in the brain of fruit flies. Yuan et al. (2006) posit that their stimulation and knockout could be used to more clearly determine the mechanisms of the serotonergic regulation of sleep.

Light is involved in circadian stimulation and entrainment via two main pathways: the photoreception of ocelli, compound eye, and the Hofbauer-Buchner eyelets (HB), as well as the blue-light photo-pigment Cryptochrome. Cryptochrome is known for its role in targeting the Timeless protein in the biochemical circadian oscillator of fruit flies (Shang et al. 2008; Fogle et al. 2011). Light promotes arousal in fruit fly brain arousal circuits, specifically by stimulating the pars intercerebralis (PI) neurons and ellipsoid body (EB) neurons of the central complex (CC). The large lateral ventral neurons (l-LNvs) and mushroom bodies are specifically implicated in arousal in their action potential firing in response to light (Shang et al. 2008).

The circadian oscillator of flies consists of negative feedback transcription cycle of the period and timeless genes, which is in part mediated by the presence of the Timeless protein. This system is mediated by 140-150 clock neurons, notably including the pigment-dispersing-factor-expressing (PDF) ventral lateral neurons (LNvs), which specifically include Evening (E cells; include 5th Lateral Neuron (5th LNv), Dorsal Lateral Neurons (LNd), some dorsal neurons (DN1s)), and Morning cells (M cells; include small LNvs (s-LNvs)) (Fogle et al. 2011). The small ventral lateral neurons (sLNvs) are critical to the maintenance of circadian rhythms. The action of period-controlling neurons controls arousal, locomotion, aggression, and reproductive tenacity in flies (Dissel et al (2015); Li et al. (2009)). This is partially accomplished by the action of 5-HT signaling: Yuan et al. (2005) reveal that 5-HT signaling is influential in maintaining sleep homeostasis, and that this regulatory signaling can be manipulated to increase 5-HT levels in a manner similar to light therapy for seasonal affective disorder (a type of depression).

Ries et al. (2017) conducted a study that established the fly as a model for MDD. Flies were exposed to a vibrational stimulus (300 Hz vibrations using a DC vibration motor, aggregate 10 hrs/day) for several days in order to stimulate 'learned helplessness.' As defined by Ries et al., 'learned helplessness' in *Drosophila melanogaster* is characterized by impaired voluntary behaviors as a result of chronic stress; for example, flies exposed to uncomfortably high temperatures will begin walking (presumably in an attempt to escape the heat) but if flies continue to be stressed by the heat, they begin showing lower signs of resistance to the stress, including slowed down paces of walking. Ries et al. (2017) find that stressed flies retain motor function, but demonstrate decreased motivation in a gap-climbing assay. The tendency of a fly to attempt to climb over an insurmountable gap assesses motivation because it tests the limits to which an organism is willing to push its voluntary behaviors (ex. spontaneous walking, as opposed to a reactive behavior, like escape from a harmful stimulus). Additionally, serotonergic signaling was reduced in flies exposed to the vibration (an often-posited symptom of MDD). Ries et al. (2017) revealed that flies exposed to vibration showed markedly inhibited 5-HT release to the alpha lobes of the *Drosophila melanogaster* mushroom bodies, which are central to the regulation of behavior. In fact, the climbing rates of flies was not influenced by knocking down critical 5-HT receptors/Kenyon cells alone at all; thus, the role of mushroom body 5-HT was suggested to be modulatory with respect to the motivation to climb or engage in other voluntary behaviors, rather than the ability to climb itself. Finally, the latency of courtship of flies exposed to vibration was

found to be greater than compared to non-exposed flies, showing yet another voluntary behavioral deficit in response to the vibrational stimulus (Ries et al., 2017).

In 2017, Qian et al. studied molecular mechanisms pertaining to the sleep homeostasis of fruit flies, specifically investigating the roles of Trh, responsible for the 5-HT synthesizing enzyme, as well as all five 5-HT receptors in the brain of flies. Using microscopy (including usage of GAL4-UAS to express fluorescent tagging proteins), genetic techniques, and a Trikinetics Drosophila Activity Monitor, Qian et al. assessed gene expression and downstream ramifications on locomotor behavior/periodicity. Using a 5-HT receptor (5HT2b) knockout line, they determined that the 5-HT2B receptor was essential for maintaining sleep homeostasis. Additionally, it was found that genetically ablating Trh, 5HT1a, or 5HT2b reduced overall sleep duration, and the ablation of Trh and 5HT2b reduced the sleep rebound of the flies after sleep deprivation.

