International Journal of Human-Computer Interaction

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/hihc20

Quantitative Physiological Assessment of Stress Via Altered Immune Functioning Following Interaction With Differing Automotive Interface Technologies

Graham K. Shelton-Rayner [a], Rubina Mian [b], Simon Chandler [c], Duncan Robertson [c] & David W. Macdonald [d]

[a] Coventry University, Coventry, UK

[b] Oxford MediStress Ltd., Birmingham, UK

[c] Jaguar Cars Limited, Engineering Centre, Coventry, UK

[d] University of Oxford, The Recanati-Kaplan Centre, Oxfordshire, UK

Available online: 27 Jun 2011

To cite this article: Graham K. Shelton-Rayner, Rubina Mian, Simon Chandler, Duncan Robertson & David W. Macdonald (2011): Quantitative Physiological Assessment of Stress Via Altered Immune Functioning Following Interaction With Differing Automotive Interface Technologies, International Journal of Human-Computer Interaction, 27:9, 900-919

To link to this article: http://dx.doi.org/10.1080/10447318.2011.555312

INTL. JOURNAL OF HUMAN–COMPUTER INTERACTION, *27*(9), 900–919, 2011

ISSN: 1044-7318 print / 1532-7590 online
DOI: 10.1080/10447318.2011.555312

Taylor & Francis
Taylor & Francis Group

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[3], and David W. Macdonald[4]
[1]Coventry University, Coventry, UK
[2]Oxford MediStress Ltd., Birmingham, UK
[3]Jaguar Cars Limited, Engineering Centre, Coventry, UK
[4]University of Oxford, The Recanati-Kaplan Centre, Oxfordshire, UK

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 utilizing differing sensory modalities while maintaining lane discipline within a computer-simulated environment was assessed. Fifteen 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 poststressor 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.

This research was sponsored by Jaguar Cars Limited, Engineering Centre, Whitley, Coventry, United Kingdom. This work formed part of a PhD program 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 Leukocyte Coping Capacity 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.

Correspondence should be addressed to 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. E-mail: david.macdonald@zoo.ox.ac.uk

1. INTRODUCTION

Quantitative assessment of the physiological changes associated with psychological stress—a threat that would not require a physiological response that elicits physiological consequences (Segerstrom & 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 & Chang, 1999; Dhabhar, Miller, McEwen, & Spencer, 1996). Short-term psychological stress characterized 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, Coe, & McCarthy, 1996; Maes, Van Der Planken, et al., 1998) 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 (BP), and the activation state of specific classes of leukocyte, notably neutrophils (Mian, Shelton-Rayner, Harkin, & Williams, 2003; Shelton-Rayner, Macdonald, Chandler, Robertson, & Mian, 2010).

Epidemiological studies demonstrate that individuals who experience increased psychological stress are more susceptible to opportunistic infection (Clover, Abell, Becker, Crawford, & Ramsey, 1989; Galinowski, 1997). Rodriguez-Galan, Correa, Cejas, and Sotomayer (2001) demonstrated how the opportunistic fungal disease *Candida albicans* proliferated in stressed rather than nonstressed 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, Song, et al., 1998; 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, Semenchuk, & O'Brien, 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 (ERPs), cerebral metabolism and blood flow (Byrne & Parasuraman, 1996; Iqbal, Adamczyk, Zheng, & Bailey, 2005; Scerbo et al., 2001; Sharit & Salvendy, 1982), and changes in the plasma concentrations of specific stress hormones (cortisol and adrenaline; Clow et al., 2006; Okutsu, Ishii, Jun Niu, & Nagatomi, 2005). The effectiveness of such techniques is limited, as studies have shown inter- and intravariability 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 was used to assess salivary cortisol concentration during periods of intense physical exertion. Enzyme-linked immunosorbant assays are complex multistep procedures requiring hours before results are known, whereas Leukocyte Coping Capacity (LCC) analysis utilizes 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, Iliadis, & Macheras, 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 behavioral changes. Psychological stress reduces the effectiveness of the immune system, thus leading to an increased risk of infection or disease (Boscarino & Chang, 1999; Dhabhar et al., 1996). Even short-term psychological stressors such as academic examinations (Kang et al., 1996; Maes, Van Der Planken, et al., 1998) 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), the purpose of which is the denaturation and removal of invading pathogens (Ruotsalainen, Hyvärinen, Nevalainen, & Savolainen, 1995). The nonspecific 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 & Smolen, 1998; Segerstrom & Miller, 2004). Leukocyte activation (primarily neutrophils) can also occur following psychological stimulation. In 2002, Atanackovic, Brunner-Weinzierl, Kröger, Serke, and Deter demonstrated how exposure to a putatively stressful event resulted in a significant reduction in ROS production compared to control. More recently, studies conducted by us and others, utilizing chemiluminescent assay of Phorbol 12-Myristate 13-Acetate (PMA) -induced ROS production by leukocytes, have demonstrated a quantitative link between psychological confusion/anxiety and immune-competency (McLaren et al., 2003; Mian et al., 2003; Montes, McLaran, Macdonald, & Mian, 2003, 2004; Shelton-Rayner, 2009; Shelton-Rayner et al., 2010). The LCC technique involves measuring the ability of leukocytes to produce a respiratory burst following chemical challenge, assayed in terms of 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 utilizes 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 more than 250 different receptors (Mian,

