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Serotonin as a Biomarker: Stress Resilience among Battlefield Airmen Trainees

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14. ABSTRACT

Epidemiologic data have shown that neuropeptide Y and dehydroepiandrosterone-sulfate have been found to predict successful performance under stress. Serotonin receptors and the serotonin transporter have been extensively studied for their efficacy in treating mental and physical disorders. In the present study, we sought to determine whether serotonin levels measured during Battlefield Airmen training were associated with stress resilience. We measured serotonin in blood samples from 21 Battlefield Airmen trainees prior to and during training for a total of six blood draws. Participants were divided into three groups: graduates, failures, and self-initiated eliminations. Failures included those who failed medically and those who failed performance. We analyzed the data using discriminant analysis. Of the 21 subjects in the analysis, all males with mean age 25 years (range, 18-35 years), 11 graduated (52.4%), 7 failed (33.3%), and 3 (14.3%) self-initiated elimination. Discriminant analysis showed that subjects with increased serotonin levels were more likely to self-initiate elimination. Although the sample was very limited, the findings demonstrate potential for using serotonin data to identify trainees who are likely to abandon training on their own. These data may improve the design of biomarker performance prediction models for stress, cognitive performance, and training ability relative to attrition rates, a U.S. Air Force School of Aerospace Medicine priority. It might also assist the design and development of real-time wearable sensor technology, an Air Force priority.

15. SUBJECT TERMS
Stress, Battlefield Airmen, resilience, biomarker, serotonin, metabolism, performance, training

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1.0 SUMMARY

Epidemiologic data have shown that neuropeptide Y and dehydroepiandrosterone-sulfate have been found to predict successful performance under stress. Serotonin receptors and the serotonin transporter have been extensively studied for their efficacy in treating mental and physical disorders. In the present study, we sought to determine whether serotonin levels measured during Battlefield Airmen training were associated with stress resilience. We measured serotonin in blood samples from 21 Battlefield Airmen trainees prior to and during training for a total of six blood draws. Participants were divided into three groups: graduates, failures, and self-initiated eliminations. Failures included those who failed medically and those who failed performance. We analyzed the data using discriminant analysis. Of the 21 subjects in the analysis, all males with mean age 25 years (range, 18-35 years), 11 graduated (52.4%), 7 failed (33.3%), and 3 (14.3%) self-initiated elimination. Discriminant analysis showed that subjects with increased serotonin levels were more likely to self-initiate elimination. Although the sample was very limited, the findings demonstrate potential for using serotonin data to identify trainees who are likely to abandon training on their own. These data may improve the design of biomarker performance prediction models for stress, cognitive performance, and training ability relative to attrition rates, a U.S. Air Force School of Aerospace Medicine priority. It might also assist the design and development of real-time wearable sensor technology, an Air Force priority.

2.0 INTRODUCTION

Repeated physical, psychological, and environmental stress can negatively affect cognitive processes, skill acquisition, and performance during military training [1]. To assess potential attrition rates as a result of stress, one would measure biomarkers such as neuropeptide Y (NPY), dehydroepiandrosterone-sulfate (DHEAS), and serotonin, which are implicated in stress resilience. The serotonergic system is considered an excellent biomarker in biological psychiatry. Increases in brain serotonin have been found to prevent the onset of various mental and physical disorders [2-7].

A large body of literature supports that fatigue during exercise is associated with elevated serotonin synthesis [1,8]. In the present study, we sought to determine whether serotonin levels measured during Battlefield Airmen training were associated with stress resilience.

3.0 MATERIALS AND METHODS

The assessment occurred during the first 10 days of Air Force Battlefield Airmen training at Lackland AFB, TX. The Institutional Review Board of the Air Force Research Laboratory approved this study protocol.

