

## Altered human neutrophil function in response to acute psychological stress.

Khanfer, R; Phillips, Anna; Carroll, Douglas; Lord, Janet

DOI:

[10.1097/PSY.0b013e3181e7fae8](https://doi.org/10.1097/PSY.0b013e3181e7fae8)

### *Document Version*

Early version, also known as pre-print

### *Citation for published version (Harvard):*

Khanfer, R, Phillips, A, Carroll, D & Lord, J 2010, 'Altered human neutrophil function in response to acute psychological stress.', *Psychosomatic Medicine*, vol. 72, no. 7, pp. 636-40.  
<https://doi.org/10.1097/PSY.0b013e3181e7fae8>

[Link to publication on Research at Birmingham portal](#)

### **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

Post-print, not final published version. Cite this article as: Khanfer, R., Phillips, A.C., Carroll, D., & Lord, J.M. (2010). Altered human neutrophil function in response to acute psychological stress. *Psychosomatic Medicine*, 72, 636-640. <http://dx.doi.org/10.1097/PSY.0b013e3181e7fae8>

## **Altered Human Neutrophil Function in Response to Acute Psychological Stress**

RIYAD KHANFER, MSC, ANNA C. PHILLIPS, PHD, DOUGLAS CARROLL, PHD, AND JANET M. LORD, PHD

From the School of Sport and Exercise Sciences (R.K., A.C.P., D.C.), University of Birmingham, United Kingdom; and School of Immunity and Infection (J.M.L.), The Medical School, University of Birmingham, United Kingdom.

Address correspondence and reprint requests to Riyad Khanfer, MSc, School of Sport and Exercise Sciences, University of Birmingham, Birmingham, United Kingdom B15 2TT. DOI: 10.1097/PSY.0b013e3181e7fae8

**Objective:** To examine the effects of an acute laboratory psychological stress task on neutrophil function, specifically phagocytosis of *Escherichia coli* and stimulated superoxide production in human neutrophils. There is mounting evidence that acute stress is associated with short-term increases in a number of immune indices.

**Methods:** Participants were 40 (n = 20 females) university students (mean age, 25.9 ± 4.56 years). Blood samples to determine neutrophil function by flow cytometry were taken at the end of resting baseline, during an acute stress task, and during recovery. The stress task was a 10-minute time-pressured mental arithmetic challenge with social evaluation.

**Results:** There was an acute increase in phagocytic ability,  $p = .047$ ,  $\eta^2p = 0.076$ , and a reduction of superoxide production,  $p = .026$ ,  $\eta^2p = 0.101$ , associated with the stress task relative to baseline.

**Conclusion:** These findings suggest that neutrophil bactericidal function may be sensitive to mental challenge tasks that provoke acute psychological stress. Further research is needed to replicate the observed psychological stress-induced changes in neutrophil function.

**Key words:** acute psychological stress, neutrophil, phagocytosis, superoxide production.

**SBP** = systolic blood pressure; **DBP** = diastolic blood pressure; **HR** = heart rate; **MFI** = mean fluorescent intensity; **EDTA** = ethylene diamine tetraacetic acid.

## **INTRODUCTION**

Acute and chronic psychological stress seem to have a differential impact on the immune system. Generally, chronic stress has been shown to have a negative effect on various aspects of immunity, whereas acute stress would seem to be immune enhancing (1). For example, acute stress has been shown to elicit lymphocytosis (2,3), increase natural killer cell cytotoxicity (4,5) and secretory immunoglobulin A secretion rate (6), stimulate aspects of the complement system (7), and boost vaccination responses (8). However, little is known about the effects of acute stress on human neutrophils and particularly their bactericidal function. This is perhaps surprising, given that neutrophils are a major component of innate immunity and are the dominant leukocyte in the circulation. These cells play a crucial role in killing invading pathogens, particularly rapidly dividing bacteria, and are key cellular components of the early phase of inflammatory responses (9). Neutrophils act quickly and without specificity and have the ability to produce a range of cytotoxic and bactericidal molecules, such as reactive oxygen species (superoxide production) and proteolytic

enzymes (10). Superoxide production is a means by which neutrophils eliminate pathogens and, thus, is key in combating infections by, for example, pneumococcal bacteria (11,12). However, extended neutrophil activation and superoxide production can cause local tissue inflammation and damage (13,14). Furthermore, tissue damage via neutrophil activation is implicated in the etiology and aggravation of chronic inflammatory disease (9,14). Finally, more recent research (9,15) suggested that neutrophils may also play a role in acquired immunity by acting as antigen presenting cells.

