Working Towards a Model of Genetic Profiling for Vulnerability/Resiliency to Sleep Loss through a Summer Research Fellowship in a Military Science Laboratory

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Abstract

Introduction: The following working model came from undergraduate students who participated in the Army Educational Outreach Program (AEOP). AEOP engages, inspires, and attracts the next generation of Science, Technology, Engineering, and Mathematics (STEM) talent by exposing STEM students to research conducted by the Department of Defense (DoD).

Methods and Materials: The students were paired with the Sleep Research Center (SRC) – the largest research center dedicated to the impact of sleep loss on mental acuity in the DoD at the Walter Reed Army Institute of Research (WRAIR). Though WRAIR is best known for developing vaccines for infectious diseases (and carrying on the legacy of Dr. Walter Reed who discovered the vector responsible for yellow fever), groundbreaking work on the functions and substrates of sleep and how sleep enhances mental acuity was also pioneered at WRAIR. Results/Discussion: During their summer fellowship, the students were inspired by two lines of research that WRAIR has pursued. First, WRAIR has developed caffeine dosing schedules used to keep Soldiers awake during combat.2 Second, WRAIR has identified new biological targets intended to predict vulnerability/resiliency to sleep loss and subsequent impact on mental acuity. 1,2 Therefore, the students made it their goal to combine these two lines of research by developing a working model of genetic profiling that could possibly help identify Soldiers whose health and safety are at-risk during combat under compromised sleep.

Introduction

Identifying the problem: lack of sufficient, restorative sleep during continuous combat operations

Seven to nine hours is the gold-standard for sleep optimization of mental acuity, although there is inter-individual variation in this amount.[1,2] Less than seven hours of sleep per night is strongly linked to lapses in attention, judgement, and emotional reactivity. If insufficient sleep persists, both morbidity and mortality increase.[1,2,3,4,5,6] Unfortunately, the United States military prides itself on achieving dominance on the battlefield by means of 'owning the night' [see current Army Posture Statement]. Even when not operating, Soldiers have constant anticipation of danger and uncertainty. These factors make it nearly impossible for Soldiers to achieve adequate and restorative sleep. Even for Army-employed sleep researchers and physicians tasked with addressing inadequate and non-restorative sleep in the operational environment, the fact remains that they too also suffer from the inability to achieve adequate and restorative sleep during deployment for reasons listed above.[7]

Identifying effective, pharmacological countermeasures for the problem: caffeine optimization

Caffeine is the most consumed drug in the United States. According to a study by National Institutes of Health (NIH) researchers in which 37,602 individuals completed comprehensive seven-day diaries, 85% of the U.S. population consumed at least one caffeinated beverage per day.[8] Caffeine consumption by military personnel is higher than the average population and is also the most common stimulant used by military personnel to stay alert and awake in the operational environment.[9] Caffeine upregulates arousal-promoting (cholinergic) pathways.[10] Caffeine can also modestly delay (also referred to as phase-shift) human sleep/wake and endocrine rhythms.[11]

The current working model was built specifically on the caffeine research completed at WRAIR. First, WRAIR researchers have developed caffeine dosing schedules using a patented, quick-release caffeinated gum: Military Energy Gum (MEG).[12] One piece of MEG contains 100 mg of caffeine. Kamimori et al. discovered in a dose-dependent clinical trial that the gum is pharmacologically active in less than 10 minutes and that the mechanisms of action were such that caffeine released from the gum quickly crossed the blood-brain barrier via the salivary buccal cavities, bypassing the digestive tract.[12] Second, WRAIR researchers have found that the ability of caffeine to preserve and/or slow the rate of degraded mental acuity with less than seven hours of nighttime sleep is obsolete after three days.[13] After three days, caffeine cannot substitute the restorative effects of sleep for next-day performance.[13] The third and most critical finding for the development of the working model is WRAIR's discovery of large inter-individual variation in responsivity (e.g., tolerance and sensitivity) to caffeine's alertness and performance-enhancing effects with sleep loss.[1,2] This data is critical because it shows that no two people respond similarly to sleep loss, recovery from sleep loss, or the ability of caffeine to mitigate the negative consequences of sleep loss. In summary, these findings make up almost two decades of research on caffeine's ability to stabilize performance under sleep loss. These findings are also the basis for caffeine dosing schedules adopted by military personnel during deployment as published in *Army Training*

Protocol (ATP) 6-22.5: A Leader's Guide to Soldier Health and Fitness (Figure 1).

