View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by CURVE/open

Coventry University

Coventry University Repository for the Virtual Environment (CURVE)

Author names: Shelton-Rayner, G.K. , Mian, R. , Chandler, S. , Robertson, D. and Macdonald, D.W.

Title: Quantitative physiological assessment of stress via altered immune functioning following interaction with differing automotive interface technologies.

Article & version: Post-print version

Original citation & hyperlink:

Shelton-Rayner, G.K. , Mian, R. , Chandler, S. , Robertson, D. and Macdonald, D.W. (2011) Quantitative physiological assessment of stress via altered immune functioning following interaction with differing automotive interface technologies. *International Journal of Human-Computer Interaction* , volume 27 (9): 900-919. http://dx.doi.org/10.1080/10447318.2011.555312

Publisher statement:

This is an electronic version of an article published in the International Journal of Human-Computer Interaction, 27 (9), 900-919. The International Journal of Human-Computer Interaction is available online http://www.tandfonline.com/doi/abs/10.1080/10447318.2011.555312

Copyright © and Moral Rights are retained by the author(s) and/ or other copyright owners. A copy can be downloaded for personal non-commercial research or study, without prior permission or charge. This item cannot be reproduced or quoted extensively from without first obtaining permission in writing from the copyright holder(s). The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the copyright holders.

This document is the author's final manuscript version of the journal article, incorporating any revisions agreed during the peer-review process. Some differences between the published version and this version may remain and you are advised to consult the published version if you wish to cite from it.

Available in the CURVE Research Collection: October 2012

http://curve.coventry.ac.uk/open

Quantitative physiological assessment of stress via altered immune functioning following interaction with differing automotive interface technologies.

Graham K. Shelton-Rayner[1], Rubina Mian[2], Simon Chandler[3], Duncan Robertson, and David W. Macdonald[4]*

[1]Department of Biomolecular and Sport Sciences, Faculty of Health and Life Sciences, Coventry University, Cox St, Coventry, CV1 5FB, United Kingdom.

[2]Oxford MediStress Ltd., Suite 125, 27 Colemore Row, Birmingham, B3 2EW, United Kingdom.

[3]Jaguar and Land Rover Research Department, Jaguar Cars Limited, Engineering Centre, Whitley, Coventry, CV3 4LF, United Kingdom.

[4]Wildlife Conservation Research Unit, Zoology Department, University of Oxford, The Recanati-Kaplan Centre, Tubney House, Abingdon Road, Tubney, Oxfordshire, OX13 5QL, United Kingdom.

This research was sponsored by Jaguar Cars Limited, Engineering Centre, Whitley, Coventry, CV3 4LF, United Kingdom.

*** Correspondence concerning this article should be addressed to**
Professor David W. Macdonald
Wildlife Conservation Research Unit, Zoology Department, University of Oxford, The Recanati-Kaplan Centre, Tubney House, Abingdon Road, Tubney, Oxfordshire, OX13 5QL, United Kingdom.

Telephone Number: +44 (0)1865 393100
Fax Number: +44 (0)1865 393101
Email Address: david.macdonald@zoo.ox.ac.uk

Abstract

Technology can enhance or diminish a user's psycho-physiological stress level; the ability to quantify these responses can help evaluate and refine design. The capability of drivers to accomplish basic tasks utilising differing sensory modalities whilst simultaneously maintaining lane discipline within a computer-simulated environment was assessed. 15 healthy subjects provided capillary blood samples before and after using three human-machine interface designs - touch-screen, voice control and multimodal. Using a chemiluminescent technique termed Leukocyte Coping Capacity, the ability of leukocytes to produce reactive oxygen species *in vitro* was assessed. Significant post-stressor changes in leukocyte activity of varying magnitude were observed following the use of all interfaces; with the multimodal interface provoking the most pronounced response and voice control the least. Although still requiring further research, the results support the proposition for using immune responsiveness as a means for quantifying psychological stress during assessment of ergonomic design and psycho-physiological and social interaction.

Keywords: Chemiluminescence, Leukocytes, Luminol, Psycho-physiological Stress, Reactive Oxygen Species

Introduction

Quantitative assessment of the physiological changes associated with psychological stress – a threat which would not require a physiological response which elicits physiological consequences (Segerstrom and Miller, 2004) - serves to increase understanding of an organism's interaction with its environment. Even mild psychological stressors can reduce the effectiveness of the immune response, thus leading to an increased risk of infection or disease (Boscarino *et al*., 1999; Dhabhar *et al*., 1996). Short-term psychological stress characterised by the taxonomic classification proposed by Elliot and Eisdorfer (1982), defined as an acute time limited event that promotes confusion or feelings of distress that consequently trigger both a psychological and/or physiological response, including academic examinations (Kang *et al*., 1996; Maes *et al*., 1998a) or psychological confusion of a magnitude encountered as part of daily life, lasting only minutes, can produce demonstrable physiological alterations to heart rate, blood pressure and the activation state of specific classes of leukocyte, notably neutrophils (Mian *et al*., 2003; Shelton-Rayner *et al*., 2010).

Epidemiological studies demonstrate that individuals who experience increased psychological stress are more susceptible to opportunistic infection (Clover *et al*., 1989; Galinowski, 1997). Rodriguez-Galan *et al*. (2001) demonstrated how the opportunistic fungal disease *Candida albicans* proliferated in stressed rather than non-stressed individuals. It therefore follows that exposure to acute psychological stressors, even for limited periods at magnitudes below perception threshold, can cause increases in both frequency and activation of circulating leukocytes (Maes *et al*., 1998b; Mian *et al*., 2003).

The ability to quantify psychological stress poses an intriguing problem with profound practical implications. Methodologies employing self assessment of perceived stress, for example Likert scales (Gaither *et al*., 2008, Hassinger *et al*., 1999), despite being subjective, are often used to provide a basic means of assessing changes in perceived stress. Traditional objective measures of stress include physiological assessment of heart rate variability, eye blink, changes in respiration, cardiovascular activity, electroencephalogram (EEG), event related potentials (ERP's), cerebral metabolism and blood flow (Byrne and Parasuraman, 1996; Iqbal *et*

al., 2005; Scerbo *et al*., 2001; Sharit and Salvendy, 1982) and changes in the plasma concentrations of specific stress hormones (cortisol and adrenaline) (Clow *et al*., 2006; Okutsu *et al*., 2005). The effectiveness of such techniques is limited as studies have shown inter and intra variability, as a consequence of differences in experimental conditions, the task itself, and from considerable biological variation and influence by feedback mechanisms designed to maintain homeostasis, all of which introduce uncertainty to comparison between individuals and populations particularly when assessing short-term stressors. The various advantages and disadvantages of each technique have been reviewed extensively (Taylor *et al.*, 2007). The hormonal changes in the plasma concentrations of specific stress hormones (cortisol and adrenaline) (Clow *et al*., 2006; Okutsu *et al*., 2005) are impractical when real time measurements are required. In addition, many of the aforementioned techniques require extensive preparation and calibration evoking significant temporal and financial implications with their use. For example a study conducted by Clow *et al*. (2006) described how an enzyme-linked immunosorbant assay (ELISA) was used to assess salivary cortisol concentration during periods of intense physical exertion. ELISAs are complex multi-step procedures requiring hours before results are known, whereas LCC analysis utilises a far simpler protocol which yields results in minutes at a fraction of the cost. Furthermore, cortisol release is subject to diurnal rhythm so differences in the rate of release are often confounded by the time of day at which the samples are taken (Dokoumetzidis *et al*., 2002). EEG, ERP and eye blink require lab based equipment that is not portable or easily affordable and is not ideal if minimally evasive detection is required.

The stress response is a complex combination of metabolic, neuroendocrine and behavioural changes. Psychological stress reduces the effectiveness of the immune system, thus leading to an increased risk of infection or disease (Boscarino and Chang, 1999; Dhabhar *et al*., 1996). Even short-term psychological stressors such as academic examinations (Kang *et al*., 1996; Maes *et al*., 1998a) can produce demonstrable physiological changes in the reactivity of specific classes of leukocyte, notably neutrophils (Mian *et al*., 2003; Shelton-Rayner *et al*., 2010).

