The Effects of Cacophony Mutations on Sleep in *Drosophila melanogaster*

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Abstract

Migraines, characterized by throbbing headaches, are often accompanied by symptoms including nausea and dizziness. Familial Hemiplegic Migraine (FHM), a subtype of migraine that runs in families, can be caused by a missense mutation in the CACNA1A gene, which is involved in the production of CA\textsubscript{v,2.1} calcium channels. Although migraine sufferers often report sleep problems, little is known about the relationship between FHM mutations and sleep. This study examines the mechanisms between two CACNA1A mutations and sleep in *Drosophila*. Lines containing the UAS-CAC-GFP\textsuperscript{S/L}(3-8m) human transgene and lines containing the UAS-CAC-GFP\textsuperscript{786C} transgene were crossed with the ELAV-GAL4 driver, which expresses these mutations across all neurons in their offspring. Using Drosophila Activity Monitors, the sleep of the offspring was monitored. Males expressing either transgene and females expressing the GFP\textsuperscript{S/L}(3-8m) transgene saw increased sleep frequency during LD and DD cycles. Both male and female *Drosophila* populations expressing the GFP\textsuperscript{S/L}(3-8m) transgene were classified as arrhythmic. The altered circadian rhythms in *Drosophila* expressing the S218L mutation may help in understanding the mechanism linking sleep issues to migraines. This is because the altered rhythms may cause the sleep issues and this should be pursued in future studies. Data from all controls varied, which in future studies may be avoided by backcrossing the flies to a common wild-type strain. Many males expressing the GFP\textsuperscript{S/L}(3-8m) transgene died during the experiment, which may have been caused by the expression across all neurons. Future studies should address this by using drivers that express the transgene in a more localized area.
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Introduction

Characterized by some degree of hemiparesis during aura, Familial hemiplegic migraine (FHM) is a rare, monogenic subtype of migraine with aura (1). In addition to this motor weakness, diagnoses of FHM, requires a first or second degree relative to have migraine with motor weakness aura (1). There are four variations of FHM, with each being categorized by the specific gene mutated (1).

In this study, a heavy focus is laid upon Familial Hemiplegic Migraine Type 1 (FHM-1). FHM-1, is caused by missense mutations in the CACNA1A gene, which encodes the CAv2.1 subunit of calcium channels on the human chromosome 19 (1,2,3,4,5). In Drosophila, the CAC gene, has been known to be involved in male courtship behavior as well as the encoding of the voltage-gated calcium channel (6,7).

The specific CACNA1A mutation targeted in this study is the S218L mutation, one of the more common FHM-1 mutations found in mammals (4).

Many migraine sufferers report many sleep problems with the most prominent being, trouble falling asleep, waking up during the night, and lack of refreshing sleep (8,9,10,11). However, little is known about the mechanisms that link FHM to sleep and circadian rhythms. The purpose of this study is to gain a better understanding of how FHM mutations affect sleep and circadian rhythms and to establish a baseline for future studies.

Prior to experimentation, it was hypothesized that abnormal sleep data will be obtained from the Drosophila expressing either of the transgenes and that this effect will take place across both genders.

Methods and Materials
**Drosophila Lines**

In this study, four separate lines of *Drosophila* were raised. The first of the experimental groups was the UAS-CAC-GFP<sup>786C</sup> mutation. This mutation was produced by a transgene insertion on the third chromosome (12). The second of the two experimental groups consisted of *Drosophila* containing the UAS-CAC-GFP<sup>S/L</sup>(3-8m) transgene mutation. This transgene was constructed by using PCR to alter the serine 161 codon of the UAS-CAC- GFP<sup>786C</sup> mutation to leucine. This substitution corresponds to the S218L mammalian CACNA1A mutation (13,14). These two *Drosophila* lines were generously provided by Dr. Andrew Frank of the University of Iowa. The driver line ELAV-GAL4 was also raised. This driver allows for the expression of transgenes in all neurons, but no other cells (15). Lastly, a *W<sub>1118</sub>*CS line was raised. This line serves as a white eyed wild type, which has no driver capabilities (16). As lines grew in population, virgin females and males were collected, and individually stored for later crosses.

**Crosses**

The GAL4/UAS system was used in this study to select which neurons would express the desired transgene (17,18). This system works by having the UAS transgenes present in all cells, but only produce in select cells where GAL4 is active (17,18). Since the ELAV-GAL4 driver is used, all neurons will express the desired transgenes (15). The gender of the lines crossed was based upon availability.