We recruited 21 healthy volunteers who had completed Basic Military Training and were entering into one of the Battlefield Airmen development courses. Only males, 18-35 years old, were eligible for this course at the time. To be included in the study, participants had to be “in generally good health” and cleared by the Air Education and Training Command physician. Volunteers were told that participation or lack thereof would not impact the status of their training. All potential participants were briefed prior to the start of their training course by the principal investigator of the study. Written informed consent was obtained from all volunteers.

The command staff and the instructors were not present during the consent process. Also, an independent witness was present to reduce the impression of coercion.

Participants were asked to do the following during the training course: (1) obtain the baseline Profile of Mood States (POMS) on day 1; (2) provide a 15-mL blood sample on day 1 to establish their individual baseline; and (3) obtain additional POMS, complete the continuous performance task, and provide five more 15-mL blood samples on days 3, 5, 8, 9, and 10.

The POMS is a 65-item questionnaire using the 5-point Likert scale to subjectively measure affect and mood on six scales: (1) tension-anxiety, (2) depression-dejection, (3) anger-hostility, (4) vigor-activity, (5) fatigue-inertia, and (6) confusion-bewilderment. Total distress was calculated using the sum of the scores of scales 1, 2, 3, 5, and 6 minus 4 above [9].

The continuous performance task tests reaction time (RT), processing, and decision making. Results were measured as continuous memory RT, math processing mean RT, and rapid decision making mean RT.

Blood samples were collected via venipuncture and performed in accordance with the Department of the Air Force venipuncture operating instruction by a certified laboratory technician, using standard antiseptic procedures and BD Vacutainers® (Becton, Dickinson and Company, Franklin Lakes, NJ) with red top tubes. Six samples were taken. For each sample, 15 mL of blood was collected into a serum separator tube and allowed to clot for 30 minutes at room temperature. Tubes were centrifuged 15 minutes at 1000 x g. Serum was removed, separated into 200-µL aliquots, and stored at -80°C. Samples were thawed on ice prior to analysis. We analyzed blood samples for the biomarker serotonin (ng/mL).

Participants were grouped in three groups: graduates, failures, and self-initiated elimination (SIE). Failures included those who failed medically and those who failed performance.

In this study, sub-scaled subjective distress variables included the following: (1) tension-anxiety, (2) depression-dejection, (3) anger-hostility, (4) vigor-activity, (5) fatigue-inertia, (6) confusion-bewilderment, and (7) friendliness (not counted as a scale in the Likert model).

Descriptive statistics, Pearson correlation coefficients, analysis of variance (ANOVA), and discriminant analysis were conducted. The relationship between serotonin levels and RT over the course of training within subjects was explored by means of a linear mixed model (repeated measures) with serotonin level and time as the fixed effect and subject as a random effect.

4.0 RESULTS

We compared serotonin levels between graduates and non-graduates (performance and medical failures and SIEs) in male participants enrolled in their first 10 days of Battlefield Airmen training.

Of the 21 subjects in the analysis, all males with mean age 25 years (range, 18-35 years), 11 graduated (52.4%), 7 failed (33.3%), and 3 (14.3%) SIE. Of the 7 failures, 5 (71.4%) were due to performance and 2 (28.6%) due to medical issues. Mean serotonin levels were 277 ng/mL (standard deviation (SD) = 90) among graduates, 506 ng/mL (SD = 264) among non-graduates (only performance and medical failures), and 472 ng/mL (SD = 301) among those with SIE. Surprisingly, graduates started with a lower mean serotonin level than non-graduates and those who SIE. A plot of serotonin level over time in the graduates showed no pattern. The data only show that the variance in graduates is much smaller than in the other groups. As shown in

Table 1, on the basis of ANOVA, the difference between graduates and non-graduates was not significantly different.

Table 1. Serotonin Level for Graduates, Failures, and SIEs – ANOVA

Variable	n	Mean (SE) (ng/mL)	95% CI	p
Graduate	9	337 (64)	201 – 473	0.4894
Failure	5	433 (85)	250 – 615	
SIE	3	472 (110)	236 – 708	

CI = confidence interval; SE = standard error.

