Development of Larvae in Higher Population Densities Increases Sleep Consolidation in Adult Drosophila melanogaster

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1. Introduction

Sleep is a behavior that is observed in all mammals, including humans. For many years the function of sleep remained a mystery until recent studies established its connection with synaptic plasticity, an important neural network for learning and memory (Fishbein & Gutwein, 1977; Levy & Steward, 1979). Although all mammals exhibit sleep, they differ between species or even individuals depending on genetic predispositions and environmental cues (Heath et al., 1998; Allison & Chicchetti, 1976). Between individuals of a species, younger animals naturally sleep more than older adult animals because sleep is essential for development (Curzi-Dascalova, 1992). The sleep pattern of a species is generally regulated by an internal biological circadian clock. Each organism has a different circadian clock that is regulated by environmental factors such as food availability and predator presence (Allison & Chicchetti, 1976). However, circadian clocks can be disrupted when these environmental conditions become extreme and inhibits proper development, leading to cognitive impairment (Banks & Dinges, 2007).

Cognitive development plays a crucial role in shaping an animal’s behavior as adults (Becker & Thoman, 1981; Beckwith & Parmelee, 1986). When certain growth stages are disrupted, behaviors adapt or even become impaired depending on the influence (Becker & Thoman, 1981). Changes in sleep behavior can be a result of genetics or environmental stresses. Individuals with sleep disorders such as insomnia suffer from sleep deprivation, leading to depression and improper memory consolidation (Roth & Ancoli-Israel, 1999). This effect is especially seen when environmental stresses affect organisms in an early stage of development. In 2012, Meredith et al. monitored cognitive development and behavioral impairments in mice. They found that early postnatal periods of life are a ‘time-restricted window’ in which early cognitive development is particularly sensitive (Meredith et al., 2012). One particular
environmental stress that has been shown to inhibit cognitive growth during the developmental phase is social isolation (Lowenthal, 1964).

Social interactions are crucial for cognitive development in many organisms. Even when the proper medical care and nutritional requirements are met, psychological problems still persist following social deprivation (Carlson & Earls, 1997). In Romania, this effect was observed in developing children who experienced severe social deprivation from birth to the age of 3, and subsequently had to be placed into institutional facilities as a result of cognitive impairments (Carlson & Earls, 1997). This effect has also been observed in animal models. In 1993, Siegel et al. performed immunocytochemistry on neurofilament proteins, an important factor in neurogenerative diseases such as Alzheimer’s, in the hippocampus of socially deprived and socially enriched prepubescent rhesus monkeys (Siegel et al., 1993). Optical density measurements revealed significant differences in the amount of phosphorylated neurofilament proteins between the two groups. They also found that the maturation of dentate gyrus granule cells in the hippocampus is particularly sensitive during the prepubescent time-frame. Abnormal development of the hippocampus is essential because it has been associated with inhibited learning and memory capabilities as well as irregular sleep behavior (Parkinson et al., 1988, Maquet, 2001).

Sleep is not limited to mammals. Insect models, such as Drosophila melanogaster (fruit fly), have been used to study sleep behaviors. Fruit flies are particularly useful model organisms to study sleep because they have shorter life cycles and are less expensive to maintain than mammalian organisms (Hendricks et al., 2000). More importantly, they have been shown to have behavioral, pharmacological, and molecular similarities to mammals including humans (Hendricks et al., 2000, Shaw et al., 2000). For decades, fruit flies have been important model
systems for studying circadian rhythms and learning and memory (Konopka & Benzer, 1971, Quinn et al., 1974). Previous research has identified mammalian paralogs for *Drosophila* clock-related genes such as *Period (Per)* and *Cryptochrome (Cry)* (Zhang & Kay, 2010). In addition, fruit fly sleep behavior is dependent on common factors with mammals such as age and availability of food (Koh et al., 2006, Keene et al., 2010). Most importantly, they also exhibit similar responses to sleep deprivation; fruit flies have increased amounts of sleep after sleep deprivation demonstrating this behavior in flies is homeostatically regulated as it is in mammals (Hendricks et al., 2000, Berger & Oswald, 1962). These similarities between fruit flies and mammals illustrate the effectiveness of *Drosophila* as a model organism.