Yuan et al (2005) studied the role of 5-HT in the circadian entrainment of *Drosophila melanogaster* using microscopy, drugs to alter circadian rhythms, and assessment of sleep architecture. They found that 5-HT plays a role in preventing dramatic adjustments to the circadian period of arousal, thus slowing entrainment, but contributing to sleep homeostasis. Additionally, immunostaining revealed that aside from lateral neurons (already established), PI neurons, mushroom bodies, and the optic lobes of the fly's brain were implicated in this serotonergic circadian regulatory mechanism (Yuan et al. 2005).

A study by Turner et al. (2008) paints a more mysterious picture of MDD than literature suggests, however. This meta-analysis revealed the role of data manipulation impacting the scientific interpretation of MDD and treatment. From 1987 to 2004, 74 studies on supporting MDD remediation centering on serotonin or associated chemicals were performed; these studies were used to validate the use of antidepressants. Out of these studies, 38 yielded positive results, and 37 of these were published. However, 36 studies declared inconclusive results; only three of these studies were published as so. 11 of the inconclusive studies were published with a 'positive outlook' on the future of research in that vein, while the majority of those studies (22 studies) went unpublished entirely (Turner et al. 2008). Clearly, this conflict in literature necessitates further investigation.

Drosophila melanogaster is an appropriate model for a behavioral study because MDD can be reliably modeled, and MDD's pathology in fruit flies has been shown to rely on similar systems to those of humans. This includes the serotonergic signaling implicated in MDD but also includes

interlocking circadian mechanisms such as the opposing dopaminergic and gamma-aminobutyric acid-releasing (GABAergic) signaling pathways, which are responsible for arousal and drowsiness respectively in flies (Martin et al. 1998; Yuan et al. 2006). In addition, the fly has great potential for genetic manipulation; for example, the use of fluorescent tagging proteins, and information about each of *Drosophila melanogaster*'s ~14,000 genes is available online (FlyBase database).

The purpose of this experiment was to elucidate the relationship between circadian oscillator modification and the severity of symptoms of MDD in the model *Drosophila melanogaster*.

The alternate hypothesis was that overexpression of *Trh* would reduce the severity of MDD-associated motivational deficits of fruit flies (ex. Climbing, reproductive tendencies), and that post-vibrational-stimulation (to mimic MDD) thermogenetic *Trh* overexpression can play a role in reducing said parameters. Additionally, the extension of the fruit fly's photophase was posited to reduce the severity of MDD-like symptoms.

The null hypothesis was that no genetic or environmental manipulation of fruit flies before or after exposure to the MDD-like vibrational stimulus would reduce the severity of motivational behavioral deficits.

II. Methodology

Wild type strains used were obtained from Carolina Biological. *Elav*-Gal4, *Pdf*-Gal4, UAS-*Trh* and UAS-*TrpA1* were obtained from the Bloomington Drosophila Stock Center. *fumin* and *w1118* controls were acquired from Rob Jackson (Tufts University School of Medicine). There were three different groups of flies: w1118 control, fmn(rec19) control, and w1118-MDD flies. The MDD flies were placed in a chamber connected to a vibrational motor that vibrated for 20 seconds at a time with 10 s in between vibrations, all controlled using an Arduino. Following stress treatment or lack thereof, all flies were placed in the Trikinetics Drosophila Activity Monitor for 48 hours. Then, the average number of sleep bouts, average number of crossings, and activity indices were calculated using custom python code. All graphs were generated using Microsoft Excel and statistical significance was determined using IBM SPSS version 25 software.

Culturing Flies

Two different strains of flies were used: w1118 control flies and recombinant fumin (fmn(rec19)) flies. The fmn(rec19) flies were sleepless mutants generated to model insomnia in fruit flies.

Food was prepared by using 1 scoop (using an included measuring cup) of Formula 4-24 Instant Drosophila Medium from Carolina Biological in a ratio of 1:1 with spring water in the vial. Then, baker's yeast was sprinkled on top. Flies were transferred to newly prepared mediums after ~1 minute to ensure that the medium is not so soggy that the flies will get stuck and die (Flagg 1988).