There was a negative but non-significant correlation between serotonin and vigor-activity (r = -0.25, p = 0.33), depression-dejection (r = -0.26, p = 0.31), fatigue-inertia (r = -0.21, p = 0.42), and confusion-bewilderment (r = -0.17, p = 0.52). The correlation with total distress (r = -0.05, p = 0.84) and friendliness (r = -0.05, p 0.84) as well as serotonin and tension-anxiety (r = 0.03, p = 0.90) was non-significant. There was a positive correlation, but non-significant, between serotonin and anger-hostility (r = 0.18, p = 0.49). There was no significant statistical relationship between serotonin and continuous memory (p = 0.88), math processing (p = 0.19), and rapid decision-making (p = 0.48) RTs.

When tested with standard t-tests and ANOVA, the overlap in the data among groups and the presence of extreme values in most variables accounted for the lack of significant differences between groups (Table 2). Friendliness had a significant p-value, but subsequent testing showed no difference among groups.

Table 2. p-Values for t-Tests for Differences between Graduates and Failures and p-values for F Tests of ANOVAs Comparing Graduates, Failures, and SIEs

Measure	Prob > t	Prob > F
Anger-Hostility	0.546	0.848
Confusion	0.535	0.777
Depression-Dejection	0.310	0.358
Fatigue	0.929	0.384
Friendliness	0.295	0.046
Serotonin	0.281	0.489
Tension-Anxiety	0.789	0.912
Total Distress	0.690	0.909
Vigor	0.165	0.066

We used discriminant analysis to look at the combination of all the variables to distinguish between the groups. Discriminant analysis showed that subjects with increased serotonin levels were more likely to SIE. Confusion-bewilderment, depression-dejection, and vigor-activity were associated with SIE, whereas friendliness and tension-anxiety were associated with graduation failure (performance and medical) (Table 3).

Table 3. Discriminant Analysis to Separate Graduates from Failures and Those Who SIE

Variable	Loading Factor	
	X Score	Y Score
Serotonin	1.20	0.70
Confusion	1.10	0.26
Depression-Dejection	1.32	0.02
Friendliness	-2.08	0.25
Tension-Anxiety	-1.22	-0.45
Vigor	1.19	0.93

5.0 DISCUSSION

This analysis of serotonin as a biomarker for assessing stress resilience is part of a larger study investigating several other biomarkers, including NPY and DHEAS. Studies have shown that DHEAS counters the effects of cortisol and may act as a resilience factor and that NPY may be a protective factor during acute stress [10]. Studies have established that low levels of serotonin are associated with depression, anxiety, anger, impulsivity, lethargy, sleepiness, and mood disorders [2-5,11,12]. Studies have also shown that prolonged exercise increases serotonin levels, which peak at fatigue [1,5].

The finding that graduates had a lower serotonin level is counterintuitive. The literature shows that lower serotonin levels are associated with depression and anxiety, a finding that is incongruent with graduating such an arduous training course. As Bouchez argues, it is not known if blood serotonin levels reflect serotonin levels in the brain [11]. It is also not known if serotonin levels cause depression or anxiety, or if depression or anxiety cause serotonin levels to drop [11].

None of the correlations for the sub-scales of subjective distress vs. serotonin achieved statistical significance. But there were four correlational observations that reveal opposing direction, although non-significant with serotonin: (1) the negative relationship with vigor-activity, (2) the lack of relationship with friendliness, (3) the positive relationship with anger-hostility, and (4) the lack of relationship with tension-anxiety. Per the POMS questionnaire used in the study, vigor-activity consists of feeling lively, active, energetic, cheerful, alert, full of pep, carefree, and vigorous. These feelings would more likely be associated with high, rather than low, serotonin levels. For the same reasons, we would not expect an inverse correlation with friendliness. In other words, we would expect low friendliness with low serotonin levels and high levels of anger-hostility and tension-anxiety with low levels of serotonin; hence, we would expect an inverse relationship.