The effect of stress on neutrophils has been examined in a series of studies of asthma (16 – 18). Academic examinations served as the source of psychological stress, and neutrophil function was assessed before, during, and after an examination period, using the superoxide dismutase-inhibitable reduction of ferricytochrome C assay to measure superoxide release. These authors found some indication that examination stress increased superoxide release. However, it has been argued that a prolonged examination period constitutes chronic rather than acute stress; whereas a single examination elicited an increase in secretory immunoglobulin A, a prolonged period of academic examinations was associated with a decrease (19). More recently, a 15-minute laboratory stress task (Raven's progressive matrices) was used to examine neutrophil activation, using the nitro-blue tetrazolium assay that indicates oxidative capacity (20). The acute stress was shown to increase neutrophil activation relative to baseline. However, neither of these assays are optimally sensitive measures of neutrophil function. The superoxide dismutase-inhibitable reduction of ferricytochrome C assay only detects superoxide release that has escaped from the cell into the medium, which may represent only a fraction of the superoxide actually generated (21). The nitro-blue tetrazolium assay measures, by microscopic inspection of staining, the percentage of nonspecifically activated neutrophils (20). Both of these measures represent a very imperfect proxy of superoxide release. In contrast, the new generation of assays that exploit flow cytometry technology to measure the effects of stimulation accordingly offer a more accurate and versatile means of examining different aspects of neutrophil function. These include phagocytosis and superoxide production in response to a range of stimuli (22–24). In addition, physiologically relevant agents, such as opsonized bacteria, can be used to elicit the neutrophil response.

Consequently, the present study examined the effects of an acute laboratory psychological stress task on neutrophil function, specifically phagocytosis and stimulated superoxide production, using this new generation of assays. Self-report and cardiovascular measurements were also taken as a manipulation check, as the effects of the stress task on these variables are well characterized (3,25). It was hypothesized that acute stress would enhance neutrophil function relative to baseline.

## **METHODS**

### **Participants**

Forty healthy participants (n = 20 females, 20 males) were recruited from the student population at the University of Birmingham between May 2008 and June 2009. Date of birth, sex, ethnicity, parental occupation, health, and smoking status were determined by standard questionnaire. Participants' mean (standard deviation) age was 25.9 (4.56) years and mean body mass index was 23.3 (2.53) kg/m<sup>2</sup>. The majority (90%) reported their ethnicity as white and that

Post-print, not final published version. Cite this article as: Khanfer, R., Phillips, A.C., Carroll, D., & Lord, J.M. (2010). Altered human neutrophil function in response to acute psychological stress. *Psychosomatic Medicine*, 72, 636-640. <http://dx.doi.org/10.1097/PSY.0b013e3181e7fae8>

they were nonsmokers, and 74% reported that they came from nonmanual occupational households. Individuals were excluded if they had an acute infection or suffered from an immune disorder.

### **Design and Procedure**

The design was a within-subjects ABB design with baseline, stress, and recovery measurements in each participant. All participants attended a 2-hour laboratory acute psychological stress session. They were asked to avoid alcohol and vigorous exercise for 12 hours, and food and caffeine for 1 hour before testing. On the testing day, participants entered the laboratory at 9.30 AM. Their height and weight were measured. An intravenous cannula was then inserted in the antecubital region of one arm, and a blood pressure cuff was attached to the other arm. Post instrumentation and laboratory adaptation, there was a baseline resting period of 20 minutes, a 10-minute psychological stress task, and a 10-minute resting recovery period.

The study was approved by the local Medical Research Ethics Committee, and all participants provided their written informed consent.

### **Acute Stress Task**

Participants undertook an acute psychological mental stress task, the Paced Auditory Serial Addition Test, which has been shown to elicit cardiovascular changes in many studies (3,7,25). Participants were presented with a series of single-digit numbers by audio-CD and requested to add each number to the immediately preceding number and say the answer aloud at the same time retaining the second of the pair in memory for addition to the next number presented. The 10-minute task consisted of five consecutive and continuous periods of 2 minutes, with numbers presented at speeds of 50, 65, 75, 100, and 125 digits at presentation rates of 2.4, 2.0, 1.6, 1.2, and 0.8 seconds, respectively. All participants were given an explanation of the task and an opportunity to practice before its commencement. To add to the challenging nature of the task, elements of competition and social evaluation were added. The social evaluation was included to increase the provocative nature of the task (26). A false leader board was in view of the participant, who was instructed to try to beat the scores on the board. A confederate experimenter scored the answers overtly at the same time sitting on a high stool at a distance of 1 meter facing the participant. Participants were seated in front of a large television screen, which allowed them to see themselves live throughout the test, and they were instructed to look at the screen at all times. They were also informed that they were being videotaped and that videos were to be assessed by "independent body language experts"; no such assessment was made. The confederate experimenter also sounded a loud aversive buzzer once during the first five of every ten trials. Participants began the test with a score of 1000 points; for each incorrect or missed answer, 5 points were deducted from their score. Immediately after the task, they rated how stressful, difficult, and engaging the task was on a 7-point scale, where 0 equaled "not at all" and 6 equaled "extremely."

