Sweating the Small Stuff: A sensor for real-time neuro-immune axis monitoring

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Although manageable with certain precautions, circadian rhythm misalignment and sleep disturbances pose a potential threat to astronaut health. It is well-known that sleep is essential for proper immunological and neurological functioning, while impairments in these functions result in risks for spaceflight success. Neuropeptides and hormones are involved in regulating the circadian clock and are used as biomarkers for circadian alignment. However, in-flight monitoring of these biomarkers is limited due to the lack of real-time sensor systems and sample collection/processing confines. Therefore, real-time measurements of biomarkers in-flight are necessary for mission success and crew health. For this, a robust biomarker involved in the neuro-immune axis of circadian rhythm cycling, physiological stress responses, and inflammation would be well-received as a viable biomarker for assessing physiological health for crew during long-duration missions, as these are currently NASA Human Research Program defined risks. Therefore, this paper describes the development of a wearable sweat biosensor to measure the biological clock neuropeptide, orexin/hypocretin. Additionally, potential for implementation of a user-friendly sensor of orexin/hypocretin to be telemetrically reported in real-time, is proposed. In brief, the proposed system has the possibility to be used as a biomarker monitor to support preventive and personalized medicine.

Additional Keywords and Phrases: Neuropeptide, Wearable, Biosensor, Biomarker, Spaceflight, Personalized Medicine

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1 INTRODUCTION

Crewmembers on long-duration missions require technologies that can safeguard health as clinical and urgent care will be inaccessible. Medications and nutritional supplementations are essential for preventative care, yet protracted monitoring of physiological disparities, and how to efficiently correct these disparities before they occur, are key to maintaining mission success and crew longevity. Currently, there is a considerable need for wearable technologies that can seamlessly support the crew's activities of the crew and provide consistent monitoring of physiological responses. Aside from the MinION DNA sequencer and MicroflowI, there is virtually no device used on the International Space Station (ISS) that can monitor biochemical markers in continuous real-time from easily collected biological samples. While wearables including Apple smartwatches are capable of monitoring heart rhythms and movement, biosensors that can analyze biofluids, such as blood, plasma, saliva, urine, and sweat are highly needed for spaceflight use and are currently utilized for athletic performance monitoring [1].

Biosensors are useful for in-flight monitoring of physiological fluctuations of the circadian cycle and stress responses and can collect biological data without the invasiveness of a blood collection that may cause faulty results of these sensitive measures. Further, analyses of these biological fluids are typically performed on Earth return, where sample storage and handling can affect processed sample quality, and during long duration, exploratory missions, measuring biological analytes back on Earth is limited and cumbersome, especially for analytes that fluctuate with sensory signals received from light. Current detection systems for biological analytes in liquids include, high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assays (ELISA), and radioimmunoassay, which detect low pg/mL concentrations. Although sensitive, these techniques require extensive instrumentation, radioactive materials, clinical laboratory settings and trained personnel to produce data. Therefore, in-flight wearable systems can be invaluable to monitoring astronaut health. Importantly, they may predict outcomes at an early stage based on trends in biomarker shifting that may be reversed by countermeasures to possibly delay or prevent the onset of an adverse event, thus protecting astronaut health and mission success.

2 PREVIOUS WORK

We have recently investigated wavelength-based sensitivity enhancement through impedance measurements for cellular biosensing using nanostructured two-terminal interdigitated electrodes. The sensitivity of the detected molecule increased by a factor of six, under light illumination compared to previously described sensing systems without light, providing evidence for the sensitivity of the proposed sensor [2]. Further work in our lab has identified elevated expression of orexin/hypocretin in brains of mice following ground-based combined low-dose radiation (LDR) and hindlimb unloading [3], implicating the potential for this neuropeptide during physiological recovery.

3 BIOSENSOR DESIGN

The sensor consists of biological recognition elements that respond to targets, chemical or biological analytes in fluids, signaling differences that are converted by a transducer into a readable datapoint. The biorecognition elements (probes) can be fabricated to detect different molecular compositions within fluids, for example, antibodies, antigens, enzymes, nucleic acids, bacteriophages, and proteins. Following biorecognition, transducers convert the physicochemical interactions between the probe and the analyte into measurable signals in electrical

domains, which provides information of the quantity of the captured molecules and analyte concentrations within a sample.