Identifying effective, biological countermeasures for the problem: genetic polymorphisms

The third step towards developing a working model to optimize Soldier health and safety was to dissect the genetic landscape of vulnerability and resiliency to sleep loss. Select genes are involved in regulation of sleep amount, sleep timing, and caffeine metabolism.[14,15,16,17,18] Furthermore, the biochemical actions of caffeine and regulation of sleepiness by adenosine are closely related. Caffeine blocks the release of adenosine, a neurotransmitter that suppresses neural activity in the brain, leading to a desire and biological need to sleep. Adenosine is also a byproduct of wakefulness due to increased production of adenosine triphosphate (ATP).[16,17] During sleep deprivation, adenosine levels in the brain will continue to rise well beyond normal physiological ranges and will not fall until an individual sleeps.[16,17]

With this knowledge, the working model was built on six single-nucleotide polymorphisms (SNPs) underlying sleep regulation by adenosine -- ADORA2A, ADA -- and by circadian clock-controlled genes -- PER2, PER3 -- as well as caffeine metabolism at the level of the liver: CYP1A2, and NAT2.[14,15,16,17,18] Each SNP has a selective predictive role/function pertinent to sleep regulation, caffeine metabolism, and next-day performance:

1. Adenosine-derived polymorphisms (ADORA2A and ADA) predict next-day performance after normal sleep and/or after sleep loss.14,15,19 The particular genetic variants of ADORA2A for this working model were rs5751862, rs5760405, rs2298383, rs3761422, rs2236624, rs35329474, and rs4822492 found on chromosome 22. The particular genetic variants of ADA for this working model were rs73598374 and rs394105 found on chromosome 20. In general, these studies have found that HT4 haplotypes were more resilient to sleep loss than non-HT4 haplotypes. Interestingly, these phenotypes were linked to "genetic trade-offs" such that non-HT4 haplotypes were more sensitive to caffeine compared to HT4 haplotypes, meaning caffeine had the ability to stabilize performance under sleep loss in non-HT4 haplotypes.

2. PER polymorphisms predict preference for early rise/bedtimes (< 0500/ < 2100, EST) or late rise/bed times (> 1000/ > 0100, EST). Preferred rise time/ bedtimes are important considerations. First, research has shown that athletes engaged in high-risk physical activity (like Soldiers) perform better in the evening.[20] Further, caffeine supplementation can elevate high-risk physical activity performed in the early morning to levels of (placebo-supplementing) evening performance.[20] Second, it has been shown that early risers are more physiologically and psychologically resilient to sleep deprivation.[21] The particular genetic variants of PER2 for this working model were rs2304672 and rs10462023 found on chromosome 2. The particular genetic variants of PER3 for this working model were rs35426314, rs228669, rs35733104, rs228696, rs35899625, rs228697, and rs17031614 found on chromosome 1. For these particular coding regions, previous studies have found, for example, that PER3 (4/4) genotypes were more resilient to sleep loss compared to PER3 (4/5) genotypes.[19] Unlike research completed with adenosine polymorphisms, the ability of caffeine to stabilize performance in PER3 (4/5) genotyped individuals under sleep loss is still unknown.

3. The two primary polymorphisms conferring inter-individual differences in caffeine metabolism, CYP1A2 and NAT2.[22,23] The particular genetic variants of CYP1A2 for this working model were rs2069514, rs12720461, and rs762551 found on chromosome 15. The particular genetic variants of NAT2 for this working model were rs1041983 and rs1801280 found on chromosome 8. The selected polymorphisms of CYP1A2 and NAT2 are linked to reduced caffeine sensitivity due to heightened metabolism of caffeine by way of heightened CYP1A2 and NAT2 ratios.[22]

Table 2-3. Using caffeine under various conditions of sleep deprivation

Condition under which caffeine is used	*Guidelines for use*
Sustained operations (no sleep)	• 200 milligrams starting at approximately midnight. • 200 milligrams again at 0400 hours and 0800 hours, if needed. • Use during daytime hours only if needed.
Night shifts with daytime sleep	• 200 milligrams starting at beginning of nighttime shift.
Restricted sleep	• 200 milligrams upon awakening. • 200 milligrams again 4 hours later. • Discontinue or reduce caffeine intake for the last 4 to 6 hours before initiating sleep.

