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Submitted in partial fulfillment of the requirements for the degree
of Master of Science in Biology from the Department of Biology
July 2007

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By

Olivita Couso

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Abstract

The highly stressful job of law enforcement personnel, rescue workers and soldiers requires them to constantly put to the test their previous training. In order to respond effectively under pressure, the training of these personnel must be performed under conditions that elicit pressure. The research proposed in this thesis evaluates established training regimens for the degree of stressfulness instilled in trainees from physiological (hypothalamic-pituitary adrenal axis, autonomic nervous system) and psychological perspectives (anxiety). Subjects participating in SWAT and military training were recruited, as well as subjects having no prior SWAT or military training. Individual physiological stress responses measured included heart rate and salivary levels for cortisol, amylase, and dehydroepiandrosterone DHEA. Psychological assessment tools used which included the visual analog scale for stress levels to determine the subjects' perception of the stressfulness of the exercise, The Borg Rating of Perceived Exertion was used to gather perceptions about how hard the body was working, and the Spielberger State Trait Anxiety Inventory (STAI) and Thayer's Activation and Deactivation Checklist (ADC) were used to determine the level of anxiety of the subjects. When the data of all trained subjects having prior SWAT and military training was pooled together significant correlations were found in DHEA vs Cortisol, as well as DHEA/Cortisol ratios vs Calmness. In contrast these correlations were not significant in the data of the subjects having no prior training recruited during a disaster drill. Furthermore the response elicited by subjects performing the same drills varied greatly, including their DHEA/cortisol ratios. A possible recommendation that may be deduced from this research is that more surprise elements should be introduced into the training

drills so as to make the training more effective for live chaotic situations since there was such a vast differences in responses and some subjects did not show any change in their salivary levels substances from the baseline both during and after the exercises were performed.

Introduction

The highly stressful job of law enforcement personnel, rescue workers and soldiers requires them to constantly put to the test their previous training. The training programs they encounter are supposed to make them ready for physiological stressors as well as psychological stress that may be encountered in certain crises. Therefore, monitoring the stress of the trained personnel during training is an extremely useful tool to determine if the current training programs are able to mimic the highly stressful situations of a real crisis. If there is no stress response elicited from the individuals during training, then certainly the current training methods used need to be reconstructed so as to better prepare and train the personnel for real situations.

The tragedies of September 11th 2001 demonstrated that terror attacks require the combined and coordinated efforts of a vast array of agencies and response units. Emergency personnel must not only be able to respond quickly to a situation, but must also arrive mentally prepared to enter an area of extreme danger. Therefore, the decision-making of the rescue workers is being constantly complicated by the uncertainty of the threat and duration of the situation. As a result, good strategies for crisis preparation and terrorists acts are those that foresee the limits in the information and knowledge that will be available and yet are still able to bring order to a chaotic situation.

Ultimately, the effectiveness of training is evaluated in terms of performance of trainees during actual events. To respond effectively under pressure, one must train under pressure. Our laboratory evaluates established training regimens for the degree of

stressfulness instilled in trainees from physiological (hypothalamic-pituitary adrenal axis, autonomic nervous system) and psychological perspectives (anxiety).

Stress elicits a stress response also known as a fight or flight response (Seyle, 1950). The stressor can be either physical, such as blunt impact or psychological, such as during a simulated combat military flight (Leino et al., 1998). Characteristics for physical stressors include intensity, duration and frequency, and characteristics for psychological stressors include controllability, predictability and the ability to escape. It should be noted that both the stressors and the stress response itself have both physical and psychological components.

The physiological response to stress has two main components: increase in activity in the sympathetic branch of the autonomic nervous system (ANS) and activation of the hypothalamic-pituitary-adrenal axis (HPAA) (Seyle, 1950). This response attempts to get more oxygen and glucose to the vital organs of the body such as the brain, heart and skeletal muscles. It also induces a release of norepinephrine (NE) from postganglionic sympathetic neurons and both epinephrine (Epi) and NE are released from the adrenal medulla leading to increases in heart rate and blood pressure; increasing blood flow to the heart, brain and skeletal muscles while at the same time decreasing blood flow to the gastrointestinal (GI) tract. In addition, there is an increase in respiratory rate so as to dilate the bronchioles in order to help increase the amount of oxygen in the blood, also glycogenolysis is increased thus increasing plasma glucose levels, and alertness levels are increased and pupils become dilate so as to get more visual information (Moldow et al, 2005).

The hypothalamic pituitary adrenal axis responds to stress. This pathway includes the hypothalamus in the brain that elicits an increase in corticotropin releasing hormone (CRH) which is then released to the anterior pituitary gland. As a result there is an increase in adrenocorticotropin hormone (ACTH) which is released into the systemic circulation which in turn leads to an increase in cortisol release from the adrenal cortex (Moldow et al., 2005). High cortisol levels then elicit an increase in plasma glucose levels, done by gluconeogenesis, which has a permissive effect on catecholamines (NE and Epi) such as vascular reactivity (Ganong, 2005) consequently, high levels of cortisol also suppress the immune system by decreasing the circulating lymphocyte count (Ganong, 2005) cortisol will also elicit an increase in gastrin release that in turn increases hydrochloric acid production (Ganong, 2005). In this network of glands and systems working together there is also an increase in plasma beta-endorphin levels (Ganong, 2005). As a result, an individual who is undergoing all these hormone fluctuations might consciously be experiencing a headache, heartburn, palpitations and/or sweating.

The psychological response to stress may be divided into the following categories: emotion, motivation, cognitive, sensory and motor. Emotion refers to a change in mood such as becoming irritable and motivation refers to the level of intensity as well as an actual drive such as being thirsty. Cognition includes attention (concentration), memory (short term and long term), and problem solving. Sensory involves increased sensitivity to stimuli, such as smell, touch and pain. (Moldow et al, 2005) The actual behavioral response associated with these stressors could be to stay and confront the situation, to

flee and avoid the situation, or to stay due to behavioral depression which includes the individual freezing up and showing no motor response.

