The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

Animal Welfare 2009, 18: 1-7
ISSN 0962-7286

*Impact of trapping and handling on Leukocyte Coping Capacity in bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*)*

M Gelling[*†], *GW McLaren*[†‡], *F Mathews*[†§], *R Mian*[#] *and DW Macdonald*[†]

[†] Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, Tubney House, Abingdon Road, Tubney, Oxon OX13 5QL, UK
[‡] Magnox North, Building B12, Berkeley Centre, Berkeley, Gloucestershire GL13 9PB, UK
[§] School of Biosciences, University of Exeter, Hatherley Laboratories, Prince of Wales Road, Exeter EX4 4PS, UK
[#] Oxford MediStress, Suite 125, 27 Colmore Row, Birmingham B3 2EW, UK
* Contact for correspondence and requests for reprints: merryl.gelling@zoo.ox.ac.uk

Abstract

*Small mammals are routinely live-trapped and subsequently handled for a range of ecological and behavioural studies. Despite the techniques commonly employed being potentially stressful for the individual animals involved, it has hitherto been difficult to quantify the physiological impact. Here, we report on the first instance of using the Leukocyte Coping Capacity technique (LCC) in bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*) to investigate the physiological impact of routine trapping and handling techniques. Twenty microlitres of blood were obtained from 40 animals, of which 25 were handled following standard protocols and 15 were not. We found that even a short period of acute stress is sufficient to trigger an immune response which was measurable using the LCC technique. These results further validate the use of the LCC technique for measuring the physiological impact of standard trapping and handling treatments on wild mammals.*

Keywords: *animal welfare, handling, Leukocyte Coping Capacity, small mammals, stress, trapping*

Introduction

Studying wild mammals often requires the use of trapping and handling techniques that are potentially stressful for the animals involved (eg Millspaugh *et al* 2000; McLaren *et al* 2004; Fletcher & Boonstra 2006; Prout & King 2006). Such studies often ignore or dismiss the potential for stress to have lasting consequences for the study animals, even though stress is known to affect a wide range of physiological parameters. For example, McLaren *et al* (2004) demonstrated that small mammals (wood mice [*Apodemus sylvaticus*] and bank voles [*Clethrionomys glareolus*]) are more likely to lose weight following an extensive handling regime including anaesthesia, than a minimal handling treatment involving brief handling and release. Weight loss in those animals exposed to the more intensive handling procedures was the likely consequence of an array of behavioural and physiological responses to stress (McLaren *et al* 2004). It is, therefore, clearly important to understand the 'cost' or 'allostatic load' (McEwen 1998) induced by stressful handling procedures to ensure that results from studies of wild animals are not biased (McLaren *et al* 2007).

McEwen (1998) defines allostasis as the normal adaptive physiological responses, occurring as a typical function of homeostasis, which enables the individual to cope with potentially stressful events. Chronic over-stimulation of these normal physiological responses may result in allostatic load, which is damaging to the body (McEwen 1998), and which may incur costs including reduced immune function.

The interaction between stress and immune function has received considerable attention in recent years (eg Romano *et al* 2004; Roberts *et al* 2007) and measures of immune function after or during stress have been suggested as direct measures of the stress response (McLaren *et al* 2003; Mian *et al* 2003; Romano *et al* 2004; Honess *et al* 2005). Measures of immune function in response to stress are particularly relevant to wild animals as any impairment of immune function is likely to impact upon an animal's future chance of survival and reproduction (Alonso-Alvarez *et al* 2004). One particular response of the immune system that has been suggested as a measure of stress is the release of oxidants by leukocytes (McLaren *et al* 2003). Some leukocytes (particularly neutrophils) can become activated and release oxygen-free radicals in response to a stressful situation (Ellard *et al* 2001; Mian *et al* 2003; Montes *et al* 2003, 2004). This process is known as the respiratory burst. The respiratory burst also occurs when neutrophils respond to agonists such as bacteria (McLaren *et al* 2003). It is thought that the stress-related release of oxygen-free radicals may be a mechanism to prepare the body for injury and thus potential attack by bacteria (McLaren *et al* 2003).

https://doi.org/10.1017/S0962728600000014 Published online by Cambridge University Press

Oxygen-free radicals are potentially damaging to the host organism as well as invading bacteria (eg see review by Kruidenier & Verspaget 2002) and are energetically costly to produce, requiring increased oxygen uptake to produce highly reactive superoxide (O_2–) and utilisation of glutamine and glucose (Lin *et al* 1995), producing lysosomal hydrolases which phagocytose microorganisms. Different leukocytes are exhausted after different time periods; neutrophils and macrophages are the primary phagocytes, but macrophages are much longer-lived (Alberts *et al* 1994).

