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Sleep-reducing effect of mutant Ken *Drosophila melanogaster* flies on virgin female flies

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Abstract

It is widely accepted that sleep plays an important role in numerous aspects of the overall functioning of organisms. It has been implicated that sleep is responsible for memory consolidation and motor function, among other aspects.

In *Drosophila melanogaster*, sexual interaction affects sleep and circadian rhythms. These flies have been shown to sleep more in densely-populated environments, while they've been shown to sleep less when socially isolated, all during the daytime. This is partially due to an increase in sleep need in socially-enriched flies. Following copulation, male fruit flies have a reduced evening activity peak and a lengthened clock period, while female flies have a decreased overall activity during the daytime. Copulation also causes a sleep-reducing effect, that takes hold during the daytime, on female flies. This effect displays how wakeful experience can affect sleep.

The Ken mutant strain causes some male flies to have internalized genitalia. There exist two variations of these types of males: one which does not seemingly have external genitalia (smooth Kens), and another which does (rough Kens). This study found that while both variations caused sleep-reducing effects on virgin females, that occurred during the daytime, they were less than those caused by Canton-S (CS) wild-type male flies. The sleep-reducing effect

caused by smooth Kens was not found to be statistically significant while rough Kens did have a statistically significant sleep-reducing effect on females.

Introduction

Various aspects of sleep, and even its primary function, still remain largely unknown (Ganguly-Fitzgerald, 2006; Dove, 2016). In search of these aspects, researchers have taken particular interest in *Drosophila melanogaster* flies. Sleep within these organisms greatly resembles that of mammals (Cirelli, 2008). Information that is learned about sleep within *Drosophila* flies, therefore, is very likely to also be true of sleep in mammals. Because these flies are immensely more simplistic creatures than mammals, they are far easier to understand through experimentation. Further, genetic manipulations can be created with ease in these organisms (Dove, 2016).

Though numerous factors regarding sleep's role are not known, sleep has been shown to play a crucial role in the consolidation of memories, the emotional recognition of certain circumstances, and adequate motor function (Walker, 2006). In Walker's 2005 article, it was expressed that learning as well as memory plasticity and consolidation are very much dependent on sleep in both humans and animals. This particular publication also emphasized how ideas and motor patterns, which are learned shortly before sleep, are likely to be remembered in greater detail than those that are learned at an earlier time before sleep (Walker, 2005). In Dove's 2016 article, whose results are displayed in Walker's 2006 publication, the significance of sleep in the recollection of memory is clearly shown. This correlation was expressed through an experiment, whereby a sleep-deprived group retained memories significantly less than another which underwent sufficient sleep. These memories were of information which was taught to both groups (Walker, 2006).

The quality of sleep, which an organism has, affects consequent wakeful behavior. Conversely, the wakeful behavior of an organism can actually alter its sleep. While the causes of both of these are not known, the latter phenomenon can be better understood through studying the effect of social interactions on the sleep of both male and female model organisms such as *Drosophila* flies (Dove, 2016).

It has been shown that fruit flies, which undertake social interaction, sleep differently from flies which lack such interaction. In Ganguly-Fitzgerald's 2006 experiment, this difference was made apparent when one group of flies underwent social enrichment while another was deprived of it. The socially enriched group, which held 30 or more flies, was found to have slept significantly more than the group consisting of individually housed flies during the daytime. When the socially isolated flies were consequently exposed to social enrichment, they showed an increase in sleep. However, social interaction was found to have no effect on the nighttime sleep of the flies, as there was no prevalent difference in the sleep of either group during the nighttime. In the same experiment, the sleep need of the flies was found to increase as the amount of flies within the socially enriched group increased by contrast to socially isolated flies. This effect on sleep need, however, did not take hold within socially enriched blind flies (Ganguly-Fitzgerald, 2006).

Social interaction, but more specifically socio-sexual interaction (SSI), has been shown to also affect the circadian rhythms of *Drosophila melanogaster*. The circadian clock is characteristic of almost every earthly organism. It drives most organisms' physiology and behavior (Peschel, 2011). In Lone's 2011 publication, a series of experiments displayed the effects of SSI on the circadian rhythms of flies. As a control group, one assortment of flies was kept from undergoing SSI, while the experimental group did undergo SSI. The control consisted of same-sex flies while the experimental had mixed-sex flies with a ratio of 1:1, each group having 30 flies. It was found that SSI reduced the evening activity peak and lengthened the clock period in males, and decreased overall activity in females under LD conditions. This study also investigated the role of functioning circadian clocks, olfactory ability, and lateral-ventral clock neurons (LNvs). The results showed that all of these factors were necessary if the same effects of SSI on the flies' circadian rhythms were to take hold (Lone, 2011). Contradictory to these results, Dove's 2016 article's concluded that sexual interactions amongst male and female fruit flies had no significant effect on their circadian rhythms (Dove, 2016). It is important to note that the effects of social interaction on sleep are not indicative of the flies' overall activity (Ganguly-Fitzgerald, 2006).

