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COMPARISON OF PHYSIOLOGICAL AND COGNITIVE PERFORMANCE IN F-22 PILOTS DURING THE TRANSITION FROM DAY TO NIGHT FLYING OPERATIONS

by

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This thesis investigated the changes in physiological and cognitive performance as F-22 pilots transitioned short-term to night-flying weeks using salivary markers of stress, cortisol and alpha amylase, wrist activity monitors, the National Aeronautics and Space Administration-Task Load Index (NASA-TLX), and a go/no-go (GNG) developed by Naval Medical Research Unit at Dayton. Seventeen fully qualified F-22 pilots took part in the two-week study. We found no differences in GNG reaction time or accuracy, NASA-TLX scores, or sleep quantity as participants transitioned to night-flying weeks. Sample cortisol levels were significantly higher than civilian levels in all experimental conditions and control days. Researchers fitted a unique participant cortisol curve and found higher-than-predicted participant cortisol levels post-flight in the day-flying condition and lower than predicted participant levels post-flight in the night-flying condition. Two negative relationships, F-22 experience by the magnitude of cortisol change (pre- to post-flight) in the day-flying condition and age by Perceived Stress Survey scores suggested stress adaptation in the F-22 community. We thought that the night-flying environment would be more stressful on the aviator. While more research is required to support the results found in this study, it appeared that day-flying is more stressful.

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COMPARISON OF PHYSIOLOGICAL AND COGNITIVE PERFORMANCE IN F-22 PILOTS DURING THE TRANSITION FROM DAY TO NIGHT FLYING OPERATIONS

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ABSTRACT

This thesis investigated the changes in physiological and cognitive performance as F-22 pilots transitioned short term to night-flying weeks using salivary markers of stress, cortisol and alpha amylase, wrist activity monitors, the National Aeronautics and Space Administration-Task Load Index (NASA-TLX), and a go/no-go (GNG) developed by Naval Medical Research Unit at Dayton. Seventeen fully qualified F-22 pilots took part in the two-week study. We found no differences in GNG reaction time or accuracy, NASA-TLX scores, or sleep quantity as participants transitioned to night-flying weeks. Sample cortisol levels were significantly higher than civilian levels in all experimental conditions and control days. Researchers fitted a unique participant cortisol curve and found higher-than-predicted participant cortisol levels post-flight in the day-flying condition and lower-than-predicted participant levels post-flight in the night-flying condition. Two negative relationships, F-22 experience by the magnitude of cortisol change (pre- to post-flight) in the day-flying condition and age by Perceived Stress Survey scores, suggested stress adaptation in the F-22 community. We thought that the night-flying environment would be more stressful on the aviator. While more research is required to support the results found in this study, it appeared that day flying is more stressful.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AFB	Air Force Base
ANS	Autonomic nervous system
Avg	average
CAR	Cortisol Awakening Response
CMR	Command Mission Ready
E	Epinephrine
ESS	Epworth Sleepiness Scale
GNG	Go/No-Go
HS	High Stress
ICU	Intensive care unit
IRB	Institutional Review Board
LCU	Life Changing Unit
LS	Low Stress
MEQ-SA	Morningness-Eveningness Questionnaire-Self Assessment
n	sample size
NASA-TLX	National Aeronautics and Space Administration-Task Load Index
NE	Norepinephrine
NHRC	Naval Health Research Center
NMRU-D	Naval Medical Research Unit Dayton
NPS	Naval Postgraduate School
p	P-value
PSS	Perceived Stress Scale
PSQI	Pittsburgh Sleep Quality Index
PVT	Psychomotor Vigilance Test
r	Correlation
RT	Reaction time
sAA	Salivary Alpha-Amylase
sCort	Salivary Cortisol
SD	Standard Deviation
SNS	Sympathetic Nervous System

SRRS	Social Readjustment Rating Scale
SWAT	Subjective Workload Assessment Technique
SWSD	Shift Work Sleep Disorder
TSST	Trier Social Stress Test
TTM	Traditional Thai Massage
USAF	United States Air Force
USCG	United States Coast Guard
WAM	Wrist activity monitor

EXECUTIVE SUMMARY

Human error is a causal factor in upward of 70%–80% of military aviation accidents (O'Hare, Wiggins, Batt, & Morrison, 1994; Shappell & Wiegmann, 1997). Understanding how and why pilots make errors is a critical part of the mishap investigation process. Moreover, recent advances in our understanding of factors such as fatigue, stress, and workload suggest that these factors often interact with one another, creating a potentially deadly synergy.

F-22 pilots are required to transition in the short-term to night-flying weeks to facilitate training. Traditional shift workers show an increase in errors and decreased performance at night; however, the implications from the transition from day- to night-flying is not well understood in the F-22 community.

This thesis captured changes in physiological and cognitive performance as F-22 pilots transitioned to night-flying weeks. Seventeen volunteer participants took part in two weeks of data collection; the first week was a normal flying week and the second week was a night-flying week. We collected saliva swabs at five pre-determined intervals over the course of three conditions: control (non-flying), day-flying and night-flying. Wrist activity monitors (WAMs) were worn continuously by participants for two weeks. A modified go/no-go (GNG) test was developed by the Naval Medical Research Unit at Dayton (NAMRU-D) and used both pre- and post-flight in both flying conditions. The National Aeronautics and Space Administration-Task Load Index (NASA-TLX) was used to quantify post-flight workload in both conditions.

Using an iPad NASA-TLX application to measure workload, we found no difference in subjective workload ratings. Participants had average workload scores of approximately 66 post-flight in both the day- and night-flying conditions. These results were similar to NASA-TLX scores of commercial airline pilots executing a landing with loss of the autopilot (Zheng, Lu, Jie, & Fu, 2017), but significantly higher than F/A-18 pilots executing an instrument landing with multiple cautions and warnings in a simulator (Mansikka, Virtanen, & Harris, 2018).

We used a two-sided paired sample *t*-test to analyze the results and found no difference in go/no-go test accuracy or reaction time as participants transitioned from day- to night-flying. Participant reaction times were similar to a civilian population using a 20% no-go test (Nieuwenhuis, Yeung, Wildenberk, & Ridderinkhof, 2003). However, the F-22 pilots had a significantly higher average inhibition accuracy in both flying conditions, 90% during day-flying and 80% during night-flying, than the 66% inhibition accuracy reported in the civilian study.

Cortisol samples were compared against 50% percentile males aged 31–40 in the CIRCORT database (Miller et al., 2016). Using time logs and sleep data, we determined predicted cortisol levels based on the CIRCORT fitted curve compared to actual cortisol levels of the participants using hours since awakening. Participants had higher cortisol levels during control days ($p < 0.001$), pre-flight ($p = 0.009$) and post-flight ($p = 0.003$) in the day-flying condition, and pre-flight ($p = 0.025$) and post-flight ($p = 0.003$) in the night-flying condition.

Because participant's cortisol levels were higher than predicted in every condition, we fit a unique participant cortisol curve using control days and hours since awakening. Participants' post-flight cortisol levels in the day-flying condition exceeded predictions ($p = 0.049$), and they had lower-than-predicted cortisol levels post-flight in the night-flying condition ($p = 0.05$).

Two negative relationships were found, suggesting stress adaptation in the F-22 community: F-22 experience and the magnitude of cortisol change (pre- to post-flight) in the day-flying condition (correlation (r) = -0.89, $p = 0.001$) and age and perceived stress survey scores ($r = -0.72$, $p = 0.005$). One possible reason for this adaptation could be the sample's characteristics, or the selection of high trait-resilient individuals. F-22 pilots are an elite group that are categorized as high-functioning, Type A personalities who are often exposed to high-threat environments and show evidence of physiological adaptation to recurring stress like similar highly resilient populations (Lu, Wang, & You, 2016).

This thesis sought to determine if F-22 pilots were experiencing increased stress as a result of different flying conditions. We expected that the night-flying environment would be more stressful on the aviator. While more research is required to support the results found in this study, it appears that the opposite may be true: day-flying is more stressful. Understanding the stress burden on F-22 aviators during the transition to night-flying operations is an important aspect of future mishap prevention efforts.

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Most importantly, this thesis is dedicated to my little brother, LT Steven “Deet” Combs Jr., who died in service to his country on 22 Nov 17. He loved to fly and was rarely seen without a smile on his face. In his own words, “you fly better happy.” Not a day goes by where I don’t miss hearing his voice or his laugh. He was one in a million, and the world will never be the same. This thesis, and all my future work, is dedicated to him.

Love you, bro.

“Once you have tasted flight, you will forever walk the earth with your eyes turned skyward, for there you have been, and there you will always long to return.”

—Leonardo da Vinci

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I. INTRODUCTION

A. BACKGROUND

Human error is a causal factor in upward of 70%-80% of military aviation accidents (O'Hare, Wiggins, Batt, & Morrison, 1994; Shappell & Wiegmann, 1997). Understanding how and why pilots make errors is a critical part of the mishap investigation process. Moreover, recent advances in our understanding of factors such as fatigue, stress, and workload suggest that these factors often interact with one another creating a potentially deadly synergy.

The normal operating cycle for F-22 squadrons cycles pilots between day and night flight operations. Daytime sorties usually commence at or around 0700. The pilots must be at the squadron roughly two hours prior to take off time to prepare for the mission. Because crew rest protocol dictates a maximum 12-hour duty day, aircrew typically return home at 1700.

Night sorties usually commence between 1300 and 1400 hours. Night flight rotations are required for aircrew to maintain night-vision goggle proficiency, night landing currency, and their Command Mission Ready rating. Night flying weeks typically occur for two weeks once a quarter.

Recent anecdotal evidence from aircrew suggests that night flights are significantly more stressful than day flights because of the lack of visual information available to the pilot. Simply stated, if a pilot can see a hazard, they can avoid it. At night, they are less likely to perceive and avoid threats to their safety. Hence, their subjective stress levels could be significantly higher for night flights. However, physiological and cognitive comparisons between day and night flying to validate that claim have not been investigated directly.

The shift from day to night flying operations serves as an ideal timeframe to investigate potential changes in pilot performance as a result of stress, fatigue, and workload. Using non-flying days as a baseline, we will be able to determine whether the transition to a night flying week contrasted with day flights causes changes in stress biomarkers that impact the cognitive performance of F-22 pilots.

1. Approach

Our quasi-experimental observational study took place over the course of two weeks. The first week of data collection was a week of normal daytime flying. The second week of data collection was a night-flying week.

We administered pre-study questionnaires prior to the start of data collection. The questionnaires included a series of questions about demographic characteristics, flight hours, tobacco and caffeine usage, and validated measures of stress and fatigue. Aspects of performance, fatigue, stress and workload were captured at predetermined intervals to monitor potential changes; in addition, salivary samples were collected from the participants. Participants completed Go/No-Go (GNG) tests pre- and post- flight in both day and night flying conditions. An additional post-flight workload assessment was completed by participants. Pilots were issued a wrist activity monitor (WAM) to be worn for the duration of the two-week study. The experimental design is discussed in greater detail in Chapter III.

2. Research Question

The following research questions form the basis for this study. Supporting material for these questions will be expanded upon in Chapter II.

- Are there differences in physiological responses (i.e., levels of alpha amylase and cortisol) in F-22 pilots following day and night sorties?
- Is there a difference in attention and executive function of F-22 pilots following day sorties and night sorties?
- Is there evidence to support stress system adaptation in the F-22 community?

Analysis of the research questions are explored in Chapter IV and Chapter V. Recommendations for future work relating to these questions are given in Chapter VI.

B. HUMAN SYSTEMS INTEGRATION

Air Force Instruction 63–1201, Life Cycle Systems Engineering drives USAF Human Systems Integration (HSI) efforts. Guidance is consolidated in the Air Force Directorate of Human Performance document (2008). This publication identifies distinct domains that the Air Force has chosen to consider in HSI efforts. The domains are as follows:

- Manpower
- Personnel
- Training
- Human Factors Engineering
- Environment, Safety, Occupational Health
- Survivability
- Habitability (Directorate of Human Performance, 2008).

These domains interact with each other, and no one domain functions in isolation. The relationships among these domains are often complex. This thesis will not explore all ways in which these domains are interrelated. Rather, it will focus on the four domains that have the most direct impact on flight operations in this context, i.e., personnel, safety, occupational health and human factors.

1. Personnel

The personnel domain focuses on having the right individuals in the right position to accomplish a task (Directorate of Human Performance, 2008). Current requirements in the aviation community rely on qualifications and flight hours. However, these general requirements fail to capture the individual differences within the population. Certain people respond differently to similar stress. Accordingly, a study of salivary biomarkers and cognitive performance may demonstrate that certain persons are more resilient to shift changes. Identification of these individuals may aid mission effectiveness through “smart scheduling” i.e., the selection of individuals for day or night sorties based on their identified strengths and or weaknesses.

2. Safety

Current safety protocols require the investigation into factors that jeopardize pilot and aircrew safety. Much of what we understand about mishaps is through a 20/20 rearview mirror. The best way to prevent future mishaps is to fully understand past mishaps. While analysis has identified certain situations in which mishaps are more likely to occur, our understanding of physiological changes, in relation to cognitive ones, are relatively uninvestigated in the aviation operational environment. This research sheds light on how physiological responses of aircrew impact cognitive performance and vice versa.

The research will determine if there is any difference in stress biomarkers, sleep quantity, or executive function between day and night flying. Where differences are found to exist within the sample, future work may be to identify more resilient individuals as an aid in smart scheduling.

3. Occupational Health

The F-22 total system includes the hardware, software, and personnel that keep the F-22 squadrons airborne. Current operations require short term night weeks to accomplish training requirements. If a pilot fails to meet these requirements, the pilot will not be Combat Mission Ready (CMR) certified. Current mission and training requirements require a high operations tempo that can result in performance and health issues for aviators.

The detrimental effects of prolonged elevated levels of stress and chronic fatigue are well known. Salivary biomarkers are a credible tool for determining stress loads in the population. Additionally, sleep monitors will illuminate how sleeping patterns are affected in the short-term shift of operations. Understanding how the current F-22 operations tempo affects pilot health can improve our understanding of the long-term health impacts to flying in the F-22.

4. Human Factors Engineering

Wiegmann and Shappell (1999) estimated that at least 75% of aviation mishaps were attributed to human error. Our efforts to create a safer aviation community require us to understand the underlying causes of error. A whole person concept has been adopted by the USAF in mishap analysis. The research outlined in this thesis studies how both the aviators' duty-day stressors and lifestyle choices can affect performance.

C. THESIS ORGANIZATION

Chapter II sets forth a review of current literature and research surrounding stress, fatigue, and workload and performance. The research components do not work in stovepipes, but rather interact with one another. The interactions are discussed in the relevant sections of Chapter II. Chapter III contains the methodology, variables, and data collection schedule. Chapter IV presents the results of the data collection. Chapter V discusses the relevant findings. Chapter VI contains conclusions, recommendations for future research, and lessons learned.

This study was improved upon by Dr. Douglas Granger and his colleagues at the Institute for Interdisciplinary Salivary Bioscience Research, University of California, Irvine.

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II. LITERATURE REVIEW

A. OVERVIEW

This thesis strives to achieve a multidimensional understanding of stress, performance, and fatigue in a select, high-functioning pilot sample. The following sections detail how each of the various areas of research contribute to understanding the physiological and psychological changes as pilots transition from day to night flight operations.

B. STRESS

1. Stress Overview

Stress, in the context of this thesis, is defined as an individual's perception to changing environmental demands and the accompanying physiological and psychological response (Ganster & Perrewe, 2011; Kahn & Byosiere, 1992). Stress affects an individual's health and disease onset (Backé, Seidler, Latza, Rossnagel, & Schumann, 2012; Steptoe, 1991; Steptoe & Kivimaki, 2012; Vita, Lapa, Trimarchi & Benvenga, 2015). Studies suggest an increase in stress, or failure to cope with increased stress, may affect long-term individual health (Alkov, Gaynor, & Boroswsky, 1985).

Physiological stress, or biological stress, is the change in bodily functions away from normal homeostasis due to internal (e.g., aging) or external (e.g., environmental) influences. Psychological stress is the perception that "environmental demands tax or exceed [their] adaptive capacity" (Cohen, Janicki-Deverts, & Miller, 2007, p. 1685).

Physiological and psychological stress is the focus of the thesis research questions. Background information on stress processes, responses and measurement techniques are discussed in this section.

2. Stress in Aviation

Research conducted by Barnes (1992) examined aircrew traits using a sample of 52 aircrew and found that pilots scored higher in anxiety than any other subset of aircrew members. This finding was attributed to the nature of their jobs, which require high levels of responsibility and accountability (Barnes, 1992).

Operational environments and requirements are not the only source of stress in aviation communities. Fiedler, Rocco, Schroeder, and Nguyen (2000) found that home stress carried over into the workplace. In a review of 19 United States Coast Guard (USCG) helicopter pilot stress questionnaires, home stress scores were positively correlated with self-reported job stress ratings with sample correlation $r = 0.81$, and p-value $(p) < 0.01$ for the two-sided test of the null hypothesis of zero correlation. The questionnaires required the aircrew to rate the importance of various stress-coping strategies and evaluate their own flight performance. The results of the study indicated that, even with coping strategies, domestic stress exhibited a carry-over effect into the workplace. While there was no evidence that the increase in home or job stress alone affected flying performance, the combination negatively impacted flying performance ($r = -0.47$, $p < 0.05$) (Fiedler, Rocco, Schroeder, & Nguyen, 2000). This research suggests that stress in a pilot's home life can carry over into the workplace and negatively impact flying performance.

3. Physiological Response to Stress

This section will first discuss a framework for physiological response to stress, two salivary analytes that can be used to quantify this response, and their combined use in research.

a. General Adaptive Syndrome

The General Adaptive Syndrome (GAS) is a common framework developed for stress response (Selye, 1950). GAS divides the stress response into three phrases: alarm response, resistance, and exhaustion (Selye, 1936). The alarm response is the body's immediate response to the stress, characterized by an increase in cortisol production, hypoglycemia, and tissue catabolism (Selye, 1950). The body attempts to compensate for these symptoms and return to homeostasis in the resistance phase. Many physiological functions will return to normal but those responding to the stressor remain alert (Lucille, 2016). Symptoms may reemerge in the exhaustion phase, which is typically associated with burnout and a continuation of the stressor beyond the body's capacity (Campbell, Johnson, & Zernicke, 2013; Lucille, 2016; Selye, 1950). This relationship is visually depicted in Figure 1.

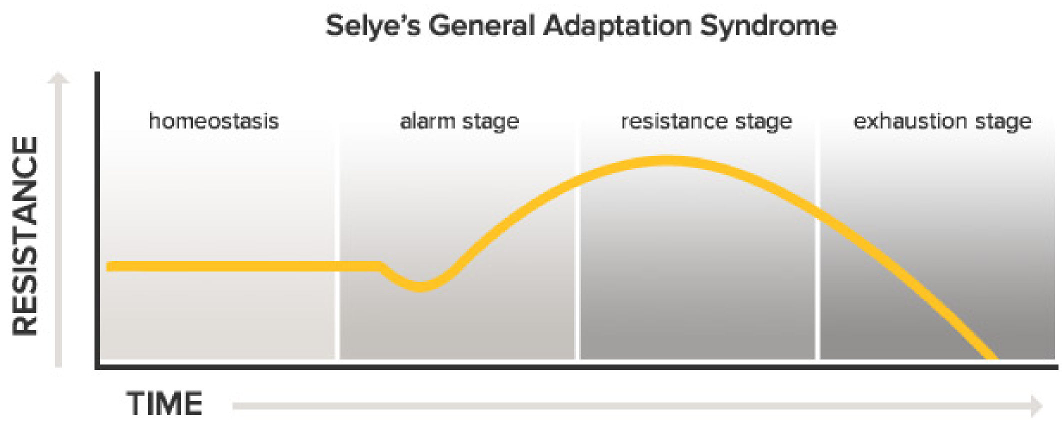


Figure 1. Selye's General Adaption Syndrome. Source: Lucille (2016).

b. Cortisol

Cortisol is a hormone released in response to stress that is easily measured to quantify the reaction. Cortisol is produced by the adrenal cortex within the adrenal gland and is released in response to both physical and psychological stress. Un-bound cortisol passively diffuses into saliva. Studies report high correlations (r = 0.83) between salivary cortisol (sCort) and serum, or blood plasma, cortisol levels (Francis et al., 1987). sCort is a reliable estimate of serum cortisol levels (Hiramatsu, 1981; Petrowski, Wintermann, Schaarschmidt, Bornstein, & Kirschbaum, 2013; Vining, McGinley, Maksvytis, & Ho, 1983).

Short-term increased release of cortisol suppresses the immune system, reduces libido, and increases hypertension, insulin resistance and hyperglycemia (Hoehn & Marieb, 2010). Long-term and prolonged elevation of cortisol can cause anxiety, depression, digestive problems, heart disease, memory and concentration impairment, sleep disturbances, and weight gain (Mayo Clinic Staff, 2016).

(1) Daily Cortisol Rhythm

Cortisol levels function on a tight diurnal rhythm which is high in the morning, gradually decreases throughout the waking day (Dorn, Lucke, Loucks, & Berga, 2007; Stone et al., 2001), and increases during sleep. This cycle is relatively constant within an individual if uninterrupted by stressors. Figure 2 shows the normal daytime cortisol rhythm of a healthy individual uninterrupted by a stressor.

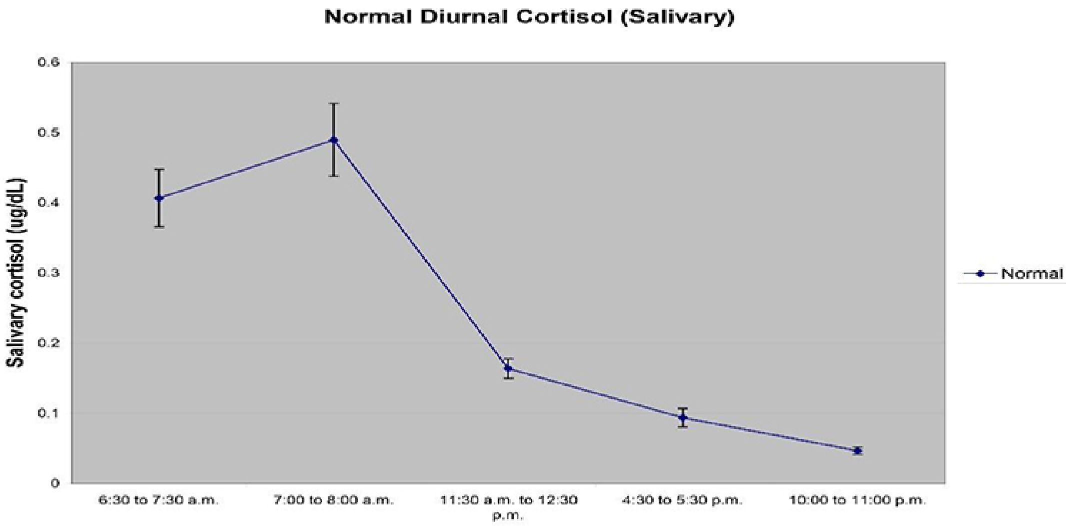


Figure 2. Normal salivary diurnal cortisol levels (n = 26).
Source: Salimetrics (2014).

The daily range of cortisol concentrations will change as individuals age. Typical daily cortisol ranges for healthy individuals as established by Salimetrics (2014) are presented in Table 1.