Figure 1: Adapted from Army Training Protocol (ATP) 6-22.5: A Leader's Guide to Soldier Health and Fitness. *Table outlines caffeine dosing schedules adopted by military personnel during deployment. These best practices were developed from over two decades of caffeine research done at the Walter Reed Army Institute of Research (WRAIR) in Washington, D.C.*

Cognitive Performance post Sleep Disruption

Caffeine Sensitivity	(A) Preserved	(B) Impaired
(1) Sensitive	A1*	B1
(2) Tolerant	A2	B2*

Figure 2: Intra-individual variation in the ability to perform is dependent on two principles. The first principle is intra-individual sensitivity to sleep disruption. Some individuals, due to genetic variants, are more resilient to sleep deprivation, meaning their mental acuity (tested using the psychomotor vigilance test) degrades at a slower rate across sleep loss compared to individuals not (genetically) resilient.[19,23] The second principle is intra-individual sensitivity to caffeine. Some individuals are highly sensitive to caffeine, allowing for performance enhancement and/or optimization under sleep loss.[10,13,19]

Methods and Results

Developing a working model to optimize Soldier health and safety in future studies through understanding of Soldier sleep, caffeine supplementation, and genetic variation in vulnerability/resiliency to sleep loss

The working model is envisioned to be used for future WRAIR- and DoD-directed studies to assist commanders with mission execution and used during the selection process of the Special Operations Command (e.g., Army Rangers). In order for the working model to have direct military application, this would require access to an individual Soldier's blood profile for each genetic variant of ADORA2A, ADA, PER2, PER3, CYP1A2, and NAT2. The four quadrants of the working model are founded on inter-individual variation in vulnerability/resiliency to sleep loss, caffeine sensitivity, and genetic environment (Figure 2). Cognitive performance in the model refers to the ability to maintain stable performance on the psychomotor vigilance test, the gold-standard for assessing real-time mental acuity (reaction time) and alertness under sleep loss.[1,2,12,13,14]

In the model, *A1 individuals (resilient*

to sleep loss and sensitive to caffeine) would be the ideal candidate to perform in military operations. According to our working model, these individuals may have a genetic predisposition that allows them to have stable performance under sleep loss coupled with possibly increased performance- and alertness-enhancing benefits from caffeine. These individuals would be characterized as what military leaders would label as elite performers and 'super Soldiers.' On the other hand, *B2 individuals (vulnerable to sleep loss and tolerant to caffeine) would be least suited to perform* military operations due to their possible decline in performance under sleep loss and an inability of caffeine to subsequently rescue performance to pre-sleep loss levels. Both traits of poor responses to sleep disruption and caffeine could impact military performance during sustained (> 24 h) missions. *A2 (resilient to sleep loss but tolerant to caffeine)* and *B1 (vulnerable to sleep loss but sensitive to caffeine)* individuals would be moderately suited for military operations. A2 individuals are able to perform normally under sleep disruption, but caffeine would not be able to give these Soldiers a competitive edge like those who fall under A1. B1 individuals would hypothetically be in danger with sleep disruption during military operations, but their high sensitivity to caffeine could hypothetically protect against decreases in military performance under sustained missions. Moreover, this chart helps to classify Soldiers into different categories of hypothetical performance, contributing to the foundations for the development of methods to help Soldiers based on their genetic background.