Dove’s 2016 publication showed the result of sexual experience on sleep in flies. It expressed that both gender and mating status, as well as the interaction between those two factors, play a significant role in altering sleep. The article’s experiment indicated that, following sexual experience, males slept more than females during the daytime. Contrarily, females were found to have a decrease in daytime sleep, which lasted six days following sexual experience (Dove, 2016).

Dove’s publication did not acknowledge or take into consideration that there are two variants of Ken flies- one of which does not seemingly have external genitalia, and another which does (Dove, 2016). These have been given the names smooth and rough flies, respectively. The research presented in this paper, on the other hand, did consider the two variants of Ken flies. It sought to find whether rough and/or smooth flies had the same daytime, sleep-diminishing effect which wild type male flies have on females following copulation.

The goal of this study was to determine whether Ken flies indeed have a sleep-reducing effect on female flies’ sleep, which is normally present in females, following copulation. More specifically, the publication sought to find whether this was true in both rough as well as smooth Kens, and if so, to what extent. Therefore, the female flies that were either kept alone or paired with CS wild-type male flies served as control groups, as those kept alone would be expected to have no sleep reduction while those paired with CS males would show the typical reduction in sleep that normally follows copulation. The sleep amount that the females which were paired with both types of Ken flies undertook would be compared to that of both control groups. Based on this comparison, the extent to which either the rough or smooth Kens had an effect on the females' sleep, after copulation, would be inferred.

Methdology

Initially, Canton-S (CS) wild type virgin female flies, CS wild type males, and Ken smooth and rough flies were collected. It was verified that the female flies were virgins by collecting females of a young age at which they do not copulate. Virgin females were collected by identifying certain traits they have: a meconium, a grey spot, on the abdomen; a generally lighter-colored abdomen; less developed, folded wings; stripes on the back. It was necessary that all of the female flies that were collected were virgin, as mated females may have had skewed effects due to copulation. Male flies' traits included genitalia, which looked like a dark patch on the males' backs, and sex combs on the legs.

The females were either put in containers without males, with CS males, with Ken smooth males, with Ken rough males, or by themselves. Nineteen virgin females were put with CS males, nine virgin females were put with smooth Ken males, and eighteen virgin females were put with rough Ken males. The ratio of females to males was 2:1 for each individual group of flies. Once the various groupings of flies were given adequate enough time to copulate, all female flies were individually placed into small, separate tubes. This was done by inserting a needle, discharging CO_2, through the styrofoam cap of the containers which covered the containers they were held in at the time. Once the CO_2 caused the flies to knock out, they were put onto a CO_2-emitting pad, on which they would not awake until taken off. On this pad, the flies were able to be distinguished from one another, and were then put into new tubes that corresponded to their purpose in the experiment.

The tubes contained agar, which the females were able to sustain themselves off of. These tubes were then put in an incubator set at 25°C and at 70% humidity.

The sleep which the flies attained during the experiment was considered in both light (LP) and dark (DP) conditions. The light and dark conditions were given a ratio of 12:12 hrs each day the flies were in the incubator. The amount of sleep attained by the female flies following sexual interaction was determined by measuring their activity. This was done through counting the amount of times they crossed a movement-sensing laser placed in the small tubes. Data from days 2-4, during which the flies were held in the incubator, was considered. This was because the sleep-effect caused by copulation began to wear off after day 4 and day 1 comprised of acclimation.

Results

As shown in Figure 1, rough and smooth Kens had a sleep-reducing effect on females, but to a lesser extent than typical male flies would have. Rough Kens had the effect on females to a greater extent than the smooth Kens. The sleep-reducing effect that smooth Kens had on females was not statistically significant while the one that rough Kens had on females was statistically significant. During the 12 hr light period, both rough and smooth Kens were found to have a sleep-reducing effect on the females. Of these two, only the rough Kens were found to have a statistically significant sleep-diminishing effect during the light cycle. None of the groups were found to have any sleep-reducing effect during the 12 hr dark period.