The actual degree of stress under training is important to ascertain. To date, there is no index that accounts for physical exertion and adequately provides an objective measure of intensity of stress. Instead there are measures that indicate that something stressful has occurred, but do not adequately track the intensity of the event (Moldow et al., 2005). In order to accurately measure in the field, scenario specific performance measures were developed and implemented so as to be noninvasive and to develop a stress profile. Individual physiological stress responses, which included heart rate and salivary levels for cortisol, amylase, and dehydroepiandrosterone DHEA, have been measured during several different scenarios. Psychological assessment tools were used which included the visual analog scale for stress levels to determine the subjects' perception of the stressfulness of the exercise (Kudeielka, et al., 2004). The Borg Rating of Perceived Exertion was used to gather perceptions about how hard the body was working (Borg, 1998) and the Spielberger State Trait Anxiety Inventory (STAI) (Spielberger, 1983) and Thayer's Activation and Deactivation Checklist (ADC) (Thayer, 1978) were used to determine the level of anxiety of the subjects.

The scenarios tested focused on finding training exercises that were specifically designed to stress the participants and/or separate out physical stress from psychological stress. To accomplish this, samples were collected in the field during training exercises for SWAT (S) teams that were devised to elicit stress. For example, verbal harassment was introduced during the live fire practice at the range as well as force on force contact

during building entry exercises. The S6 team did one exercise of building entry with limited force on force contact and flash bang grenades that consisted mostly of psychological stress as contrasted with a second exercise that was a 150 lb body drag for 50 yards that constituted mostly physical stress. Other trained personnel examined included a military (M)2 team. In addition, an exercise in which military (trained) and civilian (non-trained) participants were working together was also analyzed. This exercise was with subjects participating in a disaster drill.

The substances analyzed during these exercises included salivary cortisol, DHEA and amylase. As noted in Moldow et al. 2005, salivary DHEA levels correlate with plasma DHEA (Granger et al., 1999) and DHEA-S. DHEA-S can not cross into saliva and thus, it is not found in saliva, so instead DHEA itself is measured. An interesting note found by Morgan et al., 2004 was in stressed subjects that reported fewer symptoms of disconnection and exhibited superior military performance also exhibited a significantly higher DHEA-S cortisol ratio. These findings suggest that the degree to which an individual may be protected against the negative effects of stress may be measured by the DHEA-S cortisol ratio, as done so by (Morgan et al., 2004). Additionally, numerous studies have depicted that salivary cortisol, DHEA, and amylase all activate the HPAA and sympathetic branch of the ANS. These studies include Chatterton et al., 1996; Chatterton et al., 1997; Kugler, Hess and Haake, 1992; Morgan et al., 2002; Morgan et al., 2004; Rohleder et al, 2004; Schommer, Hellhammer and Kirschbaum, 2003. Futhermore, Chatterton et al.,1996 illustrated that after physical exercise and

psychological stress concentrations of salivary amylase are correlated with plasma norepinephrine.

In this proposed field research the levels of the tested salivary substances will be correlated against each other as well as with data obtained from questionnaires. In addition, differences in response profiles between trained personnel consisting of subjects who have received SWAT and military training and non-trained personnel consisting of civilians with no prior SWAT or military training will be explored.

Materials and Methods

Subjects – Six Groups

Groups 1(S3) and 2 (S4): These samples were obtained during training sessions for SWAT teams. The sessions consisted of all day sessions, with the morning exercises being at the firing range and the afternoon exercises consisting of building entry and force to force situations. There were four subjects recruited for each of these exercises from the S3 and S4 Swat teams.

Group 3 (S5): These samples were of a SWAT team members training. Three subjects were recruited. The exercises during the training consisted of force on force scenarios as well as building entry scenarios with the insertion of a 'flashbang' device to surprise and possibly stress the participants.

Group 4 (S6): These samples were of a SWAT team where six subjects of the SWAT team were recruited. The training was divided into two halves throughout the day. The first half was an exercise that consisted of building entry with the use of a flashbang device to surprise and possibly stress the participants, as well as limited force on force

contact in the building. The second half of the exercise was a 150 pound body drag for 50 yards.

Group 5 (M2): These samples were obtained from military group (M2) and the exercises consisted of building entry. There were seven subjects recruited.

Group 6 (NT3): These samples were of subjects participating in a disaster drill. There were thirteen subjects recruited. The scenario was release of HF gas from a train car passing by fair grounds. The exercise involved both military and civilian participants. The military component consisted of the medical group from the National Guard and the Chemical Biological Radiological Nuclear Explosives (CBRNE) Enhanced Response Team. The civilian component consisted of medical personnel.

All Subjects in all scenarios and exercises were recruited anonymously. Volunteers were read the consent form. Any questions they may have were answered. Volunteers were reminded that participation is voluntary and that they may stop participating at any time, without question or penalty. Consent form signature was not obtained as this would be the only way to identify them. All these procedure including the anonymous recruitment without the signed consent was approved by the Institutional Review Boards (IRB) from SHU, NJMS and USAMRMC.

Physiological assessment:

Physiological parameters of heart rates were continuously obtained during the sessions using Polar watches and heart rate bands. Due to technical difficulties in the collection of the heart rate data such as the interference with the polar devices by the equipment from CBRNE, cold outdoor temperatures and the necessity to further filter some of the polar

data, the polar data was not analyzed. Some representative samples are included in the appendix.

Psychological assessment:

The Borg Rating of Perceived Exertion was used to gather self perceptions from the subjects about the difficulty of scenario to which they had been exposed (Borg, 1998).

The Spielberger State Trait Anxiety Inventory (STAI) was given to each subject to determine self perception of any levels of anxiety (Spielberger, 1983). Thayer's

Activation and Deactivation Checklist (ADC) was used to gather self assessments of the subjects on information relating to calmness and tension during the exercise (Thayer, 1978).

Saliva sampling:

Volunteers spit in tubes at different times during the sessions, i.e., at the beginning of the session, immediately after the session, and 20 minutes later. Samples were stored at - 70° C (Revco) until assayed. Before the assay, samples were thawed and spun at 3000 rpm for 15 min using a Sorvall centrifuge.

Biochemical analysis:

Samples for steroid determination were assayed by ELISA and amylase was measured by enzymatic activity kits obtained from Salimetrics LLC (State College, PA). These kits are specific for saliva samples, thus, extraction was not necessary and only required small aliquots. Cortisol (25µl), DHEA (50µl), and alpha-amylase (10µl) measured in each assay.