Leukocyte activation (primarily neutrophils) results in the synthesis and release of an array of mediators and lytic agents, including reactive oxygen species (ROS), whose purpose is the denaturation and removal of invading pathogens (Ruotsalainen *et al*., 1995). The non-specific nature of the response means that the

potential exists for collateral damage to occur to surrounding healthy tissue and organs, which has been linked to the aetiology of numerous disease states (Boxer and Smolen, 1998; Segerstrom and Miller, 2004). Leukocyte activation (primarily neutrophils) can also occur following psychological stimulation. In 2002, Atanackovic *et al*. demonstrated how exposure to a putatively stressful event resulted in a significant reduction in ROS production, compared to control. More recently, studies conducted by ourselves and others, utilising chemiluminescent assay of PMA-induced ROS production by leukocytes, has demonstrated a quantitative link between psychological confusion / anxiety and immune-competency (McLaren *et al*., 2003; Mian *et al*., 2003; Montes *et al*., 2004 and 2003; Shelton-Rayner *et al*., 2010; Shelton-Rayner, 2009). The Leukocyte Coping Capacity (LCC) technique involves measuring the ability of leukocytes to produce a respiratory burst following chemical challenge, assayed in terms of Reactive Oxygen Species (ROS) and calibrated through the emission of photons via their interaction with Luminol (McLaren *et al*., 2003).

Due to their systemic distribution and responsiveness to the numerous signals of stress (illustrated in Figure 1), the LCC test utilises the body's leukocytes (primarily, but not exclusively neutrophils) to provide a bio-indication of the multifaceted effects of stress (Shelton-Rayner *et al*., 2010). Leukocytes (primarily, but not exclusively neutrophils) have over 250 different receptors (Mian *et al*., 2005) which can respond to a diverse range of factors, all of which are sensitive to stress. These include: endocrine factors in the plasma, cytokines and factors released from other cells, both circulating and non-circulating cells such as endothelial cells, changes in erythrocyte haemodynamics, changes in blood biochemistry, and changes in the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. The constant exposure to each of these stimuli pertains to their effectiveness as stress indicators. The Coping Capacity of Leukocytes (LCC), i.e. their ability to respond to an external stimulator and produce reactive oxygen species, will be affected by the immediate external environment in the blood. Leukocytes (mainly neutrophils) which have been exposed to stressors within the body will have a reduced capacity to produce reactive oxygen species in response to an external stimulator (e.g. PMA). This is the underlying technical foundation of the test (Mian *et al*., 2005).

The LCC test is a physiologically relevant blood test for objectively assessing the effect of stress. The physiological relevance is convincing since leukocytes remain suspended in whole blood which permits dynamic interaction with

surrounding erythrocytes and cell-cell interaction within and between different leukocyte cohorts; both have the potential to dramatically affect leukocyte responsiveness. Interaction and exposure to other leukocytes, hormones and cytokines released from surrounding cells can affect leukocyte responsiveness via altered shear stress and expression of cell surface receptors. As cellular integrity is maintained, the potential disruption to cell signalling pathways is limited. The LCC technique also avoids centrifugation, a process known to affect cell reactivity, and also 'plating out' cells on glass slides - as used in the NBT test (Tsukamoto *et al*., 2002). The cells are stimulated *in vitro* with PMA and their superoxide producing capacity is measured in real time. As leukocytes release reactive oxygen species in response to stress (McLaren *et al*., 2003), the stimulation allows us to evaluate the leukocyte's (predominantly, but not exclusively neutrophils) capacity to generate further reactive oxygen species. This takes into account the exposure to other stress mediators and makes the test sensitive to true stress; the reactivity of the cells is not altered by deliberate manipulation.

Every day people knowingly expose themselves to forms of psychological stressor, from caring for a sick relative (Kiecolt-Glaser *et al*., 1995) to watching a horror film (Mian *et al*., 2003). Many of these stressors are subliminal in nature, prompting the question of whether they may have immunological consequences. This study demonstrates the potential for using altered immune responsiveness to provide a rapid physiological means of objectively quantifying the effect of short-term physical and psychological stressors. The case we report here investigates the impact to an individual's psychological status following interaction with motor vehicle control systems of differing ergonomic design.

Method

Participants

Local ethical committee approval from Coventry University Ethics Committee and written informed consent was obtained before commencing the study, in accordance with the declaration of Helsinki (World Medical Association, 2004).

Subjects were 15 (7 male and 8 female) moderately fit and healthy individuals, aged between 26 and 55 years. Potential subjects were excluded on the following criteria: suffering from psychiatric illness; suffering from cardiovascular or respiratory disease; smokers; had taken prescription medicine within the previous month, and if they had prior knowledge or have owned a motor vehicle fitted with one of the interfaces to be investigated or any other sort of computer-based human-machine interface (HMI).

Materials and Procedure

The ability to interpret and act upon specific commands depends upon how the information is presented (Liu, 2001). During tasks that require high levels of concentration, including driving, interface design has the potential either to ease or to exacerbate psychological stress when the driver is required to perform secondary tasks (such as adjustment of environment temperature or changing to a different radio station). Interface configuration and the sensory modalities they are focused upon i.e. visual, auditory or a combination of the two (multimodal) are primary areas of investigation. The interfaces selected for testing in this study aimed to explore which sensory route produces the lowest increase in psychological stress.

Two test vehicles including a BMW 535D, incorporating the iDrive multimodal control interface (Interface A) and a Jaguar S-Type R fitted with a touch screen interface (Interface B) and also voice control (Interface C) were individually interfaced with a computer driving simulator (Low Cost Simulator™). The vehicles allocated for this study were provided from the sponsor's pool of test vehicles, as the majority of vehicles had already been allocated to pre-existing research projects those which remained were made available for the described study. Vehicles were therefore selected on a semi-random basis. The simulation software allowed the responses of the vehicles basic control systems to be displayed, in real-time, and used to guide the user around a computer simulated test track, viewed from the driver's perspective, that was projected onto a 2 metre square screen in front of the test vehicle. Steering inputs were received by means of a sensor placed under each of the front wheels, while acceleration and braking commands, from the respective pedals, were taken by direct feedback from the vehicles management system. Computer simulated environments have been shown to act as viable alternates to real-life scenarios, so long as they possess an adequate level of realism (Kjeldskov and Skov, 2007; Seong-

Jin Kwon *et al*., 2006). With the selected equipment, a hybrid system involving the use of production motor vehicles interfaced with a computer simulated driving environment, the aim was to create a level of immersion that was comparable to and which would result in a psycho-physiological response of a magnitude that was analogous to real-life on-road assessment.

Two days prior to testing, subjects received information outlining basic operating instructions for each interface, including how specific voice commands should be structured. The content was similar to that included in the basic tutorial which accompanies the purchase of either vehicle.

Procedure

The experimental protocols were rigorously standardised, and testing was confined to between 10am and 2pm. Subjects were required to avoid strenuous activity for at least 2 hours prior to testing (e.g. they were instructed to take the lift to the laboratory rather than climb the stairs).

Perceived Psychological Stress Perceived psychological stress was established pre- and immediately post-stressor for each interface (Interfaces A, B, and C) by means of likert scales. Subjects were provided with two identical continuous scales ranging from 1 to 10. 45 minutes prior to, and again immediately after exposure to each of the putative stressors subjects were asked to indicate, by placing a mark on the scale provided, how stressed they were feeling (with 1 representing relaxed and 10 stressed) (Gaither *et al*., 2008, Hassinger *et al*., 1999). It was expected that subjects would record an increase in perceived stress following exposure to each of the three stressors compared to pre-stressor values. The magnitude of stress would increase according to interface complexity.

Prior to obtaining resting heart rate, BP, and core body temperature following the standardised procedure outlined below and illustrated in Figure 2, subjects sat quietly and were instructed to breathe orthonasally for 15 minutes. The first pair of capillary blood samples was then taken 45 minutes before exposure to the test apparatus (45 minutes pre-stressor) (see below), these initial samples were used as a control to determine baseline leukocyte activity.

During the 45 minute pre-stressor period subjects were taken from the laboratory (Coventry University) to Jaguar Cars Ltd. research and development centre at Whitley, Coventry, United Kingdom – a 5 minute car journey.

Upon entering the first test vehicle (selected using a counterbalanced crossover design) the subject adjusted the seat and other driving controls to the correct driving position. As these actions themselves had the potential to cause increased psychological stress and subsequent changes to leukocyte activity, the examiner helped with these tasks. To eliminate all other external stimuli, all windows except the windscreen were covered. The subject then proceeded to familiarise themselves with the responsiveness of the steering and also acceleration and braking by driving around the virtual test track for 2 minutes (e.g. as engine was not running, power steering was not available which made trying to turn the steering wheel much harder than during normal operation). With the vehicle stationary within the simulated environment the examiner explained the test protocol.