Table 1. sCort range for healthy populations. Source: Salimetrics (2014).

Group	Number	AM Range (μg/dL)	PM Range (μg/dL)
Children, ages 2.5-5.5	112	0.034 - 0.645	0.053 - 0.607
Children, ages 8-11	285	0.084 - 0.839	ND - 0.215
Adolescents, ages 12-18	403	0.021 - 0.883	ND - 0.259
Adult males, ages 21-30	26	0.112 - 0.743	ND - 0.308
Adult females, ages 21-30	20	0.272 - 1.348	ND - 0.359
Adult males, ages 31-50	67	0.122 - 1.551	ND - 0.359
Adult females, ages 31-50	31	0.094 - 1.515	ND - 0.181
Adult males, ages 51-70	28	0.112 - 0.812	ND - 0.228
Adult females, ages 51-70	23	0.149 - 0.739	0.022 - 0.254
All adults	192	0.094 - 1.551	ND - 0.359

(2) Cortisol Awakening Response

Healthy individuals experience a surge of cortisol secretion in response to morning awakening. This surge is referred to as the "cortisol awakening response" (CAR) (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010). The CAR combines the reactivity of awakening and circadian regulation (Stadler et al., 2016). It only encompasses the dynamic portion of the cortisol secretion, i.e., the bump from awakening to peak response (Clow et al., 2010).

Morning cortisol levels have been associated with perceived stress in military populations (Hernandez, Markwald, Kviatkovsky, Perry, & Taylor, 2018). Hernandez and colleagues collected salivary samples from 58 active duty male Navy SEALs. CAR peaks were positively associated with stress ($r = 0.437$, $p < 0.05$). The area under the curve in terms of ground is the actual plot of cortisol concentration with respect to time and was found to be positively associated with stress ($r = 0.5$, $p < 0.01$). The average (avg) of the awakening cortisol samples ($r = 0.506$, $p < 0.01$) was also positively associated with stress.

This finding is further supported by Elder, Ellis, Barclay, and Wetherell (2016). They found that awakening cortisol levels and the magnitude of the increase were not consistent across the three days. However, as presented in Figure 3, the CAR was the same regardless of the awakening cortisol concentration (Elder et al., 2016). This result suggests that CAR remains consistent in participants irrespective of awakening cortisol levels and can help to establish participant diurnal rhythm.

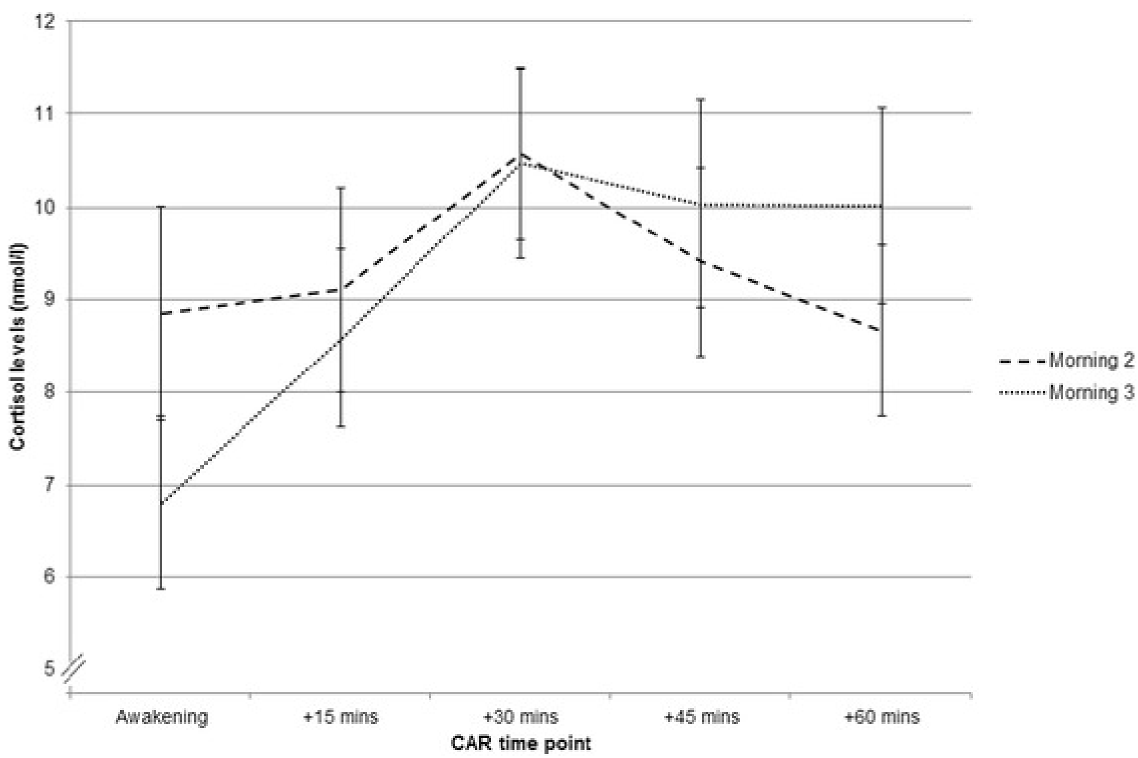


Figure 3. Mean (+- SEM) morning cortisol levels (*n* = 15).
Source: Elder, Ellis, Barclay, & Wetherell (2016).

Research suggests taking three samples post awakening to capture the CAR: at the exact time of wakening, +30 minutes awakening, and +45 minutes awakening (Stalder et al., 2016). Females have a longer and more prolonged CAR, which is the rationale for the +45 minutes of awakening sample (Stalder et al., 2016).

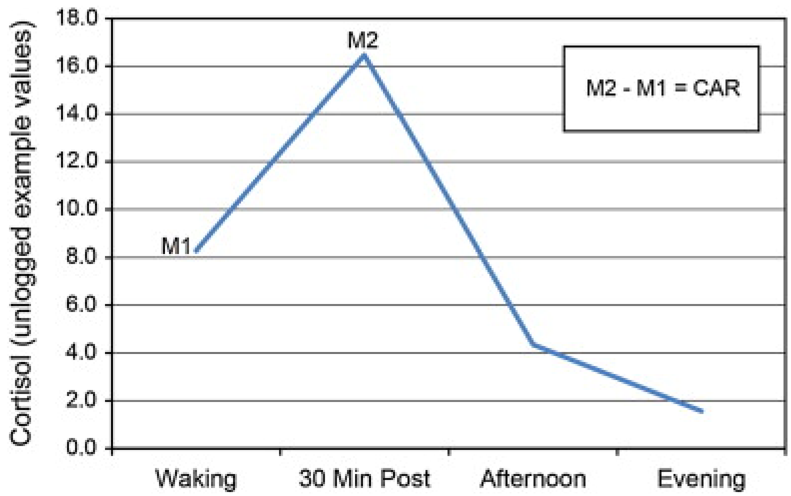


Figure 4. Calculation of CAR. Source: Corbett & Schupp (2014).

The CAR is calculated by taking the +30 awakening sample and subtracting the waking cortisol levels (Figure 4) (Corbett & Schupp, 2014). Shi and associates found that the CAR was predictive of response inhibition in a GNG test taken in the afternoon (Shi et al., 2018). This relationship will be investigated in the sample. Therefore, the CAR will be supportive to the experimental design but not imperative.

(3) Acute Cortisol Response

Cortisol concentrations increase during a stress response. Figure 5 shows the salivary cortisol concentration as it spikes following a Tier Social Stress Test (TSST) (Kirschbaum, Pirke, & Hellhammer, 1993). This type of response is common in healthy populations.

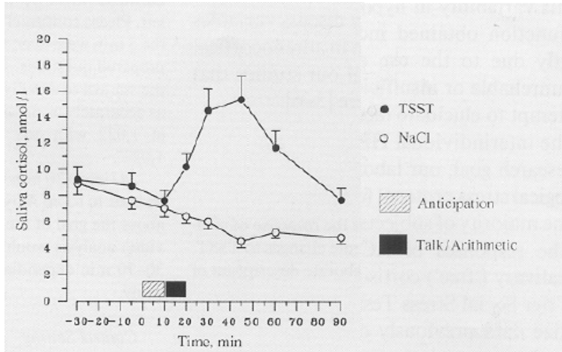


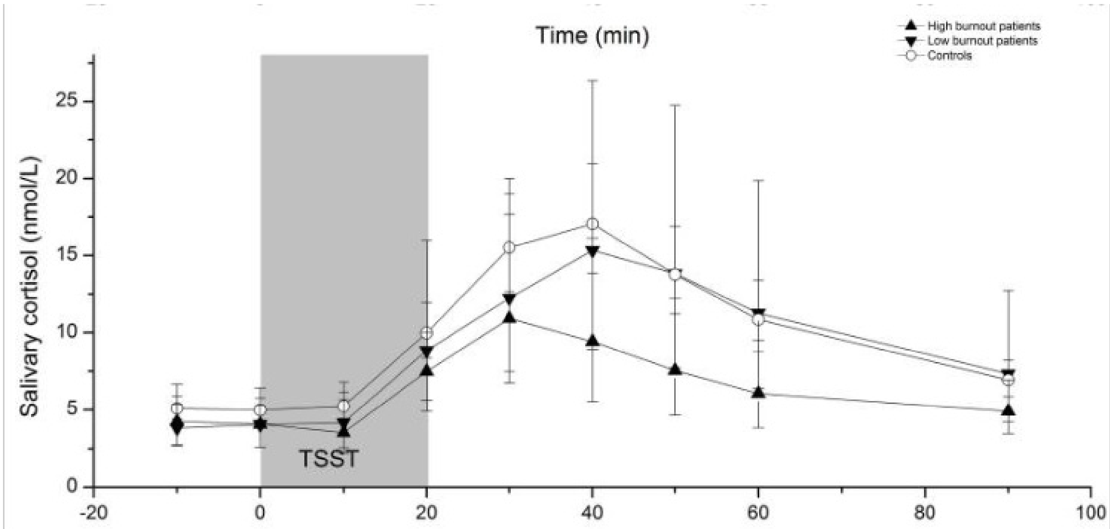
Figure 5. Salivary cortisol levels in response to TSST. Source: Kirschbaum, Pirke, & Hellhammer (1993).

Cortisol also appears to regulate based on the stressor intensity. Van Eck and Nicolson (1994) evaluated Perceived Stress Scores (PSS) against daily cortisol levels. Two groups were compared: a high stress (HS) group of 42 participants and a low stress (LS) group of 46 participants. Participants collected saliva samples ten times per day over five consecutive days. During workdays, the HS participants had higher mean cortisol levels than the LS population at each of the ten sampling times ($p < 0.02$) (van Eck & Nicolson, 1994). This suggests that cortisol levels are associated with the strength of the stressor.

(4) Burnout Cortisol Response

Burnout is the physical or mental breakdown caused by prolonged exposure to chronic workplace stress that has not been addressed (Maslach & Jackson, 1981). Burnout symptoms include exhaustion, depersonalization, and reduced satisfaction in performance (Weber & Jaekel-Reinhard, 2000).

Burnout affects adrenal production of cortisol. Lennartsson, Sjors, Wahrborg, Ljung and Jonsdottir (2015) studied cortisol and adrenocorticotropic hormone (ACTH) responses in healthy and burnout patients. Participants that reported higher burnout scores had lower serum cortisol ($p = 0.027$) and salivary cortisol responses ($p = 0.068$) than controls after the TSST. This relationship is visually depicted in Figure 6.



sCort response to stress in healthy patients, patients with low burnout scores, and patients with high burnout scores.

Figure 6. sCort levels in burnout patients. Source: Lennartsson et al. (2015).

Cortisol levels of patients with a high burnout mirror the cortisol levels of hypocortisolism patients. Patients with hypocortisolism have a blunted or flattened daytime cortisol level curve compared to healthy individuals (Edwards, 2016). Figure 7 depicts the flattening diurnal curve typically seen in patients with hypocortisolism.

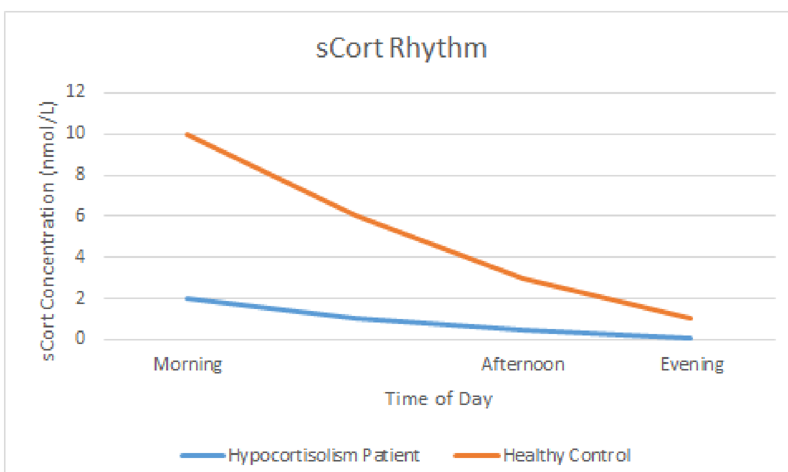


Figure 7. Sample cortisol diurnal rhythms of healthy control and patient with hypocortisolism. Source: Adapted from Edwards (2016).

Populations that are exposed to frequent activation of the fight or flight response have a blunted cortisol response to stress. Petrowski, Wintermann, Schaarschmidt, Bornstein, and Kirschbaum (2013) conducted a study with a sample of 32 healthy patients and 32 patients diagnosed with panic disorder. Both groups were exposed to TSST while sCort and serum cortisol levels were captured. Both samples had an increase in sCort and serum cortisol in response to the TSST, but patients diagnosed with panic disorder had significantly lower sCort and serum cortisol levels than the healthy sample in response to the TSST (Petrowski et al., 2013). While there was an increase in response to the TSST in both samples, those diagnosed with panic disorder had a significantly lower sCort and serum cortisol levels.

This research suggests that individuals with frequent reoccurring stress activation may experience a blunted cortisol response to a stressor compared to normal healthy populations.

c. Alpha Amylase

Alpha amylase is a protein enzyme whose primary function in the body is the breakdown of carbohydrates and starches in the digestive process (Goni, Garcia-Alonso, & Saura-Calixto, 1997). Salivary alpha amylase (sAA) is an enzyme produced in the oral cavity. It is not passively diffused nor actively transported into the saliva, which makes it unique among other salivary analytes (Granger et al., 2007). Because it is produced locally in the mouth, concentrations of sAA differ from other alpha amylase concentrations circulated throughout the body.

(1) Daily Alpha Amylase Rhythm

Alpha amylase exhibits a strong diurnal pattern that is robust against momentary influences (Nater, Rohleder, Schlottz, Ehlert, & Kirschbaum, 2007). sAA levels sharply decline 60 minutes after awakening and then continue to steadily increase throughout the waking day. This pattern is in direct opposition to the diurnal nature of cortisol and is visualized in Figure 8 (Adam, Hoyt, & Granger, 2011).

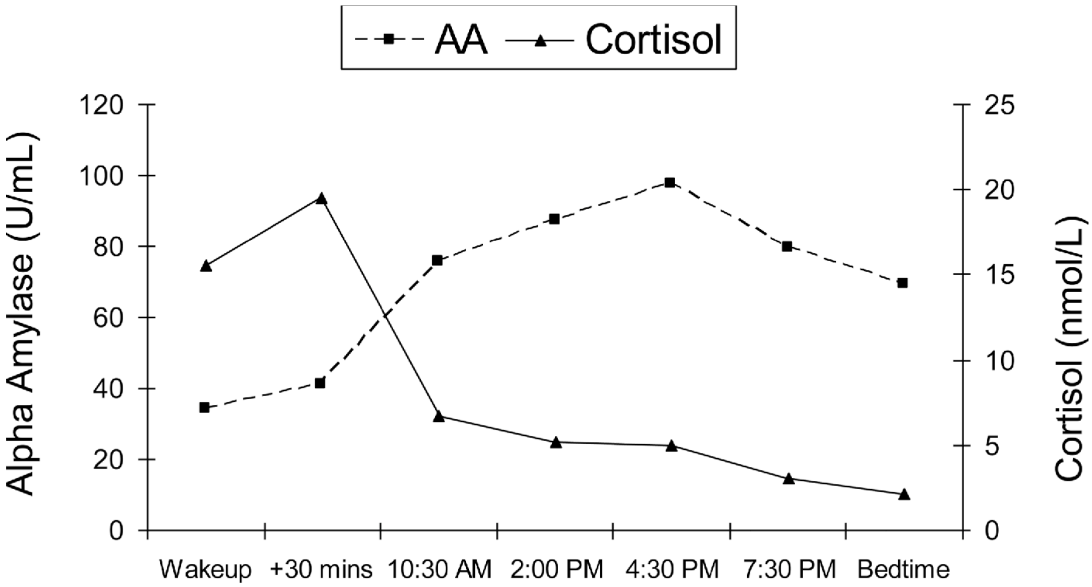


Figure 8. Alpha amylase and cortisol diurnal rhythms.
Source: Adam, Hoyt, & Granger (2011).

(2) Alpha Amylase as a Marker for Stress

sAA is correlated with psychological or physical stress (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Schumacher, Kirschbaum, Fydrich, & Strohle, 2013). sAA is used as an indicator of sympathetic activation of the autonomic nervous system (ANS).

sAA levels are not related to blood alpha-amylase levels (Schenkels, Veerman, & Nieuw-Amerongen, 1995) but do mirror norepinephrine activation (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2006). Norepinephrine (NE) is a hormone, neurotransmitter, and an indicator of sympathetic nervous system activation (Thoma, Kirschbaum, Wolf, & Rohleder, 2012; van Stegeren, Rohleder, Everaerd, & Wolf, 2006).

Plasma NE and sAA are positively associated with each other ($r = 0.54$, $p < 0.05$) (Rohleder et al., 2006). Thoma and colleagues (2012) compared the predictive power or sAA as a surrogate marker for sympathetic nervous system activity (SNS) with NE and epinephrine (E), two well-established SNS indicators. Sixty-six participants were subjected to the TSST. Saliva and blood samples were taken four times throughout the experiment and analyzed for sAA, NE and E concentrations. All three indicators showed significant increase in response to acute stress *(p* < 0.001). Researchers found that responses in sAA significantly predicted observed responses in NE (r = 0.032, p = 0.064), but failed to associate with E (p = .064). sAA does not correlate with plasma levels of alpha amylase but it has a strong association with ANS and SNS activation (Thoma et al., 2012). Figure 9 shows the similar responses of both NE and sAA to a stressor.

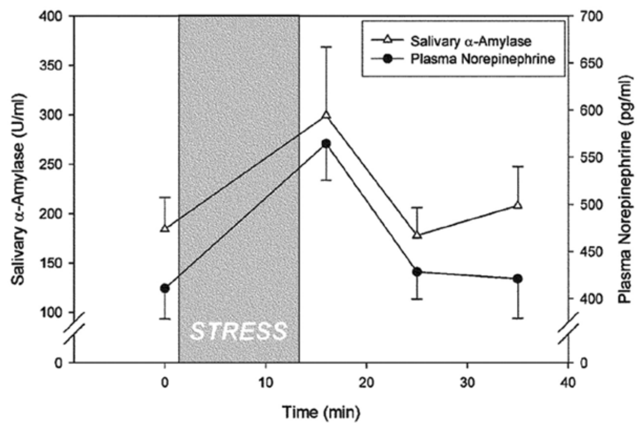


Figure 9. sAA and norepinephrine in response to stress; *gray bar* indicates the stressor. Source: Rohleder et al. (2006).

(3) sAA Acute Response

The production of alpha amylase in the salivary glands increases in response to activation of the ANS and regulate activation based off the strength of the stressor (Bosch et al., 1996; Proctor & Carpenter, 2007). Figure 10 visually demonstrates the response of sAA levels to a stressor (Nater et al., 2005).

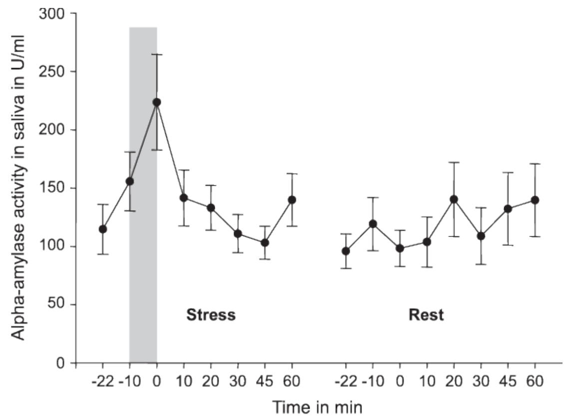


Figure 10. sAA levels in response to stress and at rest.
Source: Nater et al. (2005).

Chatterton and colleagues found that greater intensities of exercise were associated with greater increases of sAA concentrations (Chatterton et al., 996). Vineetha, Pai, Vengal, Gopalakrishna, and Narayanakurup (2014) also found that chronic stress causes statistically significant prolonged elevation of sAA compared to control samples.

This research suggests that sAA is an appropriate biomarker to evaluate ANS activation and regulates based off of stressor intensity (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Nater & Rohleder, 2009).

(4) sAA and Relaxation

sAA levels respond to relaxation techniques. Sripongngam and colleagues (2015) examined sAA levels following a traditional Thai massage (TTM). Twenty-nine participants were randomly assigned into the TTM group or the control group. After a 2-week washout period, the participants switched groups. Those exposed to a one-hour TTM following a 10-minute mentally stimulating arithmetic test had significantly lower sAA compared to the control group (p < 0.05). sAA is affected by stressful situations but also responds to relaxation techniques.

d. Biomarkers of Stress

Cortisol and sAA can be used simultaneously in research as a marker for stress system activation. Both analytes increase in response to SNS activity but differ in their response time. Cortisol exhibits a longer lag time between the onset of stress and visible increases in salivary levels than sAA (Takai et al., 2004). Takai and colleagues explored this time difference by exposing young adults to 15 minutes of a standardized stressor. Unstimulated whole sample saliva was collected every three minutes. Cortisol and sAA levels increased but sAA reacted stronger and quicker than cortisol. The researchers found that sAA levels significantly increased just after the start of the stressor, while cortisol levels exhibited a longer lag time (Takai et al., 2004).

This finding was further confirmed by Maruyama and colleagues (2012). Volunteers (*n* = 149) were exposed to both the TSST and electrical stimulation stress. Salivary cortisol and sAA measurements were taken three times; immediately before the stressor, immediately after the stressor, and 20 minutes post stress exposure. sAA levels rapidly reacted to the stressor and returned to baseline after 20 minutes. Salivary cortisol showed a delayed increase in response to the stress and remained significantly elevated 20 minutes post stressor. This research suggests that measuring both analytes provides a more complete picture of the pilot stress responses in the flight conditions.