Plate washers (Molecular Devices) and plate reader (Molecular Devices) were used to rinse the plate as stated in the protocol and to read the plates for UV data respectively.

Statistical analysis:

The statistical program, Number cruncher statistical systems (NCSS) (Hintze, 1998) was used to analysis the data. Repeated measure ANOVA followed by Tukey Kramer Test were performed to determine if there was a significant difference in the pairwise comparison of the means. Linear regression was performed, and correlation matrix generated to determine Pearson and Spearman's correlation coefficients. Spearman's provided a rank order correlation coefficient. Physiological parameters of salivary cortisol, DHEA and amylase were correlated with the questionnaire data and only the maximum values for each participant were used. The maximum levels were representative of the maximal response to stress for each participant and were correlated with the data from the questionnaires that were only obtained at one time point after the completion of the exercise.

Results

Figure 1 depicts the results of the life fire exercises with the S3 team. The time points in the figure are: (1) baseline sample taken at 10:00am, (2) 10 minutes after a live fire drill, (3) 30 minutes after the live fire drill, (4) 10 minutes after building entry exercise, (5) taken at 3:10pm after the subjects had eaten lunch and where gearing up for the afternoon exercise, (6) 10 minutes after a drill with force on force entry and (7) taken immediately after the last afternoon building entry drill. There is an increase in salivary cortisol levels at time point 2 however, it is not significant. Also, initial cortisol levels are high and there is large variance which may be due to the large differences in

individual responses during the training, the difference in responses, as well as the small number of subjects. Conversion of this data into percent of the baseline did not yield statistically significant increases. Additionally, the heart rate activity of an individual subject during S3 training exercises is depicted in Appendix Figure 1. The two sharp peaks represent a sudden increase in heart rate during building entry while subjects were running. Furthermore, the DHEA/cortisol ratios for S3 data are depicted in Figure 3 of the appendix, both by subject and by time interval.

Similar to Figure 1, Figures 2 and 3 both show no significant increases in salivary cortisol, amylase or DHEA in S4 and S5 respectively. The time points for figure 2 are: (1) baseline sample taken at 8:30am, (2) 10 minutes after live fire drill with interference such as sound, and insults, (3) 30 minutes after live fire drill, (4) 10 minutes after another fire drill with interference, (5) 10 minutes after a building entry exercise, (6) taken after lunch before an afternoon exercise at 3:20pm and (7) taken immediately afternoon drill of force on force building entry with man down. In the Appendix, Figure 2 illustrates increases in heart rate from an individual subject from S4. This figure is not representative of the entire group, instead it is an individual response. The increases were accounted for by physical activities or movement experienced at those given times. Additionally, the DHEA/cortisol ratios for S4 are depicted in Figure 4 of the Appendix, both by subject and by time interval.

The time points for figure 3 are: (1) baseline taken 10:15am, (2) taken immediately after doing physical exercises such as pushup's and abdominal crunches, (3) 10 minutes after building entry drill with shots fired, (4) 30 minutes after building entry

drill with shots fired, (5) taken after lunch before an afternoon exercise at 2:10pm and (6) taken immediately after last drill involving searching a train. The DHEA/cortisol ratios for S5 are depicted in Figure 5 of the Appendix, both by subject and by time interval.

In Figure 4, the time points are: (1) baseline before exercises started 10:30am, (2) 10 minutes after building entry exercise including the use of a flash bang device (3) 10 minutes after 150lb body drag and (4) 30 minutes after 150 lb body drag. Repeated measure ANOVA reveals that there is a significant increase in salivary cortisol [$F(3,23)=4.07$; $p=0.026$] and amylase [$F(3,23)=5.02$; $p=0.0322$] during the S6 team exercise. According to the Tukey Kramer's test, time point 4 is significantly different for cortisol from the other time points, and time point 3 is significantly different for amylase from the other time points. DHEA/cortisol ratios for S6 are depicted in Figure 6 of the Appendix, both by subject and by time interval.

Figure 5 depicts results obtained from individual members of the S6 team (Moldow et al., 2006). The time points for the S6 data are: (1) the subjects baseline before scenario started, (2) sample taken after the building entry scenario where a flash bang device was used, (3) sample taken 10 minutes after 150lb body drag and (4) sample taken 30 minutes after 150 lb body drag. Figure 5a depicts the results from a subject who did not respond to either the building entry or body drag exercise. Figure 5b is a subject whose cortisol levels increased after the building entry exercise and then remained elevated there after. Figure 5c is a subject whose salivary cortisol increased only 30 minutes after the body drag, which is physical exertion. Figure 6 of the Appendix depicts the DHEA/Cortisol ratio for the members of the S6 team.

Salivary substance of Amylase, Cortisol, and DHEA. Figure 1 S3 Training, Figure 2 S4 Training, Figure 3 S5 Training, Figure 4 S6 Training.

