ASSOCIATIONS BETWEEN CHANGES IN INTERLUKIN-8, NEUTROPHIL INTEGRIN EXPRESSION AND EXECUTIVE FUNCTION DURING ACUTE PHYSICAL AND PSYCHOLOGICAL STRESS

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Background

Lymphocyte associated antigen-1 (LFA-1/CD11a) and macrophage-1 antigen (Mac-1/CD11b) are cell adhesion molecules that mediate endothelial capture and intravascular crawling of leukocytes during inflammation. Chronically high levels of LFA-1 and Mac-1 expression on neutrophils, along with elevated concentrations of the neutrophil chemokine interleukin-8 (IL-8) have previously been associated with executive dysfunction in elderly individuals (1,2,4-6). However, whether acute perturbations to cellular or systemic mediators of neutrophil recruitment influence cognitive function is unclear. **Purpose:** To examine the relationships between changes in IL-8, LFA-1, Mac-1 and measures of executive function among young healthy individuals during a period of acute physical and psychological stress.

Methods

Participants

- Sixteen males (Age 23.1±3.5years, Body mass 80.9±11.9kg, Height) 174.4±3.8cm.

Sustained Military Operation (SUSOP) Physical and Psychological Stress

- Lecture based training
  - Participants underwent 10 hours of lecture-based training consisting of mission briefs and combat specific activities.
- Military specific physical tasks
  - Participants completed a series of physically demanding activities throughout the entire 24-hour period consisting of pull-ups, vertical jumps, 50-m litter carry, time to exhaustion assessments, and weighted ruck marches (Table 1).
- Sleep and calorie restriction
  - No sleep was permitted throughout the 24-hour time period.
  - A standard snack was provided following the blood draw at 0 Hours (0H) and again at hour 20. A standard meal ready to eat (MRE) was provided at hour 8.

Cognition and Psychological Stress Assessments

- Executive function and psychological stress was assessed at 0H and 24 hours (24H) using Automated Neuropsychological Assessment Metric (ANAM) software.
  - Throughput scores (TP), a measure of cognitive efficiency, were assessed for Mathematical Processing (MP), Matching to Sample (M2S), and Code Substitution Delayed (CSD) tasks.
  - Percent correct responses was assessed for the Go/No-Go (GNG) task.
  - Psychological stress was assessed using the ANAM concussion symptoms inventory (CSI) previously shown to be an efficacious tool to monitor psychological stress/declines (3).

Blood Draws

- Participants blood was drawn at 0H and 24H.

Cell Preparation

- Five mL of Human TruStain FcX FcR was added to 100 µL K2-EDTA-treated whole blood and was allowed to incubate for 10 minutes at room temperature in the dark. The following monoclonal antibodies were then added to the sample and allowed to incubate in the dark at room temperature for 15 minutes: 2.5-mL FITC-conjugated CD15, 5-mL PerCP-conjugated CD45, 5-mL PECy7-conjugated CD11a, 1.25-mL APC-Cy7-conjugated CD16, 5-mL BV605-conjugated CD14 and BV785-conjugated CD11b. Red blood cells were then lysed, and the remaining cells were washed in cell staining buffer. One microliter of eFlour450 fixable viability dye in 300µL phosphate buffered saline was then added to the cells and allowed to incubate for 30 minutes at 4°C. Cells were then washed a final time and resuspended in 300µL of cell staining buffer in preparation for analysis on the Novocyte 3000 cytometer.

Acute perturbations to **IL-8 and neutrophil expression of LFA-1 and Mac-1** are **not** associated with **declines in executive function**

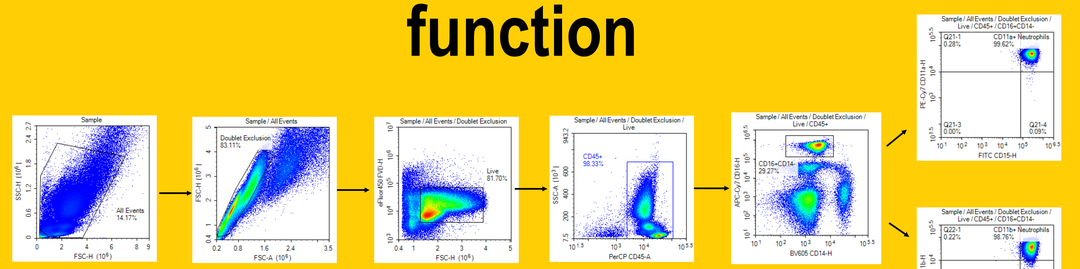

Figure 1. Gating procedure for identifying CD11a+ and CD11b + neutrophils. A doublet discrimination gate (forward scatter height [FSC-H] X forward scatter area [FSC-A] was first used to gate out doublets. Neutrophil populations were then subsequently determined as eFluor 450 FVD- (i.e. live), CD45+, CD16+/CD14-/CD15+ cells. Neutrophils were assessed for LFA-1 and Mac-1 integrin expression. Median fluorescence intensity (MFI) of the markers of interest was recorded, representing the expression per cell. Positivity was determined using quadrant gates relative to fluorescence minus one (FMO) controls.