4. Psychological Responses to Stress

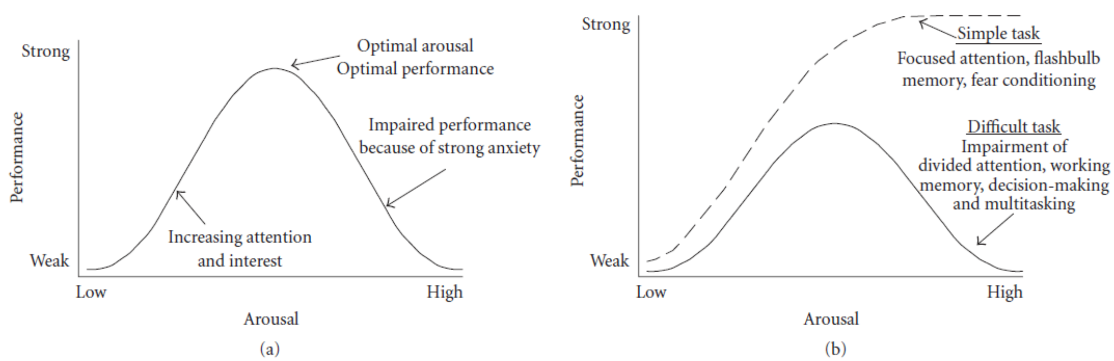
This section will focus on cognitive performance changes resulting from stress. Two models, the Yerkes-Dodson Curve and the Negative linear model, and their relevant research are discussed. The section ends with two ways to quantify psychological stress, which were used in this research protocol.

(1) Human Performance in Stressful Environments

Performance refers to the action or process of carrying out a task or function in accordance with pre-established standards. Human performance can be impacted by a variety of confounds. Stress, fatigue, circadian disruptions and experience all affect how well the individual can meet task or mission requirements (Schmidt, Hunter, & Outerbridge, 1986).

(2) The Yerkes-Dodson Curve

The Yerkes-Dodson curve is a model developed to explain how arousal affects performance. The original version identified a linear relationship between stimulus strength and habit formation for simple tasks, but a non-linear relationship with complex tasks (Diamond, Campbell, Park, Halonen & Zoladz, 2007). The Hebbian version of the Yerkes-Dodson curve did not distinguish task complexity and had a simple reverse bathtub curve which depicted an increase in performacne with an increasing arousal until an "optimal" level was reached (Yerkes & Dodson, 1908), at which point additional stress or fatigue resulted in a decrease in performance (Duffy, 1957). Figure 11 shows the original Yerkes-Dodson curve and the Hebbian variant.



The Hebbian version of the Yerkes-Dodson law (left) and the original Yerkes-Dodson law (right).

Figure 11. The Yerkes-Dodson Law. Source: Diamond, Campbell, Park, Halonen & Zoladz (2007).

The Hebbian version of the Yerkes-Dodson model eventually evolved into the more generic "stress performance curve." This inverted U-shape (shown in Figure 12) suggested that moderate levels of stress are beneficial for performance (Welford, 1973). After an arbitrary "tipping point," further increases in stress decreases performance. While this is commonly accepted, research does not support the inverted U-shape hypothesis.

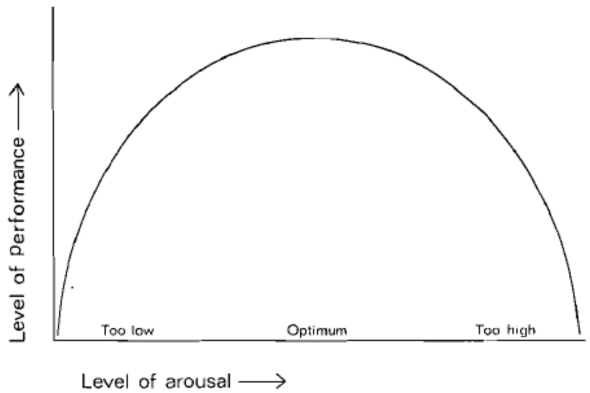


Figure 12. Relationship between stress and arousal according to the Inverted U-Hypothesis. Source: Welford (1973).

(3) Negative Linear Relationship

Current literature suggests a negative linear relationship between levels of stress and job or task performance. Job stress is the "psychological state perceived by individuals when faced with demands, constraints, and opportunities that have important but uncertain outcomes" (Yozgat, Yurtkoru, & Bilginoglu, 2013, p. 519). Job performance is a function of the individual's performance on specific tasks (Murphy & Kroeker, 1988).

Skosnik, Chatterton Jr., Swisher, and Park (2000) found that mild psychological stress altered attention processes and task performance. Fifteen minutes of exposure to a stressful video game reduced reaction time (RT) and negative priming in participants. Participants experienced a negative correlation between cortisol levels and RT immediately after stress, but not at any other time during the test (r = -0.46, $p < 0.05$) (Skosnik et al., 2000).

Yozgat, Yurtkoru, & Bilginoglu (2013) in a survey of high stress Istanbul public sector workers (n = 389) found a significant negative relationship between reported job stress and job performance (r = -0.122, $p < 0.05$). Jamal (1984) also found a negative relationship to job performance in role ambiguity (r = -0.41), role overload (r = -0.33), role conflict (r = -0.41), and resource inadequacy (r = -0.42). His research (n = 440) further supported a negative linear relationship (r = 0.11, $p < 0.01$) (Jamal, 1984).

Jamal's follow-up study further supported the negative linear relationship between job stress and performance (2007). The majority of comparisons of workers in a multinational company (n = 305 from Malaysia, n = 325 from Pakistan) supported a negative linear relationship (90%). Only 10% of the data supported a u-shaped or curvilinear relationship (Jamal, 2007).

Current research has improved upon the traditional Yerkes-Dodgson curve, suggesting a negative linear relationship between stress and overall performance.

(4) Social Readjustment Rating Scale

Several methods exist to quantify stress. The Holme-Rahe Stress Inventory, often referred to as the Social Readjustment Rating Scale (SRRS), was developed in 1967 (Holmes & Rahe, 1967). Two psychiatrists, Thomas Holmes and Richard Rahe, examined over 5,000 patients' medical records. They were looking for links between life-changing events and disease onset. Their belief was that certain life-changing, stressful events had the propensity to cause illness.

Using the results of their studies, Holmes and Rahe began development of a stress scale using naval sailors to begin scale development. Over 2,000 personnel reported life changes and illnesses during the preceding 10 years. Forty-three events were found to be identified as potentially stressful. Careful examination of the events and medical records established "scores" for events or assignment of Life Change Units (LCU). The number of LCUs was scaled for events based on the "intensity and length of time necessary to accommodate to a life event regardless of its desirability" (Rabkin & Struening, 1976, p. 1016). For example, death of a spouse (100) was assigned far more LCUs than changing jobs (36).

Further empirical research found a positive correlation (r = 0.118, p < 0.01) between LCUs and the development of an illness (McLeod, 2010; Rahe, Mahan, & Arthur, 1970). Rahe and colleagues conducted a prospective test with 2,664 sailors on board three U.S. Navy cruisers. Participants filled out a SRRS questionnaire prior to the start of a 6- to 8-month cruise. The illness data from the cruise period was evaluated against pre-cruise questionnaires. Researchers discovered a positive, though modest, relationship between pre-cruise data and illness reported at sea (Rahe et al., 1970). While small, the evidence provided a link between stressful events and illness.

The data suggested the development of LCU categories. Personnel with less than 150 LCUs for a given year reported good health for the following year, or had less than a 30% chance of developing an illness (McLeod, 2010). About half of the population with 150–300 LCUs reported an illness the following year. When LCU scores exceeded 300, over 70% of the individuals reported illness (McLeod, 2010; Rabkin & Struening, 1976).

The current SRRS is predictive of health for two years and higher scores indicate a higher likelihood of an individual developing a health breakdown. This model and subsequent validation supported the link between stress and illness (Noone, 2017) and has been cited over 14,000 times. The SRRS is useful in this research to identify pilots that are at risk for stress related health breakdowns. Usefulness of the SRRS far outweigh the limitations.

(5) Cohen Perceived Stress Scale

The Cohen Perceived Stress Scale (PSS) is another established measurement of stress and differs in several ways from the SRRS. First, it gives a snapshot of a much shorter time period. Instead of evaluating stress levels over the previous 12-month time period, the PSS focuses on just one month. The PSS measures the perception of stressor intensity by the individual and does not give just a single score per life event. The predictive quality of the PSS is also much shorter. While the SRRS is predictive of up to two years, the PSS is predictive of just four to twelve weeks post administration. The PSS allows an individual to make their own unique interpretation of a stressor rather than relying on cumulative effects of stressful events. It has been used to assess psychological stressors in a variety of settings to include disaster response (Leon, Hyre, Ompad, DeSalvo & Munter, 2007), telomere length (Parks et al., 2009), salivary cortisol (van Eck & Nicolson, 1994) and healthy lifestyle choices (Ng & Jeffery, 2003).

Ng and Jefferey (2003) found evidence to support the negative relationship between stress and lifestyle choices using the PSS. High stress in 12,110 workers was linked with higher fat intake, less frequent exercise, cigarette smoking, and recent increases in smoking

(Ng & Jeffery, 2003). Nixon, Massola, Bauer, Krueguer and Spector (2011) further supported this finding. Organizational and interpersonal conflicts had the highest relationship with job stressors and development of physical symptoms. Gastrointestinal issues and sleep disturbances were significantly related to increases in stress more than any other physical symptom (Nixon et al., 2011). There was a high correlation between the scores on the PSS scale and the development of symptomology ($r = 0.76$ and $r = 0.65$, $p < 0.001$). The PSS is a validated measure of personal stress (Cohen, Kamarck & Mermelstein, 1983).

C. WORKLOAD

This section will address how excessive workload is a source of stress. The section will define workload and possible sources of increased workload, and discuss two methods of quantifying workload that were explored in this research.

1. Workload and Performance

Workload the subjective "intersection between objective task demands and each individual's response to them" (Hart & Staveland, 1988, p. 176). Increases in workload impacts stress levels but also can also impact performance. Increasing workload was found to influence pilot performance in a study conducted by Svensson, Angelborg-Thanderez, Sjoberk and Olsson (2010). Eighteen pilots performed 72 simulated sorties with recordings of their heart rate, performance and workload levels. An increase in workload was positively correlated with increased heart rate ($r = 0.34$, $p < 0.05$). Even moderately complex tasks were found to interfere with the pilots' simulator performance (Svensson et al., 2010). This study accurately describes the relationship between workload, stress, and performance. The more demands (workload) put on the individual, the more the body reacts (stress response), thus affecting their ability to perform. This study indicates that increased workload can negatively influence performance.

2. Additional Duties

Methods used for measuring workload are unable to capture the unresourced burden, that is, the additional requirements that are not accounted for in typical manpower models in the squadron aviator. A known issue in the Naval Surface Warfare community (Fletcher, 2018), the unresourced burden caused by additional work duties has not been quantified. Pilots typically are required to perform at least one additional duty in addition to their primary Air Force Specialty Code (AFSC) that takes them away from flying. A memo, published in 2016 from Secretary of the Air Force Deborah Lee James and Air Force Chief of Staff General Dave Goldfein, cited efforts to reduce additional duties for Airmen (James & Goldfein, 2016). Citing Air Force Instruction 38–206, "Additional Duty Management," the memo attempted to eliminate 29 of 61 duties that had to be absorbed by squadrons and units with the dissolution of Commander Support Staff (CSS) functions.

A 2016 USAF Task Force titled "Airmen's Time" addressed the concerns about additional duties. As a main focus, the task force recommended reinstating the CSS functions. A secondary recommendation was a streamlined process for mandatory recurring training (Martin, 2016). The policy exists but currently, the intent and manning support has yet to reach all flying communities. Squadrons and aircrew are still being burdened with additional duties that resulted from a reduction in CSS manpower. This additional work has yet to be accounted for and is left to the existing personnel to perform in addition to their normal duties.

Quantifying workload objectively is difficult. What is a very high workload for one person may be a fairly light workload for another person. Multiple tools have been developed to objectively quantify individual workload for research purposes and two are discussed below.

3. Subjective Workload Assessment Technique

The Subjective Workload Assessment Technique, or SWAT, is a technique used to quantify workload and is based on the premise that mental workload is a multidimensional construct. SWAT uses three factors, time load, mental effort load, and psychological stress load, to score workload on identified tasks (Reid & Nygren, 1988). Individuals respond to these three factors in different ways. For example, time pressure may be extremely stressful for one individual while psychological stress may be the major driver for another individual. This scale development uses 27 cards in all possible combinations of time load, mental efforts, and psychological stress that the user must rank in terms of subjective workload. Individual SWAT users are prompted using verbal descriptors to help rate the three dimensions. This sorting process develops the interval scale, which is used in the scoring of actual events and trains the user at the exact same time. Events are scored giving a numerical value from 1 to 3 in each of the three dimensions. A score of 1–3-1 would indicate that time and psychological stress were at minimal levels, while mental effort was at the highest. This score is then compared to the interval that was established in the card sorting and results in a numerical scale of subjective workload ranging from 1 to 100.

Subjective scoring on the original SWAT test proved to be time consuming. Recent undertakings have attempted to develop a more simplified model using pairwise comparisons (Luximon & Goonetilleke, 2010). The simplified card sort process was show to significantly reduced the time to complete the training (22.08 seconds vs 476.49 seconds, $p < 0.05$) but has failed to be sensitive to low-tasks loads.

4. NASA-TLX

One of the most frequently used workload assessments is the National Aeronautics and Space Administration-Task Load Index (NASA-TLX) (Noyes & Bruneau, 2007) and it is considered a robust measurement of subjective workload (Moroney, Biers, & Eggemeier, 1995). The NASA-TLX uses six dimensions to assess mental workload: physical demand, temporal demand, performance, mental demand, effort, and frustration (Hart & Staveland, 1988). These categories are further described in Table 2 (Rubio, Diaz, Martin, & Puente, 2004). Twenty pair-wise comparisons are made amongst the dimensions to assign weight. The more times a dimension is chosen in a comparison; the more weight is applied in the overall rating. The NASA-TLX has been used to validate workload in environments from high stress hospital intensive care units (ICU) to aviation (Hoonakker et al., 2011).

Table 2. NASA-TLX category definitions. Source: Rubio, Diaz, Martin, & Puente (2004).

TITLE	ENDPOINTS	DESCRIPTIONS
MENTAL DEMAND	Low/High	How much mental and perceptual activity was required (e.g, thinking, deciding, calculating, remembering, looking, searching, etc.)? Was the task easy or demanding, simple or complex, exacting or forgiving?
PHYSICAL DEMAND	Low/High	How much physical activity was required (e.g. pushing, pulling, turning, controlling, activating, etc.)? Was the task easy or demanding, slow or brisk, slack or strenuous, restful or laborious?
TEMPORAL DEMAND	Low/High	How much time pressure did you feel due to the rate or pace at which the task or task elements occurred? Was the pace slow and leisurely or rapid and frantic?
PERFORMANCE	Good/Poor	How successful do you think you were in accomplishing the goals of the task set by the experimenter? How satisfied were you with your performance in accomplishing these goals?
EFFORT	Low/High	How hard did you have to work (mentally and physically) to accomplish your level of performance?
FRUSTRATION LEVEL	Low/High	How insecure, discouraged, irritated, stressed, and annoyed versus secure, gratified, content, relaxed, and complacent did you feel during the task?

The NASA-TLX was traditionally offered in a pencil and paper format. Recent advances in technology have allowed the NASA-TLX to be administered easily on an iPad or iPhone. One publication has suggested that NASA-TLX is superior to SWAT in terms of sensitivity especially for low mental workloads (Nygren, 1991).

D. FATIGUE

Aircrew fatigue in aviation is a known problem and a recognized source of error (Sexton, Thomas, & Helmreich, 2000). This section will discuss the causes of human fatigue, beginning by educating the reader on sleep and natural circadian rhythms. The section then addresses the relationship between sleep and performance, circadian release of hormones, and shift work. The section ends by identifying an appropriate means with which to measure sleep for the participants in the thesis research.

1. Sleep

Sleep is required to sustain life and is modulated by an individual's circadian rhythm (Fisher, Foster, & Peirson, 2013). During the sleep interval, individuals are not consciously interacting with their surroundings. Voluntary muscle control and sensory perception is diminished which facilitates rest and recovery. Once thought of as a period where the brain is "turned off," sleep studies have identified patterns of elevated brain activity during sleep intervals.

The patterns of sleep are broken down into two main divisions: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep both of which perform complementary functions. REM sleep is considered active sleep and is characterized by quick eye movement and high frequency low amplitude brain activity ("Natural Patterns of Sleep," 2007). REM sleep is responsible for memory consolidation of both short term and long-term memories (Wilhelm, Diekelmann, & Born, 2008).

NREM sleep is broken down into three intervals: N1, N2 and N3. As the individual transitions from N1 to N2 to N3 sleep there is a natural progression of slower brain waves and reduced eye movement ("Natural Patterns of Sleep," 2007). The majority of NREM sleep typically occurs at the beginning of a major sleep interval, for example, the first four hours of an eight-hour sleep period. REM sleep occurs in cycles later in the sleep interval and accounts for roughly 25% of the total time spent asleep in a healthy individual ("Natural Patterns of Sleep," 2007). The National Sleep Foundation recommends adults receive at least 7–9 hours of sleep per night (Hirshkowitz et al., 2015).

Aircrew populations are at particular risk for sleep deprivation. Long-duration missions, shifting landing and takeoff times, and high operations tempos significantly affect aviators' sleep. While fatigue has long been understood to be a causal factor in mishaps, the mission requirements often fail to accommodate sleep requirements (Hartzler, 2014).

Early show times are particularly detrimental to sleep quantity. Roach, Sargent, Darwent, and Dawson (2012) examined the effects of show time on sleep quantity in short-haul pilots using actigraphy and self-reported sleep. Short-haul pilots had significantly lower quantities of sleep with earlier show times. In their sample size (*n)* of 70 airline pilots, beginning at 0400, for each additional hour a duty-day start was delayed, the pilot received an additional 15 minutes of sleep. Self-reported fatigue levels were highest in the early duty-day start groups (0400-0500) and lowest for the later duty-day start time group (0900-1000) (Roach et al., 2012). F-22 squadron sortie times are planned in conjunction with maintenance schedules. This often produces early-duty start days, which makes the aircrew at risk for fatigue. Compounding this effect is typical workday commutes, which can lengthen the workday upwards of 2 hours in some locations.

2. Circadian Rhythm

The human circadian rhythm is the natural body clock. Controlled by the hypothalamus, it regulates many physiological processes including hormones, body temperature, and the sleep/wake cycle. Environmental factors such as light and darkness can affect the circadian rhythm ("What is Circadian Rhythm?" 2018).

Typical circadian wake/rest rhythms are slightly longer than 24 hours in the absence of light. Forced desynchrony studies found an average circadian rhythm of roughly 24.2 hours, or about a 12-minute phase shift per day (Czeisler et al., 1999). Light, either artificial or natural, is a powerful circadian synchronizer and stabilizer. While light affects hormone regulation and sleep/wake cycles, rhythmicity of core body temperature is relatively stable, even in the absence of light cues (Duffy & Wright, 2005).

3. Fatigue in Aviation

Fatigue has been accepted as part of the military aviation culture. Pilots are required to meet mission and operational demands, which include early show times, multiple flights during a week, and additional duties. But these increasing mission demands negatively impacts fatigue. Short-term fatigue in pilots has been attributed to prolonged duty periods (53%) and early wake-ups (41%) (Bourgeois-Bougrine, Carbon, Gounelle, Mollard, & Coblentz, 2003). Early wake-ups for take-offs is determined by maintenance schedules rather than the individual aircrew members. This research suggests that fatigue in aviation is not just a product of individual habits but of the squadron and mission requirements.

4. Fatigue and Performance

Failure to receive adequate sleep at night makes an individual more likely to commit errors (Johnson et al., 2014) and have a slowed reaction time (Taheri & Arabameri, 2012). Professions requiring sustained high performance, such as aviation or medicine, have found a negative relationship between fatigue and performance. In a recent study, sleep deprivation in surgical teams had a significant impact on overall patient morbidity and mortality (Parker & Parker, 2017). Similar results were found by Philip and colleagues (2005) who saw a negative relationship between driving performance and restricted sleep. Sleep restriction caused significant performance degradation evidenced by increased line crossings ($p < 0.001$), even in short performance tasks of around 105 minutes. These short driving sessions mirror average sorties times in the F-22 aircraft. Even in short duration performance tasks, such as flying, fatigue impacts performance (Philip et al., 2005).

The aviation community has long recognized that fatigue degrades performance. A study conducted by Cooper & Sloan (1985) surveyed commercial airline pilots ($n = 422$). In the self-reported survey, poor performance and fatigue were correlated ($r = 0.316$, $p < 0.001$) (Cooper & Sloan, 1985).

5. Physiological Responses to Fatigue

Sleep quality affects the normal cortisol rhythm. In a study by Wright, Valdimarsdottir, Erblich, and Bovbjerg (2007) discovered a blunted cortisol response ($n = 53$) to an experimental stressor in participants with a lower subjective sleep quality. A study by Bassett, Lupis, Gianferante, Rohleder, and Wolf (2015) further supported this finding of poor self-reported sleep associated with a blunted cortisol response. Their study showed a significant relationship in Pittsburg Sleep Quality Index (PSQI) scores and sCort responses following a Trier social Stress Test (TSST) in 73 college-aged adults (Bassett et al., 2015; Capaldi, Handwerger, Richardson, & Stroud, 2005).

Sleep quality affects the overall circadian pattern of cortisol but does not affect the cortisol awakening response (CAR). Dettenborn, Rosenloecher and Kirschbaum (2007) found that CAR of healthy women was not impacted by repeated forced awakenings. Neither time in bed, sleep efficiency, nor fatigue impacted the CAR (Hernandez et al., 2018). These results suggest that capturing perceived sleep quality is important in explaining cortisol anomalies. Failure to account for identified sleep changes or problems may bias results.

6. Shift Work and Fatigue

Shift work is often associated with work schedules outside the normal 9am-5pm and is common in many service organizations. Firefighters, police, military, and healthcare populations are traditionally engage in shift work.

Shift work is associated with "increased subjective, behavioral, and physiological sleepiness" (Akerstedt, 1988, p. 30). Shift workers experience shortened sleep intervals, drowsiness carryover effects, and insomnia (Akerstedt, 2003). These symptoms have recently been coined as shift work sleep disorder (SWSD). SWSD is a condition developed by shift workers in which periods of insomnia and excessive fatigue are caused by disruption to the normal circadian rhythm.

Not all shift workers will develop SWSD, but shift workers are more likely to be fatigued. In a poll conducted by the National Sleep Foundation, 89% of non-shift workers claimed their work schedule allowed them to get enough sleep compared to only 63% of shift workers ("What is Shift Work?" 2018). Yuan and colleagues (2011) also found that shift working nurses (n = 107) were more fatigued than traditional day shift workers (odds ratio = 2.44, $p < 0.1$) (Yuan et al., 2011).

7. Shift Work and Performance

Shift work negatively affects cognitive performance in traditional shift work populations. Gold and colleagues (1992) studied 635 nurses and nurse practitioners who were part of a hospital system. The nurses who worked an occasional night shift, defined as three or less night shifts per month, had higher automobile near miss accidents going to or from work (28.1% vs 19.6%), higher reports of nodding off while driving to or from work (40.0% vs 21.2%), higher reports of medication errors (12.1% vs 5.8%), and higher rates of medication near misses (15.5% vs 7.8%) than those on a consistent schedule (Gold et al., 1992). Nurses on a rotating schedule, defined as working an equal amount of day and night shifts, were far more likely to have near misses (50.4%) and poor quality of sleep (52.1%) than any other nursing subpopulation. Performance was degraded even in those who worked an occasional night shift but was more pronounced in the rotating shift population.