After a stressful event, and because of the costs associated with the release of oxygen-free radicals, there is a period when neutrophils reduce their usual respiratory burst response to bacterial challenge (McLaren *et al* 2003) and thus the animals are immunosuppressed. The measurement of the ability of individuals to produce a respiratory burst after a stressful event serves therefore as a potential measure of the stress response and also indicates the ability of an individual to mount an immune response. The ability of an animal's leukocytes to produce, *in vitro*, a respiratory burst in response to a bacterial-type challenge after a stressful event has been defined as 'Leukocyte Coping Capacity' (LCC) and this has been used as a measure of stress (McLaren *et al* 2003; Moorhouse *et al* 2007). The immune challenge is chemically stimulated using phorbyl myristate acetate (PMA) and compared with the individuals' basal response level of immune system response (Hu *et al* 1999; McLaren *et al* 2003). PMA works by activating NADPH oxidase, thus increasing the respiratory burst of oxygen-free radicals by leukocytes (Chen 2002). Sanidas *et al* (2002) reported that whilst some forms of physiological stress (exposure to hypoxia) produced a significant increase in the production of oxygen-free radicals within minutes *in vitro*, only a small proportion of leukocytes appear to be 'activated' (Sanidas *et al* 2000). McLaren *et al* (2003) conclude that animals with a higher LCC are more able to produce oxygen-free radicals in response to a bacterial challenge, and therefore LCC provides an assessment of an animal's current physiological condition and, thus, ability to cope with stress.

Here, we report on an experiment designed to investigate the impact of trapping and handling on stress levels in two small rodent species subjected to one of two handling treatments, using the Leukocyte Coping Capacity (McLaren *et al* 2003). Under the former, animals were trapped and handled according to conventional protocols, and then anaesthetised. Handling brings about a stress response typified by an increased heart rate and attempted escape behaviour, and is assumed to be generally stressful for wild mammals (eg Millspaugh *et al* 2000; McLaren *et al* 2004). In the second treatment, animals were anaesthetised without handling. We then measured the animals' LCC and thereby determined whether the extent of immunosuppression was affected by the handling treatment. We hypothesised that handling would be stressful and thus predicted that handled animals would have reduced LCC.

Materials and methods

Trials of alternative treatments

Small mammals were trapped overnight in Longworth live-capture traps (Penlon Ltd, Abingdon, UK) placed in pairs at 5 m intervals along hedgerows on two dairy farms in south-west England during June and July 2003. Traps were baited with approx 15 g of rabbit food (Delta Pet Foods Ltd, Llandovery, Carmarthenshire, UK), and apple (approx 5 g) as an *ad libitum* source of food and moisture. Hay was also provided in the traps for bedding. Traps were set between 1600–1630h and collected between 0800–0830h, the following morning. A single trapping session consisted of three consecutive days trapping and all licensable work was conducted under Home Office Licence PPL 30/1826.

Two species of small mammal, wood mice and bank voles, were used in the experiment; all non-target species were weighed, fur-clipped for capture-mark-recapture purposes, and released at the point of capture. Animals were randomly selected for subjection to one of two treatments: (i) handling and anaesthesia or (ii) anaesthesia without handling. Traps containing animals were taken by foot to a central sampling location less than 500 m from the trapping point, whereupon the door of each trap was opened and the animal briefly observed to ensure it was in a suitable condition to undergo anaesthesia. All occupied traps were then left covered by a blanket in a quiet area for 30 min prior to the experiment to allow the animals to rest before the experiment began. Empty traps were shut and left in place to preclude capturing any animals during the day. Handled animals were then removed from their traps by gently tipping them into a plastic bag where they were observed for species identification. Individuals were handled by their scruff to allow determination of sex and breeding condition. This is a typical handling procedure in ecological and behavioural studies of small mammals and was timed to last for 20 s. Handled animals were then returned to the plastic handling bag and anaesthetised. Non-handled animals were anaesthetised within their traps by placing the Longworth trap inside a plastic bag. The anaesthetic used was an inhalation mixture of oxygen and isoflourane (2%) delivered at a rate of 2 l min^{-1} (Isocare, Animalcare Ltd, York, UK; Mathews *et al* 2002). The non-handled animals served as a comparative sample that could be used to determine if handling caused a change in LCC. Anaesthetised animals were sexed, and their reproductive status was assessed (Gurnell & Flowerdew 1994). Animals were weighed to the nearest 0.1 g on a digital balance, their body length (snout-to-vent) measured to the nearest 1 mm, and a given a unique clip mark for capture-mark-recapture purposes. We consider only adult animals (15 g and over), in breeding condition (with scrotal testes in males, and females which are perforate) in our analyses.