Fly stocks were maintained as follows: Upon receiving the *Drosophila melanogaster*, organisms were transferred to properly prepared vials of food, with each vial labelled with the date of transfer. If the flies were age-synchronized and/or sexed, they were labelled accordingly. For age-synchronized population generation, flies were transferred daily, else 2+ age-synchronized generations would be lost (Flagg 1988). *Drosophila melanogaster* were stored in plastic vials with cotton or foam plugs that allow for air circulation as well as firm seals. In order to transfer flies from old vials to new vials, old vials were tapped briskly on a flat surface (several times if necessary) to knock flies to the bottom of the vial. Next, the plug of the old vial was quickly removed and the new vial was placed on top. Flies naturally climbed up into the new vial, but the transfer process could be hastened by flipping both vials and then vigorously tapping to knock flies to the bottom of the new vial. Finally, a new plug was inserted into the new vial (Flagg 1988).

Anesthetization

Flies in vials (with vials oriented on their sides to prevent flies from getting stuck in food if there is food in the vial) were placed in a freezer for 4 minutes. They remained largely immobilized for 2-3 minutes after removal. Care was taken not to leave flies in the freezer, for they will perish, taking into account that the older a fly is, the longer it will take to recover from the cold (Flagg 1988).

Sexing

In order to sex *Drosophila melanogaster*, first 2 fresh vials were prepared, one for males and one for females. Then, flies were anesthetized using a freezer. Next, using a fine, soft-bristled paintbrush, males and females were sorted into separate groups on top of an ice pack. Males were distinguished based on their darker, rounder, and smaller abdomens, meanwhile females were distinguished based on larger, pointier, and lighter abdomens. Males are typically smaller than

females, but this was not the sole criterion used to distinguish between males and females. After males and females were sorted, they were lightly brushed into segregated vials labeled appropriately (Flagg 1988).

Age Synchronization

To age-synchronize flies, flies (from stock population or already age-synchronized) were transferred into a fresh vial, and then transferred to another vial ~24 hours later. The vial in which flies mated for 1 day contained similarly aged *Drosophila melanogaster*, which eclosed 9-10 days later. In order to preserve age-synchronized generations, flies used for generation age-synchronized generations were transferred daily; mating periods of more than 24 hours could have led to greater discrepancies in age (Flagg 1988).

MDD Stress Treatment

15-25 males aged 3-5 days old were placed in acrylic tubes 98 mm long and 4 mm wide. Each side was plugged with cotton stoppers and a small amount of food, and these tubes were placed on top of vibration motors (Obtained from Tinkersphere, product number TS1680). Controlled using an Arduino, these motors vibrated for 20 seconds at a time with 10 s in between vibrations in order to prevent habituation. Code specifically controlling this procedure was written and uploaded to the Arduino before usage. This was repeated over 15 minutes and was followed by 30 minutes of recovery; this cycle occurred approximately 12-14 times per day (about 9-10 hours) for 3 days. Flies were transferred from standard medium to these empty tubes for vibration, and at the end of each day's worth of vibration, they were returned to standard food. Controls for vibrational stress involved placing flies in an acrylic tube on a stable tabletop or countertop instead of experiencing exposure to the vibration motor (Ries et al. 2017).

Sleep Analysis

Sleep deprivation and associated effects of locomotion were measured using a Drosophila Activity Monitor (Trikinetics) in a light-proof box (ex. cardboard). An Arduino microcontroller and an LED light shield (Adafruit) were used to control the light schedule of the flies. The monitoring system was housed in a well-ventilated and/or temperature regulated room (~25°C). It was preferable to have a dark room for the monitor; in order to see in the dark room, a lights with red filters was used (*Drosophila melanogaster* are not sensitive to red light). A PC computer was dedicated to full-time data collection, with minimal software installed to prevent crashing and loss of data. Data from the DAM was downloaded onto the device, so significant hard drive space or a

USB/similar device was also necessary. Raw binary data from when flies are in the DAM (where periods of inactivity are recorded as 0 and periods of activity are recorded as 1) was processed using DAM Filescan 102X; circadian parameters were measured in 15 and 30-minute bins, while sleep/rest parameters were measured in 1 to 5-minute bins. 5 minutes of inactivity in a row is generally accepted as sleep/rest in *Drosophila melanogaster*. Tubes were occupied by individual flies, with food plugging one end of the tube and a cap covering the other end. Adult male flies from 1-5 days of age were used to measure locomotor activity since the egg-laying of females can impede locomotion (Ali et al. 2011).