The test lasted a maximum of 15 minutes. Subjects were requested to complete the following tasks using the selected interface modality, whist simultaneously driving within the centre lane of the virtual test track at a constant speed of 60mph. Stress levels would already be high as a result of maintaining road position and speed. We hypothesise that the addition of a secondary task which involved the subject having to shift attention from the external environment to the vehicles control system would serve to further increase the subject's stress level. We propose that the extent of the additional observed stress would vary according to the interface format.

1. Whilst the vehicle was stationary within the simulated environment, program the destination – Euston Road, London NW1 - into the satellite navigation system, and initiate guidance.

2. With the vehicle in motion within the simulated environment, adjust the climate control to a temperature of 18°C with a moderate fan speed.

3. Tune the radio to a specific station (100.7 FM).

4. Turn both the radio and climate control off.

Each of the selected interfaces targets either a single sensory modality or a combination of the two. With the touch screen (Interface B), information was both provided and acted upon using a visual format via a series of menus and command screens. The selected touch screen model incorporated dedicated buttons (located to the left and right of the touch screen) allowing selection of the specific operational menus from the 'home screen' (e.g. satellite navigation, climate control, and radio menus). Once the required menu was selected all other commands were via a series of sub-menu screens displayed and accessed using the touch screen. For voice control (Interface C) all commands were provided using specific voice commands and phrases (all subjects received an information sheet detailing the commands necessary for successful completion of the task, described earlier). The system was primed for a command via depression of a button located left of the steering wheel, at the end of the indicator control stalk. To acknowledge the system was primed an audible beep was initiated and the message 'Listening' was displayed on the dashboard LCD, after which each command could be clearly spoken. The system responded to commands in the following sequence: Device, Function, Setting. For example, to turn the radio on and select 100.7 FM the command sequence would be: 'Radio on' followed by 'Radio tune' followed by '100.7 FM'. To adjust climate control the command sequence would be 'Climate control on' followed by 'Climate control temperature 18°C'. In the case of programming a destination into the satellite navigation system many of the commands could not be achieved via voice commands, subjects were instructed to attempt to use voice control first and when this proved ineffective to then resort to the use of the touch screen (previously described). The final system, Interface A (present within the BMW), employed a multimodal system incorporating both visual and audible commands that were accessed through the use of a multifunction control wheel, located next to the gear stick, which allowed the user to navigate to different control menus from the 'home screen', e.g. navigation, climate control, and entertainment menus (via up, down, left or right movements of the control wheel) and using clockwise and anticlockwise movements of the control wheel to scroll through various functions available for each control menu displayed in a sub-menu format on an LCD screen mounted within the centre stack of the dash

board. Once the desired functions and commands had been highlighted final selection was achieved via depression of the control wheel.

It was explained that once the test had begun no further verbal communication was allowed, and that assistance would only be offered after 4 minutes of attempting to complete the task. Immediately upon completion of the tasks (Immediately post-stressor) the subject was asked to come to a controlled stop in the centre of the virtual test track, whereupon heart rate, BP and core body temperature were recorded and further blood samples taken (Figure 2).

On two further occasions (ensuring a minimum interval of 2 hours existed between tests for baseline leukocyte activity to re-establish) each subject was tested using the remaining two interfaces using identical protocol (test order was decided using a counterbalanced design).

Heart Rate, Blood Pressure and Core Body Temperature Measurements. At each specified time point (Figure 2) a heart rate transceiver (Polar 610i™ Heart rate monitor, Polar Electro, Finland) attached directly to the chest, using the belt provided, monitored heart rate. Systemic blood pressure was measured using an oscillometric wrist-mounted blood pressure monitor (Omron RX-3, Omron Healthcare Inc. Illinois, 60015. U.S.A.). Core body temperature (CBT) was measured using an infra-red ear thermometer (Braun® Thermoscan™, P and G Brooklands, Waybridge, AT13 0XP. United Kingdom). It was expected that an increase in all stated parameters would be observed following stressor exposure compared to pre-stressor values. The magnitude of change would increase according to interface complexity.

Blood Samples. At each specified time point (Figure 2) two 10µl blood samples were taken using a finger lancing device (Accu-Chek® Softclix®, Roche® Ltd, East Sussex, United Kingdom) from the side of a finger of the subject's non-contractual hand. Following the procedure illustrated in Figure 3, one sample was used for the (non-stimulated) control (Sample A) and was placed into 10µl of murine heparin (concentration 0.1units) (CP Pharmaceuticals Ltd, Ash Road North, Wrexham, LL13 9UF, United Kingdom), 10µl Phosphate Buffered Saline (PBS) (Sigma Aldrich, Dorset, SP8 4XT, United Kingdom) and 90µl of 10^{-4}M Luminol ($C_8H_7N_3O_2$) (Sigma Aldrich, Dorset, SP8 4XT, United Kingdom). The second blood

sample (Sample B) was added to the same reagents, except that the 10µl of PBS was replaced by 10µl of 10^{-5}M Phorbol 12-Myristate 13-Acetate (PMA) (Sigma Aldrich, Dorset, SP8 4XT, United Kingdom). PMA stimulates leukocytes (primarily neutrophils) causing them to increase their production of oxidative metabolites. This increased production can be measured using luminol amplified light emission (chemiluminescence) (Dahlgren, 1987).

Determining Leukocyte Activity. Leukocyte Coping Capacity (LCC) is a measure, made using a Luminometer, of the concentration of Reactive Oxygen Species (ROS) calibrated through the emission of photons as a result of their interaction with Luminol. This is an indicator of the leukocyte's ability to produce a respiratory burst (McLaren *et al.*, 2003). Leukocytes (mainly neutrophils) possess a finite store of ROS available for immediate release following exposure to psychological or physiological stress stimuli. If an individual has become stressed a percentage of the available ROS has already been released *in vivo*. Therefore when the leukocytes are chemically stimulated (stressed) with PMA *in vitro*, their ability to release ROS is diminished. This is graphically illustrated in Figures 4a, 4b and 4c by a decrease in calculated RLU_{adj} values, and as an increase in the negativity of the mean values of leukocyte activity shown in Table 1. By comparing the ability of leukocytes to release ROS before and after stressor exposure, the difference in ROS concentration can be used to identify the presence of stress and also as an objective means of quantifying the magnitude of stress.

LCC is defined as the response of leukocytes (mainly neutrophils) to challenge by PMA. Subjects whose LCC score is higher have displayed a greater potential to produce a respiratory burst (in the absence of a stressor the finite store of ROS available within each leukocyte has not been released *in vivo* prior to *in vitro* PMA stimulation), and are therefore in this respect more able, physiologically, to respond to bacterial challenge (immune-competent). LCC responsiveness to *in vitro* PMA challenge is inversely related to stress level.

In this experiment, each pair of blood solutions (sample A and sample B) was simultaneously tested every 5 minutes using a Luminometer (Berthold® Technologies, Junior™ LB9509, Hertfordshire, AL3 7LZ, United Kingdom) for a total of 45 minutes (Figure 3), in order to produce a luminescence profile (Figure 4a, 4b, and 4c). Between chemiluminescence measurements the samples were incubated at 37ºC in a

water bath (JB1™ Grant Instruments, Cambridge, United Kingdom). As part of homeostasis there is a small but constant release of ROS from leukocytes which in order to ensure the accuracy of the LCC protocol during stressor analysis must be taken into account. To achieve this, one of the pair of blood solutions (sample A) was used to assess this baseline activity and did not undergo chemical stimulation with PMA. At each 5 minute interval an adjusted score, measured in Relative Light Units (RLU_{adj}) was obtained for each subject by subtracting the luminescence score of the baseline control (Sample A - without PMA stimulation) from the PMA challenge sample (Sample B).

Data Analysis. For all measured parameters data are expressed as mean post-stressor changes ± standard error of mean (S.E.M.). For T-max (time taken to reach maximum leukocyte activity), the data were classed as discontinuous as leukocyte activity was measured at 5 minute intervals for a total of 45 minutes, in this case the median ± S.E.M. is presented. Repeated measures single factor analysis of variance (ANOVA) (SPSS statistical software (release 15.0 Lead Technologies Inc.) was used to test in turn, the effect of experimental group (Interface A, B and C) on leukocyte activity, heart rate, BP, core body temperature, and perceived psychological stress rating (continuous likert scale). Tukey's honestly significant difference test for multiple comparisons were used as *post hoc* tests when applicable. Bivariate correlation was used to explore in turn, the relationship between changes in each of the measured parameters listed above and post-stressor changes in leukocyte activity. *P*-values were corrected for the use of multiple comparisons using the Truncated Product Method (Zaykin *et al*., 2002).