We used bodyweight as an index of the resources available to each animal; this was appropriate because, for small mammals, bodyweight is more closely related to the level of stored reserves than calculated body condition indices

https://doi.org/10.1017/S0962728600000014 Published online by Cambridge University Press

(Hayes & Shonkwiler 2001). We also recorded the presence or absence of fleas, since the load of these common ectoparasites could potentially influence the animal's energy balance. Post-sampling, animals were placed in recovery containers in a dark, quiet area. Once the animals had fully recovered from the procedure they were released at their point of capture. Traps were reset between 1600 and 1630h. All individuals were recaptured at some point during the study, most during the following day. All recaptured animals were re-weighed to the nearest 0.1 g to allow investigation of differences in overnight weight changes between handled and non-handled animals, and then immediately released at their point of capture.

PMA challenge and measurement of Leukocyte Coping Capacity

Both anaesthesia and LCC work was conducted from a mobile sampling unit situated at the field site. Once an animal was anaesthetised, 20 µl of blood were taken from each animal via tail venepuncture, and collected in a heparinised capillary tube. To measure the unstimulated blood chemiluminescence levels and provide a baseline with which to measure an individual's LCC response, 10 µl of heparinised whole blood was immediately transferred into a silicon anti-reflective tube (Lumivial, EG & G Berthold, Germany), to which 90 µl of 10^{-4} mol l^{-1} luminol (5-amino-2,3-dihydrophthalzine; Sigma A8511) diluted in phosphate buffered saline (PBS) was then added. The tube was then shaken gently for mixing. Luminol chemiluminesceses when combined with an oxidising agent to produce a low-intensity light reaction (Whitehead *et al* 1992). To measure the chemiluminescence emitted by the oxygen-free radicals produced by leukocytes in response to challenge, a further tube was prepared as above using the remaining 10 µl of blood, but with the addition of 10 µl phorbol 12-myristate 13-acetate (PMA; Sigma P8139) at a concentration of 10^{-4} mol l^{-1}. The PMA solution had been prepared in advance by diluting 5 mg of PMA in 500 µl of dimethyl sulfoxide (DMSO; Sigma D 5879), which was then diluted to a concentration of 10^{-4} mol l^{-1} in PBS buffer. Individual aliquots were kept frozen and dark until required. For each tube, chemiluminescence was measured in relative light units (RLU) every five minutes using a portable chemiluminometer (Junior LB 9509, E G & G Berthold, Germany) for a total of 30 min. When not in the chemiluminometer, tubes were incubated at 37°C in a water bath.

Statistical analysis

To compare differences in LCC and different handling regimes, we used a multivariate analysis of variance (MANOVA). For LCC, the difference in response at each time interval between the control and PMA measures were the dependent variables, and produced a suite of correlated results for each animal through the course of a reaction. We chose this approach because it allowed us to examine the effect of the treatment over time, giving more detail on the nature of the effect and to identify the time interval at which the treatment effect was greatest (McLaren *et al* 2003). We also examined the factors which affect peak LCC, including species, sex, bodyweight, handling treatment, overnight change in bodyweight, and presence or absence of fleas. The LCC values were log-transformed prior to the analyses to meet the GLM requirement of normality of data. This procedure was carried out on SPSS for Windows 14.0.

Results

Forty adult animals were used in this study; of these, 25 were handled (16 bank voles and 9 wood mice) and 15 were not handled (9 bank voles and 6 wood mice) prior to anaesthesia. Analysis of data for both species together revealed that handling was associated with a marked reduction in LCC, and reduced the individual variation in LCC (Figure 1). A MANOVA revealed that handling significantly reduced LCC ($F_{7,32} = 7.0$, $P \leq 0.001$). There was no effect of including species in this model, either as a variable on its own ($F_{7,30} = 0.43$, $P = 0.88$) or as an interacting variable with handling treatment ($F_{7,30} = 0.54$, $P = 0.80$), although this non-significant result could be the result of small sample size. Even though the non-handled animals had consistently higher control values than handled animals (Figure 1), this difference was not statistically significant ($F_{7,32} = 0.54$, $P = 0.80$).