Data Analysis

Graphs were generated using Microsoft Excel, and statistical significance was determined using IBM SPSS v.25 software. To analyze raw data from the Drosophila Activity Monitor, custom Python code was written to determine the average number of sleep bouts and crossings for each individual fly. Activity indices, a measure of the number of movements or beam breaks per minute of active period, were calculated for all groups.

III. Results

During both the daytime and nighttime, the *fmn* mutants had higher numbers of sleep bouts than the w1118 flies, supporting the alternate hypothesis. The w1118 control flies had higher numbers of sleep bouts during the daytime than at night, while the *fmn* flies had roughly the same number of sleep bouts during the day versus the night.

The w1118 MDD flies had a significantly higher average number of sleep bouts than the w1118 control flies, but a lower average number of sleep bouts than the *fmn* control flies. The number of crossings/beam breaks in the MDD group was less than those of the other groups, but not by a large margin. The activity index of the MDD group during the daytime was significantly higher than that of the other two groups, and its activity index during the night time significantly lower than that of the other two groups.

All graphs were generated in Microsoft Excel, and statistical significance was determined using IBM SPSS version 25 software. Asterisks denote statistical significance where $p<0.05$.

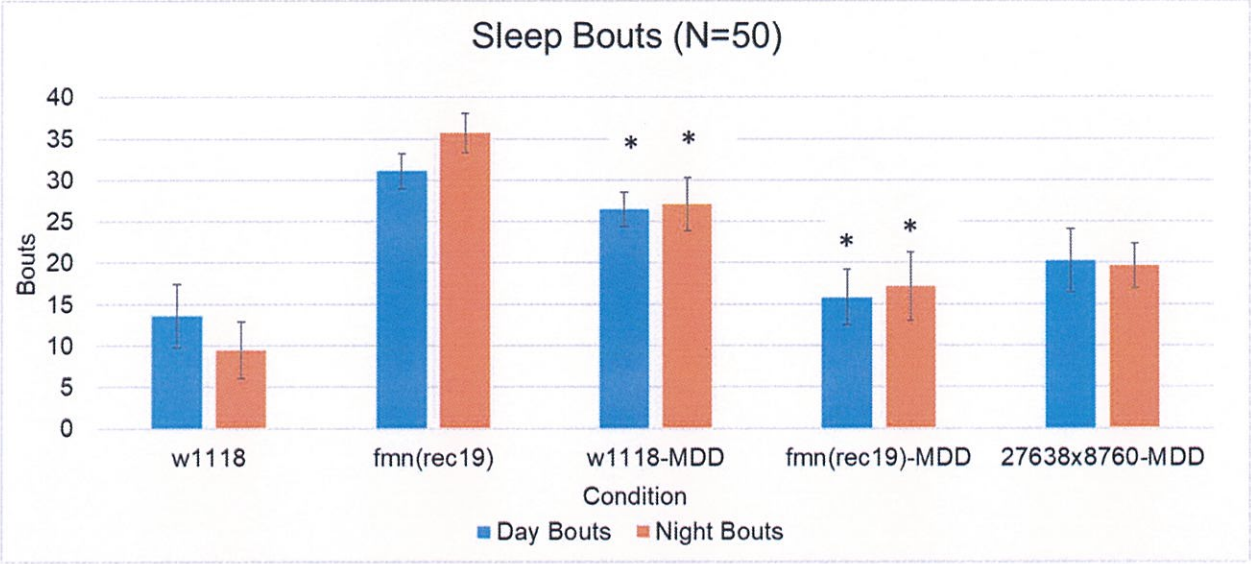

Figure 1: Sleep bouts per photophase and scotophase are shown for *w*, fmn, *w*-MDD, fmn-MDD, and 27638x8760-MDD. *Fmn flies* exhibited much higher numbers of sleep bouts and therefore had much less consolidated sleep. MDD-exposure to the w1118, fmn, and 27638x8760 flies also reduced sleep consolidation as shown by increased sleep bouts. A sleep bout is defined as 5 or more continuous minutes without movement. Asterisks denote statistical significance ($p<0.05$)