A repeated measures ANOVA model was also applied to test the effect of test order on changes in leukocyte activity. Again Tukey's honestly significant difference test for multiple comparisons was used *post hoc* when applicable (test order).

Results

Leukocyte Activity

Leukocyte activity profiles produced from blood samples taken 45 minutes pre- and immediately post-stressor displayed in Figure 4a, 4b and 4c show that following PMA challenge, maximum leukocyte activity (ROS release) for samples taken at both sampling points (45 minutes pre- and immediately post-stressor) occurred between T=15 and 20 minutes following exposure to Interfaces B (Figure 4b) and C (Figure 4c) and between T=20 and 25 minutes for Interface A (Figure 4a), after which time, all profiles showed a steady decrease in activity. The results show that a significant difference in post-stressor leukocyte activity, between treatment groups, occurred 10 minutes into the 45 minute luminescence profile (T=10 minutes) (Table 1). In general, LCC scores following the use of Interface A showed the greatest decrease in leukocyte activity with Interface C exhibiting the least (Figure 4a, 4b and 4c). The magnitude of the post-stressor change in activity following the use of Interface A was significantly greater compared to the use of Interface B ($P = 0.01$ Tukey's *post hoc* procedure) and also Interface C ($P = \leq 0.001$ Tukey's *post hoc* procedure). Whereas the use of both Interfaces B and C resulted in post-stressor changes in leukocyte activity of similar magnitude ($P = 0.47$ Tukey's *post hoc* procedure) (Table 1).

Test Order

Subjects were assigned, using a counterbalanced crossover design, to one of three test order combinations:

1) Interface A, B then C
2) Interface B, C then A
3) Interface C, B then A

Despite the use of a counterbalanced design a significant difference was found to exist between test order and adjusted post-stressor change in leukocyte activity for Hmax-RLU_{adj} ($F_{1,134} = 10.83$, $P \leq 0.001$) and T=15 minutes ($F_{1,134} = 11.02$, $P \leq 0.001$). In both cases the significant difference occurred between test order combination 1 and 3 (in each case $P = 0.02$ Tukey's *post hoc* procedure). Indicating that individuals who were first tested using Interface A found the subsequent use of Interface C significantly less mentally demanding. Conversely those who first used Interface C found Interface A significantly more demanding.

Perceived Psychological Stress

Overall perceived psychological stress was significantly different between treatment groups ($F_{1,89} = 7.73$, $P = 0.001$). The use of Interface A resulted in an increase in perceived stress (2.0 ± 0.22 units) that was significantly greater in magnitude compared to the use of Interface B (1.13 ± 0.13 units) ($P \leq 0.001$ Tukey's *post hoc* procedure) (rating based on a continuous arbitrary scale where 1 represented relaxed and 10 stressed). The use of Interface C to complete the required tasks resulted in a post-stressor increase of 0.06 ± 0.18 units, which was of a similar magnitude to that observed following the use of Interface B ($P = 0.19$ Tukey's *post hoc* procedure). Observed changes in perceived psychological stress were not found to be significantly correlated with the observed post-stressor decreases in leukocyte activity.

Core Body Temperature

Core body temperature increased significantly from baseline following the use of Interfaces A and B (Table 2). The magnitude of change between the use of Interfaces A and B was similar ($P = 0.92$ Tukey's *post hoc* procedure), whereas the use of Interface C resulted in a post-stressor increase that was significantly lower in magnitude compared to the use of Interface A ($P = 0.04$ Tukey's *post hoc* procedure).

Heart Rate, Blood Pressure

Treatment groups A and B demonstrated significant increases in heart rate compared to baseline (45 minutes pre-stressor), whereas the use of Interface C resulted in no significant change (Table 2). No significant difference in the magnitude of change between treatment groups was observed.

As with heart rate, only treatment groups A and B showed significant increases in systolic blood pressure compared to baseline (Table 2). The magnitude of the post-stressor increase was significant between treatment groups ($F_{1,44} = 7.87$, $P = 0.001$). The use of Interfaces A and B resulted in a similar magnitude of change ($P = 0.81$ Tukey's *post hoc* procedure), whereas using Interface C resulted in a post-stressor increase in systolic BP that was significantly lower in magnitude compared to Interface A ($P = 0.002$ Tukey's *post hoc* procedure) and Interface B ($P = 0.01$

Tukey's *post hoc* procedure) (Table 2). No significant change in diastolic BP was observed compared to baseline or between treatment groups.

Although the differences in post-stressor change in heart rate between treatment groups only approached significance ($F_{1,44} = 2.9$, $P = 0.07$), change in heart rate was significantly correlated with the change in time taken to reach maximum adjusted leukocyte activity (T-max) and adjusted leukocyte activity at 5 minutes into the 45 minute profile (T=5 minutes).

Discussion

This study shows that immune responsiveness is rapidly affected by an individual's psychological state, and that the magnitude of response is proportional to the intensity and duration of the psychological / physical stressor; a relationship with the potential to provide a rapid, objective means of quantifying psychological stress. The ability to accurately quantify the effect of short-term stressors is limited as many of the physical parameters utilised (heart rate, BP, respiration rate, stress hormone concentration e.g. cortisol) are highly regulated via complex biochemical feed back mechanisms designed to rapidly eliminate homeostatic imbalance. This is further confounded with the existence of considerable biological variation between individuals and populations (Brown *et al*., 2003; Hodgson *et al*., 2004). As leukocyte responsiveness is adjusted to compensate for baseline activity, as described in Figure 3, the LCC technique possesses an in-built control to compensate for biological variation, permitting the technique to quantitatively relate changes in leukocyte activity to stress level following exposure to extremely subtle short-term psychological stressors (Shelton-Rayner, 2009). Prior to the LCC technique, the only means of assessing such stressors were highly subjective, relying mainly upon self assessment questionnaires (Lemyre and Tessier, 2003). It should be noted that this study describes a novel application for LCC analysis and although returning encouraging results further investigation and comparison with other stress assessment techniques (e.g. Galvanic Skin Resistance, Heart Rate Variability, assessment of Electrocardiogram and Blood Volume Pulse Signals) would be valuable.

Leukocytes (primarily, but not exclusively, neutrophils) possess in excess of 250 different receptors capable of responding to a diverse range of factors for example, altered blood biochemistry and erythrocyte haemodynamics, endocrine factors in the plasma, cytokines and other factors released from both circulating and non-circulating cells including endothelial cells, and changes in the hypothalamic-pituitary-adrenal axis and sympathetic nervous system (illustrated in Figure 1); all of which are sensitive to stress (Mian *et al*., 2005). The constant exposure of leukocytes to each of these stress stimuli makes them ideal bio-indicators for the presence and magnitude of stress. The Coping Capacity of Leukocytes (LCC), i.e. their ability to respond to an external stimulator and produce reactive oxygen species, will be affected by the immediate external environment in the blood (Shelton-Rayner *et al*., 2010). We have previously reported how short-term stress exposure leads to a significant change in biomediator concentration (Shelton-Rayner, 2009).

The architecture and adhesiveness of a cell microenvironment is essential for the effective determination of its ability to respond *in vivo* (Théry *et al*., 2007). The deliberate suspension of leukocytes in whole blood during LCC analysis, ensures that the structural integrity and morphology of the cell remains as near to the *in vivo* condition as possible. It also permits the dynamic interaction with surrounding erythrocytes and allows cell-cell interaction within and between different leukocyte cohorts (Shelton-Rayner *et al*., 2010). During LCC analysis the process of centrifugation and 'plating out' cells on glass slides (used in the NBT test) (Tsukamoto *et al*., 2002) are both avoided, as both can potentially reduce cellular responsiveness via disruption to cellular integrity and signalling pathways.

Leukocytes release reactive oxygen species in response to stress, *in vitro* PMA stimulation allows us to ascertain (like a differential equation) the cellular potential to synthesise and release further reactive oxygen species. The process accounts for exposure to other stress mediators and provides a test that is sensitive to true stress.