8. Shift Work and Circadian Rhythm

Shift work may disrupt the normal circadian rhythm of cortisol. Touitou and colleagues conducted an early study into internal desynchronization caused by short-term shift workers (1989). Serum cortisol showed a decreased amplitude but elevated concentrations at midnight (0000). According to their research, fast rotating shift workers did not appear to have a phase shift of hormones with normal diurnal rhythms (Touitou et al., 1990). Hung, Aronson, Leung, Day, and Tranmer (2016) also found evidence of a flattened cortisol curve in shift workers. Diurnal cortisol rhythms were collected from midstream urine on traditional day hospital employees (n = 160) and rotating shift workers (n = 168). Shift workers and traditional day workers had similar cortisol production during the day. However, shift workers that were on night shift had a flatter or more muted cortisol curve, indicating a reduction in cortisol production. The research suggested that night shift work is associated with weaker cortisol production in the shift working population (Hung et al., 2016).

9. Measuring Sleep

There are three common ways to measure sleep: polysomnography (PSG), actigraphy, and self-reported fatigue ratings.

PSG remains the gold standard in quantifying sleep and is used to study sleep and diagnose sleep disorders (Douglas, Thomas, & Jan, 1992). Unfortunately, its use is not possible in field studies, as it requires specialized equipment and dozens of electrodes to monitor the sleeping patterns of participants.

Actigraphy is captured through wrist-worn activity monitors (WAMs) and gives an objective estimation of sleep quantity and quality. Actigraphy uses a software algorithm to transcribe the data into sleep intervals defined as periods of reduced movement. In a study conducted by Marino and colleagues (2013), PSG and actigraphy were simultaneously collected in a controlled sleep laboratory. Actigraphy exhibited a high accuracy (0.863) when compared to PSG readings (Marino et al., 2013).

Subjective ratings of fatigue are cheap to collect and have a high sensitivity (between 73% and 97.7%). However, of three measures discussed, subjective fatigue ratings are the least accurate and least preferable in research protocols (Ibanez, Silva, & Cauli, 2018)

E. CURRENT HYPOTHESES

The main purpose of this thesis is an investigation into the physiological and performance changes that occur during the short-term shift to night operations in F-22 pilots. This potential relationship will be explored in Hypothesis I. Current literature suggests a possible adaptation effect to repetitive stress exposure, which has not been studied in this population. This relationship will be explored in Hypothesis II.

1. Hypothesis 1

The null hypothesis is that there are no changes, in either performance or the physiological response, as the pilots' transition to night-flying operations. The null hypothesis includes comparing sleeping patterns, a go/no-go test, NASA TLX, salivary cortisol and alpha-amylase levels.

2. Hypothesis 2

Hypothesis 2 is used to investigate stress adaptation. The more an individual is exposed to a stress, the less severe a stress response may become. Experience will be evaluated in the context of flight hours. The null hypothesis is that there is no relationship between pilot experience, as determined by flight hours, and stress system activation.

III. METHODS AND EXPERIMENTAL DESIGN

This chapter will discuss the multifaceted approach in understanding the stress burden on aviators in response to different flight conditions. It begins by identifying the study design and the rationale for employing specific techniques used within the research. The last section of this chapter discusses methods for analyzing the collected data.

A. STUDY DESIGN

The research was designed as a prospective, quasi-experimental design within-participants observational study. Two weeks of sleep, stress, performance, and workload data were collected. The first week was a normal flying week in which participants flew during daytime hours. The second week of data collection was a night week in which take off times were shifted to the evening hours. Participants were instructed to not alter their natural awakening time for the purpose of this study.

1. Independent Variables

The independent variables were flight conditions (control, day, and night) and time of day. Participant descriptor characteristics identified in the pre-study questionnaire were also used as independent variables.

2. Dependent Variables

The dependent salivary variables were classified into two categories: sCort levels measured in μg/mL and sAA levels measured in nmol/L. Priority was given to sCort in the event a small sample volume was collected. The GNG test provided two dependent variables, reaction time and accuracy, which were automatically calculated by the software. WAM variables were hours asleep, average awakenings, and percentage sleep efficiency. NASA-TLX scores were also dependent variables of the study.

B. METHODS

Data was collected from February 26 to March 9 of 2018 at the 19FS and 199FS located at Hickam Air Force Base (AFB), Hawaii.

1. Participants

To reach a power of 0.80 for detecting a difference of at least 1.6 between two population means, a two-sample t-test (where both populations have a common standard deviation of 1.3) requires a minimum of 12 participants sampled from each population. The standard deviation of 1.3 for the calculation was obtained from research conducted by Vining and colleagues (1983) comparing serum and salivary cortisol levels among male participants. A participant sample of 30 was approved by Naval Health Research Center (NHRC) (Protocol #NHRC.2018.0007).

Seventeen male pilots volunteered to participate in the study. All participants were F-22 pilots with a current physical, and actively flying during the data collection period. Inclusion criteria was presented during recruitment briefs and was self-reported. No access to medical records was required. Participants could take part in all or parts of the study depending on their comfort level. The decision logic of the study can be found in Appendix A ("Study Design Logic").

2. Apparatus

The following items were used in the execution of the study design and relevant analyses.

a. Wrist Activity Monitor (WAM)

Participants were issued a WAM, which was a non-transmitting actigraph manufactured by the Philips Respironics company. WAMs were pre-programmed at the Naval Postgraduate School (NPS) for passive data collection following the recruitment brief and were worn continuously throughout the two-week data collection. Participants were instructed to wear the WAM on their non-dominant wrist and only remove for hygiene purposes. WAMs were turned in at the conclusion of the data collection period. Two participants turned in their WAMs early due to changes in their flying schedule.

b. Go/No-Go (GNG)

A GNG test was used to evaluate changes in cognitive performance pre- and post-fight in both day- and night-flying conditions. The GNG tests reaction time to one stimulus while refraining from responding to incorrect stimuli also known as response inhibition. The task is similar to decision making in the aircraft where "friend vs foe" identification is required. This task is especially important in military operating environments.

The GNG task used in the study was developed by the Naval Aviation Medical Research Unit at Dayton (NAMRU-D) to facilitate research into security forces' decision-making. The software package initially contained an individual with a gun (the "go" stimulus) and an individual with a cell phone (the "no-go" stimulus). The original test took 20 minutes to complete and evaluated reaction time and accuracy for the trials. In discussion with program designers, it was determined that the GNG could be modified to fit the proposed study. Kara Blacker, a research psychologist associated with NAMRU-D, modified the GNG. She replaced the threatening figure with two distinctly different colored squares (yellow and purple). The test time was shortened to 180 trials, 20% of which were no-go stimuli. The inter-stimulus interval, or the time between trials, was shortened from 0.5-1.5 seconds to 0.5-1.0 seconds. The random nature of the inter-stimulus interval prevented the participants from establishing a predictable response rhythm.

The benefit of using the GNG was twofold. First, it is already being used in other naval research so no license had to be purchased. Second, the GNG was administered on iPads that were used for other data collection within the experimental design. The modified GNG was appropriate for measuring executive function in the sample.

In populations of shift workers, it is important to measure elements other than just reaction time. A study by Harris and colleagues (2010) in offshore drilling shift workers compared their reaction times. Reaction times did not vary significantly in the sample on swing shift (Harris et al., 2010). Using a GNG instead of a psychomotor vigilance test (PVT) allowed for determination of response inhibition as well as reaction time, generating a more complete picture of operator performance.

The 5-minute GNG test was completed pre- and post-sortie in both day and night flying conditions. The test was administered on an NPS issued laptop using MATLAB software.

c. *NASA-TLX*

Workload was measured using the NASA-TLX. The paper and computer NASA-TLX versions vary in sensitivity. A significant difference exists, with the computer-executed assessments incurring more workload (Noyes & Bruneau, 2007). We accepted this confound in the experimental design as it reduced computational error by researchers. We used Apple iPads to administer the NASA-TLX and pre-loaded study and participant information. Weightings of each of the six dimensions in addition to a workload score were automatically calculated by the NASA-TLX app.

d. *Saliva*

Levels of cortisol and alpha amylase spike in response to physical and psychological stress (Miller et al., 2016). Therefore, it is imperative to collect samples as close to the stress exposure as possible. Participants completed sample collection during three conditions: day, night, and non-flying (control). The non-flying condition was used as a control for establishing the participants' normal diurnal rhythm of both sCort and sAA. In keeping with a recommendation by Douglas Granger (personal communication, January 25, 2018), two control samples were collected for each participant to establish a CAR and control for variability. Saliva was collected at predetermined intervals across the three conditions to control for the diurnal nature of the analytes. Initially, the data collection intervals were the same for the entire squadron. Upon discussion with the squadron schedulers, this requirement limited participant participation. The decision was made locally to have each pilot individually identify collection intervals that would accommodate their schedules, compensating for variability in the flight schedules as well as known limitations (e.g., flying during a collection time period).

Salivary alpha amylase and salivary cortisol were selected as appropriate biomarkers for this research. Saliva samples were collected using the SalivaBio Oral Swab (exclusively from Salimetrics, State College, PA). According to Salimetrics, this product is “a synthetic swab specifically designed to improve volume collection and increase participant compliance, and validated for use with salivary [Analytes]” (Salimetrics, 2018).

e. Software

(1) JMP Pro 13.1.0

The distribution graphics and regression analyses produced in support of this thesis were conducted using JMP Pro software, version 13.1.0.

(2) Microsoft Excel

The calculations in this thesis were primarily conducted using Microsoft Excel 365.

(3) MATLAB

MATLAB software was used to run the GNG test developed by NMRU-D.

3. Procedure

All computer, iPad, and pre- and post-flight saliva sampling took place at the combined squadron flight equipment room. Squadron commander approval for data collection was obtained in November 2017.

a. Recruitment

A recruitment brief was conducted in accordance with IRB protocol (NHRC.2018.0007) and took place on February 23, 2018, during squadron administration briefs with approval by the Squadron Director of Operations. Participants were briefed on the background, purpose of the study, and various components of the study. Individual recruitments were conducted until February 28, 2018, to accommodate senior leadership schedules. Study volunteers were issued consent forms, the pre-survey questionnaire, a pre-programmed WAM, salivary collection sampling materials, and instructions about the salivary collection process.

The pre-study questionnaire consisted of five validated stress and fatigue scales. Because research suggests that the interaction of home and job stress can negatively impact flying performance, a Cohen Perceived Stress Scale (PSS) and Holmes-Rahe Stress Readjustment Rating Scale (SRRS) (Fiedler, Rocco, Schroeder, & Nguyen, 2000) were administered to participants in addition to the Epworth Sleepiness Scale (ESS), Pittsburgh Sleep Quality Index (PSQI), and the Morningness-Eveningness Questionnaire-Self Assessment version (MEQ-SA).

b. Collection Overview

Data collection consisted of two distinct parts: saliva collection and performance and workload assessments. Figure 13 provides a general overview of the collection schedule.

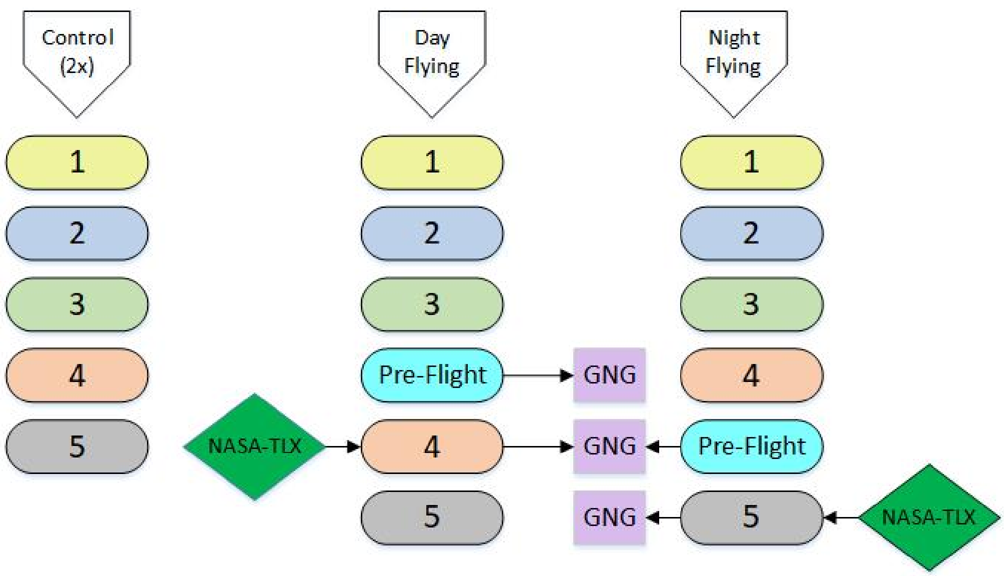


Figure 13. Two-week data collection schedule overview.

At the conclusion of the night-flying week, participants returned their pre-programed WAM and pre-survey questionnaires.

c. Saliva

Saliva sampling began February 26, 2018, and consisted of five samples per day for four days. The first sample was to be taken immediately upon waking. The second was scheduled to be collected 30 minutes after waking to establish the cortisol awakening response (CAR). Three morning samples are recommended to capture the delayed CAR in females (Stalder et al., 2016). However, only male pilots were part of the sample. In an effort to reduce participant burden, increase adherence to protocols, and decrease financial requirements associated with collection and analysis, the third CAR sample at +45 minutes was removed from the protocol.

The three additional samples were to be taken at intervals throughout the day. Participants were given flexibility in selecting the three additional collection times to allow for flight times, simulator training, and additional constraints on individual schedules. Text message reminders by research personnel were sent at the request of participants preceding a sample collection day.

Per IRB protocol, participants were not to alter their crew rest requirements nor their normal sleeping schedule for the purpose of the study. Therefore, participants were required to collect several of their own salivary samples. Participants sample self-collection is common in salivary biomarker analytics and historically have high compliance rates (Kudielka, Broderick, & Kirschbaum, 2003; Nater et al., 2007).

An additional saliva sample was collected during the day-flying and night-flying conditions. Pilots completed a pre-flight salivary sample within 30 minutes of planned engine start-up and immediately post-flight in the aircrew flight equipment room with NPS researchers.

Participants were instructed to refrain from eating or drinking prior to salivary sample collection. A list of items to avoid in addition to instructions was provided to participants for their reference. Participants collected saliva by placing the SalivaBio Oral Swab sublingually for two minutes and then placing the swab in the collection tube. Participants were instructed to not touch the swab either in the placement of the swab or in the removal of the swab to place in the tube. Participants were instructed to not chew on the swab during the sample collection. No stimulants were given to increase saliva production (Schwartz, Granger, Susman, Gunnar, & Laird, 1998). Swabs were then transferred to collection tubes and either stored in home fridges (if self-collected) until they could be turned into NPS researchers or immediately in NPS-provided coolers if collected in the presence of researchers.

Tubes were color-coded and labeled. Each participant had individually assigned tubes that were recorded on a master data sheet prior to the start of the study. Participants were provided the collection materials in a Ziploc bag containing instructions and journal to record sampling times. Figure 14 shows the Ziploc bag referred to as the “take home kit.”

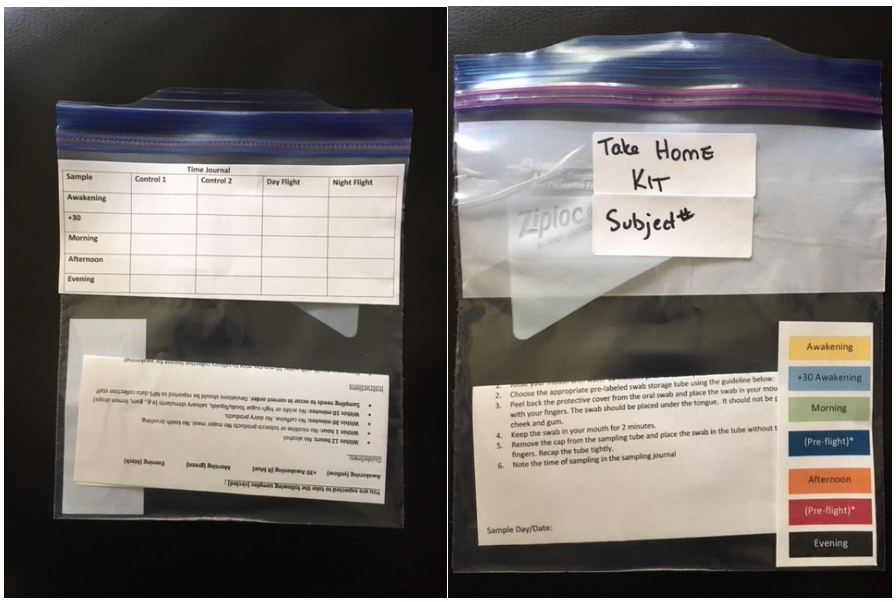


Figure 14. Participant take-home kits with time log (left) and color-coded collection schedule (right).

(1) Transportation and Storage

Salivary samples were labeled and stored in commercial off-the-shelf 3-gallon coolers. Small cooler size enabled samples to be stored in hotel refrigerators prior to shipment and maintain the cold chain. One cooler was shipped via FedEx Priority Overnight shipping to awaiting researchers at NPS. The second shipment was unable to be shipped by FedEx Overnight Priority shipping because of maintenance delays. The shipment was not expected to arrive to NPS for roughly three days, which would have disrupted the cold chain. Alternative carriers were investigated. The United Parcel Service did not offer overnight shipping from Hawaii. After discussion, we decided samples should be transported via commercial airline carrier with the returning researcher. The NPS researcher kept the samples in personal hotel fridge until transporting home the next morning using a cooler and cool packs.

At NPS, samples were stored at -40-degrees Celsius. The freezer was on an uninterrupted power supply to prevent thawing from power outages. Freezers were placed in the NPS wet lab behind a cypher-locked door.

(2) Sample Extraction and Preparation

Samples were collected using SalivaBio Oral Swab. The local lab did not have a centrifuge large enough for collection tubes for standardized extraction. We thawed samples overnight and placed swabs into sterile syringes. We pushed on the plunger to squeeze out liquid volume into marked 1.7mL tubes that were stored in 40 degrees Celsius freezers until analysis could be completed.

Saliva samples were thawed from -40-degree Celsius freezers 24 hours prior to analysis. When thawed, samples were vortexed and then centrifuged at 1500 rpms for 15 minutes prior to analysis.

(3) ELISA Kit

Salivary cortisol concentrations were found using Salimetrics Salivary Cortisol ELISA kit. The test required 25 μL of volume for analysis and had a sensitivity of 0.007 μg/dL for the concentration range of 0.012-3.00 μg/dL. The serum-saliva correlation is 0.91 (Salimetrics, 2014). The protocol can be found in Appendix B ("Cortisol Assay Protocol").

(4) Enzymatic Kit

sAA concentrations were found using the Salimetrics Salivary Alpha Amylase Enzymatic kit. The assay kit required a 10 μL sample of saliva and had a sensitivity of 0.4 U/mL (Salimetrics, 2016). The protocol can be found in Appendix C ("Salivary Alpha Amylase Protocol").

d. *GNG*

In both day- and night-flying conditions, participants were instructed to take a GNG both before and after the scheduled flight. Participants would report to the aircrew flight equipment room, we would input participant identifiers, and participants would read the instructions. Participants were instructed to press the space bar if a “go” indication was received and to refrain from pressing the space bar if a “no-go” indication was received. The indications were either a yellow or purple square on the middle of the laptop computer screen. The “go” and “no-go” indications were randomly assigned. After a brief practice session, participants would began the 5-minute timed test. This procedure was the same for both the pre- and post-flight data collection in both conditions.

e. *NASA-TLX*

Participants completed the NASA-TLX assessment following the GNG only post-sortie. The NASA-TLX was administered on a password-protected iPad provided by NPS. We would pre-load participant-identifying information prior to the participant beginning the assessment. Participants completed the pair-wise comparisons each time they were administered the NASA-TLX.

C. ANALYSIS

1. Saliva

Slopes of salivary cortisol and sAA levels were compared to civilian populations along with predicted sCort concentrations in relation to hours after awakening. The CAR was calculated and compared in each condition using a two-sided paired sample t-test.

2. Go/No-Go

Reaction time and accuracy were automatically calculated by the GNG using MATLAB software. The data were transferred to Microsoft Excel and visually examined using Pivot tables. A two-sided paired sample t-test was used to compare GNG results pre- and post-flight in both day- and night-flying conditions.

3. Actigraphy

Data from the WAMs were downloaded to allow for calculation of total daily sleep, defined as the amount of sleep obtained in a 24-hour period. One-minute epochs were used in the actigraphic recordings. The 24-hour period was calculated from midnight to midnight of each day and was separated into two categories: the average sleep for the day-flying week and for the night-flying week. The sleep amounts were compared for each day across both conditions (e.g., Monday of day-flying week was compared to Monday of night-flying week) using a two-sided paired sample t-test. Data from the WAMs were also used to establish an average awakening time in the absence of an awakening salivary sample for plotting the predicted sCort concentration curve.

4. NASA-TLX

NASA-TLX scores were calculated by the iPad NASA-TLX application. Scores were compared within subjects using a two-sided paired sample t-test on Microsoft Excel software.

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IV. RESULTS

This chapter provides results of the analyses beginning with a description of the sample, sleep and workload results, physiological testing results, and cognitive testing results. Wes used a significance level, alpha, of 0.05 for all statistical tests. Table 3 gives a summary of those hypothesis tests yielding significance at alpha = 0.05.

Table 3. Significant findings of the research described by variable, condition, method of comparison, and associated *p*-value.

Variable	*Condition*	*Comparison*	*Method*	*p-value*
Age		Flight Hours	correlation	*0.004*
		F-22 Flight Hours	correlation	*0.016*
		Night Hours	correlation	*0.012*
		PSS	correlation	*0.005*
Cortisol (ug/DL)				
	Non-Flying Day	Predicted sCort (CIRCORT Curve)	Wilcoxon Rank Sum	*<0.001*
	Pre-Flight Day	Predicted sCort (CIRCORT Curve)	paired sample t-test	*0.009*
	Post-Flight Day	Predicted sCort (CIRCORT Curve)	paired sample t-test	*0.003*
	Pre-Flight Night	Predicted sCort (CIRCORT Curve)	paired sample t-test	*0.025*
	Post-Flight Night	Predicted sCort (CIRCORT Curve)	paired sample t-test	*0.003*
	Post-Flight Day	Predicted sCort (developed curve)	paired sample t-test	*0.049*
	Post-Flight Night	Predicted sCort (developed curve)	paired sample t-test	*0.050*
Cortisol Change				
	Pre-to-Post Flight Day	F-22 Hours	correlation	*0.001*
GNG Accuracy (no-go)				
	Pre-Flight Day	Sleep Efficiency (%)	correlation	*<0.001*
	Post-Flight Night	Minutes Asleep (avg)	correlation	*0.036*
	Post-Flight Day	NASA-TLX	correlation	*0.025*
	Post-Flight Day	PSS	correlation	*0.043*
GNG Accuracy (total)				
	Pre-Flight Day	Sleep Efficiency (%)	correlation	*0.029*
	Post-Flight Day	NASA-TLX	correlation	*0.026*
GNG RT				
	Pre-Flight Day	Minutes Asleep (avg)	correlation	*0.044*

A. DESCRIPTION OF SAMPLE BY PRE-SURVEY QUESTIONNAIRE

This section outlines the sample characteristics as observed through the validated stress and fatigue scales administered during the pre-survey questionnaire. Each section discusses distribution results in addition to significant correlations.