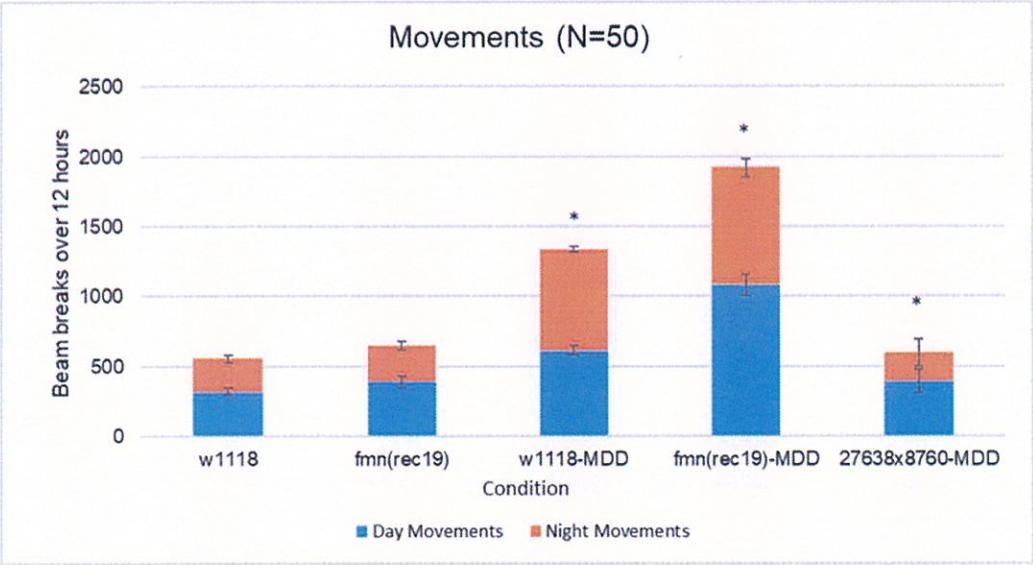

Figure 2: Daily movements are shown and are also divided into photophasal and scotophasal movements. The vibration exposure significantly increased the number of movements during both the daytime and the nighttime in the w1118 and fmn groups. A movement is defined as one time a fly breaks the infrared sensor beam inside the Drosophila Activity Monitor. Asterisks denote statistical significance ($p<0.05$)

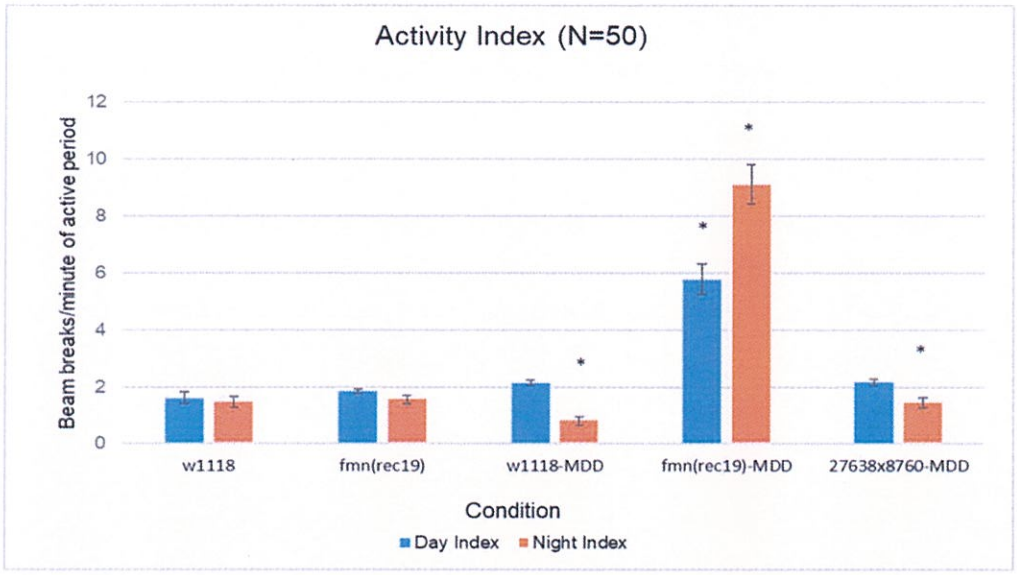

Figure 3: Activity Indices for each condition are shown. The Activity Index is the quotient of movements over time of the active period, in minutes. MDD exposure increased activity in the hyperactive fmn flies, but reduced activity in w1118 and 27638x8760 flies. Asterisks denote statistical significance ($p<0.05$)

IV. Discussion

The purpose of this experiment was to elucidate the relationship between circadian oscillator manipulation and the severity of symptoms of MDD in the model *Drosophila melanogaster*.