Walker *et al*. (2001) analysed altered psychological stress and situational awareness during the use of high feedback vehicles (employing the latest electronic driver aids designed to provide detailed information of all aspects of the internal and external driving environment), as opposed to low feedback vehicles (which provide only the bare minimum level of feedback required for safe driving practice), and concluded that situational awareness was improved and was coupled with lower perceived psychological stress. In contrast, our findings suggest that although

psychologically a person may perceive high specification driver aids as beneficial, physiologically the act of performing the simplest task (e.g. selecting a radio station) leads to short-term increased stress manifested via diminished ROS release following *in vitro* PMA challenge. The findings of this study also suggest that, while assessing the novice user, complex multimodal systems such as Interface A, designed to facilitate interaction with everything from climate control to satellite navigation, evoked greater increases in stress compared to simpler systems such as Interfaces B and C (which necessitated less hunting through layers of menus to find the correct control, thus reducing additional psychological stress). Interface C, being voice control, almost completely avoided the need for the driver to shift attention from the external environment, which meant that stress level was not significantly affected (no significant post-stressor change in leukocyte activity compared to pre-stressor values following *in vitro* PMA challenge, was observed). It should be noted that despite the process of counterbalancing an order effect was observed for the use of Interfaces A and C. Using t-tests to assess individual subject data it was concluded that the observed order trend was demonstrated in 60% of test subjects. This order effect has not been observed in other studies using the LCC technique (Honess and Marin, 2006; McLaren *et al*., 2003; Shelton-Rayner *et al*., 2010; Shelton-Rayner, 2009).

The format by which information is both received and supplied by the driver can significantly alter response time, accuracy and performance whilst undertaking basic button pushing and navigational tasks. Liu (2001) demonstrated how navigational commands supplied in a multimodal (both visual and auditory commands) or audible only formats led to improved response times and lowered subjective psychological stress ratings, compared to visual only displays. LCC analysis offers support to these findings on a psycho-physiological level. The use of Interface C (voice control), where commands were both given and received audibly, resulted in the smallest change in leukocyte reactivity (Table 1). When information was supplied visually, as with both Interfaces A and B, there was a significantly greater decrease in post-stressor leukocyte activity. As a substantial proportion of high feedback vehicles containing HMI systems, such as those tested during this study, are part of the short-term hire market (due to high purchase cost), many of these customers could be classified as being novice users. It is therefore essential to be able to objectively quantify altered psychological stress levels following interaction with such devices, to ensure that any resultant increase in stress level is

minimised and safe driving practice is maintained. The findings of this study provide an initial indication as to the effectiveness of using an aspect of the innate immune system as a means of quantifying changes in psychological stress level, we would like to emphasise that use of leukocyte responsiveness and the LCC protocol as a means of assessing and quantifying changes to psycho-physiological stress level is still in the early stages of development, clearly further work and research is needed.

In an era where ergonomic design and technological advancement aim to make everyday activities, such as driving, less demanding and more pleasurable, the ability to objectively quantify the psycho-physiological effects such changes produce is an important design tool. Our findings illustrate how a system conceived to alleviate the stresses of daily life actually provoked a quantifiable stress response. In this instance altered leukocyte responsiveness has been applied in the assessment of how ergonomic design facilitates human interaction with automotive interface technology. The results are rapid lending the LCC technique to be a quick objective indicator to assess psychological wellbeing.

Acknowledgments

This work formed part of a PhD programme of research. The PhD studentship was sponsored by financial assistance, the supply of test vehicles, and other specialist equipment including driving simulation technology by Jaguar and Land Rover Research and Development, Jaguar Cars Limited. The sole request of the sponsor was for the research team to design a simulator-based study incorporating production test vehicles for the purpose of testing and investigating the ability of the LCC protocol and other physiological parameters to act as a means for objectively quantifying changes in stress level following interaction with human-machine interfaces of differing ergonomic design. At no point did the sponsor provide any preconceptions as to the expected outcome of the study. We are grateful to Dr. Paul Johnson for statistical advice and the volunteers who gave freely of their time and good will to participate in the study.

References

Atanackovic, D., Brunner-Weinzierl, M. C., Kröger, H., Serke, S., & Deter, H. C. (2002). Acute psychological stress simultaneously alters hormone levels, recruitment of lymphocyte subsets, and production of reactive oxygen species. *Immuol. Invest., 31* (2), 73-91

Boscarino, J. A., & Chang, J. (1999). Higher abnormal leukocyte and lymphocyte counts 20 years after exposure to severe stress: research and clinical implications. *Psychosomatic Medicine, 61,* 378-386

Boxer, L. A., & Smolen, J. E. (1998). Neutrophil granule contents and their release in health and disease. *Hematol. Oncol. Clin. N., 2*, 101-134

Brown, D. E., James, G. D., Nordloh, L., & Jones, A. A. (2003). Job strain and physiological stress responses in nurses and nurse's aides: predictors of daily blood pressure variability. *Blood Press. Monit., 8* (6), 237-242

Byrne, E. A., & Parasuraman, R. (1996). Psychophysiology of Workload. *Biol. Psychol., 42* (3), 249-268

Clover, R. D., Abell, T., Becker, L. A., Crawford, S., & Ramsey, C. N. (1989). Family functioning and stress as predictors of influenza B infection. *Journal of Family Practice, 28,* 536-539

Clow, A., Edwards, S., Owen, G., Evans, G., Evan, P., Hucklebridge, F., & Casey, A. (2006). Post-awakening cortisol secretion during basic military training. *International Journal of Psychophysiology, 60* (1), 88-94

Dahlgren, C. (1987). Polymorphonuclear leukocyte chemiluminescence induced by formylmethionyl-leucyl-phenylalanine and phorbol myristate acetate: Effects of catalase and superoxide dismutase. *Inflammation Research, 21* (1-2), 104-112

Dhabhar, F. S., Miller, A. H., McEwen, B. S. & Spencer, R L. (1996). Stress induced changes in blood leukocyte distribution. *J. Immunol., 156***,** 2608–2615

Dokoumetzidis, A., IIiadis, A., & Macheras, P. (2002). Nonlinear dynamics in clinical pharmacology: the paradigm of cortisol secretion and suppression. *Br. J. Clin. Pharmacol., 54*, 21-29

Elliot, G. R., & Eisdorfer, C. (1982). Stress and human health: An analysis and implications of research. A study by the Institute of Medicine, National Academy of Sciences. New York: Springer Publishing

Gaither, C. A., Kahaleh, A. A., Doucette, W. R., Mott, D. A., Pederson, C. A., & Schammer, J. C. (2008). A modified model of pharmacist's job stress: The role of organisational, extra-role, and individual factors on work-related outcomes. *Res. Soc. Adm. Pharm., 4* (3), 231-243

Galinowski, A. (1997). Stress and Immunity, *Encephale***,** *23* (5), 18-22

Hassinger, H. J., Semenchuk, E. M., & O'Brien, W. H. (1999). Appraisal and coping responses to pain and stress in migraine headache sufferers. *J. Behav. Med., 22* (4), 327-340

Hodgson, N., Freedman, V. A., Granger, D. A., & Erno, A. (2004). Bio behavioural correlates of relocation in the frail elderly: Salivary cortisol, affect, and cognitive function. *J. Am. Geriatr. Soc., 52* (11), 1856-1862

Honess, P. E., & Marin, C. M. (2006). Behavioural and physiological aspects of stress and aggression in nonhuman primates. *Neurosci. Biobehav. Rev., 30* (3), 390-412

Iqbal, S. T., Adamczyk, P. D., Zheng, X. S., & Bailey, B. P. (2005). Towards an index of opportunity: understanding changes in mental workload during task execution. In *Conference on Human Factors in Computing Systems. Proceedings of the SIGCHI conference on Human factors in computing systems*. ISBN: 1-58113-998-5. doi: http://doi.acm.org/10.1145/1054972.1055016

Kang, D. H., Coe, C. L., & McCarthy, D. O. (1996). Academic examinations significantly impact immune responses, but not lung function, in healthy and well-managed asthmatic adolescents. *Brain, Behaviour and Immunity, 10*, 164-181

Kiecolt-Glaser, J. K., Marucha, P. T., Malarkey, W. B., Mercado, A. M., & Glaser, R. (1995). Slowing of wound healing by psychological stress. *Lancet, 346,* 1194-1196

Kjeldskov, J., & Skov, M. B. (2007). Studying usability in sitro: stimulating real world phenomena in controlled environments. *Int. J. Hum-Comput Int., 22* (1&2), 7-36

Lemyre, L., & Tessier, R. (2003). Measuring psychological stress. Concept, model, and measurement instrument in primary care research. *Canadian Family Physician, 49*, 1159-1160

Liu, Yung-Ching. (2001). Comparative study of the effects of auditory, visual and multimodality displays on drivers' performance in advanced traveller information systems. *Ergonomics, 44* (4), 425-442

Maes, M., Van Der Planken, M., Van Gastel, A., Bruyland, K., Van Hunsel, F., Neels, H., Hendriks, D., Wauters, A., Demedts, P., Janca, A., & Scharpe, S. (1998a). Influence of academic examination stress on haematological measurements in subjectively healthy volunteers. *Psychiatry Research, 80,* 201-212