### **Blood Sampling and Immune Assays**

Blood samples were taken at three time points: at the end of the resting baseline; in the final minute of the stress task; and at the end of the recovery period. At each time point, a blood

Post-print, not final published version. Cite this article as: Khanfer, R., Phillips, A.C., Carroll, D., & Lord, J.M. (2010). Altered human neutrophil function in response to acute psychological stress. *Psychosomatic Medicine*, 72, 636-640. <http://dx.doi.org/10.1097/PSY.0b013e3181e7fae8>

sample was collected into one ethylenediaminetetraacetic acid tube and one heparin tube, which was placed immediately on ice. The ethylenediaminetetraacetic acid tubes were used to count lymphocyte and granulocyte numbers, using a Coulter A<sup>®</sup> ● T diff analyzer (Beckman Coulter, Inc., Miami, Florida). The heparin tubes were used to assess neutrophil function in whole blood, specifically bacteria-induced phagocytosis and oxidative burst (superoxide production) by flow cytometry.

Phagotest kits (Orpegen Pharma GmbH, Heidelberg, Germany) measured neutrophil phagocytosis of fluorescein isothiocyanate-labeled opsonised *Escherichia coli* (*E. coli*) bacteria. Whole blood (100  $\mu$ L) was incubated with 20  $\mu$ L of bacteria ( $2 \times 10^7$ ) at 37°C for 10 minutes, whereas a negative control sample remained on ice. For further details, see the manufacturer's test protocol (<http://www.icp.ucl.ac.be/mexp/phagotst.pdf>). Using a three-laser- Dako Cyan High Performance flow cytometer (Dako, Carpinteria, California) with Summit v 4.3 software, neutrophils, among other granulocytes, were gated on forward and side scatter characteristics; the mean fluorescent intensity (MFI) corresponding to the number of bacteria engulfed by a single cell was measured, using a blue-green excitation light 488 nm argon-ion laser.

The Phagoburst test (Orpegen Pharma GmbH) determined the mean fluorescence per neutrophil from the production of reactive oxidants with or without stimulation. In summary, four tubes contained 100  $\mu$ L of heparinized whole blood that was incubated for 10 minutes with one of the following stimuli: 20  $\mu$ L of the weak control stimulus; 20  $\mu$ L of opsonized *E. coli* ( $2 \times 10^7$  bacteria); 20  $\mu$ L of the strong control stimulus; or without a stimulus at 37°C. The formation of reactive oxidants was monitored by the oxidation of the substrate, from nonfluorescent dihydrorhodamine 123 to rhodamine, which produced green fluorescence, and was analyzed with by cytometry as described above. For further details, see the test protocol (<http://www.pvi.uni-bonn.de/burstst.pdf>).

### **Cardiovascular Measurements**

Blood pressure and heart rate were assessed using a semiautomatic oscillometric blood pressure monitor (Dinamap 1846, Critikon, Tampa, FL, USA) every 2 minutes: in minutes 12, 14, 16, and 18 of the 20-minute baseline period; and minutes 2, 4, 6, and 8 of the stress task period and recovery period.

### **Statistical Analyses**

To check the psychological impact of the stress task, ratings of stress, difficulty, and engagement were scrutinized. Blood pressure and heart rate measurements were averaged separately across baseline, stress task, and recovery periods and analyzed, using a repeated-measures analysis of variance (baseline, task, recovery), with the Greenhouse-Geisser correction. The same analytic strategy was applied to lymphocyte and granulocyte numbers and to the neutrophil function data. Partial eta-squared ( $\eta^2$ ) was used as a measure of effect size. Slight variations in degrees of freedom reflect occasional missing data.

## **RESULTS**

### Psychological and Cardiovascular Impact of the Stress Task

The mean (standard deviation) ratings for the Paced Auditory Serial Addition Test were 4.0 (1.19), 4.1 (1.34), and 4.6 (1.17) for stress, difficulty, and engagement, respectively. There was a significant main effect of time, for systolic blood pressure (SBP),  $F(2,78) = 119.53$ ,  $p < .001$ ,  $\eta^2 = 0.754$ , diastolic blood pressure (DBP),  $F(2,78) = 52.05$ ,  $p < .001$ ,  $\eta^2 = 0.572$ , and heart rate (HR),  $F(2,78) = 67.83$ ,  $p < .001$ ,  $\eta^2 = 0.635$ . These effects are shown in Figure 1.