We looked at the bodyweights of each species, finding wood mice to weigh on average 23.0 g, and bank voles 22.6 g (see Table 1). Investigating the handling treatment × bodyweight interaction we found the LCC response of the heavier wood mice to be consistently higher than bank voles. LCC measures peaked in wood mice at 837.2 RLU after 20 min ($F_{2,12} = 7.19$, $P \leq 0.001$; see Figure 2), whilst vole LCC response peaked at 489.2 RLU after 15 min ($F_{2,22} = 7.84$, $P \leq 0.005$; see Figure 3). There was no significant difference in the scale of response (difference between control and PMA measures) between the two species at any time period ($t_{38} \geq 0.191$, $P \leq 0.301$, in all cases).

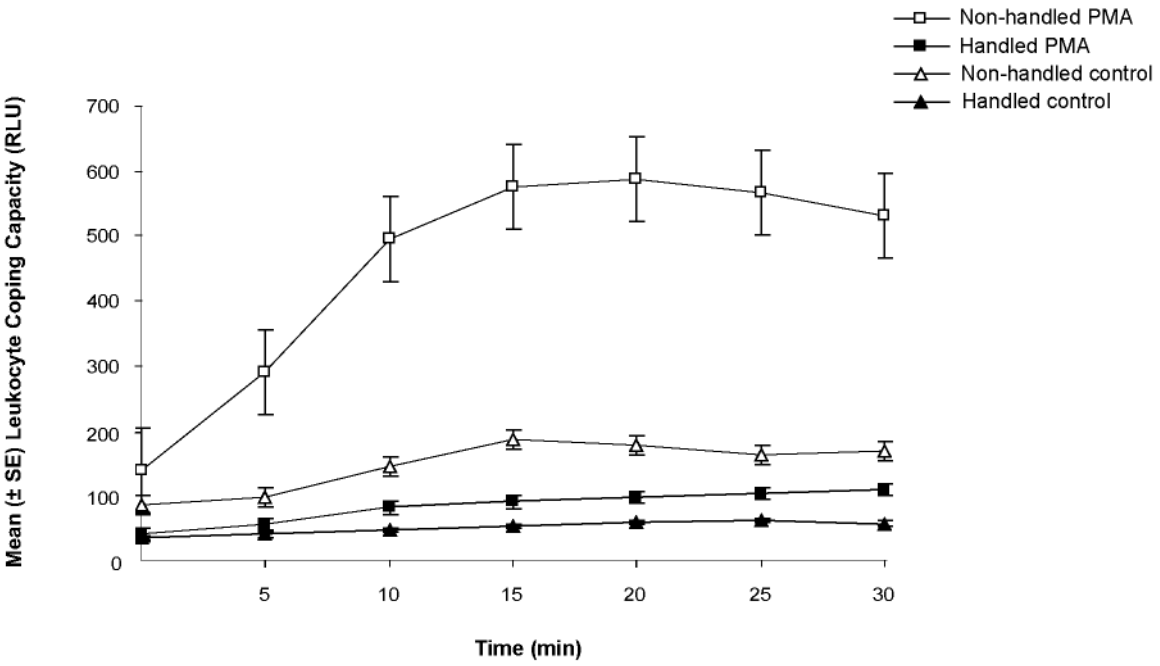
Including the incidence of fleas within the model did not have a significant impact on LCC score in wood mice, either alone ($F_{7,2} = 0.274$, $P = 0.918$), or as an interacting variable with handling treatment and bodyweight ($F_{7,2} = 0.487$, $P = 0.847$). Similar results were obtained for bank voles; fleas ($F_{7,12} = 0.683$, $P = 0.685$), fleas × handling treatment × bodyweight ($F_{7,12} = 2.43$, $P = 0.075$).

There was no significant difference between the sexes in the response of wood mice to LCC at any time period ($F_{7,7} \geq 0.03$, $P \geq 0.231$). Sex was a significant factor for bank voles at 0 min ($F_{7,17} = 0.627$, $P \leq 0.012$) but not at any other time period ($F_{7.17} \geq 0.041$, $P \geq 0.148$, in all cases).

Neither handled nor non-handled bank voles, nor handled wood mice experienced significant overnight weight change (bank voles; handled $t_8 = 1.6$, $P = 0.14$; non handled, $t_6 = 0.33$, $P = 0.75$; handled wood mice; $t_7 = –0.486$, $P = 0.642$), but non-handled wood mice did demonstrate a significant overnight weight increase ($t_3 = 6.195$, $P = 0.008$).

https://doi.org/10.1017/S0962728600000014 Published online by Cambridge University Press

Figure 1



LCC (mean [± SE] relative light units [RLU]) of handled and non-handled animals over time.