Maes, M., Song, C., Lin, A., De Jongh, R., Van Gastel, A., Kenis, G., Bosmans, E., De Meester, I., Benoy, I., Neels, H., Demedts, P., Janca, A., Scharpe, S., & Smith, R. S. (1998b). The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. *Cytokine, 10* (4), 313-318

McLaren, G. W., Macdonald, D. W., Georgiou, C., Mathews, F., Newman, C., & Mian, R. (2003). Leukocyte coping capacity: a novel technique for measuring the stress response in vertebrates. *Exp Physiol., 88* (4), 541-546

Mian, R., McLaren, G., & Macdonald, D. W. (2005). Stress: A radical approach to old problems. In K. Oxingon (Ed.), *Stress and Health: New Research* (pp.61-79). New York: Nova Science Publications

Mian, R., Shelton-Rayner, G. K., Harkin, B., & Williams, P. (2003). Observing a fictitious stressful event: haematological changes including circulating leukocyte activation. *Stress, 6*, 41-47

Montes, I., McLaren, G. W., Macdonald, D. W., & Mian, R. (2004). The effect of transport stress on neutrophil activation in wild badgers (*Meles meles*). *Anim. Welfare, 13* (3), 355-359

Montes, I., McLaren, G. W., Macdonald, D. W., & Mian, R. (2003). The effects of acute stress on leukocyte activation. *J. Physiol., 548P*, 170

Okutsu, M., Ishii, K., Jun Niu, K., & Nagatomi, R. (2005). Cortisol-induced CXCR4 augmentation mobilises T lymphocytes after acute physical stress. *Am. J. Physiol. Regul. Integr. Comp. Physiol., 288*, 591-599

Rodriguez-Galan, M. C., Correa, S. G., Cejas, H., & Sotomayer, C. E. (2001). Impaired activity of phagocytic cells in Candida albicans infection after exposure to chronic varied stress. *Neuroimmunomodulation, 9* (4), 193-202

Ruotsalainen, M., Hyvärinen, A., Nevalainen, A., & Savolainen, K. M. (1995). Production of *reactive oxygen* metabolites by opsonized fungi and bacteria isolated from indoor air, and their interactions with soluble stimuli, fMLP or PMA. *Environ. Res., 69* (2), 122-31

Scerbo, M. W., Freeman, F. G., Mikulka, P. J., Parasuraman, R., Nocero, F. D., & III Lawrence, J. P. (2001). The efficacy of psychophysiological measures for implementing adaptive technology. Technical Report: NASA-20010tp211018. NASA Langley Technical Report Server. <http://portal.acm.org/citation.cfm?id=886463>

Segerstrom, S. C., & Miller, G. E. (2004). Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychol. Bull., 130* (4), 601-630

Seong-Jin Kwon, Jee-Hoon Chun, Jong-Hyun Bae, & Myung-Won Suh. (2006). A study on the factors that improve the velocity perception of a virtual reality-based vehicle simulator. *Int. J. Hum-Comput Int., 21* (1), 36-54

Sharit, J., & Salvendy, G. (1982). Occupational Stress: Review and Reappraisal. *Human Factors: The Journal of the Human Factors and Ergonomics Society, 24* (2), 129-162

Shelton-Rayner, G. K. (2009). Quantifying Responses to Psychological and Physiological Stress in Automotive Design. PhD Thesis. Coventry University: U.K

Shelton-Rayner, G. K., Macdonald, D. W., Chandler, S., Robertson, D., & Mian, R. (2010). Leukocyte reactivity as an objective means of quantifying mental loading during ergonomic evaluation. *Cell. Immunol*., doi: 10.1016/j.cellimm.2010.02.011

Taylor, M. K., Sausen, K. P., Mujica-Parodi, L. R., Potterat, E. G., Yanagi, M. A., & Kim, H. (2007). Neurophysiologic methods to measure stress during survival, evasion, resistance and escape training. *Aviat. Space Environ. Med., 78* (5 Suppl.), B224-230

Théry, M., Jiménez-Dalmaroni, A., Racine, V., Bornens, M., & Jülicher, F. (2007). Experimental and theoretical study of mitotic spindle orientation. *Nature, 447*, 493-496

Tsukamoto, K., Suzuki, K., Machida, K., Saiki, C., Murayama, R., & Sugita, M. (2002). Relationships between lifestyle factors and neutrophil functions in the elderly. *J. Clin. Lab. Anal., 16* (5), 266-272

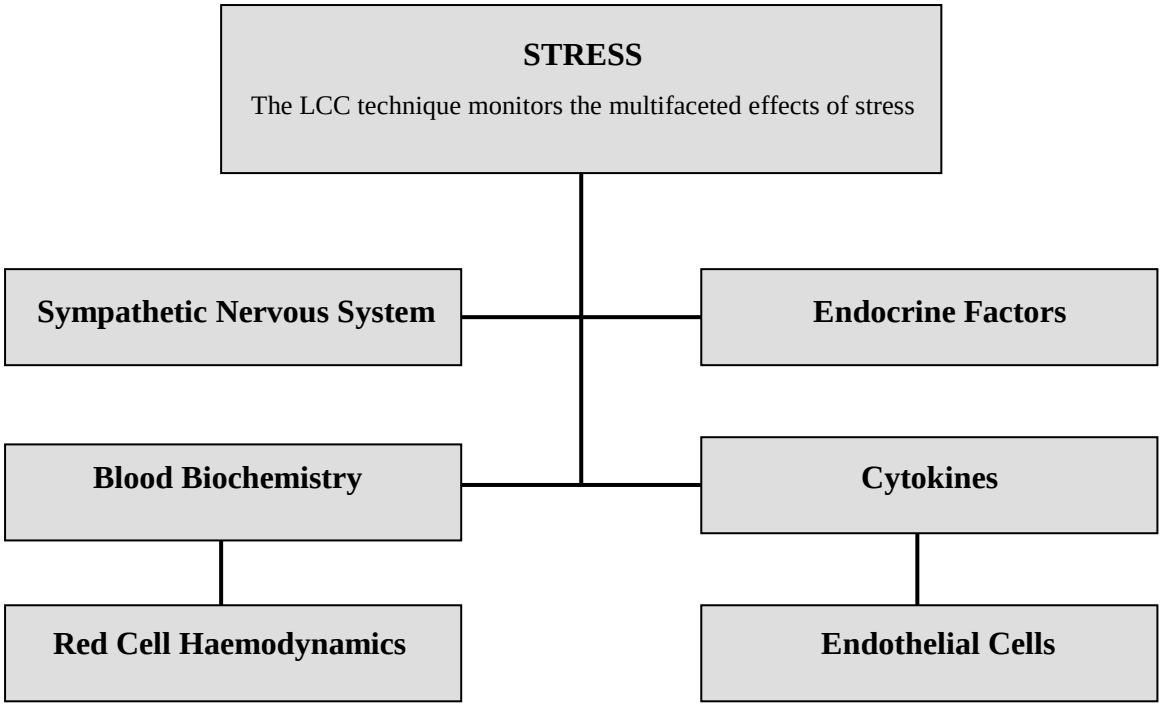
Walker, G. H., Stanton, N. A., & Young, M. S. (2001). An on-road investigation of vehicle feedback and its role in driver cognition: Implications for cognitive ergonomics. *International Journal of Cognitive Ergonomics, 5* (4), 421-444

World Medical Association (WMA) (2004). Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects WMA General Assembly, Tokyo 2004 [online] available from <http://www.wma.net/e/policy/pdf/17c.pdf> [October 2004]

Zaykin, D. V., Zhivotovsky, L. A., Westfall, P. H., & Weir, B. S. (2002). Truncated product method for combining P-values. *Genet. Epidemiol., 22* (2), 170-185.