Discussion

There were several lessons learned from the students' research fellowships at WRAIR. First, the students learned about the importance of restorative vs. non-restorative sleep during sustained (> 24 hour) military operations. With restorative sleep, individuals can maintain performance. With non-restorative sleep, performance suffers, compromising the health and safety of the Soldiers and the unit. Second, the students learned about the ability of one's genetic background to predict performance under sleep loss that can be rescued, in part, with caffeine supplementation; some individuals cognitively suffer during sleep loss (the B1s and B2s), while other individuals (the A1s and A2s) could be cognitively preserved during sleep loss as measured from gold-standard, real-time measurements of mental acuity (reaction time).

In brief, the working model may help leaders take actions towards removing subjective bias for military selection by focusing on genetic attributes of elite performers and/or non-performers. The working model may also help select individuals for military service who otherwise would not be considered. In fact, these are the exact intents of the Defense Advanced Research Program Agency (DARPA) program entitled Measuring Biological Amplitude (MBA). There are some limitations with the working model, however.

First, it would require a great deal of knowledge and coordination to align work schedule/mission requirements with knowledge of peak optimal performance predicted based on the genetic variants. Second, while optimization for when a Soldier has to work at a sub-optimal time of the day (e.g., night shift) can be achieved through caffeine supplementation, preventing tolerance to caffeine would still be a concern even with a genetic predisposition for caffeine sensitivity. Finally, even when controlling for an individual's genetic landscape, the fact remains that deployed settings do not permit for restorative sleep and so countermeasures such as frequent napping, early bedtimes, and absence of light/bedside technology (reduces the ability to achieve restorative sleep) must still be considered. To conclude, with greater knowledge of genetic profiling and benefits of restorative sleep for performance, effective countermeasures and strategies could be developed to maximize military performance.

References

[1]Belenky, G., Wesensten, N.J., Thorne, D.R., Thomas, M.L., Sing, H.C., Redmond, D.P., Russo, M.B., and Balkin, T.J. (2003) "Patterns of performance degradation and restoration during sleep restriction and subsequent recovery: A sleep dose-response study." Journal of Sleep Research. 12. Pg 1-12.

[2]Rajdev, P., Thorsley, D., Rajaraman, S., Rupp, T.L., Wesensten, N.J., Balkin, T.J., Reifman, J. (2013). "A unified mathematical model to quantify performance impairment for both chronic sleep restriction and total sleep deprivation." Journal of Theoretical Biology. 331. Pg 66-77.

[3]Czeisler, C.A., Duffy, J.F., Shanahan, T.L., Brown, E.N., Mitchell, J.F., Rimmer, D.W., Ronda, J.M., Silva, E.J., Allan, J.S., Emens, J.S., Dijk, D.J., Kronauer, R.E. (1999). "Stability, precision, and near-24- hour period of the human circadian pacemaker." Science. 284. Pg 2177-2171.

[4]McCauley, P., Kalachev, L.V., Mollicone, D.J., Banks, S., Dinges, D.F., Van Dongen, H.P. (2013). Dynamic circadian modulation in a biomathematical model for the effects of sleep and sleep loss on waking neurobehavioral performance. Sleep. 36. Pg 1987-1997.

[5]Killgore, W.D., Kahn-Greene, E.T., Lipizzi, E.L., Newman, R.A., Kamimori, G.H., and Balkin, T.J. (2008). "Sleep deprivation reduces perceived emotional intelligence and constructive thinking skills." Sleep Medicine. 31. Pg 517-526.

[6]Pace-Schott, E.F., Nave, G., Morgan, A., and Spencer, R. (2012) "Sleep-dependent modulation of affectively guided decision-making." Journal of Sleep Research. 21. Pg 30-39.

[7]Good, C., Brager, A., Capaldi, V., and Mysliwiec, V. (2020) "Sleep in the United States Military." Neuropsychopharmacology. 45. Pg 171-191.

[8]Mitchell, D., Knight, C., Hockenberry, J., Teplansky, R., Hartman, T. (2014). "Beverage caffeine intake in the U.S." Food and Chemical Toxicology. 63. Pg 136-142.

[9]Attipoe, S., Manganello, C., Scott, JM., Deuster, PA. (2017). "Usefulness of a risk assessment tool to risk stratify dietary supplements." Military Medicine. 182. Pg 2086-2091.