Crosses between UAS-CAC-GFP<sup>S/L</sup>(3-8m) males and ELAV-GAL4 virgin females, and between UAS-CAC-GFP<sup>S/L</sup>(3-8m) males and *W<sub>1118</sub>* virgin females were set up. In this case the GFP<sup>S/L</sup> x GAL4 resulting offspring will be used as the experimental group, with the control consisting of the GFP<sup>S/L</sup> x *W<sub>1118</sub>* offspring. This is because the UAS-GAL4 will cause the expression of the transgene, while the *W<sub>1118</sub>*CS will not (15,16).
Crosses between UAS-CAC- GFP\textsuperscript{786C} virgin females and ELAV-GAL4 males and between UAS-CAC- GFP\textsuperscript{786C} virgin females and W\textsubscript{1118} males were also set up. In this second set of crosses, again the GFP\textsuperscript{786C} x GAL4 resulting offspring will be used as the experimental group, with the control consisting of the GFP\textsuperscript{786C} x W\textsubscript{1118CS} offspring. This is also because the GFP\textsuperscript{786C} x GAL4 will cause the expression of the transgene, while the GFP\textsuperscript{786C} x W\textsubscript{1118CS} will not (15,16).

A final cross between ELAV-GAL4 males and W\textsubscript{1118} virgin females was set up. This cross served as another control for each of the two cases.

**Sleep Monitoring**

In this study data was obtained through the use of Drosophila Activity Monitors (DAM). These DAMs monitor the activity of individual *Drosophila* in sealed tubes. They accomplish this by making use of an infrared beam in the midpoint of the tubes, which measures fly activity by how often the fly crosses the beam. It is from this information that data regarding sleep can be obtained (19). Information obtained from these monitors was collected every minute throughout the experiment.

Prior to monitoring the sleep of the resultant offspring, all female *Drosophila* had to have mated. This is because sleep patterns in females, can be affected by whether or not mating has occurred (20,21). This variable was eliminated by leaving male *Drosophila*, with the female *Drosophila*, that will be used in the experiment, for approximately 24 hours. This ensured that mating occurred.

After this time period expired, populations of 32 male and female flies were collected from each of the crosses. This number was determined based upon the maximum capacity of Drosophila Activity Monitors (19). These flies were then placed into individual tubes, and then
into the DAMs. The DAMs were subsequently placed into incubators with stable environmental conditions.

For the first 7 days, all *Drosophila* were exposed to consistent cycle of 12 hours of light followed by 12 hours of darkness (LD cycle). The following 5 days, all *Drosophila* were kept in a constant 24 hours of Darkness (DD cycle). The LD cycle, was used to obtain data pertaining to sleep when in the presence of an external stimulus, in this case light. While the DD cycle was used to provide information on the natural circadian rhythms of the *Drosophila*, since there will be no external stimulus, such as light (22,23).

**Results**

Usable data from 17 GFP<sup>S/L</sup> x GAL4 males, 30 GFP<sup>S/L</sup> x W<sub>1118</sub> males, 30 W<sub>1118</sub>x GAL4 males, 31 GFP<sup>786C</sup> x W<sub>1118</sub> males, 31 GFP<sup>786C</sup> x GAL4 males, 32 GFP<sup>S/L</sup> x GAL4 females, 31 GFP<sup>S/L</sup> x W<sub>1118</sub> females, 29 W<sub>1118</sub>x GAL4 females, 32 GFP<sup>786C</sup> x W<sub>1118</sub> females and 31 GFP<sup>786C</sup> x GAL4 females was obtained. Data were considered usable if *Drosophila* lived throughout the entire duration of the experiment.

**Light/Dark Cycle Males**

The GFP<sup>S/L</sup> x GAL males in a LD were noted sleeping on average many more times a day than GFP<sup>S/L</sup> x W<sub>1118</sub> males or W<sub>1118</sub>CS x GAL4 males (Figure 2B). GFP<sup>S/L</sup> x GAL males also saw slightly shorter daily average episodic sleep duration than either GFP<sup>S/L</sup> x W<sub>1118</sub> males or W<sub>1118</sub>CS x GAL4 males (Figure 2C). However, GFP<sup>S/L</sup> x GAL males had a much shorter episodic sleep duration than either of its controls during the 12 hour dark period (Figure 2C). On average GFP<sup>S/L</sup> x GAL males showed small increase over its controls in total sleep duration (Figure 2A), and tended to sleep slightly more towards the end of the 12 light period than either controls (Figure 1B).
Figure 1. Male LD cycle Average Sleep per 30 minutes
A) Graph A depicts the average sleep per 30 minutes for all male flies for each cross during the LD cycle period.
B) Graph B depicts the average sleep per 30 minutes all crosses pertaining to the UAS-CAC-GFP38L(3-8m) transgene and its controls during the LD cycle period.
C) Graph C depicts the average sleep per 30 minutes all crosses pertaining to the UAS-CAC- GFP786C transgene and its controls during the LD cycle period.