Table 1 The 40 animals used in the study.

	Wood mice	Bank voles
Handled	9	16
Non handled	6	9
Mean (± SD) weight range (g)	23.0 (± 3.1)	22.6 (± 2.3)
Fleas present	7	8
Fleas absent	8	17

Discussion

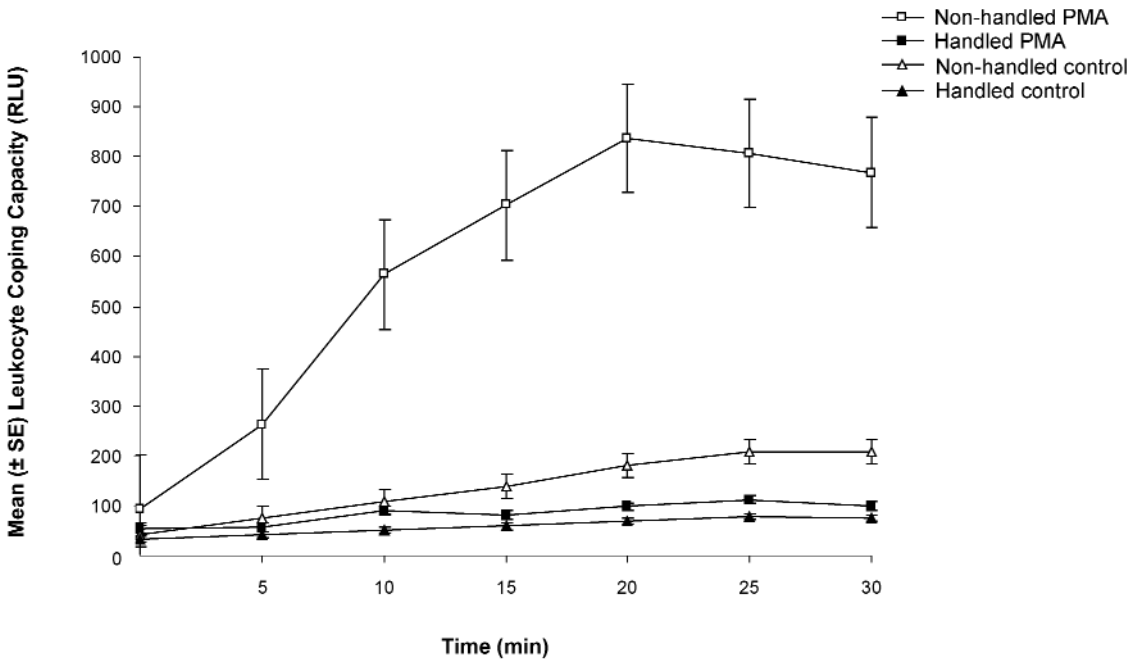
Results from this experiment show that trapping and short periods of handling, which are commonly used in small mammal studies, can result in a period of immunosuppression. After this period of leukocyte activation, the responsiveness of leukocytes to agonists, such as PMA, changes. A number of theories exist as to the mechanistics of this, including immunosuppression or immunoredistribution (Dhabhar & McEwen 1997; Braude *et al* 1998). The results from this investigation support Moorhouse *et al* (2007), who also found that handling, along with housing conditions and radio-collaring, had significant negative impacts on the immune response of water voles (*Arvicola terrestris*) as measured using the LCC technique. The reduction in immune response observed in handled animals might be explained by two hypotheses which are not mutually exclusive; 'Self-Protection' and 'Resource Limitation'. The Self-Protection hypothesis is that the immune system is suppressed in order to protect the organism from 'hyper-stimulation' of the immune system (Råberg *et al* 1998), supported by the generally reduced LCC measures found after handling. The Resource Limitation hypothesis is that immunosuppression occurs in order to allow energy and nutrients to be temporarily shifted away from the immune system and diverted into cells and tissues that are directly required to cope with the stressor.

In the case of the immune response we report here, leukocytes are activated to produce oxygen-free radicals during a presumably stressful event, as demonstrated by the LCC assay, and this response most likely occurs to prepare the body for physical damage (Mian *et al* 2003). Once oxygen-free radicals have been released, one potential reason that further production is suppressed is to protect the body from physical damage; if oxygen-free radical production exceeds the coping capacity, oxidative stress occurs which may cause functional alterations at the cellular level (Uruñuela *et al* 2002).