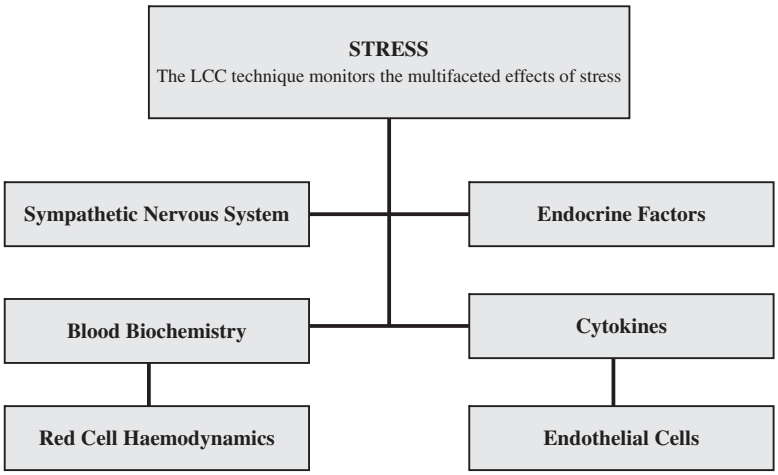

FIGURE 1 Flow diagram showing factors believed to affect the activation state of leukocytes. *Note.* From "Leukocyte Reactivity as an Objective Means of Quantifying Mental Loading During Ergonomic Evaluation," by G. K. Shelton-Rayner, D. W. Macdonald, S. Chandler, D. Robertson, and R. Mian, 2010, *Cellular Immunology, 263,* p. 23. Copyright 2010 by Elsevier. Reprinted with permission.

McLaren, & Macdonald, 2005) that 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 noncirculating 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 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) that 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 because 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 signaling 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 Nitroblne Tetrazolium (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, Marucha, Malarkey, Mercado, & Glaser, 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 on an individual's psychological status following interaction with motor vehicle control systems of differing ergonomic design.

2. METHOD

2.1. Participants

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

Subjects were 15 (7 male, 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 had prior knowledge of 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.

2.2. Materials and Procedure

The ability to interpret and act on specific commands depends on 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 the radio station). Interface configuration and the sensory modalities they are focused on (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—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 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 preexisting research projects, so those that remained were made available for the described study. Vehicles were therefore selected on a semirandom 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-m square screen in front of the test vehicle. Steering inputs were received by means of a sensor placed under each of the front wheels, whereas 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 & Skov, 2007; Kwon, Chun, Bae, & Suh, 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 that accompanies the purchase of either vehicle.

2.3. Procedure

The experimental protocols were rigorously standardized, and testing was confined to between 10 a.m. and 2 p.m. Subjects were required to avoid strenuous activity for at least 2 hr prior to testing (e.g., they were instructed to take the elevator to the laboratory rather than climb the stairs).

Perceived psychological stress. Perceived psychological stress was established pre- and immediately poststressor 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. Forty-five min 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, ranging from 1 (*relaxed*) to 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 prestressor values. The magnitude of stress would increase according to interface complexity.