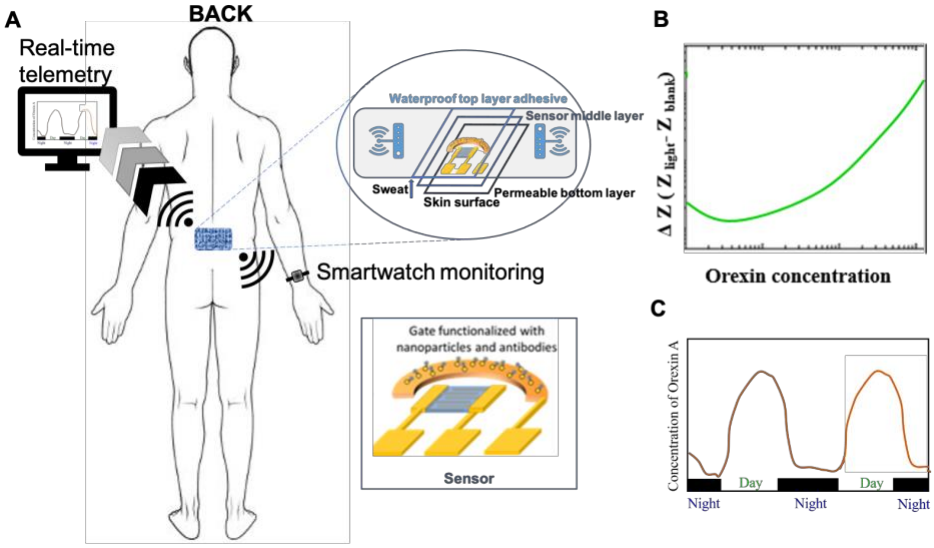

Figure 1: Proposed design of wearable sensor and output of data collected. (A) A sweat sample would be collected through a bottom layer, a permeable membrane adhered to the skin. A second layer containing the biosensor would generate a photoelectrochemical signal that would be detected and converted to a digital signal, that can be recorded via a smartwatch located on the crewmember or telemetrically to a second person monitor. Estimated size would fit the small of the back region. Inset sensor platform with interdigitated electrodes (IDEs) as source and drain and gate electrode. Biomolecules (antibodies) and gold nanoparticles are added to functionalize the sensor. (B) Predicted change in impedance value with and without light with the same concentration of analyte will be measured. (C) Representative sinusoidal plotting from continuous, real-time measurements of orexin/hypocretin will be plotted throughout diurnal timepoints.

4 NEURO-IMMUNE AXIS

Current risk factors for crew onboard the ISS include cognitive deficits, elevated risk-taking behaviors, and chronic low-grade inflammation. These risk factors can be a result of impaired circadian rhythm and are onset due to sleep disturbances [4]. Therefore, biomarkers that can address impending changes in the neuro-immune axis would be of interest and benefit to the aerospace community. Analytes involved in circadian rhythm cycling include but are not limited to [5, 6], melatonin, cortisol, and the neuropeptide, orexin/hypocretin. Although melatonin and cortisol levels have been measured in pre-, during-, and post-spaceflight [7, 8], little is known about the protein levels of the biological clock regulator, orexin/hypocretin during flight. Orexin/hypocretin is expressed from neurons in response to light signals received in the hypothalamus, can be detected in sweat at low concentrations [6, 9], and is considered a neuropeptide that regulates many different physiological systems. These include wakefulness during circadian rhythm cycling (peaking near diurnal 'night'), energy expenditure, reward, and also plays a role in suppression of inflammation [3, 9-14]. Interestingly, wake extensions result in elevated levels, and onset of sleep promotes reduced levels [15]. It acts on immune suppression via inhibition of granulopoiesis and myelopoiesis from bone marrow [14] and dampening of neuroinflammation [13]. In line with this, we and others show elevated inflammatory granulocytes and granulocyte-to-lymphocyte ratio as a biomarker for spaceflight [16], suggesting a

possible reduction of orexin/hypocretin inhibition on inflammation. In addition, work in our lab has shown orexin/hypocretin is robustly induced in brains of mice during readaptation from ground-based combined low-dose radiation (LDR) and hindlimb unloading [3], implicating the potential for this neuropeptide during physiological recovery.

5 NEURO-IMMUNE AXIS SENSOR ANTICIPATED RESULTS

We propose the development of a novel wearable, sweat sensor that can continuously measure levels of orexin/hypocretin in real-time. We hypothesize that the biological clock protein, orexin/hypocretin will be diminished in-flight, resulting in impaired wakefulness, immune differential shifting and elevated inflammation that is currently experienced by crew. We believe the use of this novel, wearable sweat biosensor will be amenable to crew in-flight monitoring of the neuro-immune axis, which is in flux with circadian cycle shifting, providing real-time measurements of orexin/hypocretin for personalized and preventative medicine on long-duration missions. For this specific orexin/hypocretin biosensor design, gold gate electrodes will be functionalized with gold nanoparticles and anti-orexin/hypocretin antibodies through covalent bonding chemistries. Confirmation of the attachment will be accomplished by standard electrochemical impedance spectroscopy and cyclic voltammetry. The photoelectrochemical impedance investigation for proof of concept will be accomplished by investigating the current/voltage curves and the impedance spectra.

6 CONSIDERATIONS OF THE END USER

We propose a photoelectrochemical sensor that can detect low levels of orexin/hypocretin in human sweat which can be used for assessment of physiological health of crew during long-duration missions. Successful implementation of this sensor is dependent, not only on the accuracy of the reported data, but also on the practicality, comfort, and user acceptance of the patch. This becomes particularly important for long-duration, long-distance missions where emergency medicine will be unavailable. The patch must be easy to apply/remove and comfortable and flexible while wearing typical in-flight gear. Data from the sensor on the smartwatch must be meaningful and intuitive as the wearer is alerted if orexin/hypocretin values are approaching inauspicious levels, signaling them to take precautions to restore health, change behavior, and maintain personalized healthcare. The potential applications include monitoring fluctuations in the sleep-wake cycle to determine (1) if this neuropeptide is affected in circadian shifting in-flight, and (2) if monitored levels were depressed an orexin/hypocretin agonist would be supplemented as a countermeasure to restore wakefulness and promote immune recovery.

7 FUTURE DIRECTIONS

Future developments for this sensor include a re-usable, multi-analyte design with a Bluetooth module for real-time telemetry to monitoring devices. In addition, the sensor could be adapted to monitor sleep performance for high-intensity professions, such as athletes or the military. Finally, this sensor design has tremendous future potential for enhanced development when coupled with prospective implementation partners.

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