In addition to environmental sleep regulatory factors such as light, temperature, and olfactory system, fruit fly sleep behavior is also shaped by the social environment (Dunlop & Loros, 2004, Eban-Rothschild & Bloch, 2012). In 2006, Ganguly-Fitzgerald et al., observed the effects of social enrichment in wild-type Canton S (CS) fruit flies (Ganguly-Fitzgerald et al., 2006). This study discovered that socially enriched adult fruit flies have a higher level of daytime sleep in comparison to socially impoverished adult fruit flies, but saw no effect during the nighttime (Ganguly-Fitzgerald et al., 2006). Not only did the socially enriched flies sleep more, but they also had more consolidated sleep. An additional study conducted by Donlea & Shaw verified this finding and saw similar results (Donlea & Shaw, 2009). These experiments have only tested social deprivation during the adult phase of *Drosophila* development. However, it has never been tested whether social enrichment during the larval phase, the “time-restricted window” for fruit flies, has similar effects on sleep behavior as social enrichment during the adult phase. In 2004, Stewart & McLean conducted a social enrichment experiment and analyzed changes in synaptic morphology. They raised *Drosophila* larvae in populations of 10
and 100 larvae and saw a relationship between larval population density and synaptic morphology, but no change in synaptic strength (Stewart & McLean, 2004). Since synaptic plasticity affects sleep, I would like to determine if social enrichment during the developmental stages of wild-type CS Drosophila affects sleep behavior as adult flies.

2. Materials & Methods

2.1. Fly Lines and Maintenance

In this experiment, I investigated the effects of varying larval population densities on the sleep behavior of adult flies for three genotypes: wild-type CS (Canton, Ohio), amn1 (Quinn et al., 1979), and amnx8 (Moore et al., 1998). Stocks of these lines were raised in a Light/Dark 25°C incubator on Bloomington Cornmeal-Sucrose-Yeast Fly medium in a stock bottle (50 mL of food). Three days after the parental flies were introduced to new bottles, they were removed and the progeny were used for egg collection.

2.2. Egg & Larvae Collection

To collect the Drosophila eggs, an egg-laying chamber was used in conjunction with apple juice plates made according to Jove’s protocol (Tran et al., 2010). Five apple juice plates were made for each fly line the day before collecting the eggs. A thin layer of yeast paste, about 3 cm in diameter, was added to each plate. Four bottles of each line from a Light/Dark 25°C incubator were transferred to an empty bottle and then transferred to separate egg-laying chambers with one of the apple juice plates to be incubated overnight at 25°C.

On the next day one hour after the lights turned on in the 25°C incubator, the incubation plates and egg-laying chambers were taken out and transferred to another apple juice plate. The flies were allowed to lay eggs for 30 minutes at a time. This was repeated with the remaining 3
plates of each group. The eggs were collected from the apple juice plates with dental picks and transferred to test tubes (10mL of food) according to population density ranging from 1, 50, 100, and 200 eggs. Before the eggs were added, the test tubes were first scored with a spatula to increase larvae survival. They were then placed into the 25°C incubator to grow.

After the eggs hatched into larvae and the larvae reached later stages of pupation, the pupae were individually transferred to separate test tubes where they eclosed into adults. The test tubes with the adult flies were put in the 25°C incubator to further develop.

2.3. Sleep Experiment

After the pupae hatched into adult flies, they continued to mature for another 3-4 days. 32 male flies and 32 female flies from each population density were collected from the test tubes and loaded onto 8 Drosophila Activity Monitoring (DAM) boards (Figure 1) (Trikinetics, Waltham, MA). These boards were put into a 25°C incubator where a 12hr/12hr LD (light-dark) sleep experiment (1-6 LD days, 7-12 DD (dark-dark) days) were used to measure the effects of various larval densities on adult sleep activity. Sleep activity was measured by an infrared light in each board that tracked movement within each Drosophila sleep tube. If there are no beam crosses for 5 minutes, the fly is considered asleep. MATLAB (MathWorks, Natick, MA) was used to analyze and compare the resulting sleep behaviors of the different larval density populations using previously published analysis scripts (Donelson et al., 2012, Levine et al., 2002). The sleep behavior types for the CS experiment were then analyzed with 1-way ANOVA tests with larval density as a factor and Tukey HSD post-hoc tests and the amnesiac vs. CS data were analyzed with 2-way ANOVA test with genotype and larval density as factors and Tukey HSD post-hoc tests.
3. Results