Although low serotonin levels are known to impair perceptual and cognitive function [2-5], there was no significant statistical relationship between serotonin and mean RT.

A perfect model in discriminant analysis would have three non-overlapping circles and all points inside the proper circle. As shown in Figure 1, in this discriminant analysis, only SIEs were separated. We could not discriminate between the graduates and the other failures (performance and medical).

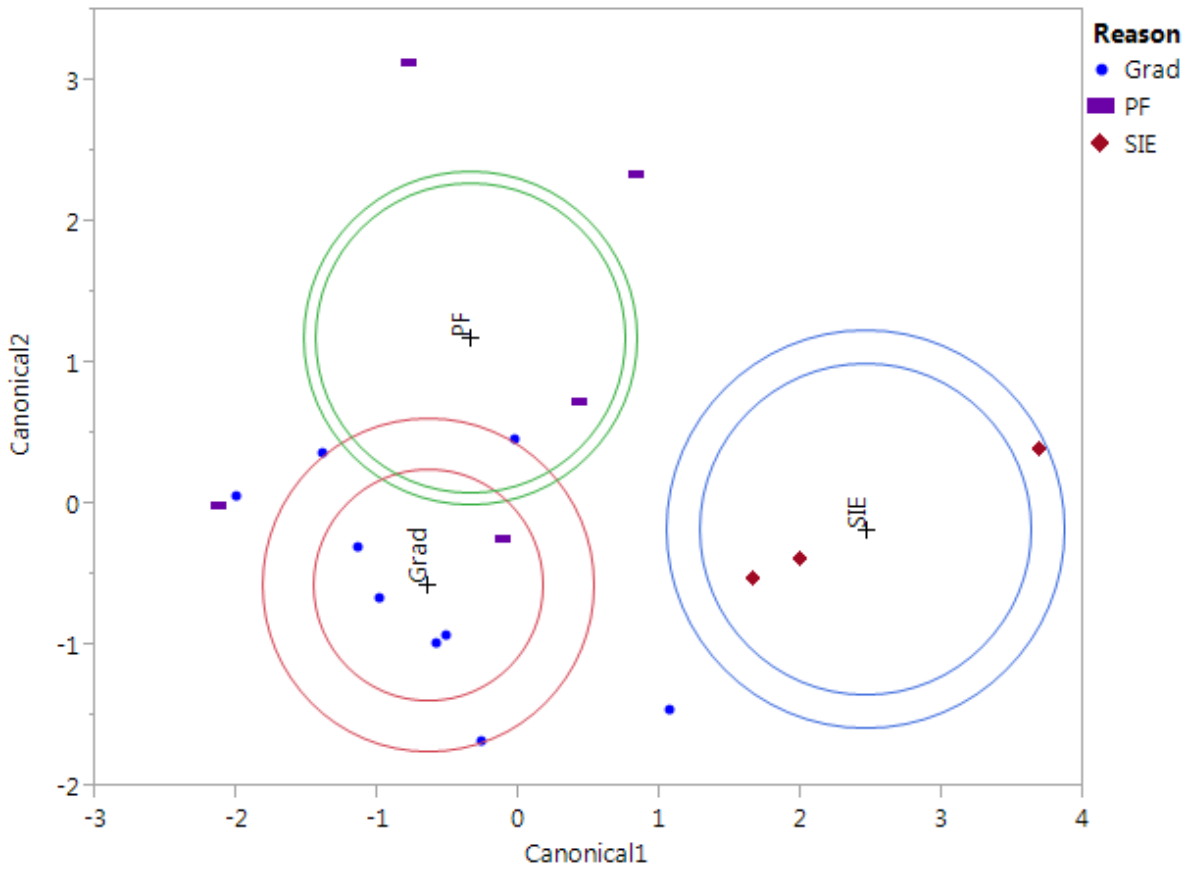

Figure 1. Discriminant analysis: graduates vs. failures vs. SIEs.