Figure 1

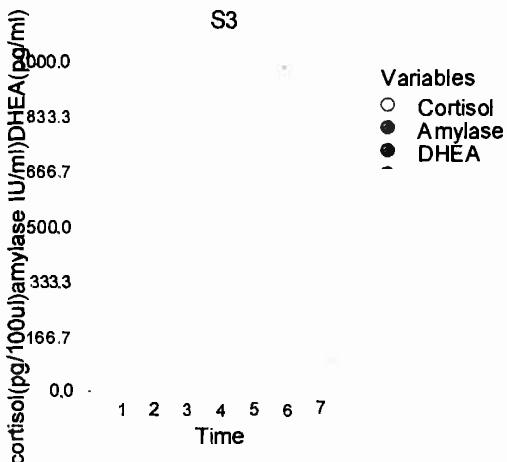


Figure 2

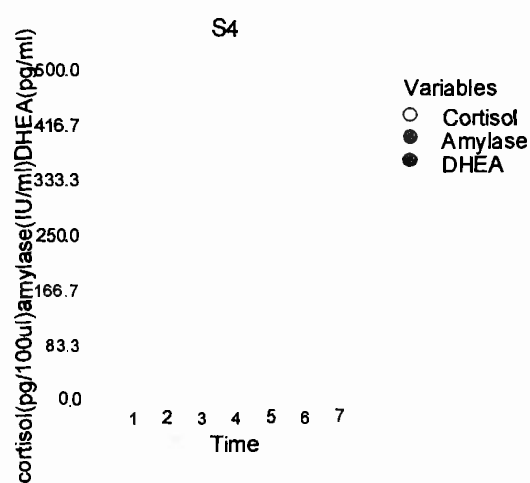


Figure 3

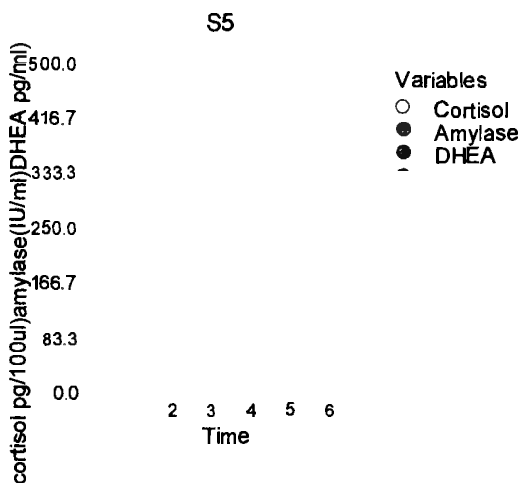
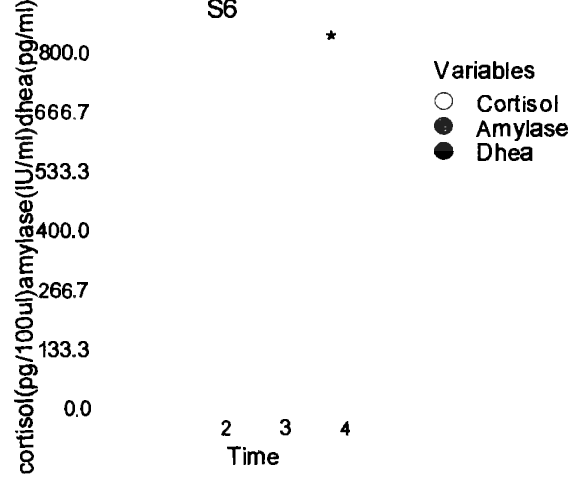


Figure 4



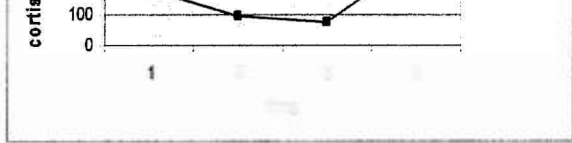


Figure 5a: Subject 1 S6

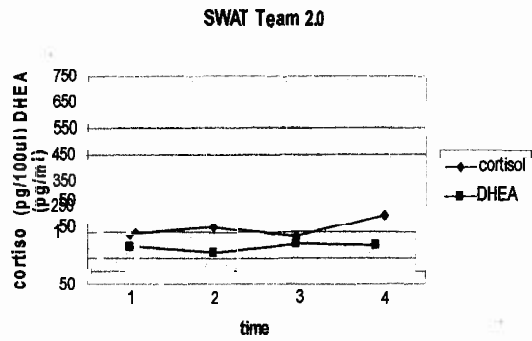


Figure 5b: Subject 2 S6

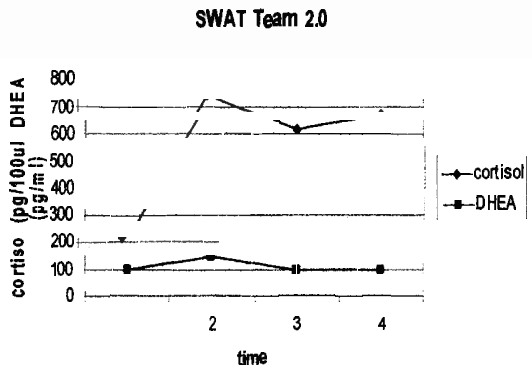


Figure 5c: Subject 3 S6



Figure 6 depicts the salivary substances in the M2 subjects. Time point 1 is the baseline before the exercise started, time point 2 is 10 minutes after drills including reloading a rifle and fighting off a bad guy, and time point 3 is 30 minutes after drills were finished. There was no significant increase in the salivary substances.

DHEA/cortisol ratios for M2 are depicted in Figure 7 of the Appendix, both by subject and by time interval.

Figures 7a and 7b illustrate the linear correlations between cortisol and DHEA (significant) and amylase (not significant) respectively in all trained personnel which includes: S3, S4, S5, S6, and M2 teams. In Figure 7a, there is a positive correlation between cortisol and DHEA. In addition, linear regression reveals that there is a significant correlation between the DHEA/cortisol ratio and calmness from the Thayer Activation Deactivation Checklist depicted in Figure 7c. However, linear regressions between amylase and cortisol did not reveal any significant correlation, as seen in Figure 7b.

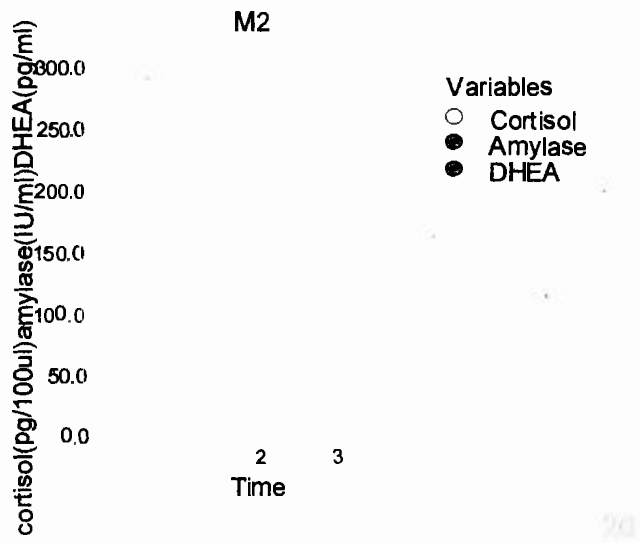
Figure 8 depicts that there are no significant increases in salivary cortisol or DHEA in the subjects during the NT3 drill. Time point 1 is at 7:00am, time point 2 is at 9:30am, and time point 3 at 10:30am after the drill was over. There is a significant increase in salivary amylase [$F(2,38)=7.05$; $p=0.0063$]. There is a significant decrease in salivary cortisol [$F(2,38)=9.67$; $p=0.0018$].

