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Leukocyte reactivity as an objective means of quantifying mental loading during ergonomic evaluation

G.K. Shelton-Rayner [a], D.W. Macdonald [b,*], S. Chandler [c], D. Robertson [c], R. Mian [d]

[a] Department of Biomolecular and Sport Sciences, Faculty of Health and Life Sciences, Coventry University, Cox St, Coventry CV1 5FB, UK
[b] Wildlife Conservation Research Unit, Zoology Department, University of Oxford, The Recanati-Kaplan Centre, Tubney House, Abingdon Road, Tubney, Oxfordshire OX13 5QL, UK
[c] Jaguar and Land Rover Research Department, Jaguar Cars Limited, Engineering Centre, Whitley, Coventry CV3 4LF, UK
[d] Oxford MediStress Ltd., Suite 125, 27 Colemore Row, Birmingham B3 2EW, UK

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ABSTRACT

Psychological stress evokes rapid changes to the cardiovascular and neuroendocrine systems, responses that can become habituated following repeated exposure. This study, comprising of two phases, suggests that the immune system follows a similar trend. Phase 1: 15 healthy subjects (aged between 26 and 56 years) provided capillary blood samples before and after completing three basic tasks using, in turn, two automotive touch screen interfaces (Interface 1—antecedent version, Interface 2—improved version). Using a chemiluminescent technique termed leukocyte coping capacity (LCC), the ability of leukocytes to produce reactive oxygen species *in vitro* was assessed. Significant differences in leukocyte activity were shown between treatment groups, where the greatest post-test decrease occurred after using Interface 1. Phase 2: a randomly selected sub-group ($n = 4$) underwent weekly repeat testing using both interfaces. Significant differences in post-test leukocyte reactivity were exhibited between test weeks for each interface—the magnitude of response decreasing with successive exposure.

1. Introduction

Quantification of psychological stress resulting from environmental challenges or social interactions is generally accomplished by one of two approaches. First, evaluation of perceived mental workload using subjective self assessment (including the NASA task load index inventory [1] and subjective workload assessment techniques (SWAT) [2]). Second, monitoring characteristics of the cardiopulmonary system and assay of specific stress hormones including salivary cortisol [3–6] and catecholamines [7]. Brown et al. [8,9] demonstrated how urinary catecholamine excretion can quantitatively indicate the effects of long-term psychological stressors—characterised by the taxonomic classification proposed by Elliot and Eisdorfer [10].

Assessing the physiological effects of short-term psychological stressors is more problematic, and is generally attempted by measurement of physical characteristics including respiration rate and skin conductance [11,12] in addition to heart rate, blood pressure and body temperature [5–8]. All are subject to considerable biological variation, introducing uncertainty to comparison between individuals and populations.

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 [13,14]. Even short-term psychological stressors such as academic examinations [15,16] can produce demonstrable physiological changes in the reactivity of specific classes of leukocyte, notably neutrophils [17].

Activated leukocytes release an array of mediators, including reactive oxygen species [18]. Although their function is to attack invading pathogens, the products of leukocytes have the potential to damage healthy tissue and organs [19,20]. A study conducted by Atanackovic et al. demonstrated how exposure to a putatively stressful event resulted in a significant reduction in ROS production, compared to control [21]. More recently, chemiluminescent assay of PMA-induced ROS production by leukocytes has been shown to provide quantitative links between psychological anxiety and immune-competency [22–24]. 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 [22].

The LCC test monitors the multifaceted effects of stress using the body's leukocytes (primarily, but not exclusively, neutrophils) as bio-indicators. These cells circulate throughout the body picking up and responding to all of the signals of stress (as indicated in

* Corresponding author. Fax: +44 1865 393101.
E-mail address: david.macdonald@zoo.ox.ac.uk (D.W. Macdonald).