There is evidence that investment in the immune system in a normal situation is dependent upon an animal's condition and nutritional status (eg male Belding's ground squirrels [*Spermophilus beldingi*] that received a food supplement had a leukocyte count three times greater than control males [Bachman 2003]). Handling can cause weight loss in both wood mice and bank voles, with heavier animals more likely to lose weight as a result of handling (McLaren *et al* 2004). Our finding that non-handled wood mice displayed an overnight increase in weight is contrary to McLaren *et al* (2004) who found that anaesthetised mice showed an overnight decrease in weight; however these animals were handled prior to anaesthesia, thus

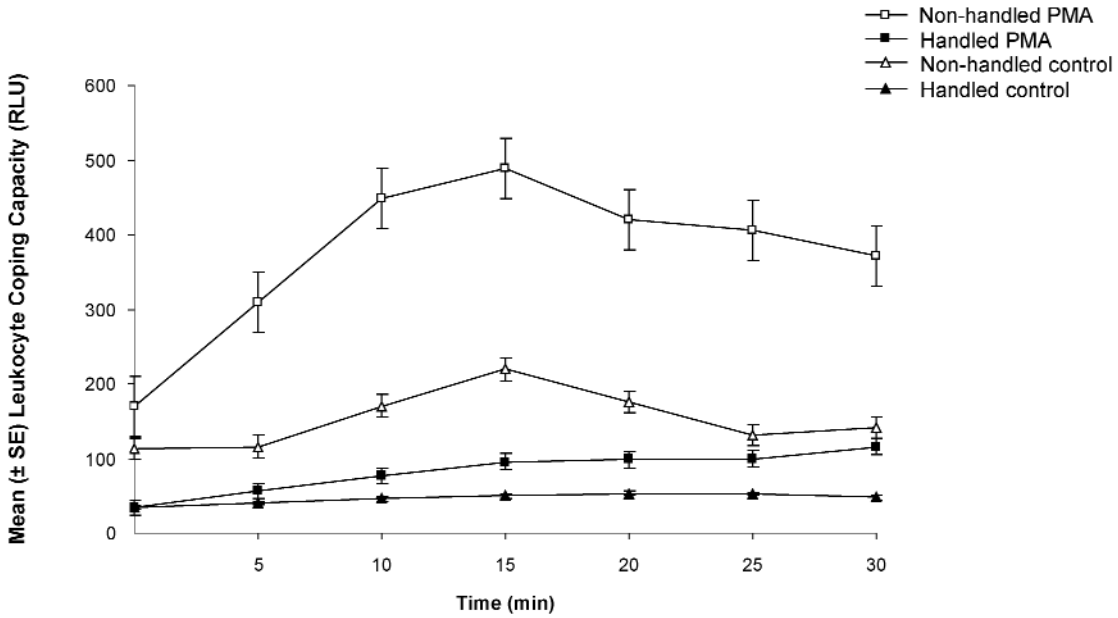
https://doi.org/10.1017/S0962728600000014 Published online by Cambridge University Press

Figure 2



LCC (mean [± SE] relative light units [RLU]) of handled and non-handled wood mice over time.

Figure 3



LCC (mean [± SE] relative light units [RLU]) of handled and non-handled bank voles over time.

potentially increasing the stress imposed upon each animal. It is thought that heavier animals may have more resources to 'invest' in the stress response (McLaren *et al* 2004). In small mammals, heavier animals are likely to benefit from a better nutritional status and thus may have more resources with which to mount an immune response, possibly explaining the higher LCC response elicited from heavier wood mice. One possibility is that heavier animals have more active immune cells, although this appears to be independent of cell numbers (McLaren *et al* 2003; Honess *et al* 2005). Unfortunately, the small volume of blood that is easily obtainable from small mammals in the field

https://doi.org/10.1017/S0962728600000014 Published online by Cambridge University Press

precluded the possibility of undertaking both LCC and leukocyte counts simultaneously in our study.

Alternatively, bodyweight- and nutrition-related differences in plasma factors, for example glutamine and glucose, could be responsible for this relationship. Glutamine is an important fuel for immune cells (Castell & Newsholme 2001; Castell 2002), and is known to enhance PMA-induced oxygen-free radical production in neutrophils (Pithon-Curi *et al* 2002). However, Fekete and Kellems (2007) suggest that there is no direct link between immunity and nutrition, although significant protein, mineral and vitamin losses may be incurred if the disease incurs malabsorption of vital nutrients and, under severe infection, the number of activated leukocytes increases at the point of infection. Another possibility is that behavioural factors related to bodyweight, such as social status, might affect this immune response (eg Avitsur *et al* 2003).