Figures 9a and 9b show the linear correlations of the salivary substances of the non-trained personnel in the NT3 drill. Figure 9c shows DHEA/cortisol ratio vs. calmness. All linear correlations of the non-trained personnel were not significant. In

addition, the DHEA/cortisol ratios for NT3 are depicted in Figure 8 of the Appendix, both by subject and by time interval.

Figure 10 depicts the DHEA/cortisol ratio in trained vs. non-trained subjects. Group 1 represents all the trained subjects consisting of S3, S4, S5, S6 and M2 teams. Group 2 represents the non-trained subjects from the NT3 disaster drill. The DHEA/cortisol ratio for Group 1 is 0.8 and 0.7 for Group 2. Therefore, in terms of DHEA/cortisol ratio there is no difference between the trained subjects and non-trained subjects.

Figure 6: M2 Salivary Substances



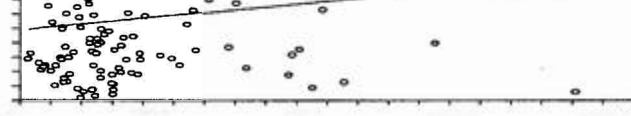


Figure 7: Linear regressions between trained participants: S3, S4, S5, S6, and M2 teams. 7a-Cortisol vs DHEA, [R2=0.1004, F(1,118)=12.83; p=0.0005] 7b= α -amylase vs cortisol [R2=0.0225, F(1, 118)=2.6483; p=0.1064], 7c-DHEA/cortisol vs Calmness [R2=0.1372, F(1, 23)=4.7722; p=0.0369]

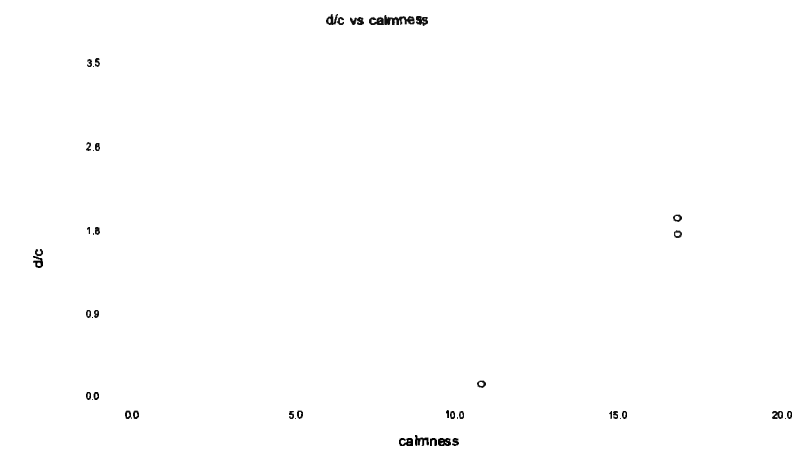
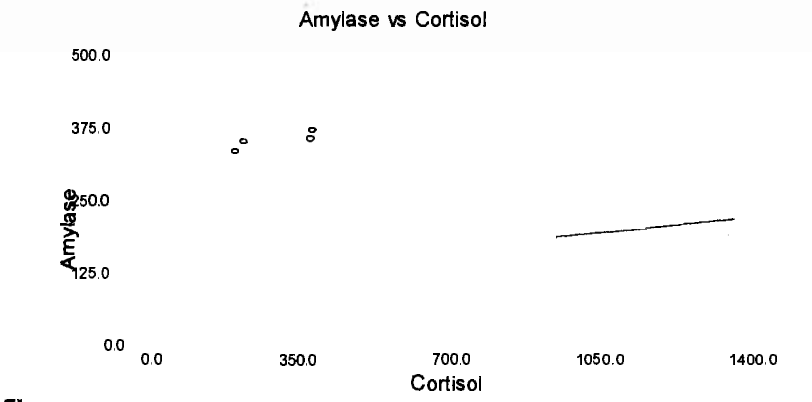
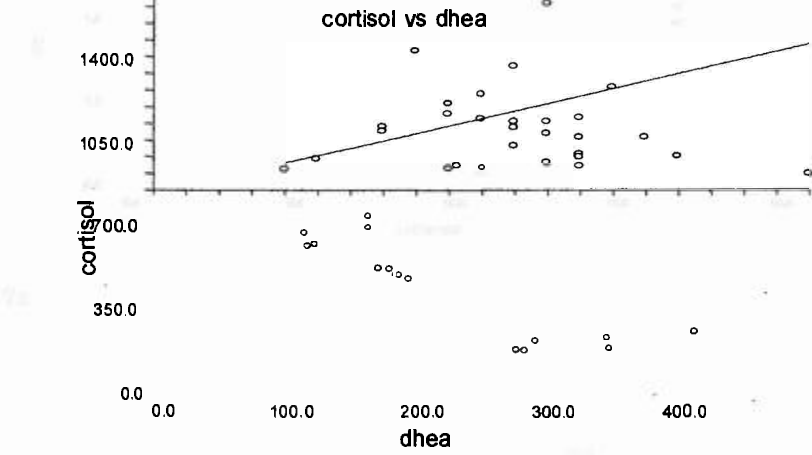
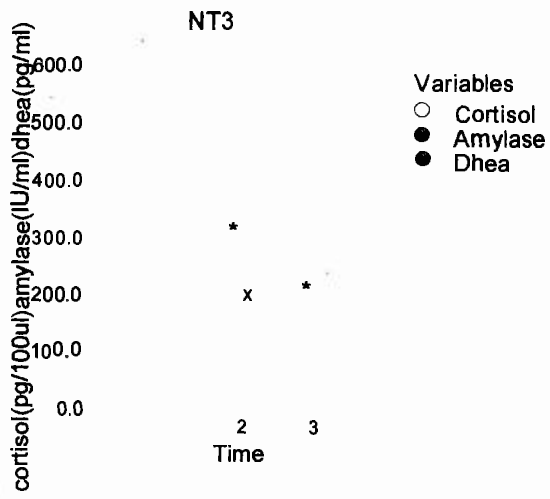
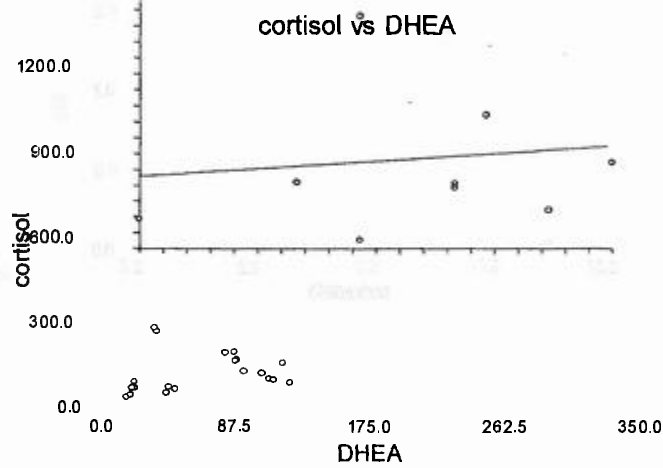