1. Sample

Seventeen F-22 pilots participated to varying degrees in the study. Participants were on average 36 years old (SD = 6.29 years), with the following averages ($\overline{X}$): total flight hours $\overline{X} =$ 1745 (SD = 944 hours), F-22 flight hours $\overline{X} =$ 576 (SD = 347 hours), and night-flying hours $\overline{X} =$ 204 (SD = 158 hours). Figure 15 illustrates the distributions.

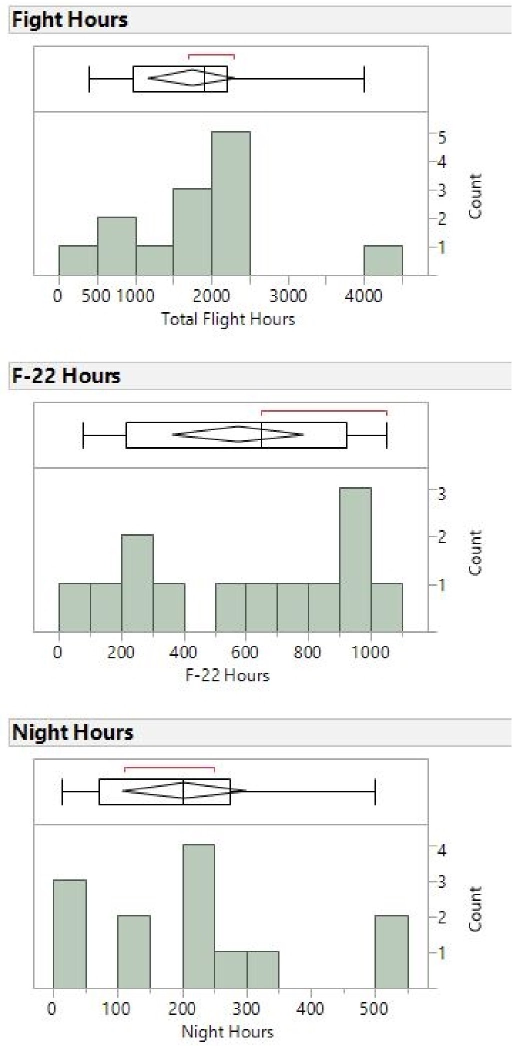

Figure 15. Flight hour distribution breakdown of total flight hours, F-22 flight hours, and night flight hours.

Age is positively correlated with total flying hours ($r = 0.75$, $p = 0.004$), age and F-22 hours, ($r = 0.65$, $p = 0.016$), and age and night flying hours ($r = 0.67$, $p = 0.012$). Figures 16, 17, and 18 visually depict these relationships.

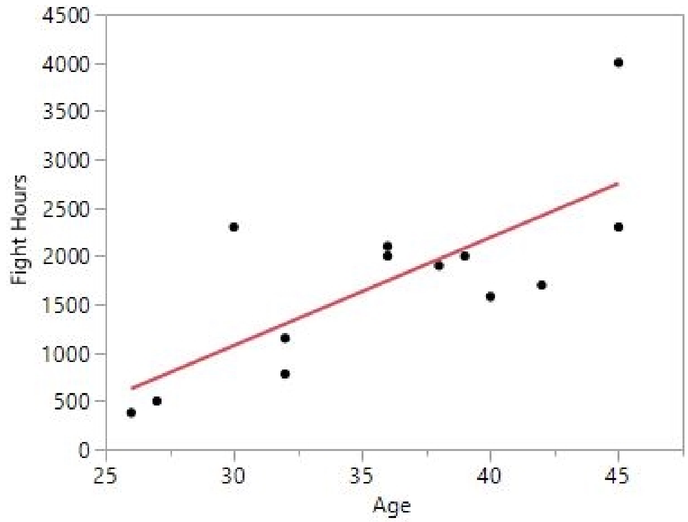

Figure 16. Relationship of age and flight hours ($r = 0.75$, $p = 0.004$)

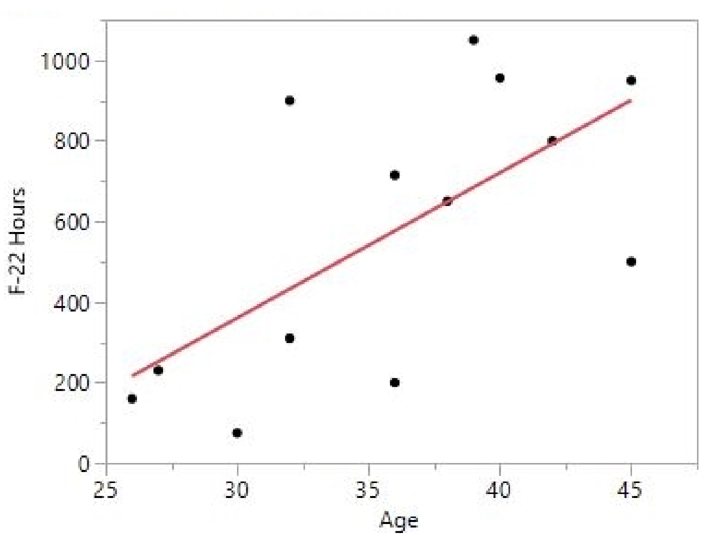

Figure 17. Relationship of age and F-22 hours ($r = 0.65$, $p = 0.016$)

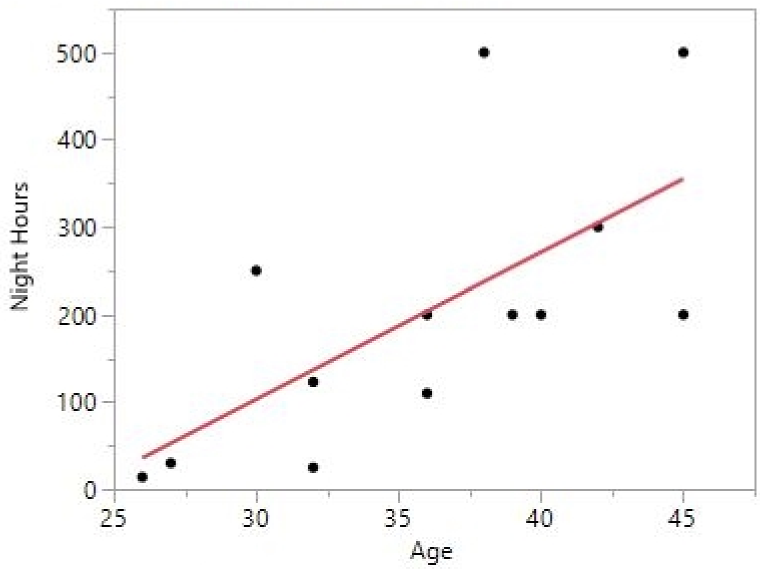

Figure 18. Relationship of age and night-flying hours (r = 0.67, p = 0.012)

2. Morningness-Eveningness Questionnaire (MEQ-SA)

Of the participants, 13 of 17 returned the MEQ-SA for a 76.5% return rate. Test scores ranged between 16 and 86. A score of 41 or below indicates an evening type. Scores of 59 and above indicate morning types. A score in the range of 42–58 indicates an intermediate type, with neither morning nor evening characteristics. Four respondents (31%) were categorized as morning types, eight respondents (61%) were intermediate types, and one (8%) respondent was categorized as an evening type. Figure 19 shows the distribution.

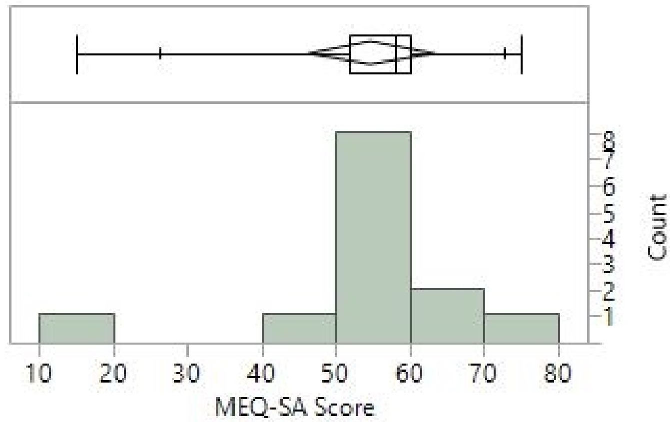

Figure 19. Distribution of MEQ-SA scores in the sample.

3. Epworth Sleepiness Scale (ESS)

Of the participants, 13 of 17 returned the ESS for a 76.5% return rate. ESS scores fall into one of the following categories: lower normal daytime sleepiness (scores 0–5), higher normal daytime sleepiness (scores 6–10), mild excessive daytime sleepiness (scores 11–12), moderate excessive daytime sleepiness (scores 13–15), and severe excessive daytime sleepiness (scores 16–24). Seven participants (54%) scores indicated normal daytime sleepiness levels. Four participants (30%) showed higher than normal daytime sleepiness. One individual showed moderately excessive daytime sleepiness (8%), and one individual showed excessive daytime sleepiness (8%). Figure 20 shows the distribution.

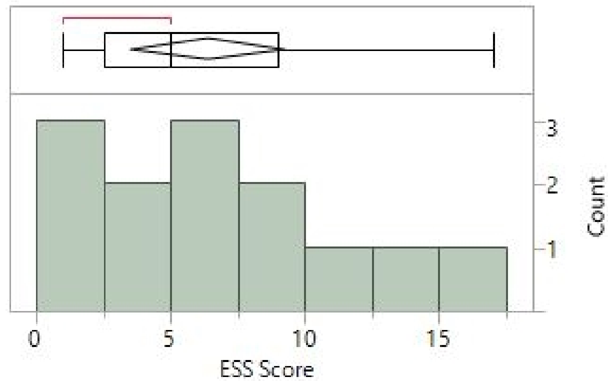

Figure 20. Distribution of ESS scores in the sample.

4. Pittsburg Sleep Quality Index (PSQI)

Of the participants, 13 of 17 returned the PSQI for a 76.5% return rate. The PSQI is rated on a 0–21 scale with higher numbers indicating increasingly poor sleep quality. Scores greater than or equal to five indicate poor sleep quality. Six participants (46%) scored five or above indicating poor sleep quality. Figure 21 shows the distribution.

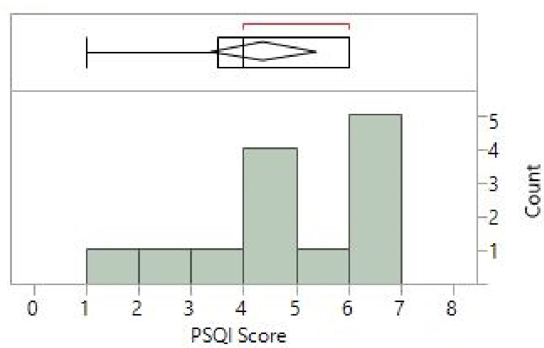

Figure 21. Distribution of PSQI scores in the sample.

5. Social Rating and Readjustment Scale (SRRS)

Of the participants, 11 of 17 returned the SRRS for a 64.7% return rate. An SRRS score of 150 or less indicates a low likelihood of developing a stress-related illness within the next two years; all but one of the respondents fell within this range. One respondent scored 164, indicating a 50% chance of developing a health related breakdown within the next two years. Figure 22 shows the distribution.

Figure 22. Distribution of SRRS scores in the sample.

6. Cohen Perceived Stress Survey (PSS)

Of the participants, 13 of 17 returned the Cohen PSS for a 76.5% return rate. PSS scores fall into the following categories: low stress (score of 0–13), moderate stress (scores of 14–26), and high stress (scores of 27–40). Twelve participants (92%) indicated low stress. Only one participant (8%) indicated moderate stress. Figure 23 shows the distribution.

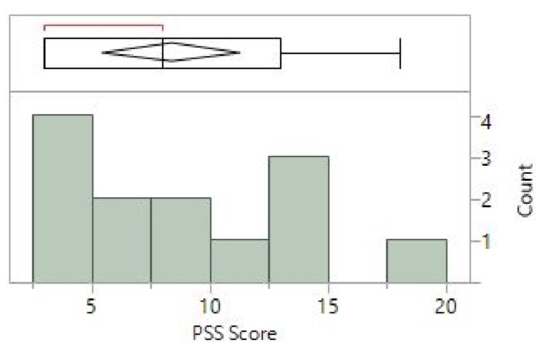

Figure 23. Distribution of PSS scores in the sample.

We evaluated the PSQI, ESS, SRRS, MEQ-SA, and PSS for a relationship with age. The PSS was the only one of the five tests that was significantly correlated with age. Scores on the PSS decreased with age (r = -0.72, p = 0.005). (See Figure 24).

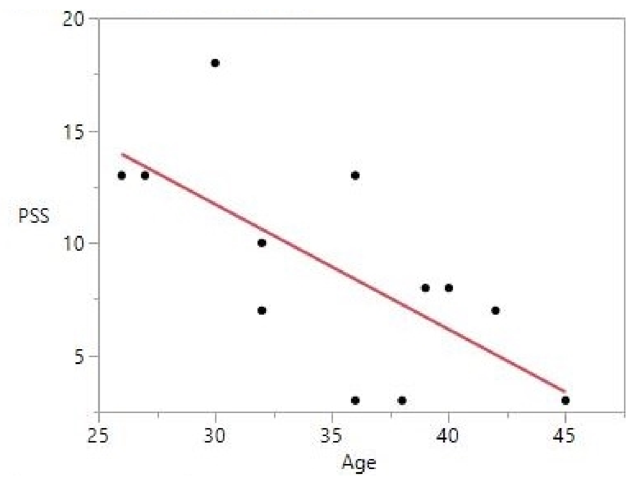

Figure 24. Relationship of PSS scores and age (r = -0.72, p = 0.005).

B. SLEEP ANALYSIS

Fifteen participants wore WAMs during the day-flight week, and fourteen participants wore WAM for all or a portion of the night-flying week. Participants slept on average 7.15 hours during the day week (SD = 52.2 minutes) and 7.01 hours during the night week (SD = 85.7 minutes). The difference in the expected number of hours slept between the two weeks was not statistically different ($t(14)$ = -0.29, p = 0.77). The notation $t(n)$ is used for a t-statistic, in this case the statistics for a paired t-test whose null distribution is t with n degrees of freedom. The average sleep efficiency was 84% across the two weeks and was positively correlated with the average minutes spent asleep (r = 0.58, p = 0.021).

C. NASA-TLX WORKLOAD ANALYSIS

The iPad application automatically calculated the NASA-TLX scores. Twelve participants completed the NASA-TLX rating post flight in the day-flight condition with an average workload score of 65.86 (SD = 14.74). Eight participants completed the NASA-

TLX post-flight in the night condition with an average workload score of 66.33 (SD = 16.83). Only six participants completed the NASA-TLX post-flight in both the day and night flying conditions. There were no significant changes in workload ratings across the two conditions ($t(5) = -0.57$, $p = 0.59$).

D. PHYSIOLOGICAL TESTING RESULTS

This section discusses the validity of the calculated cortisol concentrations, comparisons to both an established database and a participant developed cortisol curve, and analyses of the cortisol awakening response (CAR). This section ends with a brief discussion of salivary alpha amylase results.

1. Cortisol

A total of 17 pilots took part in the saliva collection with varying degrees of adherence to protocols. Only four participants completed the full sample collection (controls, day-, and night-flying) in the data collection period. We analyzed cortisol in the NPS Human Systems Integration wet-lab on nine 96-well microplates numbered 1 to 10. Plate 1 was used to pilot-test the assay protocols. Inter-assay variation was mediated by analyzing participants on the same microplate. All samples were run in duplicates when the volume allowed. Intra-assay coefficient of variation (CV) remained within an acceptable limit for all 8 plates tested. An additional plate, Plate 10, was run to test any anomalies found in the data analysis. Table 4 lists intra-assay average CV and R^2 for each plate. Each plate was within an acceptable CV and R^2 ranges.

Table 4. CV and R^2 Values for each experimental sCort microplate.

Microplate Number	Participants	CV	R2
2	10, 11, 17	4.75	0.999
3	12, 13	4.00	0.999
4	29, 31	7.16	0.998
5	20, 23, 27	8.73	0.999
6	19, 27	5.46	0.999
7	30, 15, 16	9.06	0.999
8	22, 26	8.95	0.999
9	14	5.22	0.999
10	Individual Samples	6.24	0.999

a. General Population Cortisol Comparison

We fit a non-linear curve to cortisol levels as a function of hours since awakening using the CIRCORT database 50% percentile readings for males, age 31–40, which were most representative for the sample tested (Miller et al., 2016). Figure 25 shows the fitted CIRCORT curve.

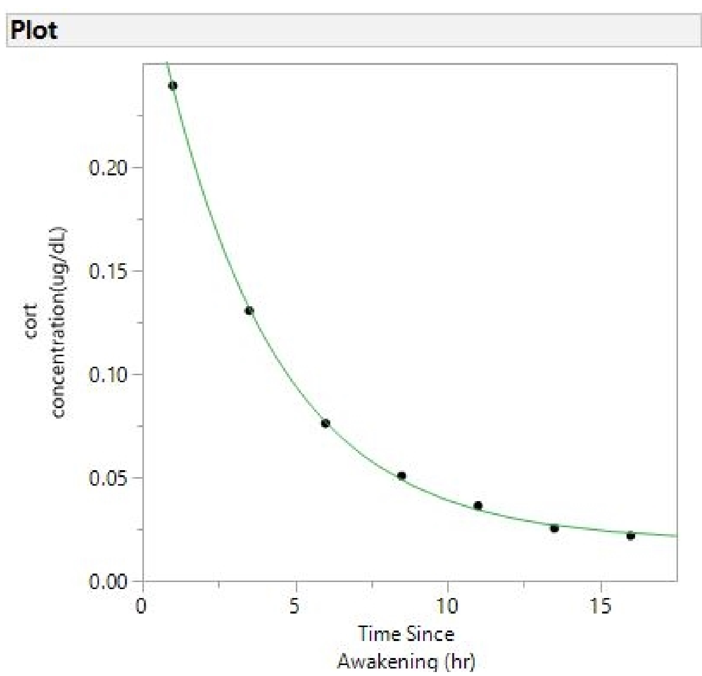

Figure 25. Cortisol curve fit using the CIRCORT database ($R^2 = 0.999$).
Source: Adapted from Miller et al. (2016).

The awakening sample established hours since awakening by each participant. In the absence of a recorded awakening time for the day, the average awakening time for the week established by WAM analysis was used. The following sections compare the participant sample sCort concentrations to those predicted by the CIRCORT fitted curve in each condition.

(1) Control Days by CIRCORT Curve

We compared participant control (non-flying) cortisol levels to the fitted CIRCORT curve. Pilots in the sample had higher than expected cortisol levels than predicted from the CIRCORT database curve.

Cortisol levels tend to increase with age (Larsson, Gullberg, Rastam, & Lindblad, 2009). As a sensitivity analysis to verify that this pattern held, we used a more conservative sample excluding participants over the age of 40. Figure 26 shows the residuals, where the residuals are the difference between the actual and predicted cortisol levels, of this comparison. Figure 27 shows the plots for actual and predicted sCort levels for the control day. To see if observed cortisol levels differ from those predicted using the CIRCORT fitted curve, we used a Wilcoxon sign test, where the test statistics, x, is the number of participants with more positive than negative residuals. For both the total sample of 13 ($x = 13$, $p < .001$) and the age restricted sample of 9 ($x = 9$, $p = .002$), the observed median cortisol levels are significantly greater than predicted.

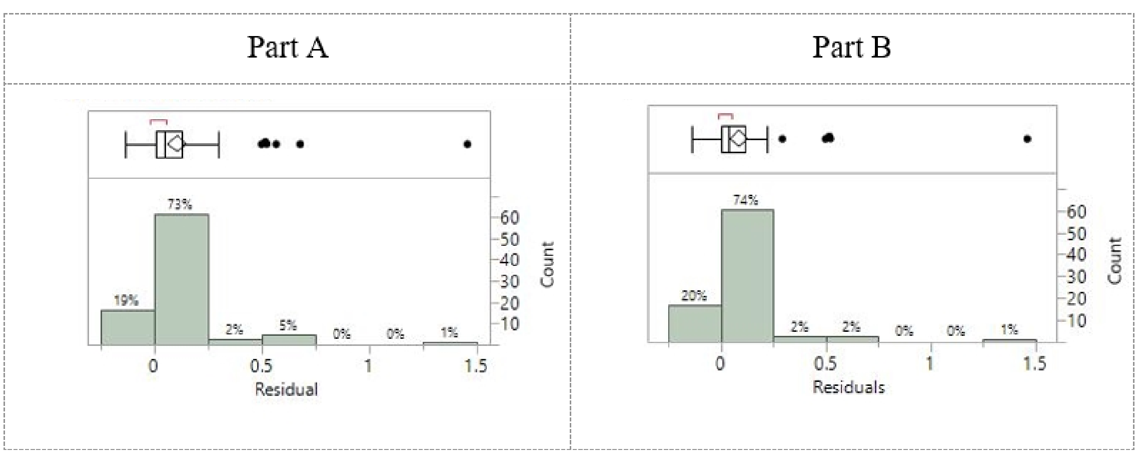

Figure 26. Residuals of control day by CIRCORT predicted levels in the total sample (Part A) and the age restricted sample (Part B).

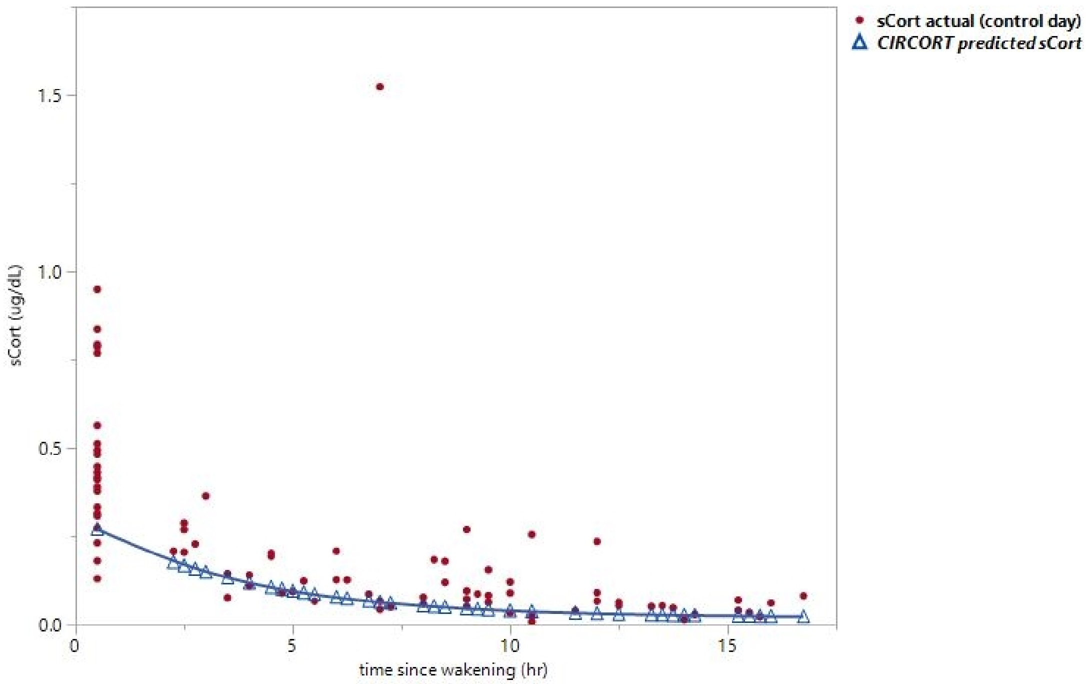

Figure 27. Plot of actual and CIRCORT predicted sCort levels (μg/dL) during control days by hours since awakening.