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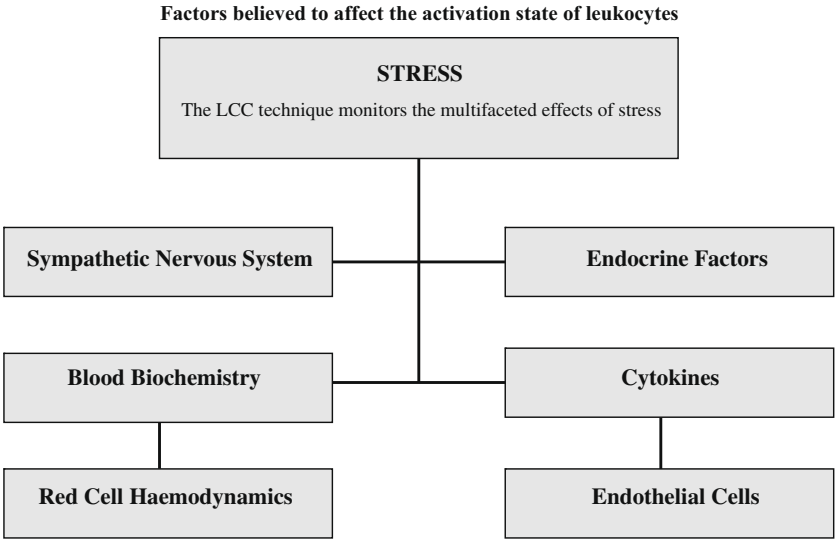

Fig. 1. Flow diagram showing factors believed to affect the activation state of leukocytes.

Fig. 1). Leukocytes (primarily, but not exclusively, neutrophils) have over 150 different receptors [25] which can respond to a diverse range of factors, all of which are sensitive to stress. These include: endocrine factors in the plasma, changes in blood biochemistry, changes in red cell haemodynamics, cytokines and factors released from other cells, both circulating and non-circulating cells such as endothelial cells, and changes in the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system. As stress affects each of these factors, leukocytes make ideal indicators of stress, being constantly exposed to a diverse range of stress stimuli. 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 [25].

The LCC test is a physiologically relevant blood test for objectively assessing the effect of stress. The physiological relevance is convincing since:

- Firstly, the leukocytes are kept in the local environment, i.e. they are suspended in the blood. The suspension of leukocytes in blood allows the cells to dynamically interact with the surrounding red cells and allows cell–cell interaction within and between different leukocyte cohorts. This can dramatically affect the responsiveness of leukocytes. The viscosity and cell–cell interaction with other leukocytes, hormones and cytokines of the surrounding cells can have a dramatic effect on shear stresses and the expression of cell surface receptors. Disruption to cell signalling pathways are minimised, and the responsiveness and integrity of cells is maintained.
- Secondly, the technique avoids centrifugation, a process known to affect cell reactivity, and also avoids 'plating out' cells on glass slides—as used in the NBT test [26]. The cells are stimulated *in vitro* with PMA and the superoxide producing capacity of the cells is measured in real time. As leukocytes release reactive oxygen species in response to stress [22], the stimulation allows us to evaluate the capacity that the cells have to produce 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.

The use of a drop of whole blood is deliberate. Leukocytes are three-dimensional entities, their ability to produce reactive oxygen species is altered by cell signalling pathways of other entities and cells. The aim of this study is to monitor the cellular capacity of leukocytes to produce superoxide radicals in real time. By deliberately leaving the cells in contact with the circulating mediators of stress within blood, the leukocytes are able to actively interact with other cellular components and mediators. Leukocytes (primarily neutrophils) have over 150 different receptors which can respond to a diverse range of factors, all of which are sensitive to stress [25]. The use of a 10 μl drop of whole blood that is maintained and which is not spread on glass or preserved, attempts to provide *in vivo* conditions within an *in vitro* environment, thus allowing three-dimensional exposure to, and interaction with hormones (which can alter the reactivity of the cells), other cells such as macrophages, other neutrophils, the haematocrit, and red blood cells (whose viscosity alter during stress).

The objectives of this study were to firstly, investigate the feasibility of using altered leukocyte responsiveness as a means of objectively assessing and discriminating between changes in psychological anxiety/mental workload, elicited as a consequence of interaction with two different touch screen interfaces from the same motor manufacturer (Interface 1—an antecedent model and Interface 2—a new version designed to facilitate ease of use and therefore reduce mental workload). Secondly, we aimed to investigate whether the concept of habituation, as observed within the cardiovascular system [27–29], can also be applied to leukocyte responsiveness, following increased psychological familiarity to a specific situation.

2. Materials and methods

2.1. The subjects

Local Ethical Committee approval from Coventry University Ethics Committee, and informed consent, was obtained before commencement of the study, in accordance with the declaration of Helsinki [30].

Subjects were 15 (7 male and 8 female) moderately fit and healthy individuals, aged between 26 and 56 years. Potential subjects were excluded on the following criteria: suffering from psychiatric illness; suffering from respiratory or cardiovascular disease; smokers; had taken prescription medicine within the pre-