3.1. The Sleep Experiment

The *Drosophila* Activity Monitoring (DAM) Board was used to measure the sleep and activity of the different fly groups. Data for the 12hr/12hr light/dark (LD) period was an average of days 2-6. Data for the 12hr/12hr dark/dark (DD) period was an average of days 8-12. The first day of LD and DD (days 1 and 7) were removed from the data analysis because they were acclimation days for the flies to adjust to the new environmental conditions.

3.2. Effects of larval population density on sleep in wild-type CS Drosophila during LD

In *Drosophila*, the sleep pattern for both males and females include morning activity at ZT=0, a siesta from ZT=2 until afternoon activity at ZT=12, and finally nighttime sleep from ZT=12 to ZT=24. However, the amount of sleep activity between males and females dramatically differ. In both the LD and DD graphs (Figure 1A and 1B) it can be seen that the males sleep considerably more than the females. In Figure 1B, the graph of male sleep activity shows a ceiling effect during the hours of siesta and nighttime sleep meaning that from days 2-6, the average amount of sleep during those times are saturated. This makes it difficult to evaluate any significant changes in sleep activity as a result of varying larval densities. Therefore, the females were used to assess any significant differences during LD for wild-type CS flies.
Figure 1. Sleep activity in wild-type CS adult flies during LD. A) Females have activity when light turn on at ZT=0, a siesta at ZT=6, an afternoon activity at ZT=12, and nighttime sleep from ZT=12 to ZT=24. B) Males sleep much more than females do. Although they follow the same sleep pattern, their daytime sleep and their nighttime sleep are saturated.

During LD, three sleep and two activity parameters were measured: total sleep duration, mean sleep episode duration, number of sleep episodes, total activity counts, and the average activity counts per minute during wakefulness (wakefulness activity). There was a significant effect of population density on total sleep duration during both the light period $F(3,168)=3.97, p=0.0091$) and the dark period ($F(3,168)=5.55, p=0.0012$). It was also significant over 24 hours ($F(3,168)=2.97, p=0.0336$), with the 200 group having a 12.9% higher duration than the 1 group. In Figure 2A, it can be seen that as larval population density increases, total sleep duration also increases.

Similarly, the mean sleep episode duration also followed a similar trend as the total sleep duration (Figure 2B). As larval population density increased, the mean sleep episode duration increased significantly over 24 hours ($F(3,168)=7.00, p=0.0002$) and also during the dark period ($F(3,168)=10.67, p=<0.0001$) with the 200 group having a 55.2% and 74.7% higher duration than the 1 group respectively. The effects of larval density on the number of sleep episodes was also significant over 24 hours ($F(3,168)=5.75, p=0.0009$) and during the dark period
(F(3,168)=9.94, p=<.0001). However, the trend for the number of sleep episodes was the inverse. As larval population density increased, the number of sleep episodes decreased with the 200 group having 21.1% and 32.8% less number of sleep episodes over 24 hours and during the dark period respectively (Figure 2C). Although the effects of larval population density on the mean sleep episode duration (F(3,168)=2.73, p=0.046) and number of sleep episodes (F(3,168)=1.59, p=0.1936) was non-significant during the light period, the relation between the groups agreed with the rest of the data. Therefore, as larval population density increases, flies have longer but less frequent sleep episodes.