Animal welfare implications

Our results support the notion that LCC can be used as an indicator of immune function following a procedure that is presumably stressful. It is noteworthy that even such a short (20 s) intervention can be detected by the LCC technique. Such measures are useful both to facilitate handling methodologies that minimise stress, and to explore the corollaries of immunosuppression. The fact that handling causes a quantifiable perturbation certainly does not mean that handling is inappropriate — we do not know how this experience translates into distress experienced by the small mammal and, in any case, this might be offset against the benefits gained from the intervention. However, this work may serve to highlight the need for researchers undertaking work using trapping and handling techniques to question the methodologies they use, and investigate whether other, less invasive methodologies are available to answer the same questions (for example; hair tube studies to investigate species composition [eg Pocock & Jennings 2006], or sign surveys as an index of abundance [eg Village & Myhill 1990]).

Further work is currently being conducted by the authors to investigate LCC measures in conjunction with other measures of stress, including glucocorticoid assays. Leukocytes are exposed to diverse factors: 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, such as endothelial cells and changes in the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system (Mian *et al* 2005). As stress affects each of these factors, leukocytes make ideal indicators of stress (Mian *et al* 2005), being constantly exposed to a diverse range of stress stimuli.

Our findings illustrate that LCC is affected rapidly and directly by stress. The LCC technique is an objective, practical technique that can be used in the field to assess the stress associated with capture and handling of small mammals.

Acknowledgements

This work was supported by grants to DWM from the PTES and DEFRA (which funded MG), and the HSUS (which funded GWM). FM was funded by a Royal Society Dorothy Hodgkin Research Fellowship.

References

Alberts B, Bray D, Lewis J, Roff M, Roberts K and Watson JD 1994 *Molecular Biology of the Cell.* Garland Publishing Inc: New York, USA

Alonso-Alvarez C, Bertrand S, Devevey G, Prost J, Faivre B and Sorci G 2004 Increased susceptibility to oxidative stress as a proximate cost of reproduction. *Ecology Letters 7*: 363-368

Avitsur R, Stark JL, Dhabhar FS, Kramer KA and Sheridan JF 2003 Social experience alters the response to social stress in mice. *Brain, Behaviour & Immunity 17*: 426-437

Bachman GC 2003 Food supplements modulate changes in leukocyte numbers in breeding male ground squirrels. *Journal of Experimental Biology 206*: 2373-2380

Braude S, Tang-Martinez Z and Taylor GT 1998 Stress, testosterone, and the immunoredistribution hypothesis. *Behaviour & Ecology 10*: 345-350

Castell LM and Newsholme EA 2001 The relation between glutamine and immunodepression observed in exercise. *Amino Acids 20*: 49-61

Castell LM 2002 Can glutamine modify the apparent immunodepression observed after prolonged, exhaustive exercise? *Nutrition 18*: 371-375

Chen CS 2002 Phorbol ester induces elevated oxidative activity and alkalization in a subset of lysosomes. *BMC Cell Biology 3*: 34

Dhabhar FS and McEwen BS 1997 Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: A potential role for leukocyte trafficking. *Brain Behavior & Immunity 11(4)*: 286-306

Ellard DR, Castle PC and Mian R 2001 The effect of a short-term mental stressor on neutrophil activation. *International Journal of Psychophysiology 41*: 93-100

Fekete SG and Kellems RO 2007 Interrelationship of feeding with immunity and parasitic infection: a review. *Veterinarni Medicina 52*: 131-143

Fletcher QE and Boonstra R 2006 Impact of live trapping on the stress response of the meadow vole (*Microtus pennsylvanicus*). *Journal of Zoology 270*: 473-378

Gurnell J and Flowerdew JR 1994 *Live Trapping of Small Mammals: A Practical Guide.* The Mammal Society: London, UK

Hayes JP and Shonkwiler JS 2001 Morphometric indicators of body condition: worthwhile or wishful thinking? In: Speakman JR (ed) *Body Composition Analysis of Animals: A Handbook of Non-Destructive Methods.* Cambridge University Press: Cambridge, UK

Honess PE, Marin C, Brown AP and Wolfensohn SE 2005 Assessment of stress in non-human primates: application of the neutrophil activation test. *Animal Welfare 14*: 291-295

Hu T, Bei L, Qian Z and Shen X 1999 Intracellular free calcium regulates the onset of the respiratory burst of human neutrophils activated by Phorbol Myristate Acetate. *Cell Signal 11*: 355-360

Kruidenier L and Verspaget HW 2002 Oxidative stress as a pathogenic factor in inflammatory bowel disease — radicals or ridiculous? *Alimentary Pharmacology & Therapeutics 16*: 1997-2015