[10]Urry, E., Landolt, HP. (2015). "Adenosine, caffeine, and performance: from cognitive neuroscience of sleep to sleep pharmacogenetics." Current Topics in Behavioral Neuroscience. 25. Pg 331-366.

[11]Burke, TM., Markwald, PR., McHill, AW., Chinoy, ED., Snider, JA., Bessman, SC., Jung, CM., O'Neil, JS., Wright, KP Jr. (2015). "Effects of caffeine on the human circadian clock in vivo and in vitro." Science Translational Medicine. 305.

[12]Kamimori, GH., Karyekar, CS., Otterstetter, R., Cox, DS., Balkin, TJ., Belenky, GL., Eddington, ND. (2002). "The rate of absorption and relative bioavailability of caffeine administered in chewing gum versus capsules to normal health volunteers." International Journal of Pharmaceutics. 234. Pg 159-167.

[13]Doty, TJ., So, CJ., Bergman EM., Trach, SK., Ratcliffe, RH., Yarnell, AM., Capaldi, VF 2nd., Moon, JE., Balkin, TJ., Quartana, PJ. (2017). "Limited efficacy of caffeine and recovery costs during and following 5 days of chronic sleep restriction." Sleep. 40.

[14]Bodenmann, S., Hohoff, C., Freitag, C., Deckert, J., Rétey, J.V., Bachmann, V., and Landolt, H.P. (2012). "Polymorphisms of ADORA2A modulate psychomotor vigilance and the effects of caffeine on neurobehavioral performance and sleep EEG after sleep deprivation." British Journal of Pharmacology. 165. Pg 1904-1913.

[15]Landolt, H.P., Rétey, J.V., and Adam, M. (2012). "Reduced neurobehavioral impairment from sleep deprivation in older adults: contribution of adenosinergic mechanisms." Frontiers in Neurology. 3. Pg 62-72.

[16]Porkka-Heiskanen, T., Strecker, R.E., Thakkar, M., Bjørkum, A.A., Greene, R.W., and McCarley, R.W. (1997). "Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness." Science. 276. Pg 1265-1268.

[17]Porkka-Heiskanen, T. and Kalinchuk, A.V. (2011). "Adenosine, energy metabolism and sleep homeostasis." Sleep Medicine Reviews. 15. Pg 123-35.

[18]Viola, A.U., Archer, S.N., James, L.M., Groeger, J.A., Lo, J.C., Skene, D.J., von Schantz, M., Dijk, D.J. (2007). "PER3 polymorphism predicts sleep structure and waking performance." Current Biology. 17.7. Pg 613.

[19]Rupp, TL., Wesensten, NJ., Newman, R., Balkin, TJ. (2013). " PER3 and ADORA2A polymorphisms impact neurobehavioral performance during sleep restriction." Journal of Sleep Research. 22. Pg 160-165.

[20]Mora-Rodriguez, R., Pallares, JG., Lopez-Guillon, JM., Lopez-Samanes, A., Fernandez-Elias, VE., Ortega, JF. (2015). "Improvements on neuromuscular performance with caffeine ingestion depend on the time-of-day." Journal of Science and Medicine in Sport. 18. Pg 338-342.

[21]Garbazza, C., Benedetti, F., (2018). "Genetic factors affecting seasonality, mood, and the circadian clock." Frontiers in Endocrinology (Lausanne). 9.

[22]Fuhr, U., Rost, KL., Engelhardt, R., Sachs, M., Liermann, D., Belloc, C. Janezic, S., Grant, D. Meyer, UA., Staib, AH. (1996). "Evolution of caffeine as a test drug for CYP1A2, NAT2, and CYP2E1 phenotyping in man by in vivo versus vitro correlations." Pharmacogenetics. 6. Pg 159-176.

[23]Brager AJ, Ratcliffe RA, Grant D, Satterfield B, Capaldi V, Balkin T, Petrovick M. (2018). "0017 Genetic polymorphisms of sleep resiliency, sleep intensity, morning preference, and caffeine sensitivity are not associated with neurobehavioral performance under repeated cycles of total sleep deprivation." Sleep. 41. A7-A8.

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