(2) Day Pre-flight

Pre-flight actual sCort levels were significantly different (*t(13)* = -3.05, *p* = 0.009) than CIRCORT predicted pre-flight sCort levels. Figure 28 shows the residual distribution and Figure 29 plots the actual day pre-flight levels and the predicted CIRCORT curve.

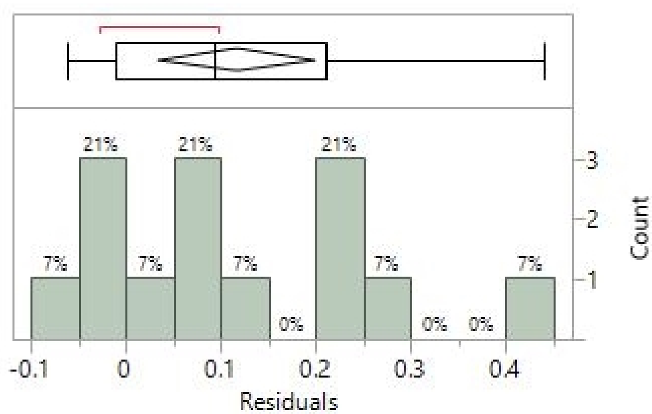

Figure 28. Residuals of day pre-flight sCort levels by CIRCORT predicted levels.

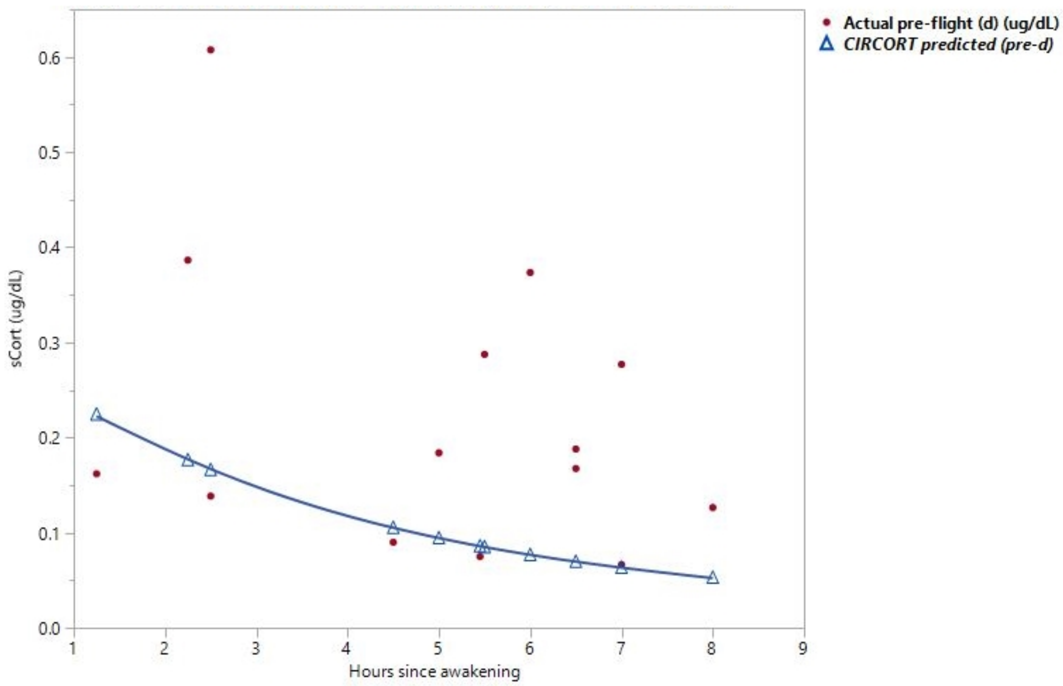

Figure 29. Plot of actual and CIRCORT predicted sCort levels (μg/dL) during day pre-flight days by hours since awakening.

(3) Day Post-flight

The day post-flight actual sCort levels differed significantly (*t(12)* = -3.63, *p* = 0.003) from the CIRCORT predicted day post-flight levels. Figure 30 shows the distribution of residuals. Figure 31 shows the plotted post-flight day actual sCort levels by CIRCORT database predicted levels.

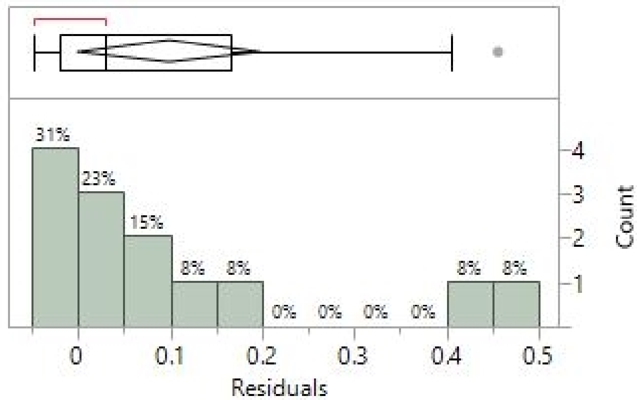

Figure 30. Residuals of day post-flight sCort levels by CIRCORT predicted levels.

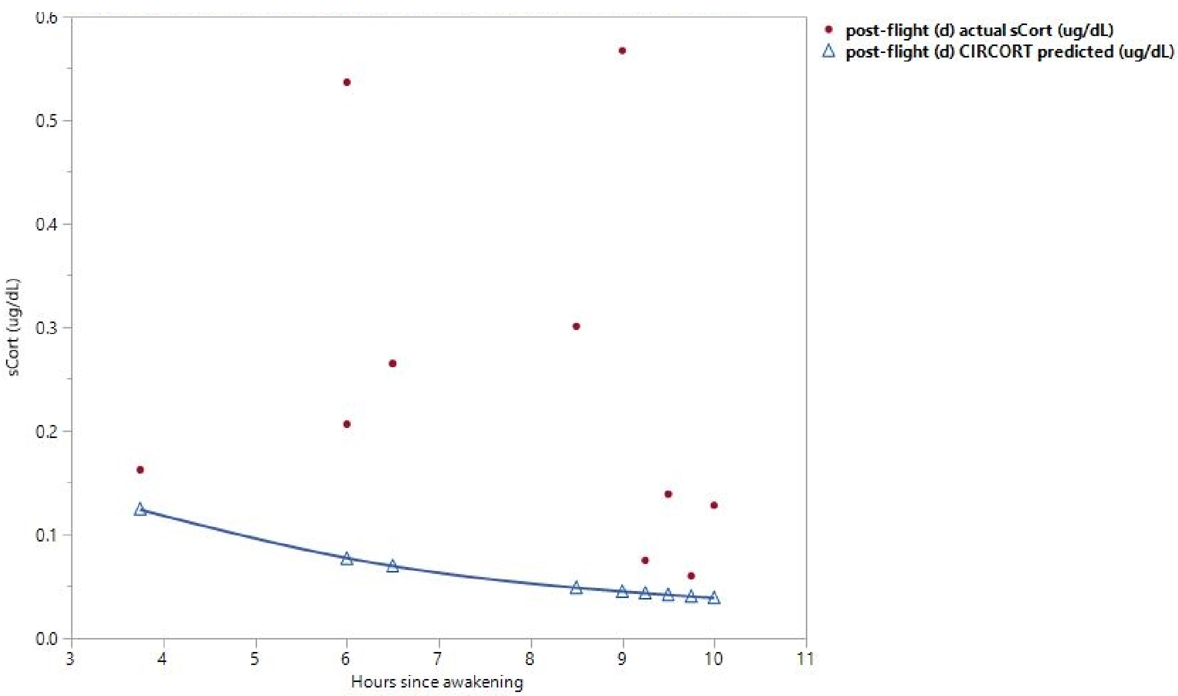

Figure 31. Plot of actual and CIRCORT predicted sCort levels (μg/dL) during day post-flight by hours since awakening.

(4) Night Pre-flight

We compared night pre-flight actual cortisol levels to expected CIRCORT database pre-flight sCort levels. Actual sCort levels were significantly higher than predicted CIRCORT levels (*t(10)* = -2.64, *p* = 0.025). Figure 32 shows the residuals from pre-flight in the night-flying condition. Figure 33 shows the plot of actual cortisol levels as compared to predicted CIRCORT cortisol levels.

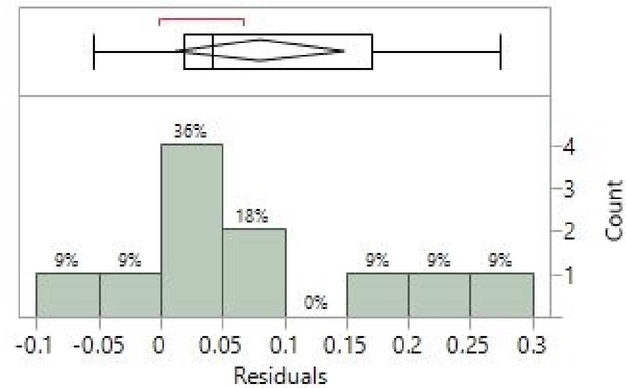

Figure 32. Residuals of night pre-flight sCort levels and CIRCORT predicted levels.

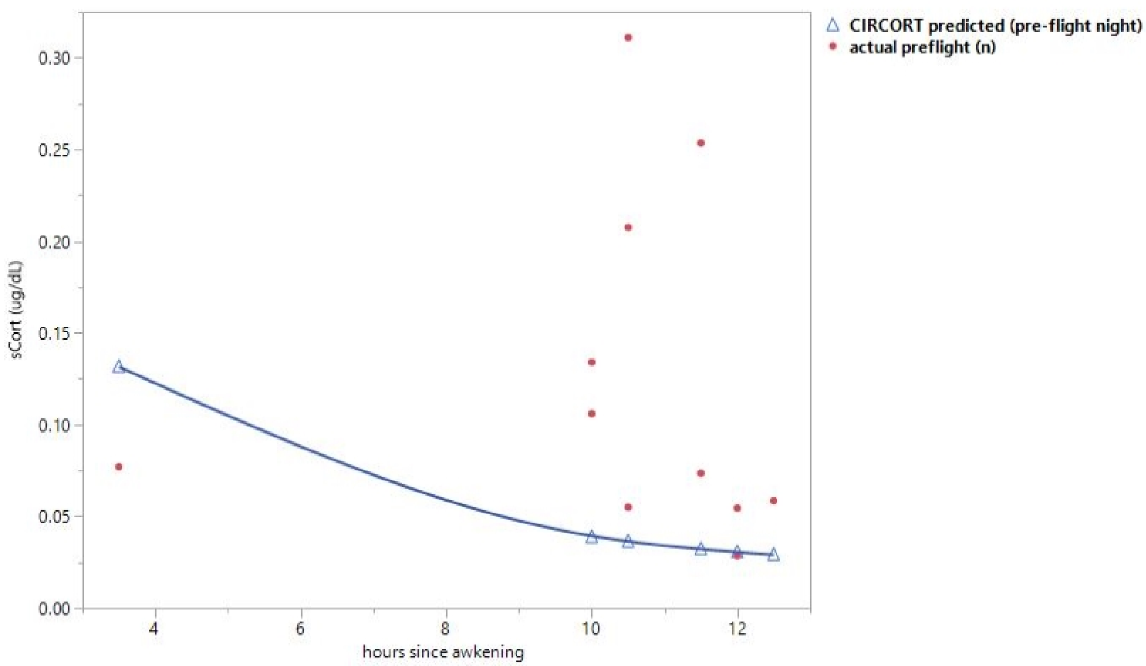

Figure 33. Plot of actual and CIRCORT predicted sCort levels (μg/dL) during night pre-flight by hours since awakening.

(5) Night Post-flight

Night post-flight actual sCort levels were also significantly higher than CIRCORT predicted sCort levels ($t(10) = -3.78$, $p = 0.003$). Figure 34 shows the distribution of the residuals. Figure 35 shows the fit line of the CIRCORT database predicted sCort levels by the actual sCort levels of the sample.

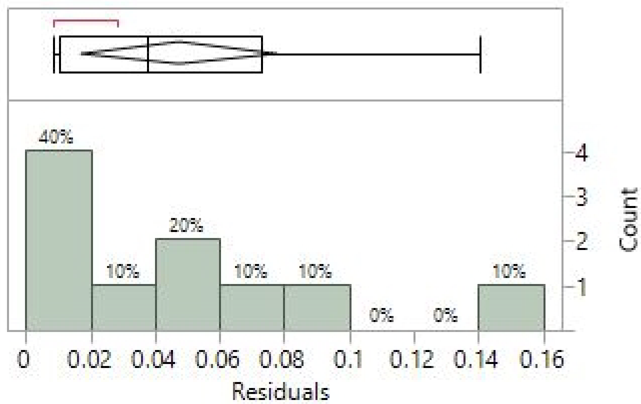

Figure 34. Residuals of night post-flight sCort levels by CIRCORT predicted levels

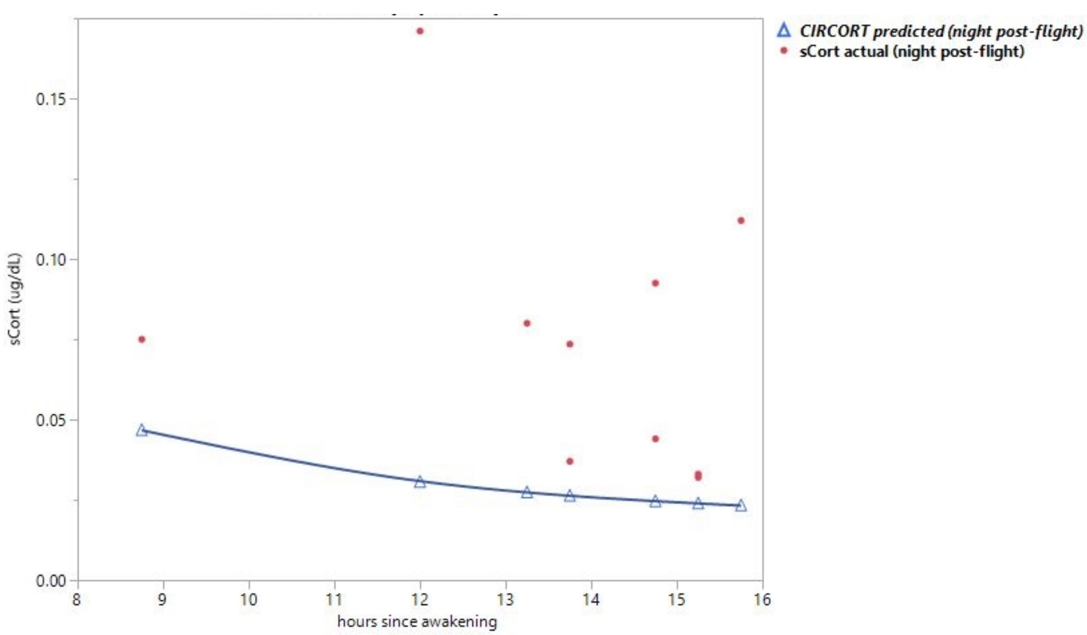

Figure 35. Plot of actual and CIRCORT predicted sCort levels (μg/dL) during night post-flight by hours since awakening.

b. Fitted Pilot Sample Curve

We captured the sample by fitting sCort levels for the control (non-flying) samples as a function of hours since awakening. A total of 83 data points were used. Figure 36 shows the curvilinear relationship.

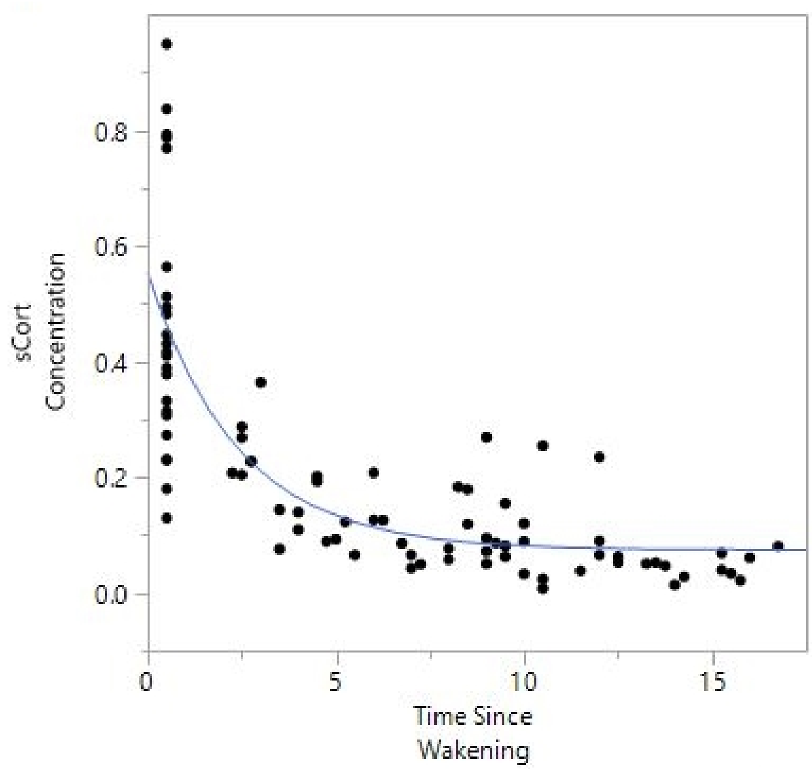

Figure 36. Fitted diurnal curve for sample by hours since wakening.

We calculated the day- and night-flying pre-flight and post-flight predicted sCort concentrations using the fitted curve and compared the results using a two-sided paired sample t-test.

(1) Pre-flight

Pre-flight actual and predicted sCort levels were not different in the day-flying ($t(13) = 0.96$, $p = 0.35$) or night-flying condition ($t(10) = 0.25$, $p = 0.8$).

(2) Post-flight

Post-flight actual sCort levels were significantly higher in the day-flying condition ($t(12) = -2.19$, $p = 0.049$) and significantly lower in the night-flying condition ($t(9) = -2.3$, $p = 0.05$). Figures 37 and 38 show the residuals. Figures 37 and 39 show the plotted actual and predicted sCort levels.

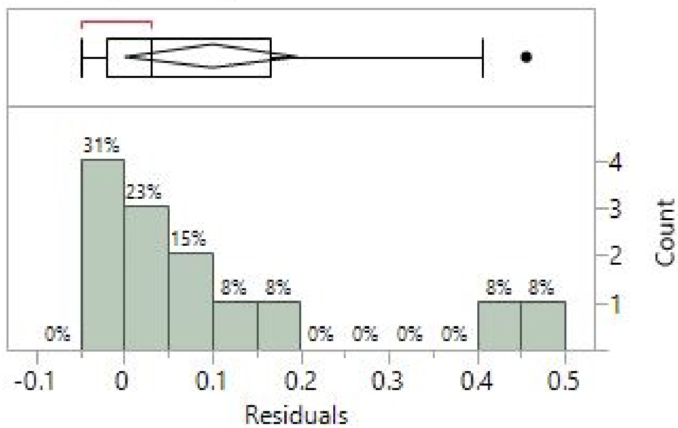

Figure 37. Residuals of day post-flight sCort levels by predicted in participant fitted curve

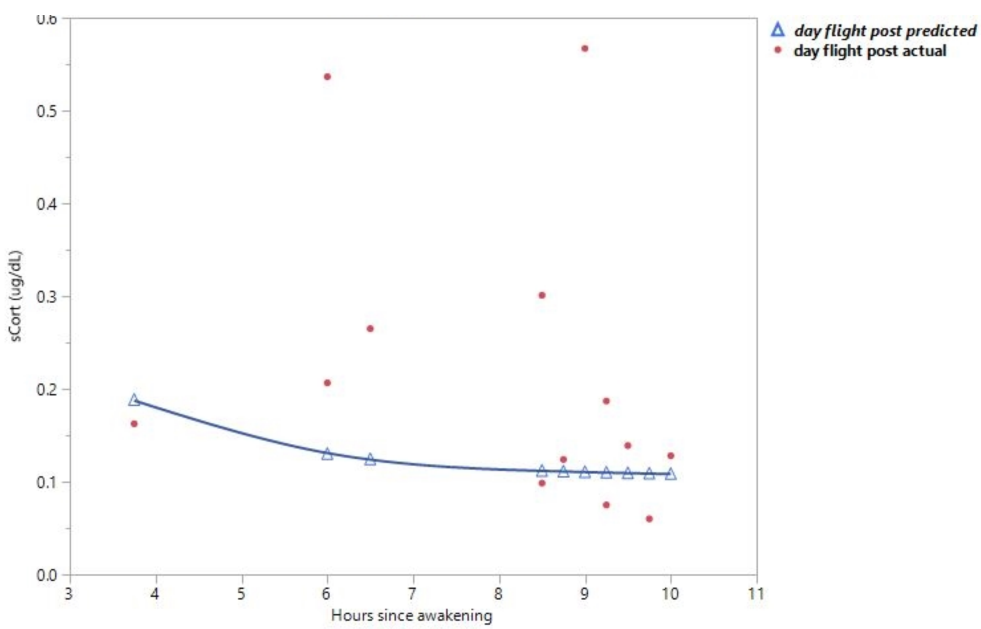

Figure 38. Plot of actual and participant-developed curve predicted sCort levels (μg/dL) during day post-flight by hours since awakening.

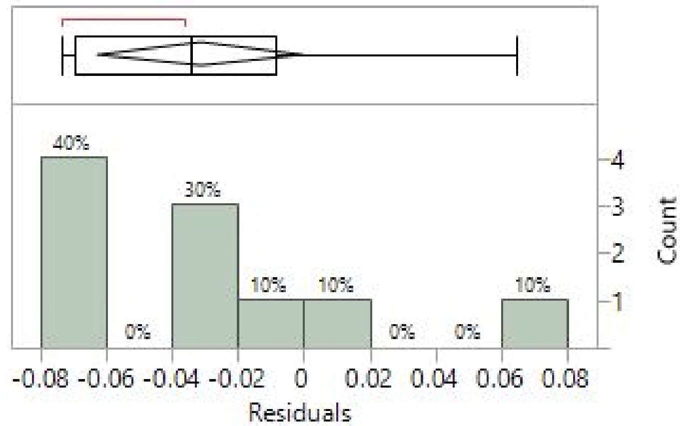

Figure 39. Residuals of night post-flight sCort levels by predicted in participant-developed curve.