### Lymphocyte and Granulocyte Numbers

The summary lymphocyte and granulocyte number data are also presented in Figure 1. For lymphocytes, there was a significant main effect of time,  $F(2,78) = 14.21$ ,  $p < .001$ ,  $\eta^2 = 0.267$ . For granulocytes, there was also a significant main effect of time,  $F(2,78) = 10.22$ ,  $p < .001$ ,  $\eta^2 = 0.208$ . In both cases, it is clear that the acute stress was associated with a transient increase in circulating leukocytes, which decreased after cessation of the task.

### Neutrophil Function

For neutrophil phagocytosis measurements, all samples showed that 100% of the neutrophils were able to phagocytose the *E. coli*, and differences seen were in the number of bacteria ingested (indicated by differences in MFI). Stimulated and unstimulated immune fluorescent intensities at the three time points are shown in Table 1. There was a significant main effect of time on neutrophil phagocytic capacity,  $F(2,78) = 3.20$ ,  $p = .047$ ,  $\eta^2 = 0.076$ , as shown in Figure 2. Thus, the stress task seemed to have enhanced phagocytosis. For superoxide production, when granulocytes were stimulated by *E. coli*, the opposite pattern of effects emerged. There was a significant reduction in superoxide production across the session,  $F(2,72) = 4.03$ ,  $p = .026$ ,  $\eta^2 = 0.101$ , as illustrated in Figure 2.

Neutrophil reactivity was not associated with gender (women: mean, 6.30; men: mean, 5.66,  $p = .91$ , for phagotest; and women: mean, -10.40; men: mean, -1.46,  $p = .41$ , for phagoburst to *E. coli*), body mass index ( $r = .16$ ,  $p = .33$ , for phagotest, and  $r = .02$ ,  $p = .88$ , for the phagoburst to *E. coli*), or smoking status (current smokers: mean, 8.37; nonsmokers: mean, 5.71,  $p = .79$ ). Baseline neutrophil function was not related to neutrophil reactivity with the exception of superoxide production to *E. coli*, where there was a negative association and baseline accounted for 27% of the variation in neutrophil reactivity,  $r = -.48$ ,  $p = .003$ .

### Sensitivity Analysis

Additional analyses were run, using phagotest and phagoburst values with the subtraction of the unstimulated MFIs in order to examine any influence of psychological stress on unstimulated neutrophil function. For phagocytosis, the main effect of time was attenuated,  $F(2,74) = 1.98$ ,  $p = .14$ ,  $\eta^2 = 0.51$ . For phagoburst to *E. coli*, the significant time effect remained,  $F(2,72) = 4.03$ ,  $p = .026$ ,  $\eta^2 = 0.101$ . However, there were no significant main effects of time on neutrophil function in the control/unstimulated tubes.

### Association Between Cardiovascular and Neutrophil

### Activity During Acute Stress

There were no significant correlations between cardiovascular activity during stress and neutrophil function: SBP:  $r = -.05, p = .75$ ; DBP:  $r = .06, p = .67$ ; HR:  $r = -.03, p = .83$  for phagotest; and SBP:  $r = .19, p = .24$ ; DBP:  $r = .22, p = .17$ ; HR:  $r = -.007, p = .96$  for phagoburst to *E. coli*.

### DISCUSSION

The self-report and cardiovascular data confirmed that the acute stress task provoked emotional and physiological responses. Similarly, as expected, there was an acute stress induced increase in lymphocyte and granulocyte numbers. The effects of stress on neutrophil function, however, were more complex. For neutrophil phagocytosis, there was an acute increase in phagocytosis associated with the stress task. This was attenuated in analyses where the MFI of the control tubes are incorporated into the dependent variables, but this could reflect a reduction in degrees of freedom, as there was no significant effect of time on phagocytic ability in the control/unstimulated tubes. However, the acute stress task was associated with reduction in neutrophil superoxide production when stimulated with *E. coli*.

Given the preliminary nature of this study, further research is needed to replicate the observed psychological stress-induced changes in neutrophil function. However, the present study is the first we know of to examine the effects of psychological stress in humans, using the new generation of assays that measure neutrophil function via flow cytometry techniques and in response to physiologically relevant stimuli. Accordingly, the existing literature offers little help in establishing a precedent for our findings. However, the effects of the acute stress task on neutrophil phagocytosis observed here are consistent with the increase in neutrophil activation to a short mental stressor reported previously (20). Superoxide production in the present study decreased in contrast to the increase with examination stress shown in previous reports (17,18). However, it should be noted that academic examination stress over several weeks cannot be considered to be acute psychological stress. Furthermore, studies evaluating neutrophil function and regulation have shown that phagocytosis and intracellular bacterial killing are independent activities of neutrophil-mediated antibacterial defense (27,28). Accordingly, it is feasible that phagocytosis and stimulated superoxide production could be differentially affected by acute stress.

