Do Male *C. elegans* Sleep?

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Background:

All animals sleep, indicating that sleep must serve an important function. In humans, sleep loss is associated with defects in cognitive performance and cardio-metabolic function (Mullington et al., 2009). However, it is not yet clear why sleep loss has these effects, and whether sleep might be dispensable under certain conditions. To investigate these questions, we are using the nematode worm *C. elegans.* Interestingly, these animals do not sleep at regular intervals, like we do, but rather, they sleep only following exposure to damaging conditions, such as noxious heat, ultraviolet light, and bacterial toxin (Hill et al., 2014; DeBardeleben et al., 2017), a phenomenon known as stress-induced sleep (SIS). Importantly, following noxious heat exposure, failure to sleep is associated with reduced survival (Hill et al., 2014). These data indicate that, at least in *C. elegans*, cellular damage contributes to sleep drive, and sleep promotes cellular repair.

C. elegans are hermaphroditic, but males can arise at a low frequency. Because males are rare, their sleep behavior hasn't been previously examined. Here we present the surprising results of our analysis of sleep behavior in *C. elegans* males.

Results: *C. elegans* males are sleepless

To characterize male sleep behavior, we subjected them to multiple stressors known to trigger stress-induced sleep (SIS). We examined the sleep response following exposure to Cry5B, a known SIS-inducing toxin. When exposed to this damaging toxin, most hermaphrodites fall asleep, but surprisingly, almost none of the males do, demonstrating that males were sleepless in certain stressful situations. (Fig 2A). We hypothesized that the male sleep defect might be due to a competing drive to mate, which may override SIS. To examine this possibility, we assayed sleep in the absence of hermaphrodites and found that males were similarly sleepless (Fig 2B), indicating that a competing drive to mate is not the cause of male sleeplessness.

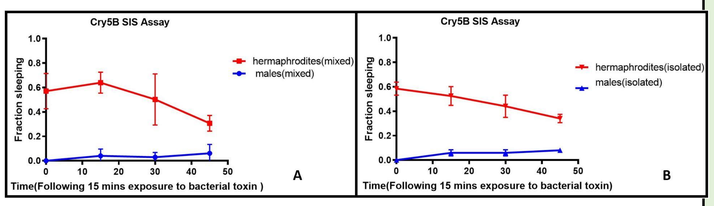


Figure 2 C. elegans males are sleepless and this is not attributable to the chance of imminent mating. (A,B) WT hermaphrodites and males were examined for SIS following Cry5B exposure with the initial scoring performed at the end of the exposure.

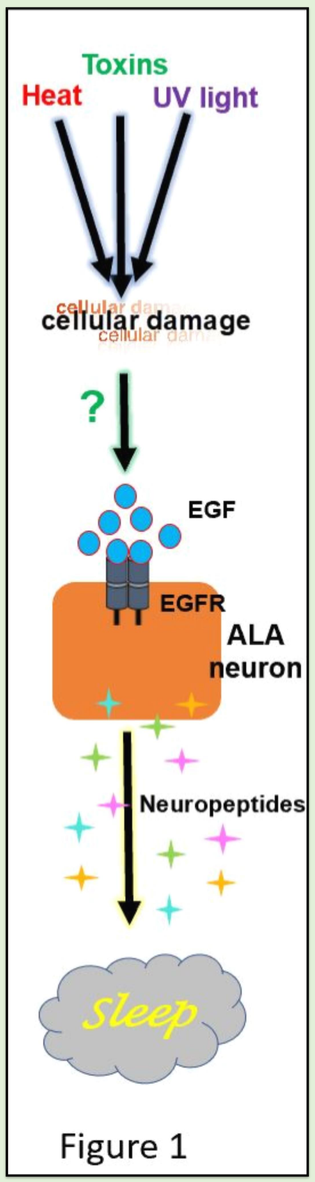
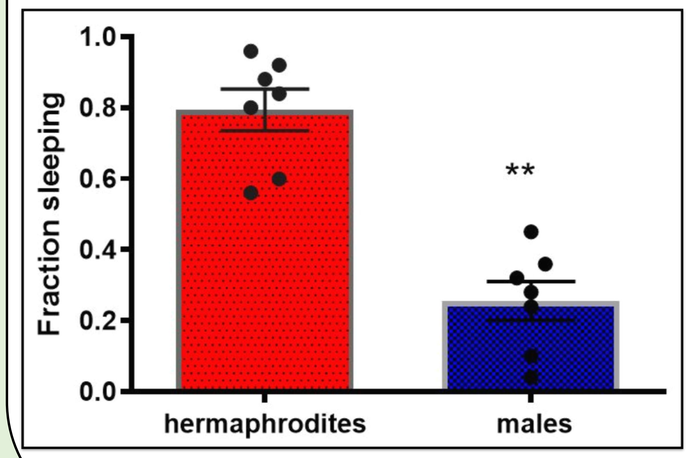


Figure 1

Results: Male sleeplessness is likely not due to stress resistance

We thought the cause of male sleeplessness was attributed to stress resistance or a defect in the SIS pathway (Fig 1). To determine if the males were stress resistant, we forced EGF expression using an inducible transgene. This allowed us to put the animal to sleep anytime, even in the absence of cellular damage and to determine whether males differ in a component upstream or downstream of EGF. (Fig 3) We found that males were defective in EGF-induced sleep compared to hermaphrodites, suggesting there is something defective downstream of EGF otherwise males would respond to damage similarly to hermaphrodites. This rules out the hypothesis that males have stress resistance.



Materials and Methods:

For the Cry5B SIS assays, WT hermaphrodites and males were examined for SIS following a 15-minute Cry5B exposure. Animals were scored every 15 mins for sleep, as defined by immobility. The initial scoring was performed at the end of the exposure and immediately before transferring the animals to an NG recovery plate for the remainder of the time course.

For the EGF-induced sleep assay, animals were placed on small plates and placed in a 35 ° C water bath for 20 minutes. Animals were scored every 15 minutes. The initial scoring was performed at the end of 60 minutes.

Discussion:

In this study we show that *C. elegans* males do not engage in stress-induced sleep (SIS). The SIS defect doesn't appear to be attributable to an overriding drive to mate, as males are similarly sleep-defective whether hermaphrodites are present or not, and it is also not likely due to stress resistance. Instead, the sleeplessness is likely attributable to sexual dimorphism in the SIS pathway, as evidenced by the male sleep defect observed following EGF expression. We are very interested in characterizing the nature of this sleep defect as well as identifying any conditions under which these males will sleep . While our findings indicate that *C. elegans* males do not sleep, we do not yet know if they still need to sleep. This question can be addressed only if we first find a condition under which males sleep, as this would reveal whether sleep improves male survival following near-lethal heat exposure, as has been observed in hermaphrodites. If these males are sleepless without consequence, while hermaphrodites die without sleep, this would represent an extremely valuable model system for the study of sleep need. As components of SIS have been conserved across species, we speculate that our studies of sleep need in *C. elegans* may have relevance for humans.

References:

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