Figure 2. Male LD Cycle Sleep Graphs
A) Graph A depicts the average total sleep duration across all male flies for each cross during the LD cycle period.
B) Graph B depicts the average number of sleep episodes across all male flies for each cross during the LD cycle period.
C) Graph C depicts the average episodic sleep duration across all male flies for each cross during the LD cycle period.
GFP\textsuperscript{786C} x GAL4 males were seen sleeping on average a few more times daily than either the GFP\textsuperscript{786C} x W\textsubscript{1118} males or the W\textsubscript{1118}x GAL4 males. The largest difference occurred during the 12 dark period (Figure 2B). GFP\textsuperscript{786C} x GAL4 males also showed a shorter average daily episodic sleep duration than either of the controls, with the largest discrepancy occurring during the 12 hour dark period (Figure 2C). GFP\textsuperscript{786C} x GAL4 males were seen sleeping on average more than the W\textsubscript{1118}x GAL4 males during the 12 hour light cycle, but less than GFP\textsuperscript{786C} x W\textsubscript{1118} males during both the light and dark cycles (Figure 1C, Figure 2A).

Dark/Dark Cycle Males

The GFP\textsuperscript{S/L} x GAL4 males in DD had on average a much lower rhythmicity index (RI) than either GFP\textsuperscript{S/L} x W\textsubscript{1118} males or W\textsubscript{1118}CS x GAL4 males (Figure 4A). In fact, 70.6\% of the GFP\textsuperscript{S/L} x GAL4 males were classified as arrhythmic (RI<0.2), meaning the population studied was arrhythmic. Neither of its controls was classified as arrhythmic and each had a much lower percentage of arrhythmic flies (Figure 4B). The GFP\textsuperscript{S/L} x GAL4 males are noted as having a much flatter line in the average sleep per 30 minutes graph than either of their controls, showing their weaker rhythmicity (Figure 3B). These flies also had a slightly shorter circadian period length than their controls (Figure 4C). GFP\textsuperscript{S/L} x GAL4 males were noted sleeping many times more per day than GFP\textsuperscript{S/L} x W\textsubscript{1118} males or W\textsubscript{1118}CS x GAL4 males (Figure 5A) and having a much shorter average episodic sleep duration, especially during the second period of darkness, than these controls (Figure 5C). The average total sleep duration of GFP\textsuperscript{S/L} x GAL4 males, was slightly less than their controls (Figure 5A).
Figure 3. Male DD cycle Average Sleep per 30 minutes
A) Graph A depicts the average sleep per 30 minutes for all male flies for each cross during the DD cycle period.
B) Graph B depicts the average sleep per 30 minutes all crosses pertaining to the UAS-CAC-GFP\textsuperscript{5/7}(3-8m) transgene and its controls during the DD cycle period.
C) Graph C depicts the average sleep per 30 minutes all crosses pertaining to the UAS-CAC- GFP\textsuperscript{7/86C} transgene and its controls during the DD cycle period.
All error bars are based on SEM.

Figure 4: Male Circadian Data
A) Graph A depicts the average Rhythmicity Index (RI) across all male flies for each cross.
B) Graph B depicts the percentage of male flies of each cross with an RI<0.2.
C) Graph C depicts the average circadian period length across all male flies for each cross.
The GFP$^{786C}$ x GAL4 males showed only a slightly lower average RI when compared to GFP-$^{786C}$ x W$^{1118}$ males or W$^{1118}$x GAL4 males (Figure 4A). The average circadian period length of GFP$^{786C}$ x GAL4 males was longer than that of W$^{1118}$x GAL4 males, but shorter than that of GFP$^{786C}$ x W$^{1118}$ males (4C). GFP$^{786C}$ x GAL4 males slept on average more times per day and for a shorter average episodic duration than either of the controls groups (Figure 5B, Figure 5C), the larger difference for both cases was between GFP$^{786C}$ x GAL4 males and W$^{1118}$x GAL4 males.
Figure 6. Female LD cycle Average Sleep per 30 minutes
A) Graph A depicts the average sleep per 30 minutes for all female flies for each cross during the LD cycle period.
B) Graph B depicts the average sleep per 30 minutes all crosses pertaining to the UAS-CAC-GFP $^{3L}$/(3-8m) transgene and its controls during the LD cycle period.
C) Graph C depicts the average sleep per 30 minutes all crosses pertaining to the UAS-CAC-GFP $^{786C}$ transgene and its controls during the LD cycle period.
All error bars are based on SEM.