Figure 8 NT3 Salivary Substance

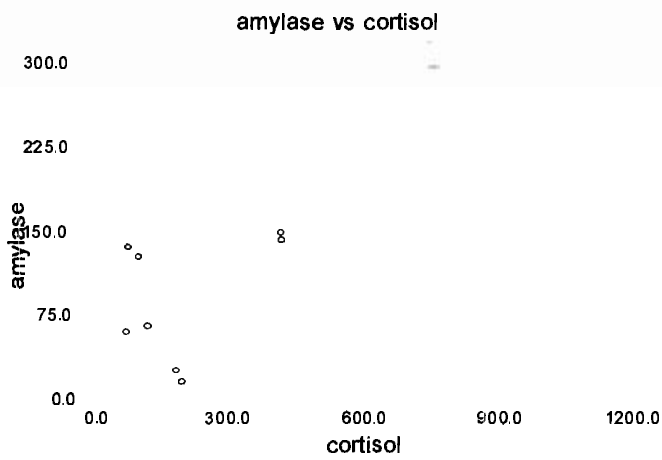


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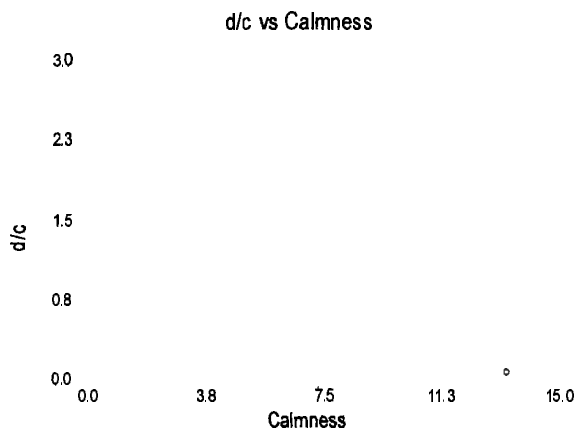
Figure 9: NT3 Drill Linear regressions in non trained participants. 9a-Cortisol and DHEA [R2=0.03, F(1, 38)=1.05; p=0.31], 9b- Amylase and cortisol [R2=0.0014 F(1, 38)=0.0378; p=0.8472], 9c- DHEA/cortisol ratio and calmness in [R2=0.0092 F(1, 12)=0.0833; p=0.7795]



9a

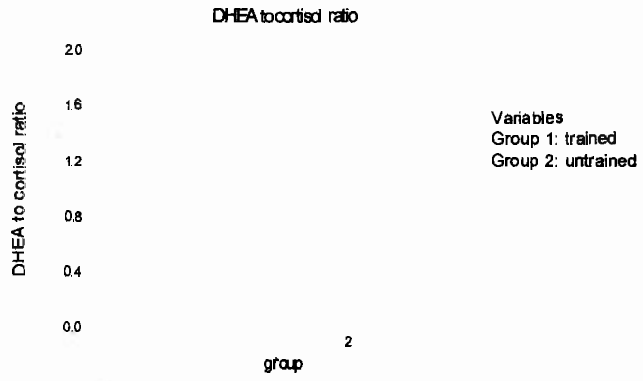


9b



9c

Figure 10: DHEA/Cortisol Ratio Trained vs. Non-Trained Subjects



Discussion

Figures 1-4 illustrate the salivary substances responses in four of distinct scenarios studied. Interestingly, as a whole these four scenarios all involved trained SWAT team professionals from different units, however only Figure 4 showed significant results. It should be noted that different individuals performing the same exercise or drill will react to their environment differently and therefore vast differences amongst individuals may lead to insignificant results. In Figure 4, repeated measure ANOVA reveals that there is a significant increase in salivary cortisol during the S6 team training exercise at 30 minutes after 150 lb body drag for 50 yards. In addition, there is also a significant increase in salivary amylase at 10 minutes after 150 lb body drag for 50 yards. It was fortunate that in this particular training exercise the samples were able to be obtained at exactly the time course for the expected peak in salivary amylase and salivary cortisol, thereby yielding the desired results.

Figure 5 represents individual responses from three different subjects during the S6 team drills. When separated out individually, it becomes quite clear exactly how every individual responds differently in a given situation. In Figure 5a, the subject showed no increase in salivary cortisol following the building entry exercise nor following the body drag exercises. Figure 5b depicts a different subject having an increase in cortisol immediate following building entry exercise and then the cortisol levels remaining high the rest of the drills. It should be noted, that this subject had recently become a member of the SWAT team and during the drill the subject accidentally shot the ‘good guy’ in the building entry exercise. The accidental shot may be a one possible cause for the cortisol levels remaining elevated throughout the rest of the training exercises. Figure 5c depicts a

third subject's different response to the same drills and it reveals the subject's cortisol levels increased 30 minutes after the body drag drill and interestingly there was also an increase in the DHEA levels at the same time of the cortisol increase. The rise in DHEA with cortisol levels may indicate a better trained individual since DHEA has been reported to be an anxiolytic in times of stress (Morgan et al., 2004). In addition, as seen in Figure 3 of the appendix subjects who had the lowest D/C ratio exhibited a stress response (tripling of cortisol levels) to building entry, which represents a form of psychological stress. In contrast, subjects who had the highest D/C ratio had no response (no increase in cortisol levels) to either building entry or body drag exercises or they only exhibited a response to the physical stress but not a response to the psychological stress.