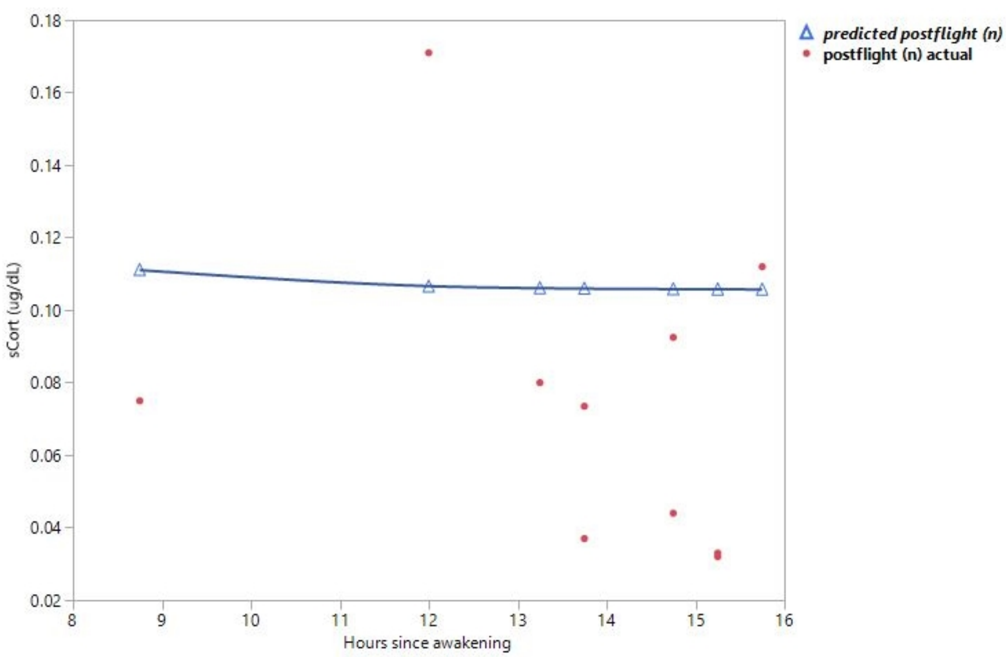

Figure 40. Plot of actual and participant-developed curve predicted sCort levels (μg/dL) during night post-flight by hours since awakening.

c. Cortisol Awakening Response (CAR) Magnitude

Varying degrees of compliance for the CAR samples are shown in Table 5.

Table 5. CAR compliance by participant and condition.

CAR Compliance				
Subject ID	Control 1	Control 2	Day	Night
10			X	
11	X	X	X	
12		X		X
13	X			
14	X	X	X	X
15	X	X		
16				
17	X	X		
19	X	X	X	
20	X	X		X
22	X	X	X	
23	X	X		X
26	X	X	X	X
27	X	X		
29	X	X	X	X
30	X	X	X	
31				

We calculated the CAR by taking the concentration of the awakening +30 minutes' sample and subtracting the awakening sCort concentration (in μg/dL). Table 6 shows the calculated CARs.

Table 6. CAR magnitude by participant and condition.

Subject ID	CAR (control)	CAR (day)	CAR (night)
10		0.7285	
11	2.358	0.4835	
12	0.265		0.274
13	0.064		
14	0.555*	0.3545	-0.3835
15	0.489*		
16			
17	0.409		
19	0.769	0.6155	
20	0.141		-0.087
22	0.113	0.0435	
23	0.69*		0.287
26	0.389	0.085	0.0675
27	0.102*		
29	0.092	0.511	0.126
30	0.3015	0.748	
31			

* Indicates an average was taken.

There was no difference in the control to day flight CAR responses ($t(6) = -0.85$, $p = 0.43$), the control to night CAR responses ($t(5) = -2.12$, $p = 0.087$) nor the day to night CAR responses ($t(2) = -1.83$, $p = 0.209$).

d. Pre- to Post-flight Cortisol Change

The change in cortisol levels, pre-flight to post-flight, was not significant in the day-flying condition ($t(11) = -1.33$, $p = 0.21$) nor the night-flying condition ($t(10) = -1.19$, $p = 0.26$).

We evaluated flying hours, PSQI, PSS, ESS, MEQ-SA, SRRS and NASA-TLX for a relationship with the cortisol response change from pre- to post-flight. A correlation exists only between the magnitude of the cortisol change in the day flying condition and F-22 hours, $r = -0.89$, $p = 0.001$, such that individuals with fewer hours in the F-22 experienced a larger increase in cortisol from pre-flight to post-flight. Figure 41 shows this relationship. This relationship was only found with F-22 hours in one flight condition.

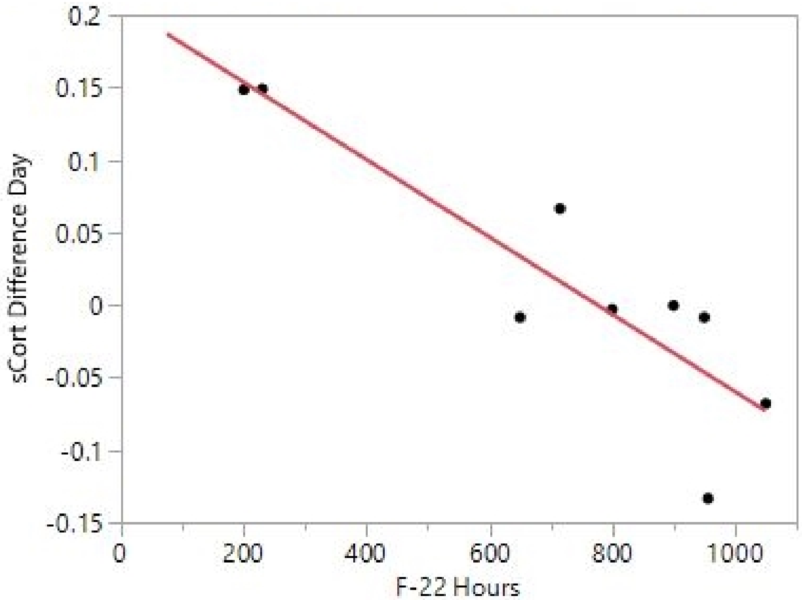

Figure 41. F-22 hours by difference in sCort levels pre- and post-flight in the day-flying condition (*r* = -0.89, *p* = 0.001).

2. Alpha Amylase

Salivary alpha amylase analysis was limited due to placing priority on the cortisol analysis, which thereby limited the amount of saliva available. Only comparison of pre- and post-flight concentrations in the day- and night-flying conditions could be analyzed. There were no significant differences between the pre- and post-flight conditions in the day-flying condition ($t(11) = -0.361$, $p = 0.73$), night-flying condition ($t(7) = -1.227$, $p = 0.24$) or the magnitude of the difference, measured pre- to post-flight, in the day- or night-flying operations ($t(5) = 1.195$, $p = 0.29$).

E. COGNITIVE TESTING RESULTS

This section focuses on results and analysis of the Go/No-Go outputs in the different conditions. Each section discusses analyses with supporting graphics provided for significant findings. The section ends with discussion of several significant correlations that existed with various components of the GNG tests.

Fourteen individuals completed the GNG with varying levels of compliance (Table 7). We compared the conditions using a two-sided paired sample t-test.

Table 7. Compliance for GNG test across both day and night conditions.

Go/No-Go Compliance				
Subject	Pre-Flight (Day)	Post-Flight (Day)	Pre-Flight (Night)	Post-Flight (Night)
10				
11	X	X		
12	X	X	X	X
14	X	X	X	X
15		X		X
16		X		X
17		X		
19	X	X		
20			X	X
22		X	X	
23	X	X	X	X
26		X	X	X
27			X	X
29	X	X	X	X
30	X	X		
31				

We calculated the average no-go accuracy, total test accuracy, and reaction time (RT) in seconds (s) in both the day and night conditions (see Table 8).

Table 8. Averages for accuracy and reaction time across two flying conditions.

Time	Avg Accuracy (no-go)	Avg Total Test accuracy	Avg RT (s) (go)	Avg RT (s) (incorrect responses)
Pre-Flight (d) (*n* = 8)	0.9166 (SD = 0.088)	0.9825 (SD = 0.017)	0.3135 (SD = 0.025)	0.253 (SD = 0.031)
Post-Flight (d) (*n* = 12)	0.8957 (SD = 0.072)	0.9768 (SD = 0.014)	0.3149 (SD = 0.03)	0.2631 (SD = 0.031)
Pre-Flight (n) (*n* = 7)	0.8134 (SD = 0.13)	0.9602 (SD = 0.029)	0.3049 (SD = 0.04)	0.2566 (SD = 0.028)
Post-Flight (n) (*n* = 9)	0.7931 (SD = 0.175)	0.9555 (SD = 0.034)	0.2994 (SD = 0.033)	0.2595 (SD = 0.025)

Eight participants completed both a pre- and post- flight GNG test in the day-flying condition. There were no significant changes in no-go accuracy ($t(7)$ = -0.57, p = 0.58), total test accuracy, ($t(7)$ = -1.13, p = 0.29), reaction time of the correct response in seconds (s) ($t(7)$ = 0.38, p = 0.71), or reaction time of an incorrect response ($t(7)$ = -0.19, p = 0.85).

Seven participants participated in both the pre- and post-flight night GNG test. There were no differences in the accuracy of the inhibitory response ($t(6)$ = -0.63, p = 0.54), total test accuracy ($t(6)$ = -0.82, p = 0.44), reaction time of the correct response ($t(6)$ = -0.11, p = 0.91), or reaction time of an incorrect response ($t(6)$ = 0.459, p = 0.66).

Seven participants completed both post-flight GNG tests in day- and night-flying conditions. There were no significant changes in the post-flight inhibitory accuracy ($t(6)$ = -1.37, p = 0.21), total test accuracy ($t(6)$ = -1.47, p = 0.19), reaction time of a correct response ($t(6)$ = -0.74, p = 0.48), nor reaction time of an incorrect response ($t(6)$ = 0.865, p = 0.42) in either the day or night post-flying condition.

Four participants completed the pre-flight GNG tests in both the day- and night-flying conditions. The pre-flight inhibitory accuracy was suggestive of a change ($t(3)$ = -2.49, p = 0.09) but did not meet the threshold for significance. Neither total test pre-flight accuracy ($t(3)$ = -1.93, p = 0.14), pre-flight reaction time of a correct response ($t(3)$ = -.318, p = 0.77), nor pre-flight reaction time of an incorrect response ($t(3)$ = 0.34, p = 0.76) varied across the conditions.

We found several significant correlations with various components of the GNG test. These correlations are discussed below.

1. Sleep and Go/No-Go (GNG)

We evaluated average minutes asleep and percentages of sleep efficiency for a relationship with the GNG results. As sleep efficiency increases, so does GNG accuracy in the no-go response (r = 0.97, p < 0.0001) and total test accuracy (r = 0.93, p = 0.029) in the day-flying condition. Figures 42 and 43 show these relationships.

As sleep increased so did the GNG RT in the day-flying condition (r = 0.63, p = 0.044). In the night post-flight condition, GNG accuracy of the no-go response increased with sleep (r = 0.87, p = 0.036). Figures 44 and 45 show these relationships. No other correlations exist between the GNG and sleep.

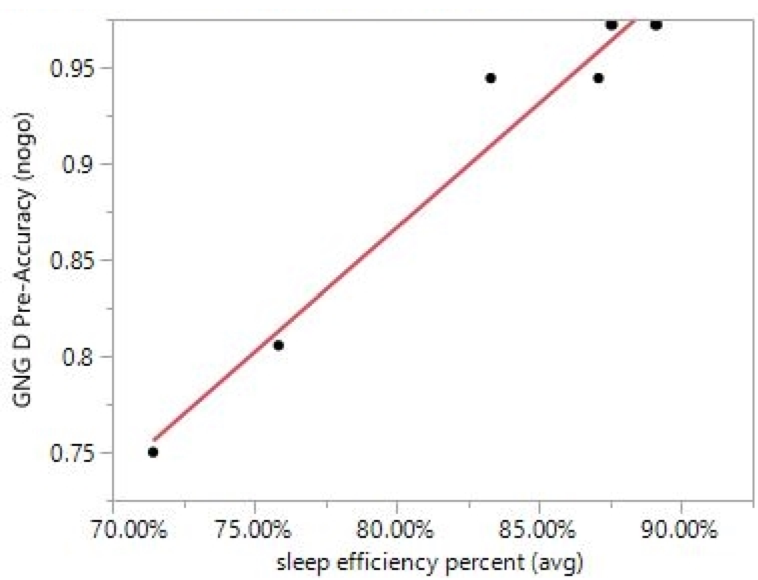

Figure 42. Relationship of GNG day pre-flight accuracy of "no-go" response and avg sleep efficiency (r = 0.97, p < 0.001).

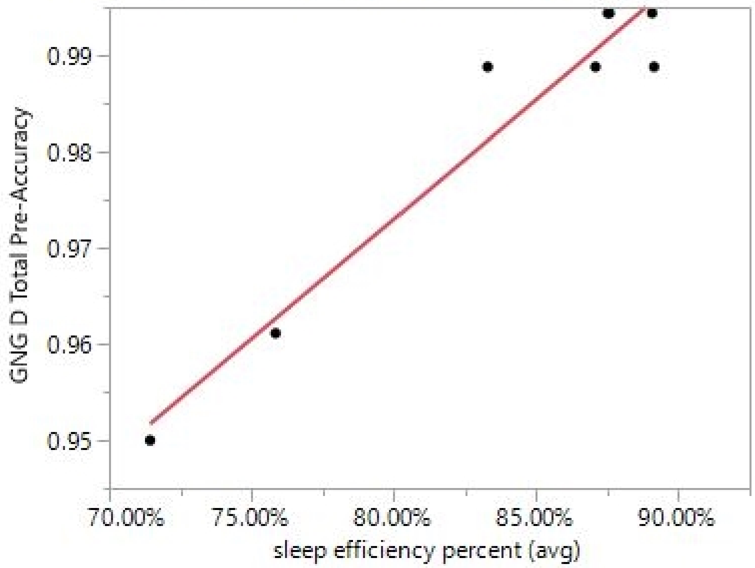

Figure 43. Relationship of GNG day pre-flight total accuracy and avg sleep efficiency ($r = 0.93$, $p = 0.029$).

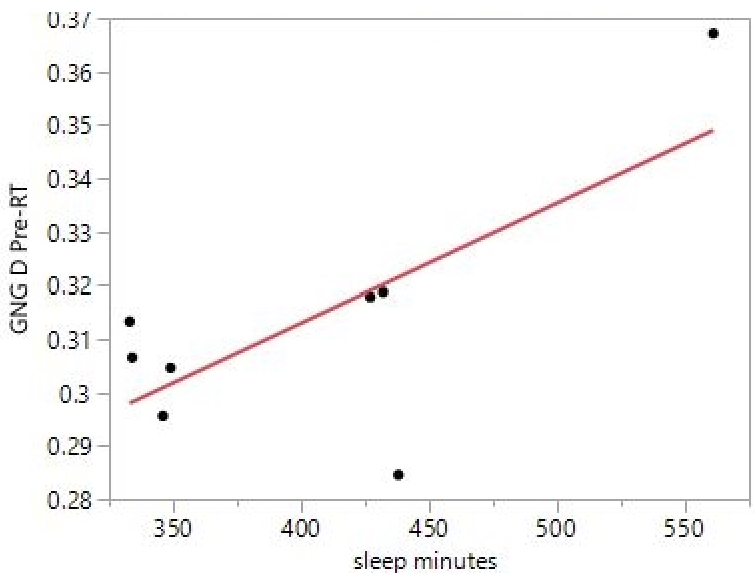

Figure 44. Relationship of avg minutes asleep and GNG RT (s) in day pre-flight condition ($r = 0.63$, $p = 0.044$).

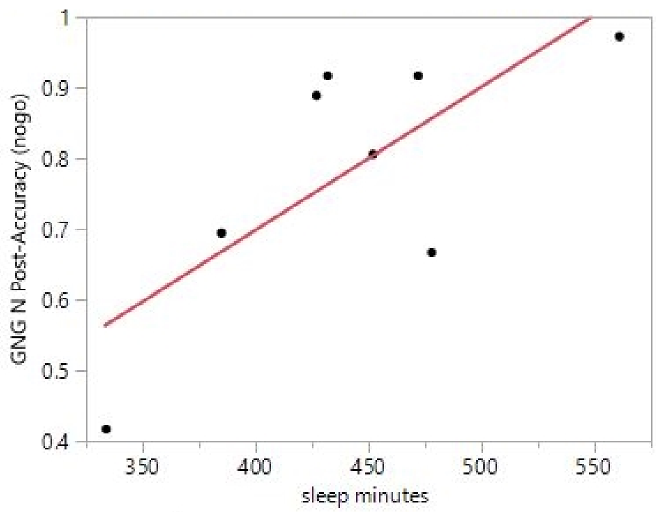

Figure 45. Relationship of GNG night post-flight accuracy of no-go response by avg minutes asleep ($r = 0.87$, $p = 0.036$).

2. NASA-TLX and Go/No-Go

We evaluated physiological changes, sleep, and the GNG test results for a relationship with the NASA-TLX. GNG post-flight accuracy of the no-go response ($r = 0.63$, $p = 0.025$) and of total test accuracy ($r = 0.64$, $p = 0.026$) increased with NASA-TLX scores in the day-flying condition. Figures 46 and 47 show these relationships. No other relationships exist with the NASA-TLX.

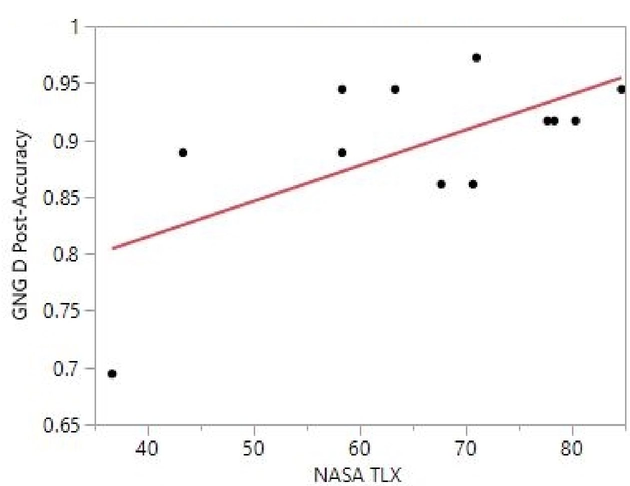

Figure 46. Relationship of GNG post-flight accuracy in the day flying condition by post-flight day NASA TLX scores ($r = 0.63$, $p = 0.025$).

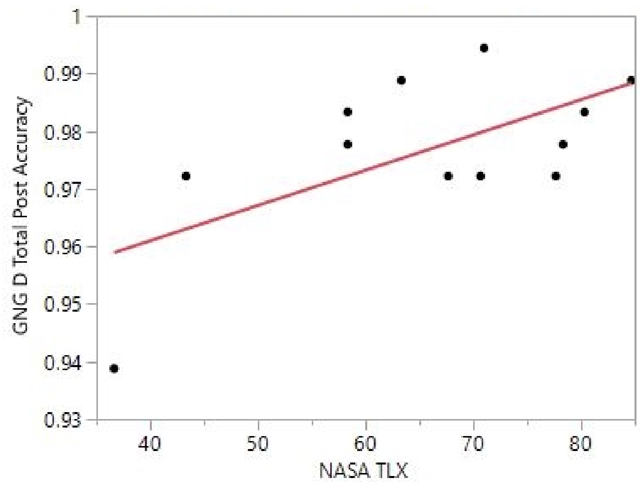

Figure 47. Relationship of GNG post-flight total accuracy in the day flying condition by post-flight day NASA TLX scores ($r = 0.64$, $p = 0.026$).

3. Perceived Stress Survey and Go/No-Go

We evaluated the PSQI, ESS, SRRS, MEQ-SA and PSS for a relationship with the GNG. The PSS was the only one of the five tests that was significantly correlated with the GNG. GNG RT in seconds decreased with increasing PSS scores in the night post-flight condition ($r = -0.61$, $p = 0.043$). Figure 48 shows this relationship.

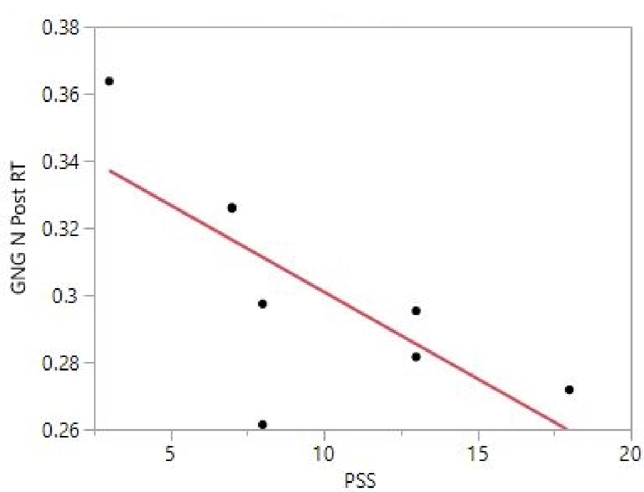

Figure 48. Relationship of night post-flight GNG RT and PSS scores ($r = -0.61$, $p = 0.043$).

4. Cortisol Awakening Response (CAR) and Go/No-Go

We evaluated the magnitude of the CAR for a relationship with GNG test results. In the night post-flight condition, accuracy of the no-go response ($r = 0.88$, $p = 0.011$) and total accuracy ($r = 0.92$, $p = 0.005$) increased with the CAR magnitude. Figures 49 and 50 show these relationships. No other relationship exists with the CAR and GNG test results in either condition.

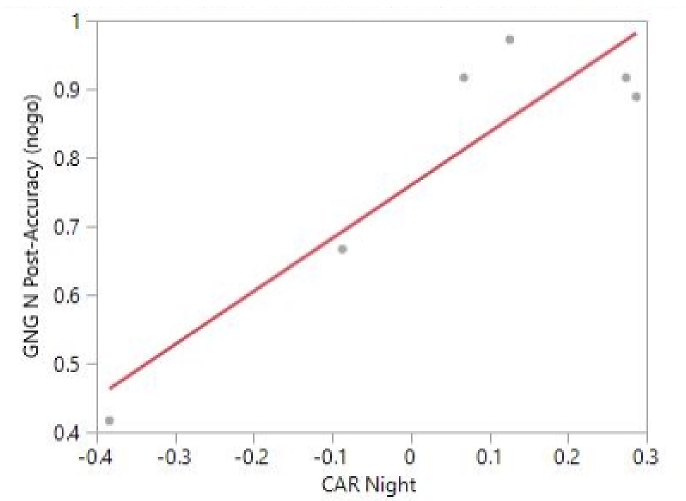

Figure 49. Relationship of CAR magnitude and GNG post-flight accuracy of no-go in the night-flying condition ($r = 0.88$, $p = 0.011$).

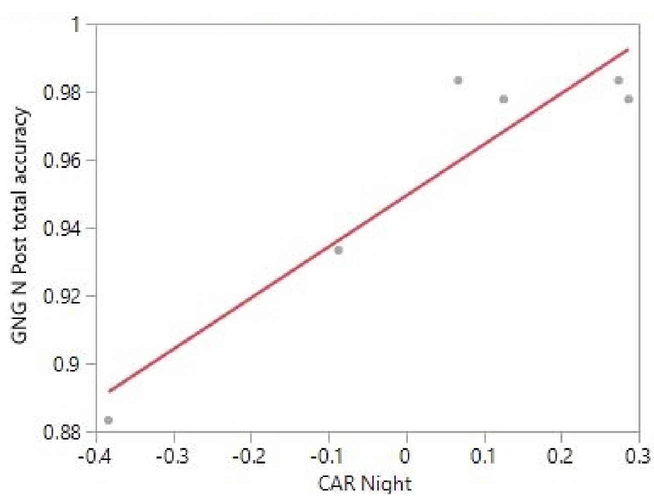

Figure 50. Relationship of CAR magnitude and GNG post-flight total accuracy in the night-flying condition ($r = 0.92$, $p = 0.005$).