Fly activity is interesting to observe because it measures the locomotor ability of flies as a result of development under varying larval population densities. If overall activity is changed, sleep will also be affected because sleep is defined by 5 minutes of inactivity. The total activity count (Figure 2D) indicates the total number of beam breaks across days 2-6. The effect of larval population density on the total activity count was non-significant across 24 hours (F(3,168)=1.89, p=0.1324) and during the light period (F(3,168)=2.58, p=0.0550). It was only significant during the dark period (F(3,168)=3.41, p=0.0190) with the 200 group having 21.5% less total activity counts than the 1 group. Although the effects on wakefulness activity (Figure 2E) was significant during the light period (F(3,168)=5.57, p=0.0012), the dark period (F(3,168)=10.60, p=<0.0001) and over 24 hours (F(3,168)=5.23, p=0.0018), the trend observed in the total activity counts was only seen during the dark period. During the dark period, the flies that developed in a higher larval density were 8.1% less active per minute than the flies that developed in social isolation. However, during the light period, the socially enriched flies were 12.1% more active per minute than the socially deprived flies. The 200 group seems to be more
active during the day and less active at night in comparison to the 1 group. This suggests that the presence of light may play a factor in regulating activity during the light period.

Figure 2. Sleep and activity parameters of wild-type CS female flies during LD. A-E) Quantification of sleep parameters for the same average LD day, across all 24 hours, across the light period (LP), or across the dark period (DP). Error bars represent standard error of the mean. n represents the number of flies in each group. Letters above columns are present in cases where a significant interaction between genotype and population density was observed, and indicate significance groups from post-hoc analysis. Columns within each group of four that do not have any letter in common are significantly different, B) Total sleep duration, C) Mean sleep episode duration, D) Number of sleep episodes, E) Total activity counts, F) Wakefulness activity.
3.3. Effects of larval population density on sleep in wild-type CS Drosophila during DD

Similar to the LD period, the sleep and activity parameters of the DD period were averaged across days 2-6. The sleep pattern during the DD days differed from the LD days (Figure 3). Without the presence of light to modulate sleep behavior, the flies followed their innate circadian rhythm. Differences between the male flies were visible during DD because their sleep was no longer saturated as it was during LD. During the first 12 hours of each day, the females lost their siesta but still slept more during the last 12 hours of each day (Figure 3A). The males still maintained its siesta, but it seems to be a continuation from its nighttime sleep (Figure 3B).

![Figure 3. Sleep activity in wild-type CS adult flies during DD. A) During DD, females have lost the siesta seen in LD. They continue to exhibit more sleep at night than during the day time, B) During DD, males still sleep much more than females do. Unlike LD, their sleep is no longer saturated.](image)

During DD, the trends for the female flies were not as apparent across the groups as they were in LD. However, the comparison between the 200 group and the 1 group retains the same trends as LD. The effect of larval population density on the total sleep duration (Figure 4A) was significant over the subjective light period (F(3,168)=7.92, p=<0.0001), the subjective dark period (F(3,168)=8.36, p=<0.0001), and also over 24 hours (F(3,168)=9.14, p=<0.0001) with the
200 group having a higher duration of total sleep than the 1 group by 62.0%, 34.9%, and 43.6% respectively.

The mean sleep duration (Figure 4B) was also significantly higher in the 200 group than in the 1 group. During the subjective light period (F(3,168)=10.54, p=<0.0001), the subjective dark period (F(3,168)=12.84, p=<0.0001), and over 24 hours (F(3,168)=14.55, p=<0.0001), there was a significant increased duration of sleep for each episode by 86.1%, 110.5%, and 82.0% respectively. The number of sleep episodes (Figure 4C) in the 200 group was significantly less than the 1 group during the subjective light period (F(3,168)=3.21, p=0.0245) and non-significant during the subject dark period (F(3,168)=2.52, p=0.0600) and over 24 hours (F(3,168)=2.65, p=0.0503). The relationship between longer sleep episode and less frequent sleep was also seen in LD.