Prior to obtaining resting heart rate, BP, and core body temperature following the standardized procedure outlined next and illustrated in Figure 2, subjects sat quietly and were instructed to breathe orthonasally for 15 min. The first pair of capillary blood samples was then taken 45 min before exposure to the test

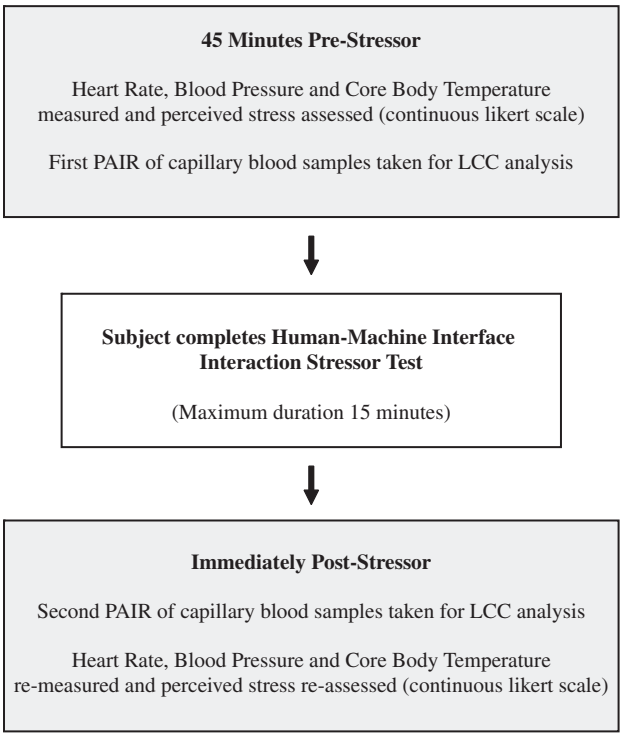

FIGURE 2 Flow diagram illustrating the time line for blood sample collection, for Leukocyte Coping Capacity (LCC) analysis, and measurement of other physiological (heart rate, systolic and diastolic blood pressure, and core body temperature) and psychological (perceived psychological stress rating) parameters. *Note.* From "Leukocyte Reactivity as an Objective Means of Quantifying Mental Loading During Ergonomic Evaluation," by G. K. Shelton-Rayner, D. W. Macdonald, S. Chandler, D. Robertson, and R. Mian, 2010, *Cellular Immunology, 263,* p. 24. Copyright 2010 by Elsevier. Adapted with permission.

apparatus (45 min prestressor; see next); these initial samples were used as a control to determine baseline leukocyte activity.

During the 45-min prestressor period, subjects were taken from the laboratory (Coventry University) to Jaguar Cars Ltd. research and development center at Whitley, Coventry, United Kingdom—a 5-min journey by car.

Upon entering the first test vehicle (selected using a counterbalanced crossover design), subjects 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 subjects then proceeded to familiarize themselves with the responsiveness of the steering and the acceleration and braking by driving around the virtual test track for 2 min (e.g., as the 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 min. Subjects were asked to complete the following tasks using the selected interface modality while driving within the center lane of the virtual test track at a constant speed of 60 miles per hour. Stress levels would already be high as a result of maintaining road position and speed. We hypothesize 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. While 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 on 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 submenu 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 to the 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 degrees Celcius." In the case of programming a destination into the satellite navigation system, many of the commands could not be achieved via voice commands, so 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—for example, 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 submenu format on an LCD screen mounted within the center stack of the dashboard. 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 be offered only after 4 min of attempting to complete the task. Immediately upon completion of the tasks (immediately poststressor), the subject was asked to come to a controlled stop in the center 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 hr existed between tests for baseline leukocyte activity to reestablish), each subject was tested using the remaining two interfaces using identical protocol (test order was decided using a counterbalanced design).

Heart rate, BP, 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 BP was measured using an oscillometric wrist-mounted BP monitor (Omron RX-3, Omron Healthcare Inc., Deerfield, Illinois). Core body temperature was measured using an infrared ear thermometer (Braun® Thermoscan™, P and G Brooklands, Waybridge, UK). It was expected that an increase in all stated parameters would be observed following stressor exposure compared to prestressor 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, UK) from the side of a finger of the subject's noncontractual hand. Following the procedure illustrated in Figure 3, one sample was used for the (nonstimulated) control (Sample A) and was placed into 10μl of murine heparin (concentration 0.1 units; CP Pharmaceuticals Ltd, Wrexham, UK), 10μl Phosphate Buffered Saline (Sigma Aldrich, Dorset, UK) and 90μl of 10^{-4}M Luminol ($C_8H_7N_3O_2$; Sigma Aldrich, Dorset, UK). The second blood sample (Sample B) was added to the same reagents, except that the 10μl of Phosphate Buffered Saline was replaced by 10μl of 10^{-5}M PMA (Sigma Aldrich, Dorset, UK). 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).