FIGURE 1 Factors believed to affect the activation state of leukocytes

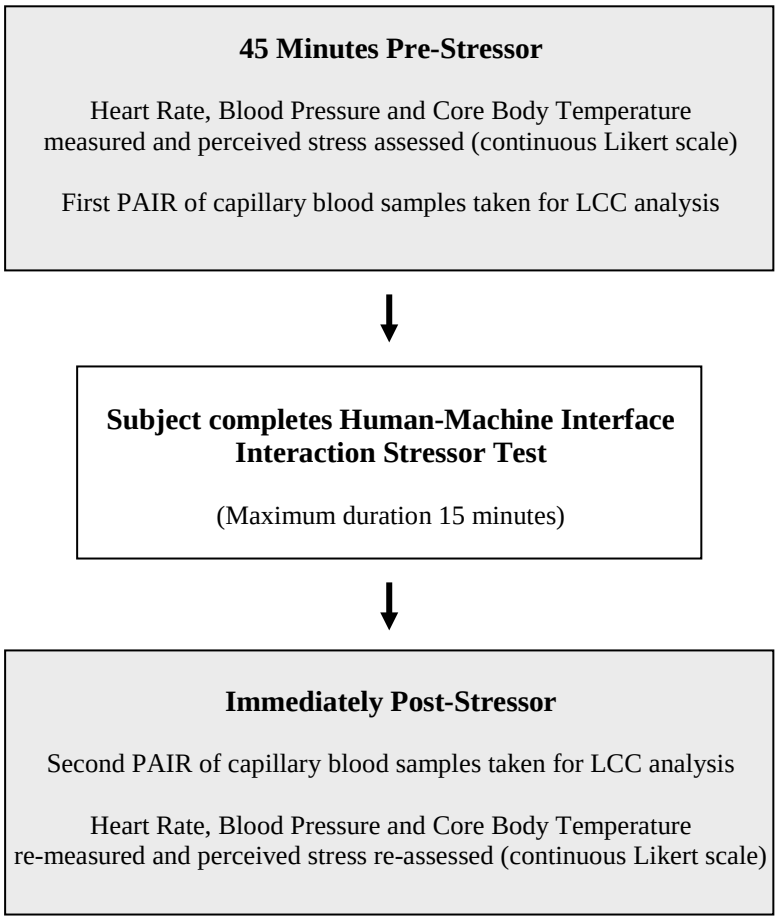
(Taken from Shelton-Rayner *et al*., 2010)



Flow diagram showing factors believed to affect the activation state of leukocytes.

FIGURE 2 Sampling Protocol

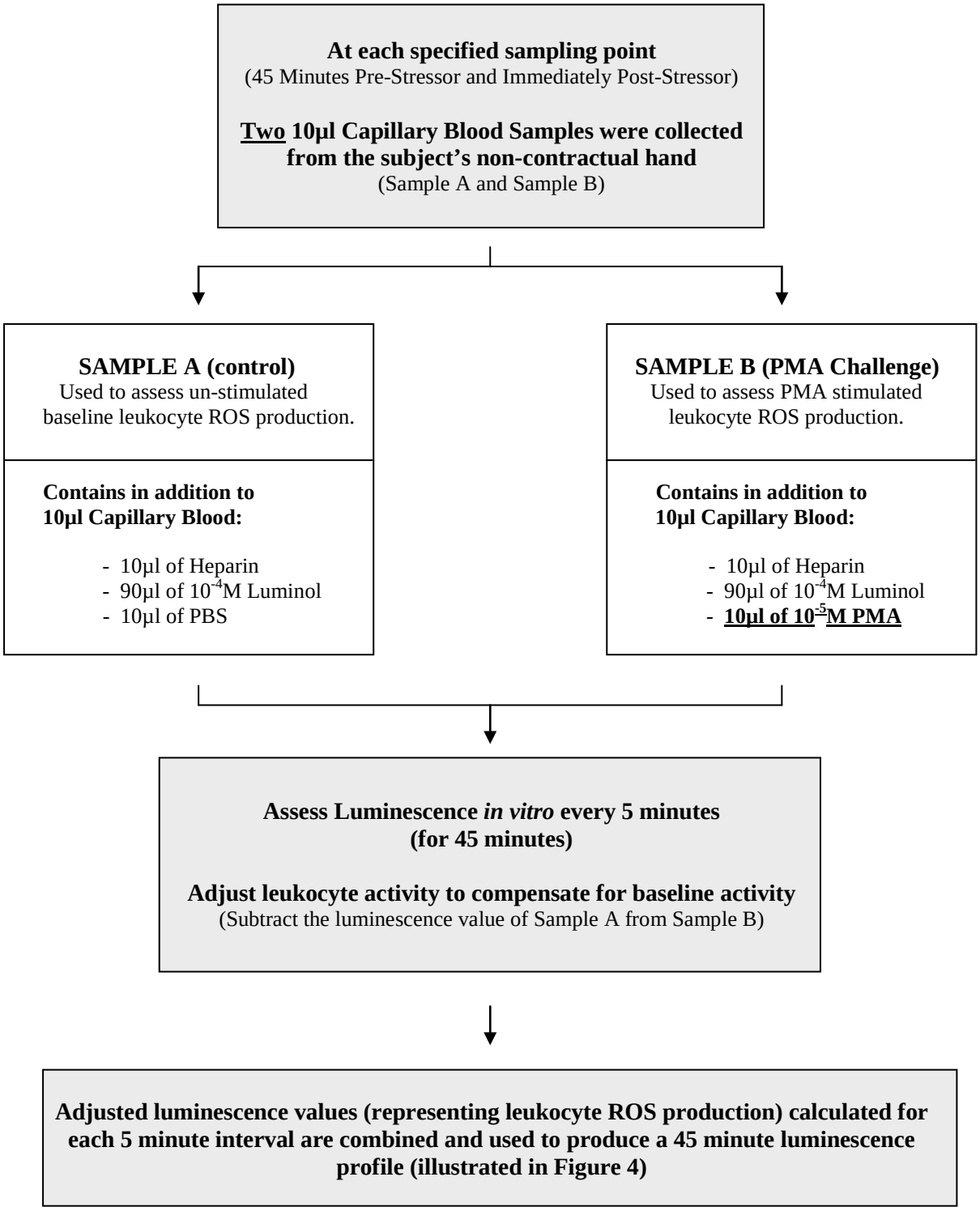
(Adapted from Shelton-Rayner *et al*., 2010).



Flow diagram illustrating the time line for blood sample collection, for Leukocyte Coping Capacity (LCC) analysis, and measurement of other physiological (Heart Rate, Systolic and Diastolic Blood Pressure, and Core Body Temperature) and psychological (Perceived Psychological Stress Rating) parameters.

FIGURE 3 Leukocyte Coping Capacity (LCC) Protocol

(Taken from Shelton-Rayner *et al*., 2010).



Leukocyte activity was assessed using whole blood samples taken 45 minutes pre- and immediately post-stressor, following the protocol illustrated.

Figure 4a. Interface A (n=15)

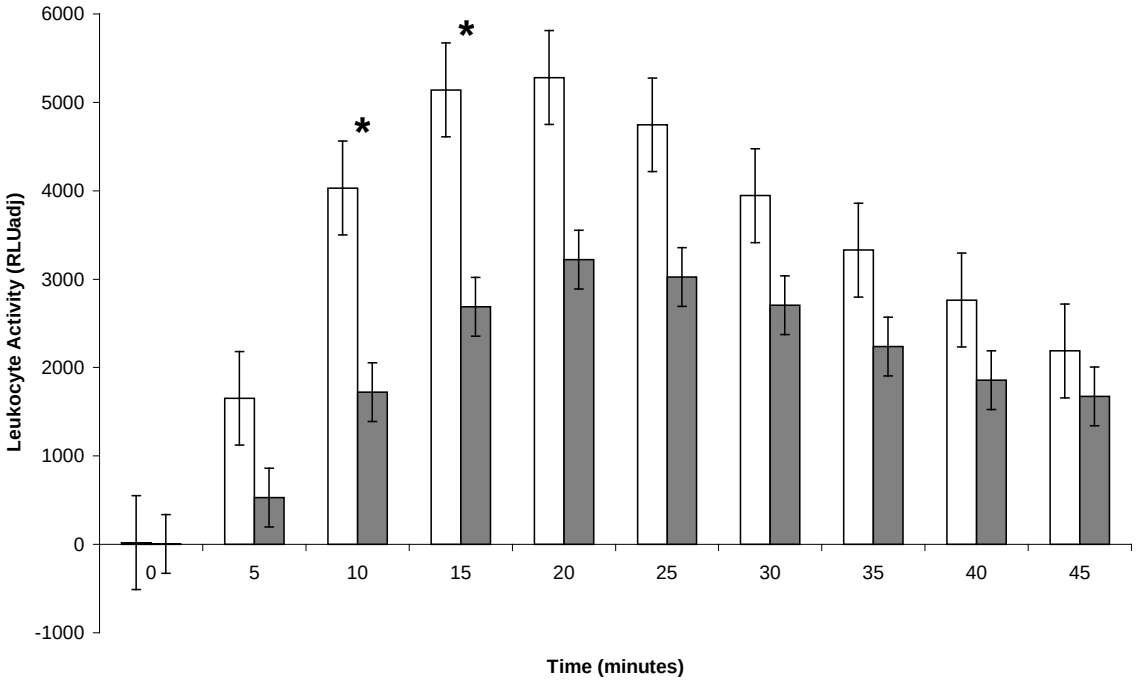


Figure 4b. Interface B (n=15)