Neutrophil function was differentially sensitive to acute psychological stress in the present study. However, cardiovascular activity did not seem to be related to neutrophil function. This is surprising, given that neutrophils have been shown to have  $\alpha$ - and  $\beta$ -adrenergic receptors (29,30), and changes in adrenalin and noradrenalin have been shown to relate to circulating neutrophil numbers (30). However, it is possible that the effects observed in the present study are mediated by other stress pathways, such as the hypothalamic-pituitary-adrenal axis. For example, others (31) have shown in an animal model study that dexamethasone (acting as a cortisollike synthetic stimulant) can significantly decrease neutrophil superoxide production in cats. In vitro studies (32,33) of human neutrophils have also shown that cortisol is able to suppress neutrophil superoxide generation directly. Consequently, the declining superoxide production to *E. coli* observed here might relate to the effects of the acute stress task on cortisol levels, although measurements of serum cortisol would be needed to confirm this. It is difficult to elucidate the exact mechanisms behind the

different phagocytosis and superoxide production results observed here, given that the regulation of neutrophil function is under the control of many different receptors for a variety of cytokines (27), which are also differentially affected by stress (34,35).

The present study might be considered to have some limitations. First, the sample size was fairly modest. However, it was of the same order of magnitude as previous studies of stress and human neutrophil function (17,20,36) and larger than studies examining the effects of acute psychological stress on other immune outcomes (4,7). Furthermore, study numbers were reasonable, given the temporal demands of the current protocol, with cannulation, time of testing session, and same-day flow cytometry assays. Second, despite the advantages of the flow cytometry assays, they are sensitive to diurnal and day-to-day variation (22,37). However, such effects were minimized by testing at the same time of day and the using internal controls for each assay. Finally, the design would have been stronger with an appropriate no stress control group to unequivocally discount alternative explanations for our findings, such as the effects of cannulation. However, with our pretask baseline, the most parsimonious explanation for the pattern observed is perturbation by acute stress. Neutrophils are the first cells to infiltrate sites of wounding/inflammation; thus, any effect of cannulation would have been evident at baseline, although an additional baseline measure would have confirmed a lack of cannulation effect.

In conclusion, the present study revealed the effects of psychological stress on neutrophil function. The acute stress task elicited a short-term elevation in phagocytosis and a reduction of superoxide production. The present results must be regarded as preliminary, and replication is necessary. Given that neutrophil function has been shown to decline with age (38), future studies might consider the effects of age on the effects of acute psychological stress. In addition, the underlying mechanisms of acute stress effects on neutrophil function warrant further investigation.

## REFERENCES

1. Segerstrom SC, Miller GE. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychol Bull* 2004;130:601–30.
2. Anane LH, Edwards KM, Burns VE, Drayson MT, Riddell NE, van Zanten JJ, Wallace GR, Mills PJ, Bosch JA. Mobilization of gammadelta T lymphocytes in response to psychological stress, exercise, and betaagonist infusion. *Brain Behav Immun* 2009;23:823–9.
3. Willemsen G, Carroll D, Ring C, Drayson M. Cellular and mucosal immune reactions to mental and cold stress: associations with gender and cardiovascular reactivity. *Psychophysiology* 2002;39:222–8.
4. Bosch JA, Berntson GG, Cacioppo JT, Marucha PT. Differential mobilization of functionally distinct natural killer subsets during acute psychologic stress. *Psychosom Med* 2005;67:366 –75.
5. Sgoutas-Emch SA, Cacioppo JT, Uchino BN, Malarkey W, Pearl D, Kiecolt-Glaser JK, Glaser R. The effects of an acute psychological stressor on cardiovascular, endocrine, and cellular immune response: a prospective study of individuals high and low in heart rate reactivity. *Psychophysiology* 1994;31:264 –71.

Post-print, not final published version. Cite this article as: Khanfer, R., Phillips, A.C., Carroll, D., & Lord, J.M. (2010). Altered human neutrophil function in response to acute psychological stress. *Psychosomatic Medicine*, 72, 636-640. <http://dx.doi.org/10.1097/PSY.0b013e3181e7fae8>