5. Awakening Cortisol Levels and GNG

We evaluated the awakening cortisol levels for a relationship with GNG responses. A relationship only existed in the night pre-flight reaction time. GNG RT (s) was positively correlated with awakening cortisol levels (r = 0.84, p = 0.038). Figure 51 shows this relationship.

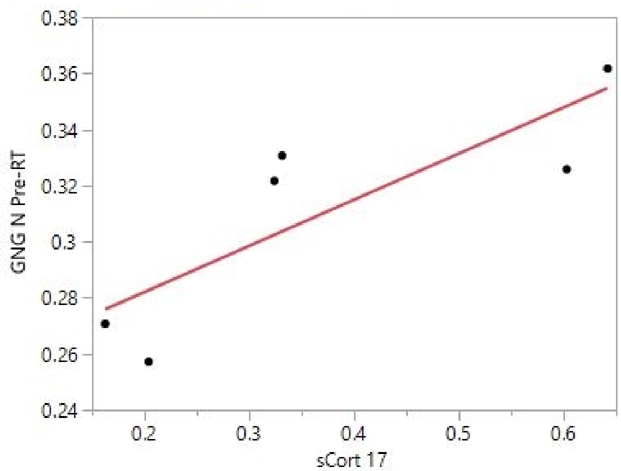

Figure 51. Relationship of awakening cortisol levels and GNG RT (s) pre-flight in day-flying condition (r = 0.84, p = 0.038).

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V. DISCUSSION

This thesis studies the overall stress burden to F-22 aviators as they transition from day- to night-flying. This chapter begins by addressing the hypotheses posited in Chapter II with relevant findings, and ends by identifying other findings that resulted as a result of the research. We rejected both null hypotheses posed in Chapter II in favor of the alternatives based on the findings that are discussed in this chapter.

A. HYPOTHESIS 1: EVIDENCE OF CHANGES

We found evidence to support the alternative hypothesis that a difference in stress system activation exists between the day- and night-flying conditions. This finding stands in contrast to the null hypothesis posed in Chapter II that no changes exist in the physiological or performance measures as the pilots transition from day- to night-flying.

Day-flying appeared to cause a stress response based on the lack of change of pre- to post-flight cortisol levels and the higher than predicted cortisol levels based off the participant developed curve. Pre- and post-flight levels of cortisol were not different in the day-flying condition ($t(11) = -1.33$, $p = 0.21$). This finding, in itself, is evidence of a stress response. Normal cortisol levels decrease throughout the waking day. In the absence of a stress response, participant cortisol levels would have dropped post-flight, following their normal diurnal rhythm. However, participant cortisol levels did not change during the flight interval and support the theory that a stress response occurred.

The participants had significantly elevated cortisol levels post-flight in the day-flying condition ($t(12) = -2.19$, $p = 0.049$) compared to predicted levels from the developed curve providing evidence of a stress response. In keeping with expected behavior, neither day ($t(13) = 0.96$, $p = 0.35$) nor night-flying ($t(10) = 0.25$, $p = 0.8$) showed elevated cortisol levels pre-flight and failed to support an anticipatory effect in the stress response. Participants' day-flight cortisol levels followed a predictable rhythm on a developed curve up until post-flight, at which point they became elevated. Similar analyses failed to support a stress system activation in the night-flying condition as participants had significantly lower than predicted cortisol levels post-flight ($t(9) = -2.3$, $p = 0.05$). These results suggest an increased stress system activation caused by the daytime flying environment but not the night-flying environment.

This change cannot be definitively attributed to any one factor or a combination of factors, but the results were surprising. Several factors could have influenced these results. First, the sample shared their runway with an international airport that was commonly congested with commercial air traffic during the day. Additionally, because Hawaii is a tourist destination, sightseeing aircraft also used the airspace. It is possible that the increase in air traffic may have contributed to a stress response. Second, day-flying requires a visual scan to "see and avoid." Radar assists in the identification of threats, but the visual system is the primary sensory input device in the daytime flight environment. Pressure on the pilot to visually identify objects in an already congested airspace may have contributed to activation of the stress response.

Visual requirements differ at night and could also be responsible for the lack of a response in the night post-flight condition. Less commercial air traffic at night may require less attention devoted to aircraft deconfliction. Additionally, at night visual scans are limited to instrumentation cues. While some maneuvers need to be confirmed under night vision goggles, the majority of the visual information about the environment is digitally processed. Pilots are both assisted by and restricted to their instrument feedback. These factors may have contributed to the lower than expected cortisol levels in the night-flying condition.

The physical requirements of day-flying are demanding and also may have played a role the cortisol spike. The F-22 is a 9Gz capable aircraft. Several pilots reported experiencing high or repeated G-forces during their day-flight sorties. G-forces are physically demanding and a sustained muscle contraction, termed the anti-g straining maneuver, to maintain consciousness. Because night-flying is G-limited, it does not require the same physical effort. The additional physical effort of day-flying may have caused the higher than predicted cortisol levels post-flight.

There are a number of gaps in our understanding of just why cortisol is elevated post-flight in day-flight and further investigation is warranted.

B. HYPOTHESIS 2: EVIDENCE OF STRESS ADAPTATION

We found evidence to support the alternative hypothesis that evidence of stress adaptation exists in the F-22 community. This finding stands in contrast to the null hypothesis posed in Chapter II that no relationship between experience and stress system activation exists in the F-22 community.

Two significant correlations suggest some form of stress adaptation within the F-22 sample: age as it relates to Perceived Stress Survey (PSS) scores and the magnitude of the cortisol change as it relates to F-22 hours. The PSS is a validated measure of personal, perceived stress (Cohen, Kamarck & Mermelstein, 1983). In the pre-survey questionnaire, participants reported their perceived level of stress. Participants age was negatively correlated with their level of perceived stress, that is younger participants reported higher levels of stress ($r = -0.72$, $p = 0.005$). This finding is interesting for two reasons. First, it confirms the high stress levels that was communicated by participants to the research team during the data collection. Participants, in casual conversation, would discuss life stressors. Several participants had young children and were struggling to find appropriate schooling on the island, childcare issues, or had a new infant at home. These participants all had relatively higher (within this sample) PSS scores. Three participants held highly visible positions of responsibility on the base. These three individuals were on the extreme right end of the age distribution of the participants and, though arguably being taxed with more responsibility, had lower PSS scores. Second, the PSS generally has shown a trend to decrease with age (Cohen & Janicki-Deverts, 2012). The participant distribution of PSS scores is lower than the distribution averages of both men ($n = 968$, $\overline{X} = 15.52$, SD = 7.44) and of 35–44 year olds ($n = 331$, $\overline{X} = 16.38$, SD = 7.07).

We also found a negative correlation between the magnitude of the cortisol change and the number of flight hours in the F-22, further suggesting an adaptation effect. As participants flew more hours in the F-22, the magnitude of their stress response was less ($r = -0.89$, $p = 0.001$). Some experienced participants were non-reactors and maintained a steady cortisol decline even during flight. This suggests that with increased exposure to the stressor, in this case flying, the less stressed the individual would become.

These two points suggest adaptation, not burnout, within the F-22 sample. This physiological adaptation falls in line with other research into repeated stress exposures. One possible reason for the adaptation would be the sample's characteristics, or the pilot selection process that brings in individuals with high resilience. F-22 pilots are an elite group who are categorized as high-functioning, Type A personalities who are often exposed to high-threat environments and show evidence of physiological adaptation to recurring stress like similar highly resilient populations (Lu, Wang, & You, 2016).

C. OTHER FINDINGS

We found several significant relationships that did not support either hypothesis but warrant discussion. These findings are described below.

1. Demographics and Age

We found a significant linear relationship between age and total flying hours, F-22 hours, and night hours. The older the pilot, the more total flying hours, F-22 hours and night flying hours. This result is intuitively easy to follow, and while significant, is to be expected.

2. Cortisol Levels

There was not enough evidence to show a relationship between participant cortisol awakening levels and the magnitude of the cortisol response in either the day- or night-flying condition. This finding was surprising as awakening levels were found to be predictive of stress in research by Elder, Elliss, Barclay, and Wetherell (2016). The small participant sample size may have been a factor in this finding.

An interesting relationship in physiological stress and perceived stress was noted in the sample. Physiological stress indicators were higher than civilian populations but perceived stress scores were lower than civilian populations. We attempted to compare the F-22 pilot sample to an established CIRCORT database of similar-aged males. Unexpectedly, the participant sample had significantly higher cortisol levels across all conditions and days. PSS scores were lower than similar populations. Future investigations should attempt to understand the relationship between perceived stress and cortisol level in the flying environment.

3. Go/No-Go

Participant reaction time in the go/no-go test did not vary across the different flight conditions. This finding reflects the research by Harris and colleagues (2010) who found consistent reaction times in populations who transitioned to shift work.

Some correlations exist between sleep and the go/no-go results; however, these correlations were not consistent across conditions or tests. The small participant sample size may be responsible for lack of consistent correlations across the conditions.

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VI. SYNTHESIS, RECOMMENDATIONS AND CONCLUSION

This thesis explored how the short-term shift in operations affects F-22 pilots in an attempt to aid future mishap prevention efforts. Studies show that traditional shift workers, such as nurses and military personnel, suffer from an increase in fatigue, disruption of cortisol, and decreased performance. Until now, this shift has not been investigated in the F-22 community. Previous chapters have focused on the research, experimental methods, and results of the thesis research. This chapter synthesizes the current study and speaks to its relevancy. It concludes by providing recommendations for future research projects to quantify the stress burden to aviators.

A. SYNTHESIS

F-22 pilots are high-functioning, resilient individuals tasked with operating the USAF's 5th generation fighter in support of missions across the world. Pilots are required to transition from day operations to short-term night operations to ensure that their training remains current; however, the impact on aviator performance from this short-term shift remains unknown. We quantified the stress burden on 17 volunteer participants as they made a short-term transition to night flights to maintain their readiness status. Performance, workload, fatigue, and stress patterns were monitored in the participants over two weeks as the F-22 aviators transitioned from day- to night-flying operations.

Using a modified go/no-go test (GNG) to measure reaction time and response accuracy in the participants, we found no difference as participants transitioned from day- to night-flying operations. On average, participants had an inhibition test accuracy of about 90% in both pre- and post-flight in the day-flying condition. Reaction time of the correct response averaged approximately 0.31 seconds and reaction time of the incorrect response averaged approximately 0.26 seconds pre-flight and post-flight in the day-flying condition. In the night-flying condition, participants scored on average approximately 80% inhibition accuracy, 0.30 seconds reaction time of the correct response, and approximately 0.25 seconds of the incorrect response in both pre-and post-flight. Reaction times were similar to a civilian population using a 20% no-go test (Nieuwenhuis, Yeung, Wildenberk, &

Ridderinkhof, 2003). However, the F-22 pilots had a significantly higher average inhibition accuracy in both flying conditions (90% and 80%) than the 66% inhibition accuracy reported in the civilian population (Nieuwenhuis et al., 2003).

Using a NASA-TLX (an iPad application version) to measure workload, we found no difference when comparing the subjective workload ratings. Participants had average workload scores of approximately 66 post-flight in both the day- and night-flying condition. These results were similar to NASA-TLX scores of commercial airline pilots executing a landing with the loss of the autopilot (Zheng, Lu, Jie, & Fu, 2017) but significantly higher than F/A-18 pilots executing an instrument landing with multiple cautions and warnings in a simulator ($\overline{X}$ = 39.04, SD = 7.86) (Mansikka, Virtanen, & Harris, 2018).

Attempts to compare F-22 participants to civilian populations of similar gender and age revealed an unexpected uniqueness. F-22 pilots had significantly elevated cortisol levels compared to the CIRCORT database, a repository of civilian cortisol levels, in all measured conditions. We then developed a unique participant curve and compared the actual cortisol to the new predicted levels. Participants' cortisol levels were different from predictions in both day and night post-flying conditions. Based on their own unique curve, F-22 pilots had significantly higher-than-predicted cortisol levels in the post-flight day condition and significantly lower-than-predicted cortisol levels in the post-flight night condition.

Two relationships suggest there may be some form of stress adaptation within the F-22 sample: the first is age as it relates to Perceived Stress Survey (PSS) scores and, secondly, the magnitude of the cortisol change as it relates to F-22 hours. Additionally, PSS scores were lower than average PSS scores for similar age and demographic populations. These findings suggest that F-22 pilots may have a different psychological threshold for perceived stress.

Using validated measures of stress, performance, sleep and workload, we studied changes that could ultimately affect aircrew safety. Sleep, reaction time, accuracy, and perceived workload did not change across the conditions; however, the sample size restricts the generalizability of the finding due to the lower test power.

More questions than answers emerged from this study; continued research should focus on the impact of elevated cortisol and long term health risk it could pose in this participant sample. If, indeed, further research confirms that job requirements are placing pilots' health at risk, we need to delve deeper. Are F-22 pilots required to accept stress as a normal part of their duty? What are the long-term health implications of elevated cortisol levels? Are these changes unique to military aviation? That question and others likely need further examination. The results of this thesis suggest that the full burden of F-22 flying on pilots is not yet understood.

B. RECOMMENDATIONS FOR FUTURE RESEARCH

This section focuses on two dimensions of future research: replication efforts and identifying stress patterns in other populations.

1. Replication of Research

The following section outlines some recommendations for research replication efforts as this thesis only achieved a 31% test power given the small number of participants. Many of these recommendations come from lessons learned in the current research and include the rationale for these changes.

a. Go/No-Go (GNG)

Future experimental design considerations should not interfere with pre-sortie time constraints. The window of time after the Top 3 brief (in which weather conditions and other notifications are discussed) and before "stepping" to the aircraft was a limiting factor for several participant participants. While the saliva sample could be undertaken with minimal interference, administering the GNG test took 5 minutes. Several pilots were unable to complete the GNG test under the time constraints. As the data collection week progressed, pilots would take the test in the period between the mission brief and Top 3 brief. This adjustment allowed for before and after GNG sample collection. Some pilots were too time-pressed to attempt this test prior to flying but accomplished it post-sortie.

Aircrew were much more compliant and willing to participate in the go/no-go test when not otherwise pressured by mission demands.

b. Data Collection Window

Future research into comparisons of day- and night-flying operations should include more night flights to account for scheduling changes. F-22 pilots fly an average of five times per month. While one week was adequate to get participation for the day flights, night flying posed a unique problem. Personnel issues caused several pilots to have to drop from the schedule and prevented some individuals from participating in the night-flying data collection. The squadron had additional night-flying weeks available but we did not plan for data collection during that timeframe.

c. Method of Saliva Collection

Future efforts into stress measurement in the pilot population should utilize passive drool kits. While slightly more time-consuming, the volume obtained from a passive drool kit would have allowed for multiple analyses. Swabs were used instead of passive drool to allow for saliva collection while pilots donned their flight gear. We gave priority in the analysis to cortisol, which limited the amount of analysis conducted on alpha amylase due to gaps in the data.

d. Stress Scale

A more appropriate measure of daily stress may be the “Daily Hassles and Uplifts” by Kanner, Coyne, Schaefer, and Lazarus (1981). This scale identifies 117 hassles and 135 uplifts that occur in everyday life. While the Perceived Stress Survey (PSS) and Social Readjustment and Rating Scale (SRRS) are common in stress research, they do not capture the traffic jams, lost items, or arguments that are a daily part of life and contribute to the stress burden. These vague annoyances are captured in the “Daily Hassles and Uplifts” scale and may be more predictive of short-term stress response than either the PSS or SRRS.

2. Other Populations

Results of this study need to be shored up by studies that look at a larger sample size, but evidence suggests that the F-22 sample does not conform to typical stress patterns. Efforts should focus on additional aviation communities to determine if this change is unique to F-22s or is also prevalent in other aircraft communities.

Additionally, personnel in support fields, such as maintenance and aircrew flight equipment, also transition to accommodate the flying schedule. Efforts should be made to investigate if similar changes exist in these support career fields.

3. Eye-Tracking

Future researchers could use eye-tracking software to confirm if see-and-avoid procedures contribute to the cortisol spike post-flight in the day-flying condition. Increased traffic and efforts on deconfliction have been suggested as a potential contributor to the elevated post-flight cortisol levels.

C. CONCLUSION

This thesis sought to determine if F-22 pilots were experiencing increased stress as a result of different flying conditions. We thought that the night-flying environment would be more stressful on the aviator. While more research is required to support the results found this in this study, it appeared the opposite is true: day-flying is more stressful. Understanding the stress burden to F-22 aviators during the transition to night-flying operations is an important aspect of future mishap prevention efforts.

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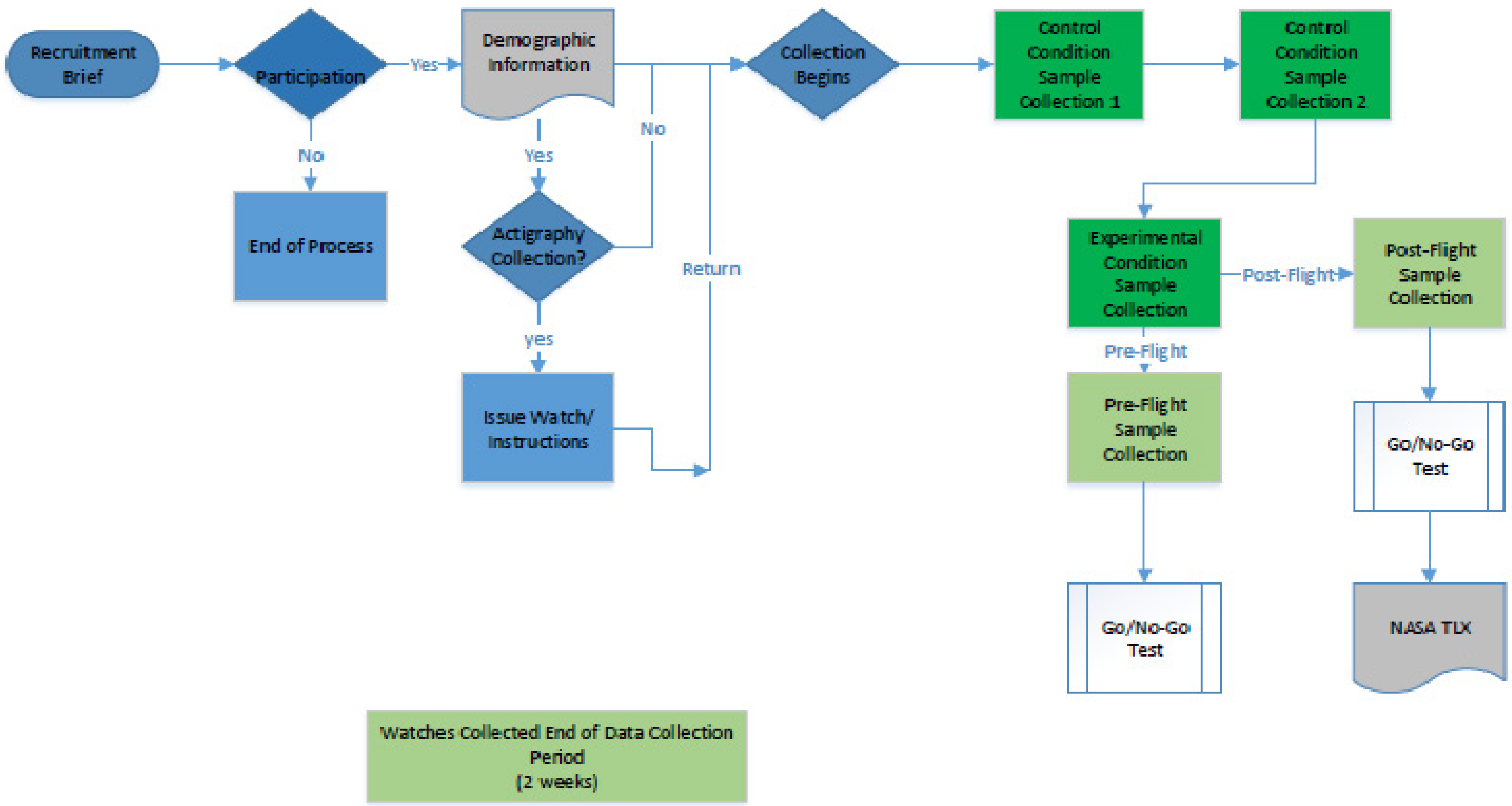
APPENDIX A. STUDY DESIGN LOGIC



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APPENDIX B. CORTISOL ASSAY PROTOCOL

Task	Description	Note
Prepare reagents	Bring reagents to RT and mix. Bring plate to RT. Dilute wash buffer by adding 100 mL concentrate to 900 mL DI water. Pipette 24 mL diluent into disposable tube provided. Set aside.	min 1.5hrs @RT Keep plate pouch closed. Use serological pipette
Prepare plate	Remove strip 1 & 2 and break off bottom wells. Break off 2 NSB wells and place in H1 and H2 position.	Store unused wells at 2–8°C.
Plate	Use the plate layout to: Pipette 25 µL standards into wells. Pipette 25 µL diluent to ZERO and NSB wells. Pipette 25 µL controls (high and low). Pipette 25 µL saliva samples to plate.	Vortex standards and controls before pipetting. Change tips between standards/controls/samples. Use same tip for duplicates. Missing samples – pipette diluent to wells
Add enzyme conjugate	Dilute enzyme conjugate by adding 15 µL conjugate to the 24 mL diluent tube. Vortex. Pipette 200 µL into each well.	
Incubate	Mix on plate shaker 5 min @500 rpm. Incubate @RT 55 min	Start 1 hour timer.
Wash plate	Pipette 300–350 µL wash buffer into each well. Discard over sink or kill bucket. Blot plate thoroughly on paper towel. Repeat 3 more times.	A 1 min soak is recommended for next to last wash

Task	Description	Note
Add substrate	Pipette 200 µL TMB substrate solution into each well. Cover plate with foil.	
Incubate	Mix on plate shaker 5 min @500 rpm. Incubate @RT 25 min.	Start 30 min timer.
Add stop solution	Pipette 50 µL stop solution into each well. Mix on plate rotator 3 min @ 500 rpm.	If green color remains, continue mixing until yellow.
Read plate	Wipe off plate with damp kimwipe then dry with fresh kimwipe. Run cortisol experiment on plate reader.	Read within 10 min of adding stop solution.

APPENDIX C. SALIVARY ALPHA AMYLASE PROTOCOL

Task	Description	Note
Prepare reagents	Bring to RT and mix	min 1.5hrs @RT
Start incubator heat	Set plate shaker/incubator to 37°C Start plate reader heat 37°C	
Heat substrate	Put substrate in trough provided, cover and heat on plate incubator @37°C >20 min	Pre-heat incubator
Prepare dilution tubes	Number one 0.6 mL and one 1.7 mL tube for each sample. Pipette 90 µL diluent into 0.6 mL tubes. Pipette 190 µL diluent into 1.7mL tubes	Use plate sample number 1–40, not study sample number.
Dilute samples	Pipette 10 µL sample into 0.6 mL tube. Vortex. Pipette 10 µL diluted sample into 1.7 mL tube. Vortex	First dilution step is 1:10, second step becomes 1:200. Missing samples - diluent only.
Plate	Pipette 8 µL of high and low controls to plate. Pipette 8 µL diluted sample to plate.	Vortex controls before pipetting. Use reverse pipetting to avoid bubbles.
Add substrate	Use electronic pipette to add 320 µL pre-heated substrate to each well.	Discard tips and leftover substrate after every use.
Read plate	Run SAA experiment on the plate reader.	Check for bubbles when the plate is ejected between reads.

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