The trend seen for total activity counts and the wakefulness activity during LD was also seen during DD. The 200 group had 25.7% less total activity than the 1 group during the subjective light period (F(3,168)=9.88, p=<0.0001), 28.8% less total activity (Figure 4D) during the subjective dark period (F(3,168)=9.47, p=<0.0001), and 27.1% less total activity over 24 hours (F(3,168)=10.32, p=<0.0001). Similarly, the wakefulness activity (Figure 4E) was also significantly lower in the 200 group than the 1 group by ~20% in the subjective light period (F(3,168)=12.33, p=<0.0001), the subjective dark period (F(3,168)=12.80, p=<0.0001) and also over 24 hours (F(3,168)=12.92, p=<0.0001). However, the results do not show clear trends across the different densities as seen in LD.
Figure 4. Sleep and activity parameters of wild-type CS female flies during DD. A-E) Quantification of sleep parameters for the same average DD day, across all 24 hours, across the subjective light period, or across the subjective dark period. Error bars represent standard error of the mean. n represents the number of flies in each group. Letters above columns are present in cases where a significant interaction between genotype and population density was observed, and indicate significance groups from post-hoc analysis. Columns within each group of four that do not have any letter in common are significantly different. A) Total sleep duration, B) Mean sleep duration, C) Number of sleep episodes, D) Total activity counts, E) Wakefulness activity.

During DD, the sleep activity of the males was not saturated as it was in LD, allowing differences to be analyzed between the sleep activities as a result of varying larval densities. Although the total sleep duration for the males during DD was non-significant between the
groups (Figure 5A), the average sleep episode duration (Figure 5B) of the 200 group was significantly longer than the 1 group during the subjective light period (F(3,211)=2.97, p=0.033), subjective dark period (F(3,211)=4.41, p=0.0049), and over 24 hours (F(3,211)=7.01, p=0.0002). The 200 group also experienced less sleep episodes than the 1 group (Figure 5C) during the subjective light period (F(3,211)=8.70, p=<0.0001), the subjective dark period (F(3,211)=5.14, p=0.0019), and over 24 hours (F(3,211)=9.36, p=<0.0001). This was also seen between the 200 group and 1 group females during DD.

The effect of larval population density on total activity counts (Figure 5D) was significant during the subjective light period (F(3,211)=7.68, p=<0.0001), subjective dark period (F(3,211)=7.62, p=<0.0001), and over 24 hours (F(3,211)=8.06, p=<0.0001). This is also seen in the average activity counts per minute of wakefulness (Figure 5E) (F(3,211)=15.46, p=<0.0001), (F(3,211)=15.00, p=<0.0001), (F(3,211)=15.68, p=<0.0001). In both the activity parameters, the activity counts of the 200 group were >30% less than the 1 group. This continues to support the relation between increased population density and less activity in the absence of light during both LD and DD.
3.4. Effects of larval population density on sleep in amn1 Drosophila during LD

Previous studies have shown that larval density affects development of the calyx, a structure in the mushroom body that integrates olfactory, visual, and tactile information (Hitier et
al, 1998, Strausfeld et al., 1998). In *amnesiac* mutants, the calyx was observed to be larger than in wild-type CS flies (Hitier et al., 1998). However, development in varying larval densities only affected the size of the calyx in wild-type CS and not *amnesiac* mutants (Hitier et al., 1998). Since the mushroom body is involved in regulating sleep, we decided to test the effect of larval density in two lines of *amnesiac* mutants, *amn1* and *amn^{x8}* , to see if *amnesiac* is integral in adult sleep (Joiner et al., 2006). I hypothesize that the effect of larval density seen in the wild-type CS flies will be nullified in the *amnesiac* mutants because of the loss of plasticity in the calyx (Hitier et al., 1998). Given that the trends were quite consistent across the varying larval densities and the largest difference was observed between the 200 group and the 1 group in wild-type CS, the effect of larval density on adult sleep in *amn1* and *amn^{x8}* flies were observed by comparing only the 200 group and the 1 group.

Of the two *amnesiac* lines, only *amn1* was analyzed because of the 100% death rate of the socially isolated *amn^{x8}* larvae. From Figure 6A, differences in sleep pattern between *amn1* and wild-type CS could already be observed. LD analysis quantified the differences in sleep parameter trends between *amn1* and the wild-type CS line. For total sleep duration (Figure 6B), there was a significant interaction between genotypes and larval densities during the dark period ($F(1,189)=17.25$, $p=<0.0001$) and also over 24 hours ($F(1,189)=9.10$, $p=0.0029$), but it was non-significant during the light period ($F(1,189)=0.70$, $p=0.4054$). The wild-type CS flies continued to exhibit the same trend as before; higher larval population densities lead to an increased amount of sleep. However, this effect was negated when examining the difference between the *amn1* groups.