Critically, analysis of sleep bouts revealed that *w*-MDD flies - flies exposed to a MDD-like symptom-inducing vibrational stimulus - exhibited greater counts of sleep bouts for both day and night compared to *w* flies. Generally, lower numbers of sleep bouts confer more consolidated sleep, because the flies are waking up less often and thus are sleeping for longer periods of time. Lower numbers of sleep bouts usually correlate to longer sleep bouts and a lower frequency of crossings as well as a lower activity index. Higher numbers of sleep bouts indicate less consolidated sleep, which generally correlates to a higher frequency of crossings and a higher activity index. (Kume 2005) This general principle held true for the w1118 flies and most of the *fmn* flies. However, it was observed that the data from some *fmn* flies demonstrated apparent activity so frequently over the time periods that very low bout counts were observed, but it was also evident that flies were not sleeping. This variation in *fmn(rec19)* flies may account for some of the variation in the data in that aspect.

The results supported the alternate hypothesis that the *fmn* flies would have higher numbers of sleep bouts than the w1118 flies. This makes sense considering that the *fmn* flies are hyperactive; they are mutants that specifically exhibit increased levels of locomotor activity, an alteration of rest (sleep) arousal threshold, and decreased rest rebound in response to deprivation. The *fmn* flies experience less consolidated sleep than the w1118 flies do, as they are hyper-responsive to mechanical stimuli compared to the w1118 flies. They also demonstrated a lower rest rebound than the w1118 strain. Rest rebound is a phenomenon by which more sleep than usual is exhibited following sleep deprivation. Although *fmn* flies rest less than w1118 flies, they do not exhibit more of a rest rebound as would be expected. (Kume 2005) However, there was no significant difference between the activity indices for the w1118 flies compared to the *fmn* mutants.

Fmn flies are hyperactive because they carry a mutation in the *Drosophila dopamine transporter* gene (dDAT). The observed hyperactivity in *fmn* flies can reasonably be attributed to increased dopamine signaling in *Drosophila*, because the dDAT protein in *Drosophila* is comparable to mammalian dopamine transporters and DAT mutant mice also exhibited spontaneous hyperactivity and hyperlocomotion, very similar to the behaviors that the flies exhibited. (Kume 2005)

The arousal state of *fmn* flies is characterized by enhanced alertness because of the higher amount of extracellular dopamine, indicated by the fact that *fmn* flies have a decreased arousal threshold and are more sensitive to mechanical stimulation. Arousal threshold is the amount of energy or stimulation required to cause an organism to wake. A lower arousal threshold means that the organism, in this case *Drosophila*, will wake up more easily. This explains why the *fmn* flies have less consolidated sleep, as they are more easily awoken and have a less consolidated sleep than the control flies. (Kume 2005).

The vibration stress caused by the MDD vibration motor induces a depression-like state in fruit flies, as depression-like symptoms can be induced by chronic stress as well as learned helplessness. Continuous stress application has this impact because it induces reduced serotonin (5-HT) signaling in flies, typically by compromising 5-HT release at the α-lobes of the mushroom bodies (MBs) in *Drosophila*. The MB compartments of the *Drosophila* brain utilizes serotonergic activation of the α-/β- and γ-lobes to modulate motivational behaviors, such as climbing. The receptors 5-HT-1A and 5-HT-1B, found in the α-/β- and γ-Kenyon cells, respectively, are the two main types of serotonin receptors responsible for 5-HT regulation in the MBs of *Drosophila*.