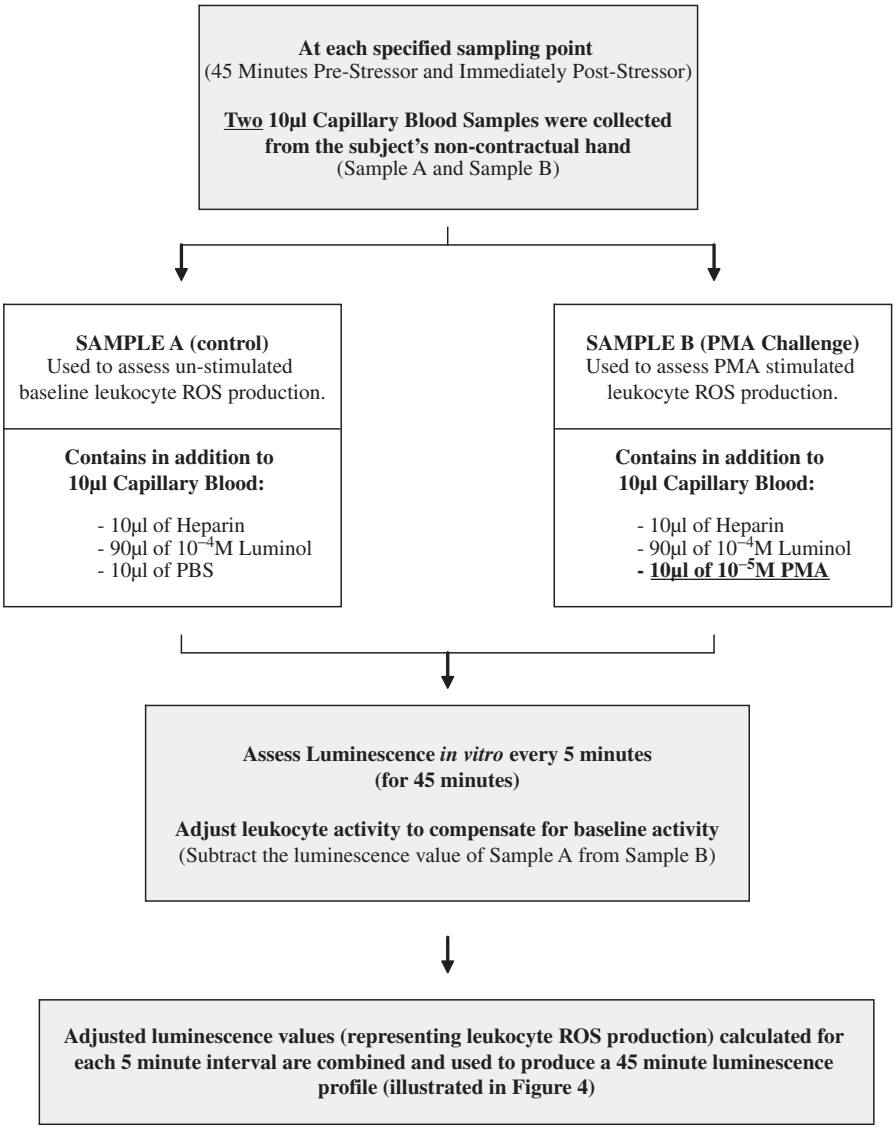

FIGURE 3 Leukocyte activity was assessed using whole blood samples taken 45 min pre- and immediately poststressor, following the protocol illustrated. *Note.* ROS = reactive oxygen species; PMA = Phorbol 12-Myristate 13-Acetate. From "Leukocyte Reactivity as an Objective Means of Quantifying Mental Loading During Ergonomic Evaluation," by G. K. Shelton-Rayner, D. W. Macdonald, S. Chandler, D. Robertson, and R. Mian, 2010, *Cellular Immunology, 263,* p. 25. Copyright 2010 by Elsevier. Reprinted with permission.