A similar trend was observed in the average sleep episode duration (Figure 6C). We continued to see the 200 group have a longer average sleep episode durations compared to the 1
group in the wild-type CS flies, but not the amn1 mutants. There was a significant interaction during the light period (F(1,189)=11.63, p=0.0008), the dark period (F(1,189)=6.34, p=0.0126), and over 24 hours (F(1,189)=22.27, p=<0.0001). Again, the same trend for the number of sleep episodes (Figure 6D) was seen in the wild-type CS flies, with the 200 group having less sleep episodes than the 1 group. However, this effect was also nullified by the amn1 mutants. The overall interaction for the number of sleep episodes between the two genotypes and densities were significant during the light period (F(1,189)=10.45, p=0.0014), the dark period (F(1,189)=8.29, p=0.0044), and over 24 hours (F(1,189)=4.17, p=0.0426). Our results for amn1 agrees with previous studies that also observed more fragmented sleep and shorter mean sleep episode duration in amnesiac mutants (Liu et al., 2008).

For the total activity counts (Figure 6E), the overall interaction was non-significant during the light period (F(1,189)=1.30, p=0.2550), but was significant during the dark period (F(1,189)=4.82, p=0.0294), and over 24 hours (F(1,189)=4.41, p=0.0370). The wakefulness activity (Figure 6F) had a non-significant interaction during the light period (F(1,189)=0.79, p=0.3739), the dark period (F(1,189)=0.36, p=0.5514), or over 24 hours (F(1,189)=0.47, p=0.4958). Figure 6F reveals that the pattern between the densities of the wild-type CS flies and the amn1 mutants are very similar.
Figure 6. Sleep and activity parameters of amn1 female flies during LD. A) Minutes of sleep per 30-minute bin, across an LD day. Zeitgeber time (ZT) indicates the time after the lights turn on. This plot corresponds to the legend on the left. B-F) Quantification of sleep parameters for the same average LD day, across all 24 hours, across the light period (LP), or across the dark period (DP). Error bars represent standard error of the mean. n represents the number of flies in each group. Letters above columns are present in cases where a significant interaction between genotype and population density was observed, and indicate significance groups from post-hoc analysis. Columns within each group of four that do not have any letter in common are significantly different. The bar graphs correspond to the legend on the right. B) Total sleep duration, C) Mean sleep episode duration, D) Number of sleep episodes, E) Total activity counts, F) Wakefulness activity.
4. Discussion

Investigating the effects of social isolation during the developmental phase of life is important because social interactions influence the cognitive development of an organism as it matures into an adult. Previous studies have shown that synaptic plasticity is essential for functions such as learning and memory as well as sleep (Fishbein & Gutwein, 1977, Levy & Steward, 1979). In this experiment, we investigated the effects of larval density on the sleep behavior during LD and DD periods in two lines of flies, wild-type CS and amn1.

During LD, the sleep pattern of a wild-type CS fly includes activity at ZT=0, a sieta at ZT=6, afternoon activity at ZT=12, and nighttime sleep from ZT=12 to ZT=24. This pattern was still present across varying larval population densities. However, the amount of sleep and activity varied between population density groups. Our results show that as population density increases, the number of sleep episodes decreases but each episode is longer. This suggests that sleep becomes more consolidated in adults that develop in higher population densities. This relationship was seen throughout the experiment in both males and females. Past studies have also observed consolidated sleep in socially enriched adult flies, but only during the light period (Ganguly-Fitzgerald et al., 2006). However, they only isolated the flies during adulthood, not during larval development. The effect of larval density on locomotor activity was non-significant during the light period but significant during the dark period. The difference in activity may be regulated by the presence of light. Since flies are extremely sensitive to light, it is natural for them to be more active during the light period than the dark period.

Under DD conditions, the entire 24 hours is absent of light. However, under these conditions the clearest trends in the various sleep parameters across larval population densities were observed potentially because sleep patterns rely solely on innate sleep behavior. We saw the most consistent effect in the male flies during DD. Similar to the female flies in LD, we saw
that the male flies that developed in larger population densities had more consolidated sleep than the ones that developed in social isolation. In fact, different degrees of population densities resulted in different degrees of sleep consolidation. This phenomenon could be a result of differences in synaptic development as observed by Stewart & McLean in 2004.