Behavioral activity in *Drosophila* is tightly regulated by serotonergic signaling: the activation of 5-HT-1A in α-lobe Kenyon cells enhances motivational behaviors and the activation of 5-HT-1B in γ-lobe Kenyon cells inhibits them. This push-pull mechanism of serotonin regulation ensures that consistency in behavioral activity is maintained. Since repeated vibrational stress causes the inhibition of 5-HT release to the α-lobes of the MBs, these neurons are depleted of serotonin. Therefore, the 5-HT-1A receptors of the MB α-lobes are unable to enhance behavioral activity, and motivational behavioral deficits occur. (Ries et al 2017)

The MBs of the *Drosophila* brain are also critical in the regulation of sleep and circadian rhythmicity in the fruit fly. (Joiner et al 2006) The serotonin receptor 1A has been implicated in sleep regulation in *Drosophila*, as flies that have a truncated 5-HT-1A receptor exhibit shorter, more fragmented sleep than normal. Because serotonin has a conserved function in sleep regulation, it can be concluded that the presence of 5-HT-1A in the mushroom bodies is necessary for the maintenance of sleep stability in fruit flies. (Yuan et al 2006)

Since the 5-HT-1A receptors of the MB α-lobes are responsible for both the regulation of motivation-based behaviors used to model MDD in *Drosophila* as well as the regulation of sleep in *Drosophila*, a link can be made between vibrational stress and sleep regulation. The w1118 flies that had been exposed to the vibrational stress had a significantly higher number of sleep bouts than the control w1118 flies that had not been exposed to it. This indicates that the vibrational stress caused more fragmented sleep and reduced sleep consolidation in the MDD model of the flies. Additionally, the difference between the daytime and nighttime activity indices was most dramatic in the MDD group, revealing that vibrational stress caused a reduction of activity during the night and an increase in activity during the daytime. Lastly, the group of flies exposed to the vibrational stimulus had a lower average number of crossings than the control w1118 group, indicating that the stimulus decreased the overall activity of the flies.

Elevating *trh* appeared to partially resolve sleep consolidation, partially supporting the alternate hypothesis, by fixing irregular serotonin levels. 5-hydroxy-L-tryptophan is a precursor tryptophan hydroxylase, which is a precursor to 5-HT, so increasing expression of *trh* in the MBs of *Drosophila* would consequently increase serotonin expression, which enhances behavioral activity. (Ries et al. 2017)

Limitations included the fact that the high vibration output of the MDD apparatus severely impacted survivorship of the flies. The vibration motor output had to be significantly reduced in

order to prevent fly mortality in the MDD apparatus. Additionally, *fmn* mutants are characterized by enhanced sensitivity to mechanical stimuli, which could have also played a role affected their mortality rates. (Kume 2005)

The Drosophila Activity Monitor could have also impacted results because of the way the apparatus works. It is possible that flies could have been moving around in the tubes without crossing the infrared sensor of the monitor, and therefore this movement would not be reflected in the data. This could have potentially led to error in the data from the Activity Monitor.

V. Conclusion

Fmn flies had a higher average number of sleep bouts because their arousal is characterized by enhanced alertness, due to the fact that they have a higher amount of extracellular dopamine and a decreased arousal threshold, and they are more sensitive to mechanical stimulation. This causes reduced sleep consolidation.

The repeated vibrational stress increased the number of sleep bouts because it causes the inhibition of 5-HT release to the 5-HT-1A receptors in the α-lobes of the MBs, and these neurons are depleted of serotonin as a result, also causing less consolidated sleep.

Driving the synthesis of tryptophan hydroxylase partially ameliorated irregularity in sleep architecture as a result of vibrational stress, which may be attributed to the likely increased brain serotonin levels (tryptophan hydroxylase is rate-limiting in 5-HT biosynthesis).

VI. Future Studies

To further characterize how learned helplessness can affect circadian patterns different methods of perturbations can be used. For example, Yang et al. (2013) detail how the inability to escape from uncomfortable heat can modulate cognitive deficits in the fruit fly. Further observations suggested a 'mood-like' component of this behavioral change; it was observed that these flies were less likely to make walking attempts to escape the disturbing stimulus (Yang et al. 2013). Thus, it will be instructive to investigate how different methods of inducing learned helplessness may affect behaviors differentially, including observation of sleep architecture and cognitive ability. Per Kume et al. (2005), *fmn* flies are particularly susceptible to mechanical stress, so testing heat-induced learned helplessness may eliminate mechanical stress as a potential confounding factor based on the MDD-stimulation used in this study.

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