Lin X, Tan WC and Candlish JK 1995 Effect of glucose on the respiratory burst of circulating neutrophils from asthmatics. *Experimental Molecular Pathology 62*: 1-11

Mathews F, Honess P and Wolfensohn S 2002 Use of inhalation anaesthesia for wild mammals in the field. *Veterinary Record 150*: 785-787

McEwen BS 1998 Stress, adaptation and disease — allostasis and allostatic load. *Annals of the New York Academy of Sciences 840*: 33-44

McLaren GW, Macdonald DW, Georgiou C, Mathews F, Newman C and Mian R 2003 Leukocyte coping capacity: a novel technique for measuring the stress response in vertebrates. *Experimental Physiology 88*: 541-546

https://doi.org/10.1017/S0962728600000014 Published online by Cambridge University Press

McLaren GW, Mathews F, Fell R, Gelling M and Macdonald DW 2004 Body weight change as a measure of stress: a practical test. *Animal Welfare 13*: 337-341

McLaren GW, Bonacic C and Rowan A 2007 Animal welfare and conservation: measuring stress in the wild. In: Macdonald DW and Service K (eds) *Key Topics in Conservation Biology*. Blackwell Publishing Ltd: Oxford, UK

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

Mian R, McLaren G and Macdonald DW 2005 Of stress, mice and men: a radical approach to old problems. In: Oxington K (ed) *Stress and Health: New Research*. Nova Science Publications: New York, USA

Millspaugh JJ, Coleman MA, Bauman BJ, Raedeke KJ and Brundige GC 2000 Serum profiles of American elk at the time of handling for three capture methods. *Canadian Field Naturalist 114*: 196-200

Montes I, McLaren GW, Macdonald DW and Mian R 2003 The effects of acute stress on leukocyte activation. *Journal of Physiology 54*: 88

Montes I, McLaren GW, Macdonald DW and Mian R 2004 The effect of transport stress on neutrophil activation in wild badgers (*Meles meles*). *Animal Welfare 13*: 355-359

Moorhouse TP, Gelling M, McLaren GW, Mian R and Macdonald DW 2007 Physiological consequences of captive conditions in water voles (*Arvicola terrestris*). *Journal of Zoology 271*: 19-26

Pithon-Curi TC, Levada AC, Lopes LR, Doi SQ and Curi R 2002 Glutamine plays a role in superoxide production and the expression of p47 phox p22 phox and gp91 phox in rat neutrophils. *Clinical Science 103*: 403-408

Pocock MJO and Jennings N 2006 Use of hair tubes to survey for shrews: new methods for identification and quantification of abundance. *Mammal Review 36*: 299-308

Prout DM and King CM 2006 The effect of handling under anaesthetic on the recapture rate of wild ship rats (*Rattus rattus*). *Animal Welfare 15*: 63-66

Råberg L, Grahn M, Hasselquist D and Svensson E 1998 On the adaptive significance of stress induced immunosuppression. *Proceedings of the Royal Society of London B265*: 1637-1641

Roberts ML, Buchanan KL, Hasselquist D and Evans MR 2007 Effects of testosterone and costicosterone on immunocompetence in the zebra finch. *Hormones and Behavior 51*: 126-134

Romano TA, Keogh MJ, Kelly C, Feng P, Schlundt CE, Carder DA and Finneran JJ 2004 Anthropogenic sound and marine mammal health: measures of the nervous and immune systems before and after intense sound exposure. *Canadian Journal of Fisheries and Aquatic Sciences 61*: 1124-1134

Sanidas D, Garnham A and Mian R 2000 Activation of human leukocytes by hypoxia. *Experimental Physiology 85*: 263-266

Sanidas D, Garnham, A and Mian R 2002 Hypoxia-induced chemiluminescence in human leukocytes; the role of calcium. *European Journal of Pharmacology 453*: 183-187

Uruňuela A, Sevillano S, de la Mano AM, Manso MA, Orfao A and de Dios I 2002 Time-course of oxygen free radical production in acinar cells during acute pancreatitis induced by pancreatic duct obstruction. *Biochimica et Biophysica Acta 1588*: 159-164

Village A and Myhill D 1990 Estimating abundance for predator studies – snap-trapping versus sign indexes. *Journal of Zoology 222*: 681-689

Whitehead TP, Thorpe GHG and Maxwell SRJ 1992 Enhanced chemiluminescent assay for antioxidant capacity in biological fluids. *Analytica Chimica Acta, 266*: 265-277

https://doi.org/10.1017/S0962728600000014 Published online by Cambridge University Press