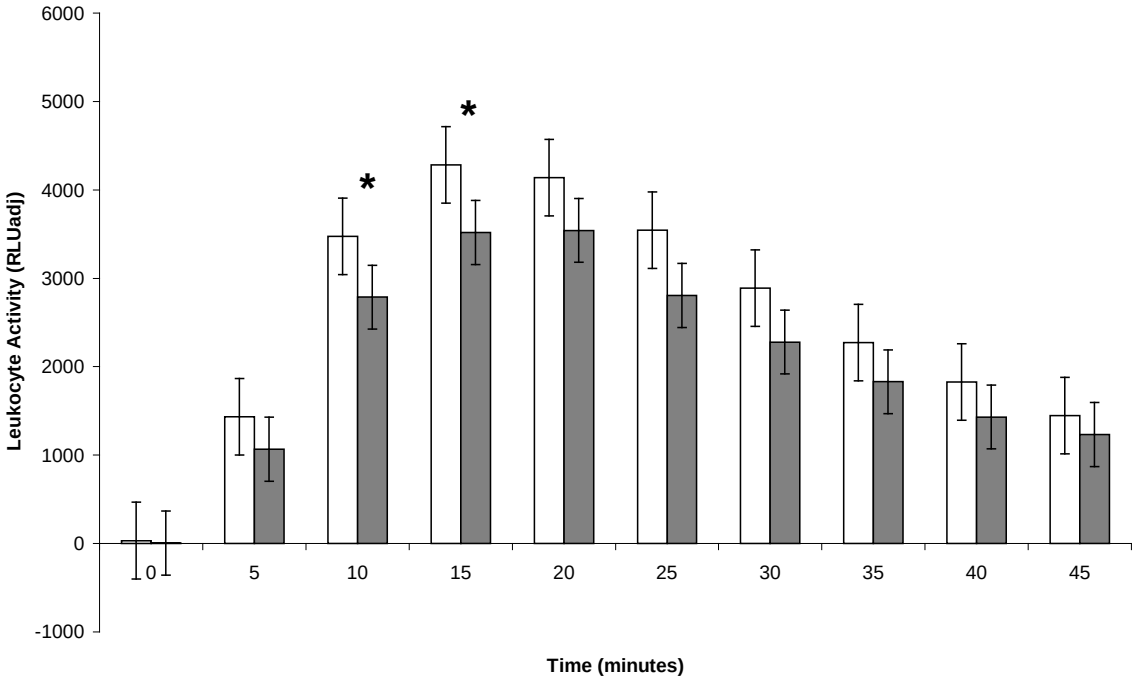
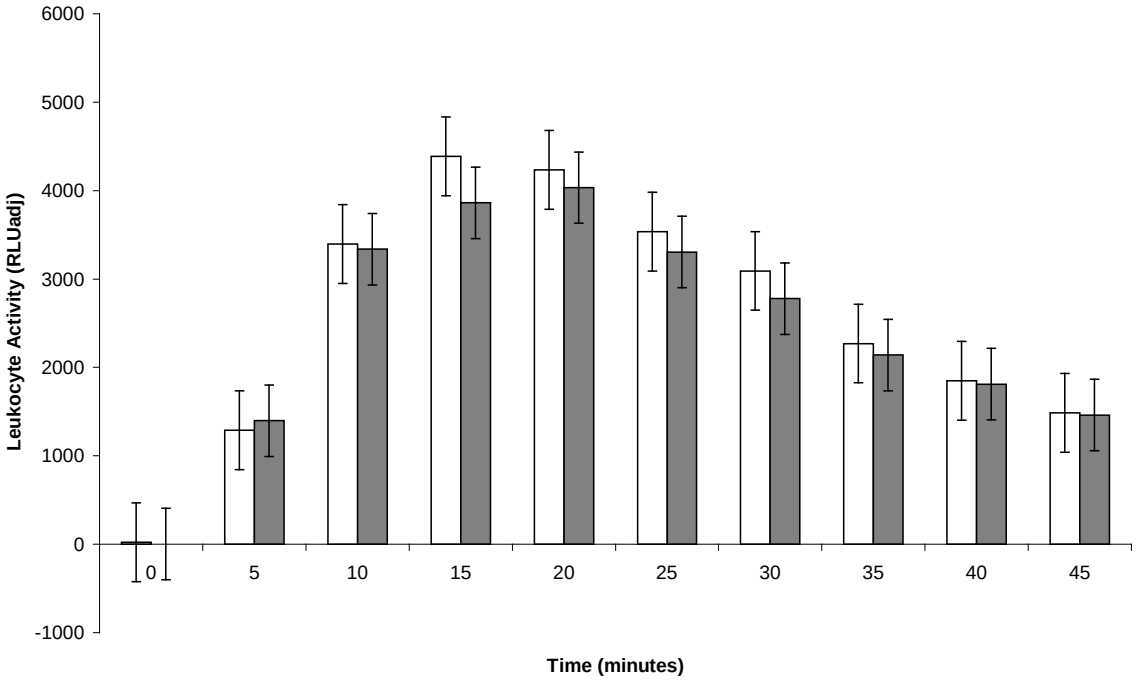


Figure 4c. Interface C (n=15)



Mean adjusted Leukocyte Coping Capacity (RLU_{adj}) ± S.E.M. for treatment groups A, B and C (n=15 for each). Open bars represents mean adjusted leukocyte activity 45 minutes pre-stressor and closed bars represents activity immediately post-stressor. * indicates significant difference in activity between 45 minutes pre-and immediately post-stressor ($P < 0.05$).

TABLE 1 Effect of stressor on leukocyte activity

	INTERFACE A	INTERFACE B	INTERFACE C	*P* (F)
Δ Hmax (RLUadj)	-2418.06 ± 714.24 •	-775.8 ± 229.43	-518.26 ± 219.44	0.07 (2.82)
Δ T-max (minutes)	-5.0 ± 2.01 •	0.0 ± 0.48	0.0 ± 0.48	0.15 (2.01)
Δ T=5 minutes (RLUadj)	-1124.0 ± 348.71 •	-369.06 ± 121.88	-107.0 ± 169.8	0.09 (2.65)
Δ T=10 minutes (RLUadj)	-688.8 ± 182.55 •	-308.73 ± 689.16 •	-57.4 ± 120.15	0.04* (3.46)
Δ T=15 minutes (RLUadj)	-2453.93 ± 705.31 •	-763.33 ± 313.34 •	-525.13 ± 411.97	0.12 (2.26)

Mean and standard error of the mean (S.E.M.) are presented for the change (Δ) in leukocyte activity (difference between 45 minutes pre- and immediately post-stressor samples) (Leukocyte Activity - Adjusted Relative Light Units – RLU_{adj}) for each treatment group (n = 15 for each). Repeated measures single factor ANOVA was used to investigate the effect of treatment on leukocyte activity (*P*) (*d.f.* = 44). For T=10 minutes a significant difference was observed between all treatment groups.

▲Difference between treatment groups ($P < 0.05$) (Tukey's *post hoc* procedure)

• Difference between Pre- and Post-Stressor Leukocyte Activity ($P < 0.05$)

* $P < 0.05$ Statistically Significant

TABLE 2 Effect of stressor on heart rate, core body temperature and blood pressure

	INTERFACE A	INTERFACE B	INTERFACE C	*P* (F)
Δ Heart rate (bpm)	3.0 ± 1.0 •	3.0 ± 1.0 •	1.0 ± 0.0	0.07 (2.9)
Δ Core Body Temperature (°C)	0.3 ± 0.1 •	0.2 ± 0.1 •	0.1 ± 0.1	0.03* (3.85)
Δ Systolic Blood Pressure (mmHg)	2.0 ± 1.0 •	1.0 ± 1.0 •	0.0 ± 0.0	0.001* (7.87)
Δ Diastolic Blood Pressure (mmHg)	0.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.72 (0.33)

Mean and standard error of the mean (S.E.M.) are presented for the change (Δ) in heart rate, core body temperature and systolic and diastolic blood pressure (difference between 45 minutes pre- and immediately post-stressor samples) for each treatment group (n = 15 for each). Repeated measures single factor ANOVA was used to investigate the effect of treatment on each of the stated parameters (*P*) (*d.f.* = 44).

▲Difference between treatment groups ($P < 0.05$) (Tukey's *post hoc* procedure).

• Difference between Pre- and Post-Stressor ($P < 0.05$)

* $P < 0.05$ Statistically Significant