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Changes in Gene Expression with Sleep

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There is general agreement within the sleep community and among public health officials of the need for an accessible biomarker of sleepiness. As the foregoing discussions emphasize, however, it may be more difficult to reach consensus on how to define such a biomarker than to identify candidate molecules that can be then evaluated to determine if they might be useful to solve a variety of real-world problems related to insufficient sleep. With that in mind, a goal of our laboratories has been to develop a rational strategy to expedite the identification of candidate biomarkers.[1] We began with the assumption that since both the genetic and environmental context of a gene can influence its behavior, an effective test of sleep loss will likely be composed of a panel of multiple biomarkers. That is, we believe that it is premature to exclude a candidate analyte simply because it might also be modulated in response to other conditions (e.g., illness, metabolism, sympathetic tone, etc.). Our next assumption was that an easily accessible biomarker would be more useful in real-world settings. Thus, we have focused on saliva, as opposed to urine or blood, as a rich source of biological analytes that can be mined to optimize the chances of bringing a biomarker out into the field. Finally, we recognize that conducting validation studies in humans can be expensive and time consuming. Thus, we have exploited genetic and pharmacological tools in the model organism *Drosophila melanogaster* to more fully characterize the behavior of the most exciting candidate biomarkers.

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SUPPLEMENT

Although the fly has only been used to study sleep for ~10 yr, several tools have been developed in flies that are well suited for rapidly identifying biomarkers of sleepiness. Sleepiness in flies is operationally defined as the presence of sleep homeostasis, the increase in sleep observed following extended waking. Like mammals, flies display a large sleep rebound following a night of sleep deprivation indicating that they are sleepy.[2-4] Accordingly, a biomarker should be increased in flies following periods of waking that are associated with a homeostatic response but should remain unchanged in flies following periods of waking that do not produce a sleep rebound. In flies, the disassociation between wake time and sleep homeostasis can be accomplished in several ways. For example, during periods of starvation, flies remain spontaneously awake but, in contrast to sleep deprivation, do not display a homeostatic response and do not show learning impairments.[5,6] That is, despite experiencing similar amounts of waking to their sleep-deprived siblings, starved flies do not appear to be sleepy or cognitively impaired. A similar disassociation between wake time and sleep homeostasis can be achieved by comparing flies treated with caffeine or methamphetamine.[1,7] Both caffeine and methamphetamine each produce sustained periods of waking and similar locomoter activity profiles. However, unlike caffeine, flies do not compensate for the lost sleep accrued during methamphetamine-induced waking. Finally, wake time and sleep homeostasis can be disassociated genetically. For example, flies mutant for *timeless* (*tim*^{01}) are specifically resistant to short-term sleep deprivation (3 and 6 h) but exhibit a normal homeostatic response following 9 and 12 h of sleep deprivation.[3,8] Thus, if a biomarker is truly associated with being sleepy, it should remain unchanged following 3 and 5 h of sleep deprivation in *tim*^{01}mutants but should be significantly altered following 9 and 12 h of sleep deprivation.

Using a variety of gene discovery techniques, we have identified several candidate biomarkers in flies that are only increased when waking is accompanied by a sleep rebound (e.g., sleep deprivation, caffeine administration, 9 and 12 h of sleep deprivation in *tim*^{01}mutants) and are not modified when waking does not initiate a homeostatic response (e.g., starvation, methamphetamine, 3 and 5 h of sleep deprivation in *tim*^{01}mutants). Candidate biomarkers that pass these tests are then further characterized to determine if they are activated by "stress" or to non-specific effects of the sleep deprivation apparatus. This is accomplished in three ways. First, we stress the animals by feeding them the toxin, paraquat. Second we expose the animals to the same mechanical stimulus during their primary waking period. And, third, we evaluate the analyte during ontogeny, a naturally occurring condition during which sleep drive is high and thus there is no need to intervene to enforce waking.[2,9,10] The first gene that we identified using this approach was Amylase. Thus, in flies, Amylase is a *bonafide* biomarker of sleepiness: it is unregulated only during conditions that are accompanied by a sleep rebound and is not modified by a variety of "stressors."

To determine whether Amylase might also be modified by sleep deprivation in humans, we examined salivary Amylase levels after 28 h of sustained wakefulness compared to untreated circadian-matched controls.[1] We found that both Amylase protein and Amylase mRNA were increased following sleep deprivation. In contrast, salivary cortisol was not altered by sleep deprivation. Thus "stress" is as unlikely an explanation

for the changes in Amylase after sleep deprivation in humans as it is in flies. Indeed, a recent study has shown that genetic reduction of adenosine deaminase (ADA) activity results in more slow wave sleep, increased feelings of being sleepy, impaired performance on the psychomotor vigilance task (PVT) and enhanced Amylase activity.[11] Together with the fly data, these results indicate that Amylase may be a useful tool for assessing sleep drive in humans.

It is important to acknowledge that while Amylase levels are clearly responsive to sleep drive, Amylase activity is also modulated by the circadian system and is acutely increased by sympathetic activation.[11-14] The extent to which these, and other variables, might influence Amylase levels in the field remains unknown. As a consequence, the utility of Amylase as a biomarker of sleepiness in humans will require further validation under a variety of experimental conditions. As mentioned, we believe that a true test of sleepiness will require a panel of independent biomarkers, each of which has been fully validated. There is no question that the sleep community possesses the skills to validate potential biomarkers. Indeed, several different strategies for validating a biomarker have been discussed during this meeting. The problem, then, is not determining how to validate a biomarker but rather, to first identify candidate biomarkers that need to be validated.

We have outlined a simple and effective strategy for identifying biomarkers of sleepiness using unique genetic and pharmacological tools in *Drosophila*. With this approach it is possible to evaluate the behavior of a candidate molecule under a variety of experimental conditions and determine whether it is consistently associated with high sleep drive or whether it is nonspecifically activated by a particular experimental protocol. In addition, we have shown that it is possible to identify candidate biomarkers of sleepiness in human subjects from samples derived from a readily accessible biological fluid using noninvasive procedures.

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DISCLOSURE STATEMENT

The authors have indicated no financial conflicts of interest.