6. Ring C, Drayson M, Walkey DG, Dale S, Carroll D. Secretory immunoglobulin A reactions to prolonged mental arithmetic stress: inter-session and intra-session reliability. *Biol Psychol* 2002;59:1–13.
7. Burns VE, Edwards KM, Ring C, Drayson M, Carroll D. Complement cascade activation after an acute psychological stress task. *Psychosom Med* 2008;70:387–96.
8. Edwards KM, Burns VE, Adkins AE, Carroll D, Drayson M, Ring C. Meningococcal A vaccination response is enhanced by acute stress in men. *Psychosom Med* 2008;70:147–51.
9. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* 2006;6:173–82.
10. Nauseef WM. How human neutrophils kill and degrade microbes: an integrated view. *Immunol Rev* 2007;219:88–102.
11. Segal AW. How superoxide production by neutrophil leukocytes kills microbes. *Novartis Found Symp* 2006;279:92–8.
12. Segal AW. How neutrophils kill microbes. *Annu Rev Immunol* 2005;23: 197–223.
13. Cossette C, Patel P, Anumolu JR, Sivendran S, Lee GJ, Gravel S, Graham FD, Lesimple A, Mamer OA, Rokach J, Powell WS. Human neutrophils convert the sebum-derived polyunsaturated fatty acid sebaleic acid to a potent granulocyte chemoattractant. *J Biol Chem* 2008;283:11234–43.
14. Mayadas TN, Tsokos GC, Tsuboi N. Mechanisms of immune complex-mediated neutrophil recruitment and tissue injury. *Circulation* 2009;120: 2012–24.
15. Cowburn AS, Condliffe AM, Farahi N, Summers C, Chilvers ER. Advances in neutrophil biology: clinical implications. *Chest* 2008;134: 606–12.
16. Kang DH, Coe CL, Karaszewski J, McCarthy DO. Relationship of social support to stress responses and immune function in healthy and asthmatic adolescents. *Res Nurs Health* 1998;21:117–28.
17. Kang DH, Coe CL, McCarthy DO. Academic examinations significantly impact immune responses, but not lung function, in healthy and well-managed asthmatic adolescents. *Brain Behav Immun* 1996;10:164–81.
18. Kang DH, Coe CL, McCarthy DO, Ershler WB. Immune responses to final exams in healthy and asthmatic adolescents. *Nurs Res* 1997;46:12–9.
19. Bosch JA, Carroll D. Stress and mucosal secretory immunity. In: Fink E, editor. *Encyclopaedia of stress*, 2nd ed. London: Elsevier, 2007.
20. Ellard DR, Castle PC, Mian R. The effect of a short-term mental stressor on neutrophil activation. *Int J Psychophysiol* 2001;41:93–100.
21. Kang DH, McCarthy DO. The effect of psychological stress on neutrophil superoxide release. *Res Nurs Health* 1994;17:363–70.

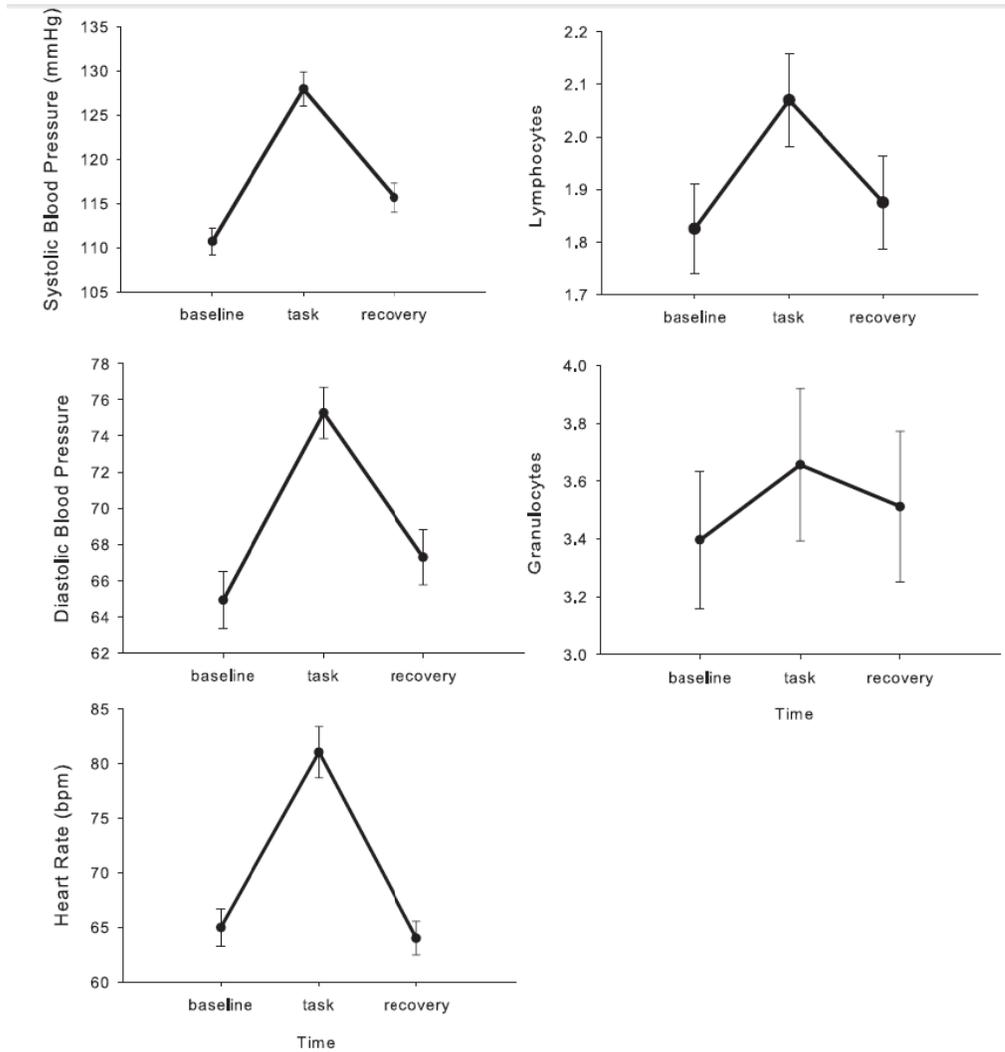
Post-print, not final published version. Cite this article as: Khanfer, R., Phillips, A.C., Carroll, D., & Lord, J.M. (2010). Altered human neutrophil function in response to acute psychological stress. *Psychosomatic Medicine*, 72, 636-640. <http://dx.doi.org/10.1097/PSY.0b013e3181e7fae8>