A similar effect was observed in the male activity counts during DD. As population density increased, the amount of total activity and also wakefulness activity decreased. Again, different degrees of population density resulted in different degrees of activity. The same effect was observed in the female flies during DD between the 200 group and the 1 group. However, groups 50 and 100 did not follow the same trend. Therefore, I would like to continue to investigate the female flies to verify the trends seen in the males or to establish trends specifically seen in females. This could be done by swapping the days of LD with the days of DD. The discrepancy in the trends across larval population densities could potentially be due to the difference in age of the flies; the flies in the start of DD are 6 days older than the flies in the start of LD. Therefore, it would be interesting to see if the same effects are observed if the LD and DD periods are switched.

The effect of larval population density on sleep behavior was also conducted in amnesiac mutant amn1. Unlike the wild-type CS flies, the development in varying population densities for amn1 flies did not have an effect on sleep behavior. This could be due to the loss of synaptic plasticity of the calyx in amnesiac mutants as observed by Hitier et al. (1998). Although it was clear that the amn1 mutants did not have an increased amount of sleep in the 200 group as the wild-type CS flies did, it was still unclear whether the amnesiac mutation was an effect on sleep or activity. This became clear when observing the wakefulness activity. In this activity parameter, both densities of the amnesiac groups seem to follow the same trends as the wild-type
CS groups. This suggests that *amnesiac* does not play a role in activity and only affects sleep behavior. This could be investigated further by analyzing the effects of larval population density on the sleep behavior of *amn1* flies under DD conditions.

In this study, we have established a relationship between development in varying larval population densities and sleep behavior in adult flies. Our results showed that larvae that developed in higher population densities had more consolidated sleep as adult flies. Past social deprivation studies have discovered that the variability in sleep is related to the plasticity of the mushroom bodies, an important regulator of sleep (Technau, 1984, Campbell & Turner, 2010). Therefore, one possibility for more consolidated sleep in flies that developed in a higher larval population density may be the social interactions it experiences during development. By having more interactions, it would increase in the number of fibers in the Kenyon cells, allowing for more consolidated sleep, while socially isolated larvae would have more fragmented sleep. The *amnesiac* mutation did not follow this trend. Figure 6 shows little changes in sleep behavior as a result of population density. Hitier et al., saw no significant change in size of the calyx as a result of population density in larvae (1998). Since there was a loss of synaptic plasticity in response to development in varying larval population densities, *amnesiac* must play an important role in the parts of the brain that are influenced by varying population densities.

In this experiment, development during the larval period did not distinguish between the three larval development stages (1<sup>st</sup> instar, 2<sup>nd</sup> instar, and 3<sup>rd</sup> instar) but was constant until pupation. In the future, I would like to isolate the stage of larval development or adult fly development that would result in the greatest effect of larval density on adult sleep. In addition, I would like to determine other factors that may influence this effect by altering environmental variables such as temperature, humidity, light-intensity, or even food type. Raising larvae on
food that has already been exposed to larvae churning and adult fly feeding may develop differently than larvae that were raised on fresh food, possibly because the olfactory system would be sensitive to the pheromone residues. Differences can be evaluated by conducting the same experiment in mutants of or47b, an important gene for the olfactory system (Keller & Vosshall, 2003) and comparing its effects with wild-type CS. It would also be interesting to conduct the same experiment with other mutants that have a loss of sight or touch.

In fruit flies, larval population density has been shown to affect the number of fibers in the Kenyon cells of the mushroom body; as population density decreases, the number of fibers in the Kenyon cells decreases as well (Technau, 1984). A similar effect was seen in rhesus monkeys. Siegel et al. observed differences in the amount of phosphorylated neurofilament proteins in socially enriched and socially deprived prepubescent monkeys (1993). The similarity in synaptic plasticity as a result of social enrichment in fruit flies and rhesus monkeys may stem from gene analogs that could even be relevant to humans.
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