The model shows that high values for serotonin, confusion-bewilderment, depression-dejection, and vigor-activity push the score to the right on the x-axis toward the SIE group. High scores for friendliness and tension-anxiety push the score to the left toward the graduate and the other failure groups. This demonstrates that participants with higher levels of serotonin, confusion-bewilderment, depression-dejection, and vigor-activity were more likely to quit, while those with higher levels of friendliness and tension-anxiety were more likely to graduate or fail. The small sample size and outliers had a strong influence in the analysis, and we could not differentiate between the latter two (graduates and the rest of the failures).

The small sample size is a limitation of this study. But this study demonstrated that serotonin might be a good predictor of participants most likely to SIE (quit), which could translate into significant cost savings. For example, considering that the complete 24-month training costs $250,000 per participant, 3 SIEs out of 21 subjects carried a potentially significant opportunity cost, resources that could have been invested on Battlefield Airmen trainees most likely to graduate. Additional classes of Battlefield Airmen trainees need to be tested to identify trends for this course to examine the role of serotonin as a predictive biomarker for attrition rates.

6.0 REFERENCES

1. Friedl KE. What is behind the fatigue concept? Proceedings of the RTO Human Factors and Medicine Panel Workshop on Operational Fatigue; 2007 Feb 7-9; Paris, France. Neuilly-sur-Seine, France: North Atlantic Treaty Organisation, Research and Technology Organisation; 2007. RTO/HFM-WS-151.
2. Bowirrat A, Chen TJ, Blum K, Madigan M, Bailey JA, et al. Neuro-psychopharmacogenetics and neurological antecedents of posttraumatic stress disorder: unlocking the mysteries of resilience and vulnerability. Curr Neuropharmacol. 2010; 8(4):335-358.
3. Corchs F, Nutt DJ, Hood S, Bernik M. Serotonin and sensitivity to trauma-related exposure in selective serotonin reuptake inhibitors-recovered posttraumatic stress disorder. Biol Psychiatry. 2009; 66(1):17-24.
4. Kim JJ. Serotonin, stress, and conditioning. Biol Psychiatry. 2008; 63(9):819-820.
5. Southwick SM, Vythilingam M, Charney DS. The psychobiology of depression and resilience to stress: implications for prevention and treatment. Annu Rev Clin Psychol. 2005; 1:255-291.
6. Trevor AJ, Katzung BG, Masters SB. Katzung & Trevor's pharmacology examination & board review, 7th ed. San Francisco (CA): Lange Medical Publications/McGraw-Hill; 2005:149-155.
7. Young SN. How to increase serotonin in the human brain without drugs. J Psychiatry Neurosci. 2007; 32(6):394-399.
8. Davis JM, Alderson NL, Welsh RS. Serotonin and central nervous system fatigue: nutritional consideration. Am J Clin Nutr. 2000; 72(2 Suppl):573S-578S.
9. McNair DM, Lorr M, Droppleman LF. Manual for the Profile of Mood States. San Diego (CA): Educational and Industrial Testing Services; 1981.
10. Russo SJ, Murrough JW, Han MH, Charney DS, Nestler EJ. Neurobiology of resilience. Nat Neurosci. 2012; 15(11):1475-1484.
11. Bouchez C. Serotonin: 9 questions and answers. [Accessed 31 Jan. 2016]. Available from http://www.webmd.com/depression/features/serotonin?page=1.
12. Haglund ME, Nestadt PS, Cooper NS, Southwick SM, Charney DS. Psychobiological mechanisms of resilience: relevance to prevention and treatment of stress-related psychopathology. Dev Psychopathol. 2007; 19(3):889-920.

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	analysis of variance
DHEAS	dehydroepiandrosterone-sulfate
NPY	neuropeptide Y
POMS	Profile of Mood States
RT	reaction time
SD	standard deviation
SIE	self-initiated elimination