Interestingly, there were significant correlations obtained when the data of all trained personnel from S3, S4, S5, S6, and M2 teams was pooled together, as seen in figures 7a-7c, however, this was not the case for the non trained personnel in the NT3 disaster drill. In the NT3 disaster drill there was a significant increase in salivary amylase as well as a significant decrease in salivary cortisol in the subjects as depicted in Figure 8. However, the linear regressions of the data revealed there was no significant correlation between cortisol and DHEA in the civilian subjects who participated in this drill (Figure 9a). In Figure 9c, the linear regression of the DHEA/cortisol ratio and calmness from the Thayer Activation Deactivation Checklist revealed there was no significant correlation. These two findings were in complete contrast with the trained personnel data, in which there was a significant correlation. Lastly, linear regressions of

the amylase and cortisol from the subjects in the drill had no significant correlation (Figure 9b).

It should be noted that the circadian rhythms of both cortisol and DHEA may confound the interpretations of the training sessions being performed at different times of day (Moldow et al., 2005). In a study performed by Young, et al., 2002, they found that DHEA levels remain constant throughout a 12 hour period. The study showed salivary cortisol levels significantly dipped from 16 to 3.6nm/l throughout the day, however the DHEA levels remained consistent averaging a mean of 2.5nm/l throughout the day. The highest peak for DHEA levels was 2.7nm/l at 8:00am and therefore the change in DHEA levels yielded no significant results throughout the day.

In contrast, other circadian rhythm studies indicate there is a peak of DHEA levels immediately upon awakening followed by a drop off in levels which then remains consistent throughout the rest of the day. A study performed by Hucklebridge et al., 2005 found that both cortisol and DHEA levels dropped off from high peaks throughout the day. On average upon awakening cortisol averaged 14nmol/l and 12 hours later it had consistently dropped off to 5nmol/l. DHEA on the other hand dropped off much faster from the peak of 1.4nmol/l upon wakening to 0.6nmol/l after just 3 hours and then remained at that level throughout the rest of day. Furthermore, in a study performed by Netherton et al., 2004 a dip in salivary cortisol levels from 330 to 43pg/100µl was observed throughout a 12 hour period and a decrease in salivary DHEA levels from 250 to 130pg/ml was also noted for the 12 hour period. Both Hucklebridge et al. and

Netherton et al. used the same methods of 3 hour time intervals for a period of 12 hours to gather there data.

Salivary amylase levels have been reported to peak approximately 10 minutes after a stressful event (Nater et al., 2005; Takai et al., 2004) and salivary cortisol levels are known to peak approximately 20 minutes (Gaab et al., 2003; Kirschbaum et al., 1999; Kudielka et al., 2004; Nater et al., 2005; Schommer, Hellhammer and Kirschbaum, 2003; Takai et al., 2004) after initiation of stress stimulus. Recent reports indicate that salivary amylase and cortisol do not correlate (Nater et al., 2006) which is consistent with the data reported in this research study. However, it is important to note the differences in the time courses when looking at the correlations. In addition, Nater et al., 2006 noted that salivary amylase levels do correlate with sympathetic tone which could be a further area of research in future studies.

The ratios of salivary DHEA to salivary cortisol were taken into account for all the scenarios examined as depicted in Figures 3 through 8 of the Appendix. For each exercise there were vast differences of DHEA/cortisol ratios among the subjects. In addition, as depicted in Figure 10, there was no difference between trained and non-trained subjects in terms of DHEA/cortisol ratios. This is consistent with the observations reported by Moldow et al., 2005. However, these findings are in contrast with the study performed by Morgan et al., 2004 that reported the ratio of DHEA-S to salivary cortisol levels correlate with military performance. However, as depicted in Figures 7a there was a significant linear correlation between salivary cortisol and DHEA in trained subjects in contrast to the lack of correlation observed in the non-trained group from NT3 (Figures

9a). It is not possible to rule out the effect of the different exercises on the subjects or the older age of the NT3 participants.

In the literature, there exist many correlations between salivary cortisol or amylase and the assessment tools such as VAS for stress (Kudielka et al., 2004), STAI (Takai et al., 2004), ADC (Nejtek, 2002). Peak amylase levels have been reported to correlate with STAI for trait (Takai et al., 2004). Furthermore, Vedhara et al., 2003 suggested that patterns of changes in cortisol may correlate with levels of anxiety.

When pooled together the data of all trained personnel, the trait of calmness from the Thayer ADC was correlated with the ratio of DHEA/cortisol. In contrast, this correlation was not depicted in the group that did not receive any training. It remains to be elucidated why there is a correlation between calmness and DHEA in trained vs non trained subjects.

The separation of the effect of physical exertion from psychological stress is an important goal reached by this project. Building entry provides us with essentially a psychological stress in contrast to body drag which is essentially a physical stress. It is interesting to note that salivary cortisol can increase in both cases and thus demonstrates the necessity of adding to the profile in order to differentiate physical from psychological stress. In future studies, the literature suggests dividing the subjects into high and low responders which might be a more effective way to analyze the data (Schommer, Hellhammer and Kirschbaum, 2003; Singh et al., 1999).

In addition, increasing the number of physiological parameters, such as measuring Neuropeptide Y (NPY) which is co-released with norepinephrine and Vasoactive

Intestinal Peptide (VIP) which is co-released with acetylcholine might be of interest. In 1986, Pernow et al. demonstrate the release of Neuropeptide Y during prolonged or intense stress. There are references in the literature indicating possible detection of these peptides in saliva (Dawidson et al., 1997; Dawidson et al., 1998; Naito, Itoh and Takeyama, 2003). The scenarios examined throughout this thesis repeated for neuropeptide Y will prove useful in further distinction between psychological and physical stress. Currently in the lab of Roberta L. Moldow, 2007 neuropeptide Y and VIP have been detected in saliva samples taken from students after final examinations and presentations. Therefore, it is probable to assume both neuropeptide Y and VIP should be able to be detected in saliva samples taken from trained personnel after stress. Currently preservation of the peptides in saliva and in extraction using solid phase techniques has been optimized for Neuropeptide Y and VIP.