Figure 7. Female LD Cycle Sleep Graphs
A) Graph A depicts the average total sleep duration across all female flies for each cross during the LD cycle period.
B) Graph B depicts the average number of sleep episodes across all female flies for each cross during the LD cycle period.
C) Graph C depicts the average episodic sleep duration across all female flies for each cross during the LD cycle period.
All error bars are based on SEM.
GFP$^{786C}$ x GAL4 males were also noted having a slightly lower average total sleep duration than their controls (Figure 5A).

**Light/Dark Cycle Females**

In LD the GFP$^{S/L}$ x GAL4 females were seen having a much higher average total sleep than their controls, which were the GFP$^{S/L}$ x W$^{1118}$ females and the W$^{1118}$CS x GAL4 females (Figure 6b, Figure 7A). This is likely caused by the GFP$^{S/L}$ x GAL4 females having a higher number of average sleep episodes per day (Figure 7B), while also having a slight increase in average daily episodic sleep duration than either of their controls (7C).

The GFP$^{786C}$ x GAL4 females were noted having an increase in average number of sleep episodes per day, when compared to the GFP$^{786C}$ x W$^{1118}$ females or the W$^{1118}$x GAL4 females (Figure 7B). The average episodic sleep duration for GFP$^{786C}$ x GAL4 females, was slightly less than their controls over the course of a day, and much less during the second 12 dark periods (Figure 7C). Lastly, the average total sleep of the GFP$^{786C}$ x GAL4 females was slightly more than that of the W$^{1118}$x GAL4 females and slightly less than that of the GFP$^{786C}$ x W$^{1118}$ females (Figure 7A).

**Dark/Dark Cycle Females**

In DD the GFP$^{S/L}$ x GAL4 females had an average RI value much lower than that of the GFP$^{S/L}$ x W$^{1118}$ females or the W$^{1118}$CS x GAL4 females (Figure 9). This lower average RI translate to approximately 80.4% of GFP$^{S/L}$ x GAL4 females being classified as arrhythmic (RI<0.2) (Figure 9B). This percentage is much higher than either of the controls, which is likely the reason for the GFP$^{S/L}$ x GAL4 females showing a much flatter curve in the average sleep per 30 minutes graph than the two controls (figure 8B). However, the average circadian period
Figure 8. Female DD cycle Average Sleep per 30 minutes
A) Graph A depicts the average sleep per 30 minutes for all female flies for each cross during the DD cycle period.
B) Graph B depicts the average sleep per 30 minutes all crosses pertaining to the UAS-CAC-GFP<sub>SL</sub>(3-8m) transgene and its controls during the DD cycle period.
C) Graph C depicts the average sleep per 30 minutes all crosses pertaining to the UAS-CAC-GFP<sub>786C</sub> transgene and its controls during the DD cycle period.
All error bars are based on SEM.

Figure 9: Female Circadian Data
A) Graph A depicts the average Rhythmicity Index (RI) across all female flies for each cross.
B) Graph B depicts the percentage of female flies of each cross with an RI<0.2.
C) Graph C depicts the average circadian period length across all female flies for each cross.
All error bars are based on SEM.
length of the GFP<sup>S/L</sup> x GAL4 females was slightly shorter than that of the GFP<sup>S/L</sup> x W<sub>1118</sub> females but slightly longer than that of W<sub>1118CS</sub> x GAL4 females (Figure 9C). The GFP<sup>S/L</sup> x GAL4 females also had a much higher average number of sleep episodes per day and a slightly higher average daily total sleep duration over the two control groups (Figure 10B, Figure 10A). The average episodic sleep duration of the GFP<sup>S/L</sup> x GAL4 females, was slightly higher than that of the GFP<sup>S/L</sup> x W<sub>1118</sub> females, but slightly lower than that of the W<sub>1118CS</sub> x GAL4 females.
The GFP$^{786C}$ x GAL4 females had an average RI that was slightly lower than that of GFP$^{786C}$ x W$^{1118}$ females but slightly higher than that of W$^{1118}$CS x GAL4 females (Figure 9A). The GFP$^{786C}$ x GAL4 females also had an average circadian period length that was slightly shorter than that of GFP$^{786C}$ x W$^{1118}$ females but slightly longer than that of W$^{1118}$CS x GAL4 females (Figure 9C). The GFP$^{786C}$ x GAL4 females had a higher average number of sleep episodes per day (Figure 10B), but a shorter average episodic sleep duration than their two controls (Figure 10C). This ultimately leads to the GFP$^{786C}$ x GAL4 females having a lower average total sleep duration than their controls (10A).