22. Kampen AH, Tollersrud T, Larsen S, Roth JA, Frank DE, Lund A. Repeatability of flow cytometric and classical measurement of phagocytosis and respiratory burst in bovine polymorphonuclear leukocytes. *Vet Immunol Immunopathol* 2004;97:105–14.
23. Lehmann AK, Sornes S, Halstensen A. Phagocytosis: measurement by flow cytometry. *J Immunol Methods* 2000;243:229–42.
24. Panasiuk A, Wysocka J, Maciorkowska E, Panasiuk B, Prokopowicz D, Zak J, Radomski K. Phagocytic and oxidative burst activity of neutrophils in the end stage of liver cirrhosis. *World J Gastroenterol* 2005;11:7661–5.
25. Phillips AC, Gallagher S, Carroll D. Social support, social intimacy, and cardiovascular reactions to acute psychological stress. *Ann Behav Med* 2009;37:38–45.
26. Dickerson SS, Kemeny ME. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull* 2004;130:355–91.
27. Pechkovsky DV, Potapnev MP, Zalutskaya OM. Different patterns of cytokine regulation of phagocytosis and bacterial killing by human neutrophils. *Int J Antimicrob Agents* 1996;7:33– 40.
28. Vollebregt M, Hampton MB, Winterbourn CC. Activation of NF-kappaB in human neutrophils during phagocytosis of bacteria independently of oxidant generation. *FEBS Lett* 1998;432:40–4.
29. Gosain A, Gamelli RL, DiPietro LA. Norepinephrine-mediated suppression of phagocytosis by wound neutrophils. *J Surg Res* 2009;152:311– 8.
30. Abraham E, Kaneko DJ, Shenkar R. Effects of endogenous and exogenous catecholamines on LPS-induced neutrophil trafficking and activation. *Am J Physiol* 1999;276:L1– 8.
31. Hoffmann-Jagielska M, Winnicka A, Jagielski D, Lechowski R. Influence of dexamethasone on some cellular aspects of the immune system incats. *Vet Res Commun* 2003;27:643–52.
32. Bekesi G, Kakucs R, Varbiro S, Racz K, Sprintz D, Feher J, Szekacs B. In vitro effects of different steroid hormones on superoxide anion production of human neutrophil granulocytes. *Steroids* 2000;65:889 –94.
33. Butcher SK, Killampalli V, Lascelles D, Wang K, Alpar EK, Lord JM. Raised cortisol:DHEAS ratios in the elderly after injury: potential impact upon neutrophil function and immunity. *Aging Cell* 2005;4:319 –24.
34. Steptoe A, Willemsen G, Owen N, Flower L, Mohamed-Ali V. Acute mental stress elicits delayed increases in circulating inflammatory cytokine levels. *Clin Sci (Lond)* 2001;101:185–92.
35. Yamakawa K, Matsunaga M, Isowa T, Kimura K, Kasugai K, Yoneda M, Kaneko H, Ohira H. Transient responses of inflammatory cytokines in acute stress. *Biol Psychol* 2009;82:25–32.
36. Kang DH, Fox C. Neuroendocrine and leukocyte responses and pulmonary function to acute stressors. *Ann Behav Med* 2000;22:276–85.