Determining leukocyte activity. LCC is a measure, made using a Luminometer, of the concentration of 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 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

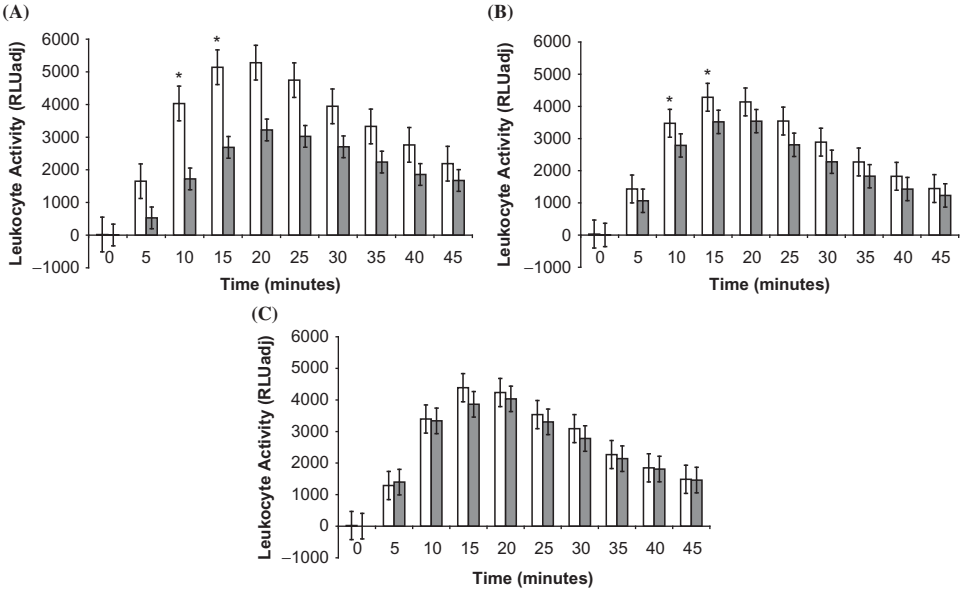

FIGURE 4 Mean adjusted Leukocyte Coping Capacity (RLU_{adj}) ± *SEM* for Treatment Groups A, B, and C ($n = 15$ for each). *Note.* Open bars represents mean adjusted leukocyte activity 45 min prestressor, and closed bars represents activity immediately poststressor. Asterisk indicates significant difference in activity between 45 min pre- and immediately poststressor ($p < .05$).

Table 1: Effect of Stressor on Leukocyte Activity

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

Note. Mean and standard error of the mean are presented for the change (△) in leukocyte activity (difference between 45 min pre- and immediately poststressor samples) (Leukocyte Activity – Adjusted Relative Light Units – RLU_{adj}) for each treatment group ($n = 15$ for each). Repeated measures single factor analysis of variance was used to investigate the effect of treatment on leukocyte activity (p) ($df = 44$). For T = 10 min, a significant difference was observed between all treatment groups. ▲Difference between treatment groups ($p < .05$, Tukey's post hoc procedure). • Difference between pre- and poststressor leukocyte activity ($p < .05$). *$p < .05$ statistically significant.

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 min using a Luminometer (Berthold® Technologies, Junior™ LB9509, Hertfordshire, UK) for a total of 45 min (Figure 3) to produce a luminescence profile (Figure 4a, 4b, 4c). Between chemiluminescence measurements the samples were incubated at 37 °C in a water bath (JB1™ Grant Instruments, Cambridge, UK). As part of homeostasis, there is a small but constant release of ROS from leukocytes that, 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-min 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 poststressor changes ± standard error of mean (*SEM*). For T-max (time taken to reach maximum leukocyte activity), the data were classed as discontinuous as leukocyte activity was measured at 5-min intervals for a total of 45 min, in this case the median ± *SEM* is presented. Repeated measures, single-factor analysis of variance (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 was 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 just listed and poststressor changes in leukocyte

activity. The p values were corrected for the use of multiple comparisons using the Truncated Product Method (Zaykin, Zhivotovsky, Westfall, & Weir, 2002).

A repeated measures analysis of variance 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).