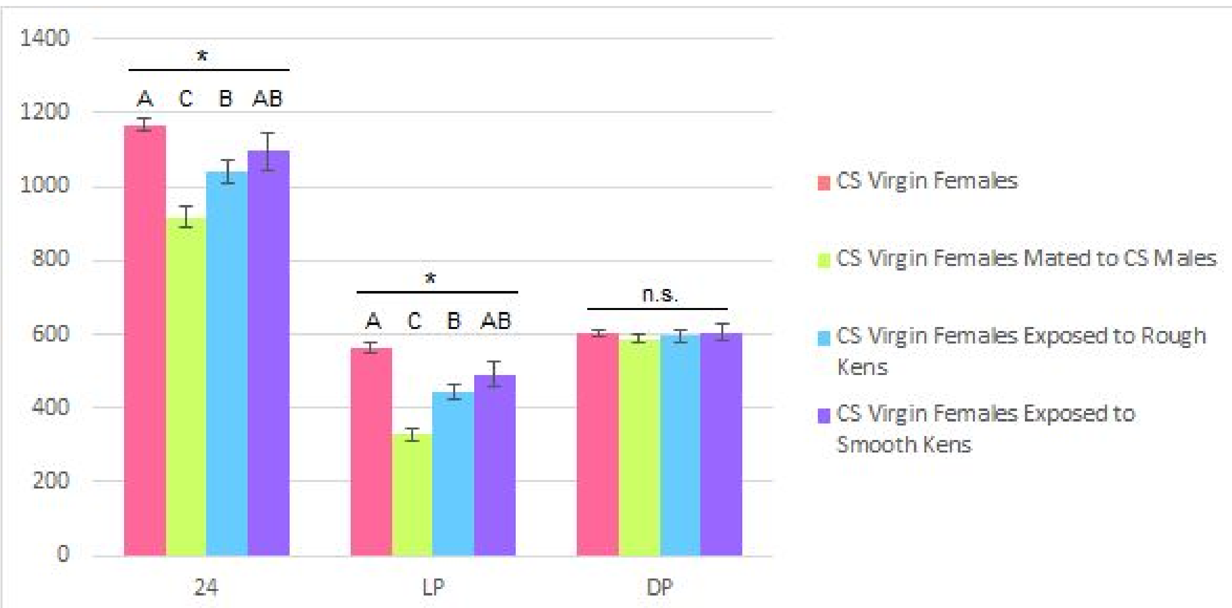

Figure 1- The CS Virgin Females (A) were found to have no sleep-diminishing effect due to copulation in both light and dark conditions. CS Virgin Females which mated with CS Males (C) had a typical, expected sleep-diminishing effect on sleep. Those results from groups A and C served as controls. The CS Virgin Females that were exposed to Rough Kens (B) and those

that were exposed to Smooth Kens (AB) both had less sleep than the CS Virgin Females during LP, while having more than CS Virgin Females mated to CS Males. However, only the daytime sleep-reduction in CS Virgin Females that were exposed to Rough Kens was significant of both B and AB.

Conclusion

The “rough” and “smooth” Kens both caused a sleep-reducing effect in females following copulation. However, the “rough” Kens did so to a greater extent than did the “smooth” Kens while both types of Kens caused the effect to a lesser extent than the CS wild-type males. The “smooth” Kens did not have a statistically significant effect on the females’ sleep.

To better this experiment in the future, and perhaps gain more accurate findings as a result, it should be made sure that: a more proportional ratio of male to female flies is made, and whether the female flies underwent copulation or not, in the incubator, is made apparent.

If a similar study were made, which would seek to re-test this experiment, then it would likely have more accurate results if it had a greater sample size of flies, had a ratio which had more males than females, to ensure the females would undergo copulation, and was re-done using multiple generations of flies. Researchers could try to find other factors that may have some relevance in the degree to which copulation causes the sleep-reducing effect in female flies. Studies can also be made involving the effect that social interactions generally have on flies and how they sleep.

Some researchers wonder what the evolutionary reason behind the daytime sleep-reduction in females due to copulation is. Some theorize that this sleep-reduction is caused to promote foraging behavior for egg-laying sites. One other question is why the sleep-effects brought on by copulation, and social interaction in general, in *Drosophila melanogaster*, seemingly only takes hold during the daytime.

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