**Discussion/Conclusion**

The main purpose of this study was to gain a better understanding of the mechanism linking FHM-1 to sleep and circadian rhythms. The sleep patterns of *Drosophila* with a common FHM-1 mutation were monitored and analyzed, and compared to that of *Drosophila* with a different cacophony mutation that doesn’t result in FHM-1. Perhaps the most promising data pertains to circadian rhythms. The GFP$^{S/L}$ x GAL4 cross produced both males and female populations that were classified as arrhythmic, having RI of less than 0.2. This finding may prove to be significant, because mutations in cacophony may lead to altered circadian rhythms. Also, nearly 50% of males expressing the S218L mutation died over the course of the experiment. This may be a result of broadly expressing this gene in all neurons. Future studies may want to potentially use drivers that do not express the transgene across all neurons to look for any significance in this increase mortality rate. Overall the data supported the original hypothesis. This is because lines expressing either of the transgenes tended to show different sleep data, across the three major sleep categories analyzed, while abnormal circadian rhythms were observed in both male and female flies expressing the S218L. In the future, an ANOVA test will be conducted on this
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data to gain a better understanding of the significance of the data. From the data the most
pertinent problem faced, was the discrepancies between the control groups. The three control
groups GFP<sub>S/L</sub> x W<sub>1118</sub>, W<sub>1118</sub>x GAL4, GFP<sub>786C</sub> x W<sub>1118</sub>, should have produced similar results.
This is because none of the controls had both a UAS and a GAL4 group which would cause the
expression of the mutated transgene. Despite this the controls produced varying results, which
different greatly in many portions of the data. Potential explanation for the cause of the control
discrepancies could have been the lack of backcrossing, prior to sleep examination (24). This
backcrossing would help to reduce the effects of the flies’ genetic background on the study, and
help to block out some background genes which could alter sleep patterns in <i>Drosophila</i> (24).
This may be a potential avenue for further research, and using the same experiment but
implementing backcrossing into the procedure. A second cause of potential issues, particularly
for the males, would be the inheritance of the desired gene. Being able to only inherit one X
chromosomes, male flies may not inherit the proper genes to express the desired phenotype,
which could lead to varying data. Again, this should be addressed by future studies, in order to
increase the reliability of the controls. Another interesting future area of study would be locating
the specific neurons which may connect these cacophony mutations to sleep and circadian
rhythms would be to implement different drivers. Different drivers, will express these mutations
in different cells in the <i>Drosophila</i>, and by observing the effects of different drivers the
mechanism linking FHM to sleep and circadian rhythms could be further localized and a better
understanding of it be had. The expression of the S218L mutation, the most common mammalian
FHM-1 mutation, resulted in altered circadian rhythms in <i>Drosophila</i>. The fact that a mutation in
the same gene as the S218L, which does not result in FHM, did not alter circadian rhythms
strengthened the case that FHM alter circadian rhythms. This is because only cacophony
mutations that result in FHM resulted in altered circadian rhythms in this study. This finding may also expand to humans who suffer from FHM-1. Migraines have been known to negatively impact sleep in humans, but the underline reasons behind this negative impact is not fully understood. This finding that S218L is linked to altered circadian rhythms may help to explain the negative affects migraines and in particular FHM have on sleep. The worsened sleep of those suffering from FHM may be attributed to their altered circadian rhythms, and this avenue for future research should be explored. To do this, mammalian species, such as rats, containing the S218L mutation should have their sleep monitored, and potential alterations in circadian rhythms should be looked for. If the same results are duplicated in mammalian species, then a new explanation for why those with FHM experience worsened sleep may have been discovered. That discovery would be that FHM does not affect sleep directly; rather it affects circadian rhythms, which in turn has a negative impact on individuals sleep.

References