37. Hirt W, Nebe T, Birr C. [Phagotest and Bursttest (Phagoburst), test kits for study of phagocyte functions]. *Wien Klin Wochenschr* 1994;106:250–2.

38. Lord JM, Butcher S, Killampali V, Lascelles D, Salmon M. Neutrophil ageing and immunesenescence. *Mech Ageing Dev* 2001;122:1521–35.

### Tables and Figures



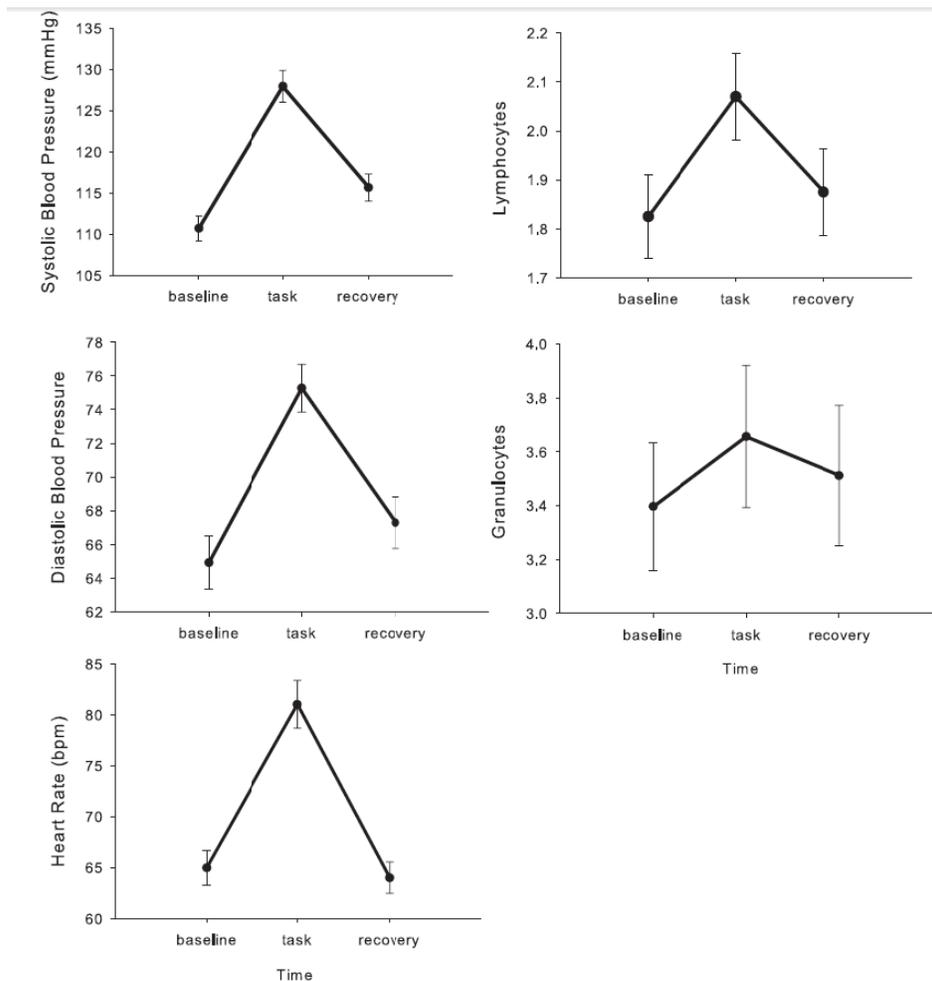
**Figure 1.** Cardiovascular activity and lymphocyte and granulocyte numbers during baseline, stress, and recovery (*error bars* are standard error of the mean).

**TABLE 1. Measurements of Mean Fluorescent Intensities of Unstimulated and Stimulated Neutrophil at Baseline, Task, and Recovery**

Stimulation	Mean (SD)		
	Baseline	Task	Recovery
Phagocytosis unstimulated	4.5 (2.18)	4.8 (2.46)	4.8 (2.26)
Phagocytosis <i>Escherichia coli</i>	143.0 (55.17)	150.3 (56.68)	149.9 (59.85)
Phagoburst unstimulated	6.2 (1.57)	6.1 (1.46)	6.2 (1.43)
Phagoburst <i>E. coli</i>	182.7 (87.40)	177.2 (75.98)	169.4 (72.22)

SD = standard deviation.

**Table 1.** Measurements of Mean Fluorescent Intensities of Unstimulated and Stimulated Neutrophil at Baseline, Task, and Recovery



**Figure 2.** Neutrophil phagocytosis of *Escherichia coli* and superoxide production on stimulation with *E. coli* during baseline, stress, and recovery (error bars are standard error of the mean).