Time	Event	Details
0800	0H	Hydration, blood draw, snack[a], ANAM™, and military specific tasks
0900		
1000		
1100		
1200	Lecture-based training, mission briefs, combat-specific activities	Introduction/basic views on leadership
1300		Introduction to insurgency
1400		Infrastructure of an insurgency
1500		MRE[b], questions
1600		Counter-guerilla operations
1700		Foreign internal defense
1800		Fundamentals of being a military advisor
1900		Reconnaissance
2000		
2100		Hydration and military Specific tasks
2200		
2300		
2400	Equipment fitting	
0100	Ruck March 1	
0200		Reconnaissance activity and report
0300	Lecture based training	
0400		Hydration, Snack[a]
0500	Lecture based training	Enter and clear a room (U.S. Army Battle Drill 6)
0600	Ruck March 2	
0700		
0800	24H	Hydration blood draw, and ANAM™

Table 1. Time course and details of the 24-hour SUSOP. ANAM™ = Automated Neuropsychological Assessment Metric. [a]Standardized diet bar (kcal: 190; protein: 7g; carbohydrates: 19g; fat: 13g; [b]Selection of different "Meals, Ready-to-Eat" (kcal: 1106±90; protein: 28±6g; carbohydrates: 167±19 g; fat: 33±11g).

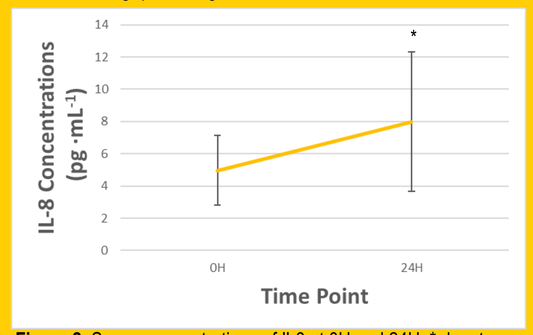

Figure 2. Serum concentrations of Il-8 at 0H and 24H. * denotes significant difference from 0H (p=.007). pg · mL^{-1} = picograms per milliliter.

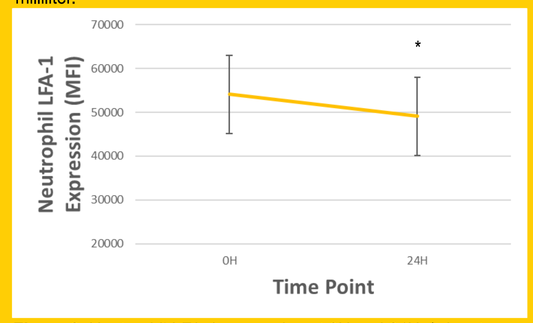

Figure 3. Neutrophil LFA-1 expression at 0H and 24H. * denotes significant difference from 0H (p=.004). MFI = Median Fluorescence Intensity.

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Methods cont.

Gating Procedure

- Shown in Figure 1, neutrophil integrin expression was analyzed using NOVOExpress software for the 0H and 24H time points.

Blood Assays

- Serum concentrations of IL-8 were assayed using a Luminex Human XL Magnetic Performance Assay (R&D Systems, Cat No. LUXLM000) on a MAGPIX instrument (CV = 1.38%).

Statistical Analysis

- A paired sample t-test was used to compare the changes in each variable at 0H and 24 hours.
- Delta scores for all values were calculated as the change from 0H to 24H.
- Stepwise multiple linear regression analysis was used to examine the relationships of the delta scores for IL-8, LFA-1, and Mac-1 with the delta scores of each executive function variable (GNG, MP, CSD, and M2S).
- An alpha value of $p \leq .05$ was considered statistically significant.

Results

- Frequency ($p<.001$) and severity ($p<.001$) of psychological stress symptoms increased significantly from 0H to 24H.
- IL-8 significantly increased from 0H to 24H ($p=.007$) (Figure 2).
- Neutrophil LFA-1 expression significantly decreased from 0H to 24H ($p<.004$) (Figure 3).
- Significant decreases in M2S ($p = .001$) and CSD TP scores ($p=.009$), as well as GNG percent correct ($p<.001$) were observed from 0H to 24H.
- No significant changes were seen in neutrophil Mac-1 expression ($p=0.84$).
- No significant associations between changes in IL-8, LFA-1, or Mac-1 and in measures of executive function were found.

Conclusion

Acute perturbations in serum IL-8 along with changes in degree of neutrophil integrin expression of LFA-1 and Mac-1 do not appear to be associated with impairments in executive function and increases in psychological stress seen during a SUSOP.

Practical applications: Perturbations to cellular and systemic mediators of neutrophil recruitment do not appear to influence executive function under conditions of acute physical and psychological stress. The influence of other components of the innate immune response on cognitive function during acute physical and psychological stress may warrant further investigation.

Acknowledgments

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations or trade names do not constitute an official Army endorsement of approval of the products or services.

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