3. RESULTS

3.1. Leukocyte Activity

Leukocyte activity profiles produced from blood samples taken 45 min pre- and immediately poststressor 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 min pre- and immediately poststressor) occurred between T = 15 and 20 min following exposure to Interfaces B (Figure 4b) and C (Figure 4c) and between T = 20 and 25 min for Interface A (Figure 4a), after which time all profiles showed a steady decrease in activity. The results show that a significant difference in poststressor leukocyte activity, between treatment groups, occurred 10 min into the 45-min luminescence profile (T = 10 min; 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, 4c). The magnitude of the poststressor change in activity following the use of Interface A was significantly greater compared to the use of Interface B ($p = .01$, Tukey's post hoc procedure) and Interface C ($p = \leq .001$, Tukey's post hoc procedure), whereas the use of both Interfaces B and C resulted in poststressor changes in leukocyte activity of similar magnitude ($p = .47$, Tukey's post hoc procedure; Table 1).

3.2. 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 poststressor change in leukocyte activity for Hmax-RLU_{adj}, $F(1, 134) = 10.83$, $p \leq .001$, and T = 15 min, $F(1, 134) = 11.02$, $p \leq .001$. In both cases the significant difference occurred between test order combination 1 and 3 (in each case $p = .02$, 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.

3.3. Perceived Psychological Stress

Overall, perceived psychological stress was significantly different between treatment groups, $F(1, 89) = 7.73$, $p = .001$. 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, $p \leq .001$, Tukey's post hoc procedure; rating based on a continuous arbitrary scale where 1 represented *relaxed* and 10 represented *stressed*). The use of Interface C to complete the required tasks resulted in a poststressor increase of 0.06 ± 0.18 units, which was of a similar magnitude to that observed following the use of Interface B ($p = .19$, Tukey's post hoc procedure). Observed changes in perceived psychological stress were not found to be significantly correlated with the observed poststressor decreases in leukocyte activity.

3.4. 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 ($p = .92$, Tukey's post hoc procedure), whereas the use of Interface C resulted in a poststressor increase that was significantly lower in magnitude compared to the use of Interface A ($p = .04$, Tukey's post hoc procedure).

3.5. Heart Rate, BP

Treatment Groups A and B demonstrated significant increases in heart rate compared to baseline (45 min prestressor), 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.

Table 2: Effect of Stressor on Heart Rate, Core Body Temperature, and Blood Pressure

	Interface A	*Interface B*	*Interface C*	*p (F)*
△ Heart rate (bpm)	3.0 ± 1.0 •	3.0 ± 1.0 •	1.0 ± 0.0	0.07 (2.9)
△ Core body temperature (°C)	0.3 ± 0.1 •	0.2 ± 0.1 •	0.1 ± 0.1	0.03* (3.85)
△ Systolic blood pressure (mmHg)	2.0 ± 1.0 •	1.0 ± 1.0 •	0.0 ± 0.0	0.001* (7.87)
△ Diastolic blood pressure (mmHg)	0.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.72 (0.33)

Note. Mean and standard error of the mean are presented for the change (△) in heart rate, core body temperature and systolic and diastolic blood pressure (difference between 45 min pre- and immediately poststressor samples) for each treatment group ($n = 15$ for each). Repeated measures single factor analysis of variance was used to investigate the effect of treatment on each of the stated parameters (p) ($df = 44$). ▲Difference between treatment groups ($p < .05$; Tukey's post hoc procedure). •Difference between pre- and poststressor ($p < .05$). *$p < .05$ statistically significant.

As with heart rate, only Treatment Groups A and B showed significant increases in systolic BP compared to baseline (Table 2). The magnitude of the poststressor increase was significant between treatment groups, $F(1, 44) = 7.87$, $p = .001$. The use of Interfaces A and B resulted in a similar magnitude of change ($p = .81$, Tukey's post hoc procedure), whereas using Interface C resulted in a poststressor increase in systolic BP that was significantly lower in magnitude compared to Interface A ($p = .002$, Tukey's post hoc procedure) and Interface B ($p = .01$, 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 poststressor change in heart rate between treatment groups only approached significance, $F(1, 44) = 2.9$, $p = .07$, 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 min into the 45-min profile (T = 5 min).

4. 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 utilized (heart rate, BP, respiration rate, stress hormone concentration, e.g., cortisol) are highly regulated via complex biochemical feedback mechanisms designed to rapidly eliminate homeostatic imbalance. This is further confounded with the existence of considerable biological variation between individuals and populations (Brown, James, Nordloh, & Jones, 2003; Hodgson, Freedman, Granger, & Erno, 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 & Tessier, 2003). It should be noted that this study describes a novel application for LCC analysis, 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 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, Jiménez-Dalmaroni, Racine, Bornens, & Jülicher, 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, and 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, Stanton, and Young (2001) analyzed 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 an 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 prestressor 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 &

Marin, 2006; McLaren et al., 2003; Shelton-Rayner, 2009; Shelton-Rayner et al., 2010).