In conclusion, we have demonstrated the feasibility of collecting saliva samples in the field during training exercises. It is particularly interesting to note that cortisol and DHEA release correlate in trained subjects but not nontrained subjects. It is important to note that there were vast differences amongst the individuals in the S3, S4, S5, S6 and M2 teams in their responses to the drills, where some showed no rise in salivary substances levels. In the future, data should be analyzed on an individual and group basis during the same scenarios. A possible recommendation that may be deduced is that more surprise elements should be introduced into the drills so as to make the training more effective for live chaotic situations since some subjects did not show any change in their salivary levels substances from the baseline both during and after the exercises were

performed.

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Appendix

Figure 4: S4 DHEA/Cortisol ratio

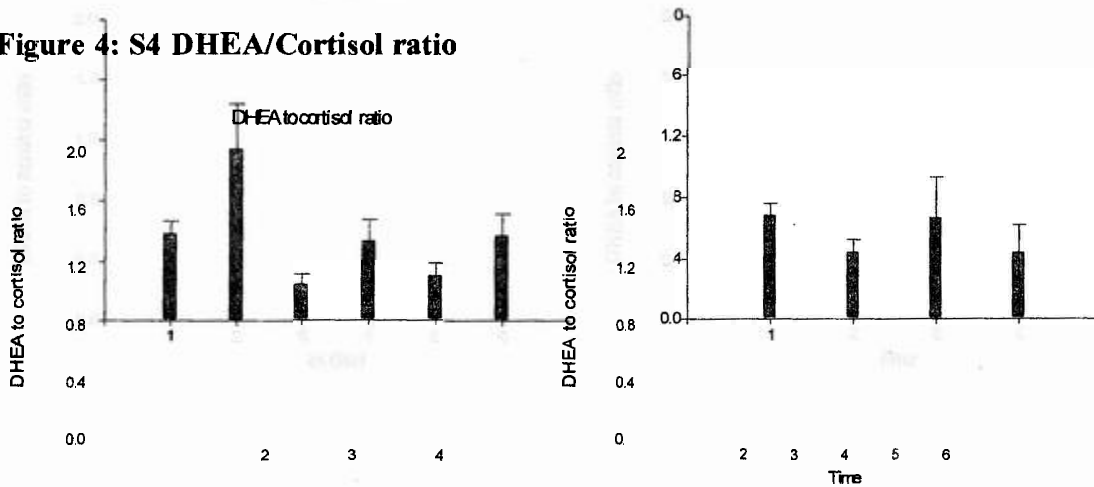


Figure 5: S5 DHEA/Cortisol ratio

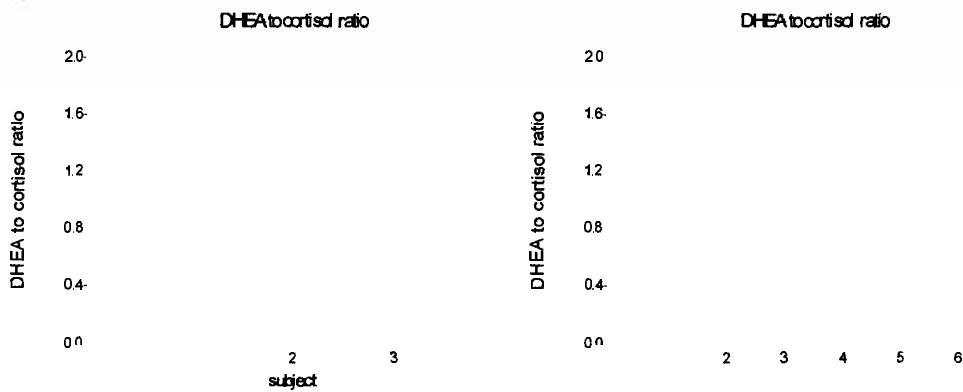


Figure 6: S6 DHEA/Cortisol ratio

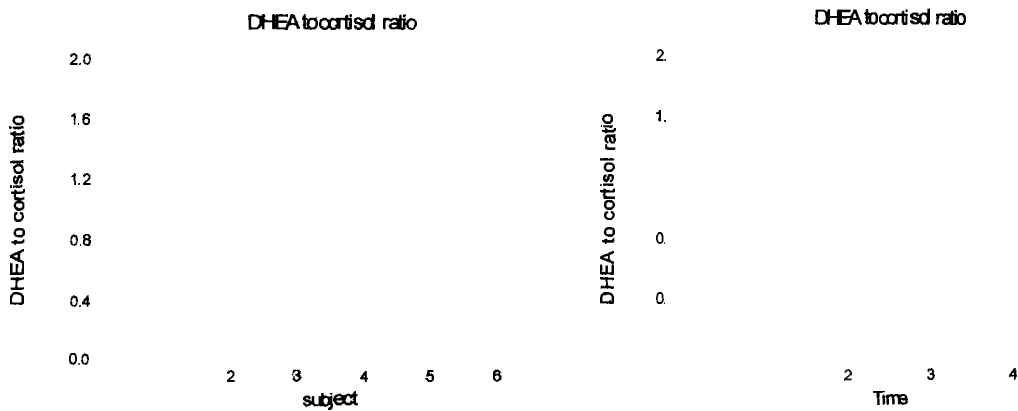




Figure 7: M2 DHEA/Cortisol ratio

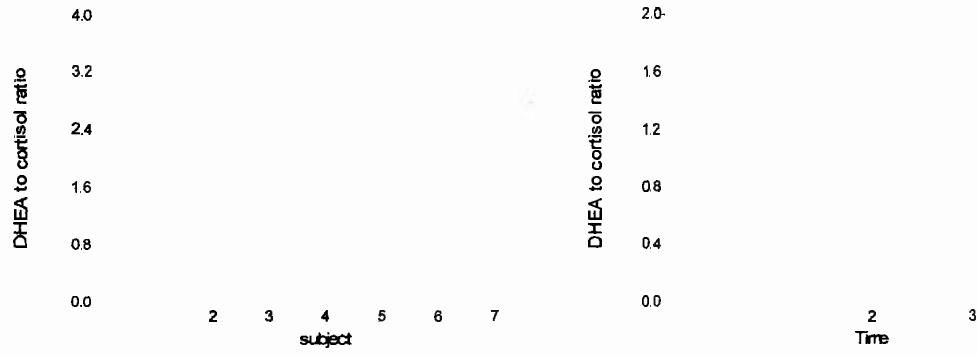


Figure 8: NT3 DHEA/Cortisol ratio