The format by which information is both received and supplied by the driver can significantly alter response time, accuracy, and performance while 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 poststressor leukocyte activity. As a substantial proportion of high-feedback vehicles containing human–machine interface 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 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 minimized 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, and 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 that 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 well-being.

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. *Immunological Investigations*, *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. *Hematology/Oncology Clinics of North America*, *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 Pressure Monitoring*, *8*, 237–242.

Byrne, E. A., & Parasuraman, R. (1996). Psychophysiology of workload. *Biological Psychology*, *42*, 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. *Journal of Immunology*, *156*, 2608–2615.

Dokoumetzidis, A., Iliadis, A., & Macheras, P. (2002). Nonlinear dynamics in clinical pharmacology: the paradigm of cortisol secretion and suppression. *British Journal of Clinical Pharmacology*, *54*, 21–29.

Elliot, G. R., & Eisdorfer, C. (1982). *Stress and human health: An analysis and implications of research*. New York, NY: Springer.

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. *Research in Social & Administrative Pharmacy*, *4*, 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. *Journal of Behavioral Medicine*, *22*, 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. *Journal of the American Geriatrics Society*, *52*, 1856–1862.

Honess, P. E., & Marin, C. M. (2006). Behavioural and physiological aspects of stress and aggression in nonhuman primates. *Neuroscience & Biobehavioral Reviews*, *30*, 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. *Proceedings of the ACM Conference on Human Factors in Computing Systems*, 311–320.

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. *International Journal of Human–Computer Interaction*, *22*(1&2), 7–36.

Kwon, S-J., Chun, J-H., Bae, J-H., & Suh, M-W. (2006). A study on the factors that improve the velocity perception of a virtual reality-based vehicle simulator. *International Journal of Human–Computer Interaction*, *21*, 36–54.

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

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

Maes, M., Song, C., Lin, A., De Jongh, R., Van Gastel, A., Kenis, G., . . . Smith, R. S. (1998). The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. *Cytokine*, *10*, 313–318.

Maes, M., Van Der Planken, M., Van Gastel, A., Bruyland, K., Van Hunsel, F., Neels, H., . . . Scharpe, S. (1998). Influence of academic examination stress on haematological measurements in subjectively healthy volunteers. *Psychiatry Research*, *80*, 201–212.

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. *Experimental Physiology*, *88*, 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, NY: Nova Science.

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. (2003). The effects of acute stress on leukocyte activation. *Journal of Physiology*, *548P*, 170.

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

Okutsu, M., Ishii, K., Jun Niu, K., & Nagatomi, R. (2005). Cortisol-induced CXCR4 augmentation mobilises T lymphocytes after acute physical stress. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, *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*, 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. *Environmental Research*, *69*, 122–131.

Scerbo, M. W., Freeman, F. G., Mikulka, P. J., Parasuraman, R., Nocero, F. D., & Lawrence, J. P., III. (2001). *The efficacy of psychophysiological measures for implementing adaptive technology* (Tech. Rep. NASA-20010tp211018). NASA Langley Technical Report Server. Retrieved from http://portal.acm.org/citation.cfm?id=886463 (last accessed May 2010)

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

Sharit, J., & Salvendy, G. (1982). Occupational stress: Review and reappraisal. *Human Factors: The Journal of the Human Factors and Ergonomics Society*, *24*, 129–162.

Shelton-Rayner, G. K. (2009). *Quantifying responses to psychological and physiological stress in automotive design*. Unpublished doctoral dissertation, Coventry University, Coventry, UK.

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. *Cellular Immunology*, *263*, 22–30. 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. *Aviation, Space, and Environmental Medicine*, *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. *Journal of Clinical Laboratory Analysis*, *16*, 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*, 421–444.

World Medical Association. (2004, October). *Declaration of Helsinki*. Ethical Principles for Medical Research Involving Human Subjects WMA General Assembly, Tokyo 2004. Available from http://www.wma.net/en/30publications/10policies/b3/17c.pdf (last accessed May 2010)

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