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ORIGINAL ARTICLE

Combat-training stress in soldiers increases S100B, a marker of increased blood-brain-barrier permeability, and induces immune activation

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Abstract

BACKGROUND: Experimental data suggest stress-related cognitive dysfunction may be associated with increased blood-brain-barrier (BBB) permeability secondary to immune activation.
METHODS: We investigated the relationship between prolonged and intense physical and psychological combat-training stress, immune activation and blood-brain-barrier permeability in 37 healthy male army medical rapid response troops.
RESULTS: Soldiers during intense combat training showed greater self-reported stress, anxiety and depression levels than at rest, as assessed by specific questionnaires. S100B, a marker of BBB permeability, as well as serum cortisol, IL-6 and TNF-α concentrations, were significantly increased in soldiers during combat training compared to rest (all $p<0.05$). Serum S100B correlated negatively with morning serum cortisol in soldiers during combat training, but not during the rest period ($r=-0.387$, $p<0.05$).
CONCLUSION: We conclude that combat training inducing significant levels of stress, depression and anxiety is accompanied by evidence of increased blood-brain barrier permeability and by increases in systemic pro-inflammatory mediators.

INTRODUCTION

Clinical data suggests that cognitive dysfunction, including emotional, memory and mental changes may be related to increased blood-brain-barrier (BBB) permeability in several human diseases, as well as in clinical conditions with increased immune activation (Davies & Hardy 1988; Huber 2008; Huber *et al.* 2001; Weiss *et al.* 2009). Increased 'leakiness' of this dynamic protective barrier of the brain has also been associated with several situations relevant to stressful combat settings, including psychological and physical stress, exercise in hot environments and inflammatory illnesses (Abbott *et al.* 2010; Baumgart & Dignass 2002; Esposito *et al.* 2001; Farrall & Wardlaw 2009; Huber *et al.* 2002; Trojano *et al.* 1992; Watson *et al.* 2006; Weiss *et al.* 2009; Wright

& Merchant 1994). BBB permeability changes have generally been assessed using either *in vitro* or invasive animal methodologies. S100B protein is the best validated non-invasive, peripheral marker of BBB permeability changes in humans and has been tested in a wide range of conditions including brain trauma, inflammation, pharmacological BBB disruption and in response to stress, and blood levels correlate with BBB opening rather than neuronal damage (Marchi *et al.* 2004; Diebel *et al.* 2005; Gerlach *et al.* 2006; Kleindienst *et al.* 2007; Scaccianoce *et al.* 2004; Tanaka *et al.* 2008; Teepker *et al.* 2009).

Immune activation and inflammatory markers are associated with cognitive dysfunction in various clinical settings (Bower *et al.* 2009; Jehn *et al.* 2006; Lutgendorf *et al.* 2008; Meyers *et al.* 2005; Musselman *et al.* 2001). Furthermore, circulating immune mediators, especially cytokines, can directly affect brain endothelial cells to increase their permeability (de Vries *et al.* 1996). Previous studies in mice have demonstrated acute stress-induced increases in BBB permeability, which are dependent on the release of CRH and on the activation of brain mast cells with selective release of inflammatory cytokines. The result was uncontrolled access of macromolecules, neurotoxins, inflammatory and other circulating mediators to the brain during the inflammatory process (Abbott 2000; Argaw *et al.* 2006; Esposito *et al.* 2001; Esposito *et al.* 2002; Theoharides & Konstantinidou 2007).

Based on this experimental data our hypothesis was that humans in situations of sustained operational stress experience increased BBB permeability, which may be an underlying pathological mechanism in the cognitive dysfunction observed in such situations. Hitherto most stress studies in humans have for ethical reasons been restricted to experimental and acute paradigms, with obvious limitations on the clinical applicability of the results given the well-known differences between acute and chronic stress (Leonard 2005). Combat-training exercises generate high levels of stress, providing a unique opportunity to examine psychological and physiologic responses of normal humans to intense real-life stress. In this prospective study, we therefore investigated the effects of the prolonged, mixed physical and psychological stress experienced during intense combat-training on BBB permeability and immune activation in comparison with a resting period.

MATERIALS AND METHODS

Subjects

Thirty-nine young male non-smoking soldiers undergoing a standardized 6-week combat-training were recruited from the Medical Response Force (MRF) of the Singapore Armed Forces (SAF). All soldiers were physically healthy and did not have a significant medical history, chronic neurological or inflammatory disease based on detailed medical history questionnaires and examination, or abnormal pre-training hematology and biochemistry tests. Participation in this study was voluntary, written informed consent was obtained from all the subjects and written parental consent was also obtained for those under the age of 21 years. The study protocol was approved by the Ethics Committees of the Singapore National Healthcare Group and the DSO National Laboratories Institutional Review boards.

Combat-training course and study design

The MRF combat-training conversion course is a 6-week period of all-day high-intensity stress and serves as a transition from the relatively stress-free classroom-based medical course to an environment with high physical and psychological demand, comprising combat-training missions, some in heavy anti-chemical suits. These identical activities for all soldiers are carried out at average ambient temperatures above 30 °C and humidity above 80%. Blood sampling for stress and immune mediators was performed at identical times (see below) after 4 weeks of combat-training and on the twelfth full day of rest after the end of the combat-training. All subjects refrained from ingestion of alcohol for at least 5 days prior to test days and from smoking in the morning before testing, and fasted from 10 pm to 6 am before the tests.

Questionnaires: Anxiety, Depression, Stress and Bowel function

The Perceived Stress Scale-10 item (PSS-10) questionnaire was used for quantification of stress (Cohen *et al.* 1983), the Hospital Anxiety and Depression (HAD) scale for anxiety and depression (Zigmond & Snaith 1983). Ratings were performed independently by all subjects before blood sampling during the combat-training and rest periods.

Stress markers and inflammatory mediators

Fasting blood samples for cortisol quantification were collected between 6:00 and 6:15 AM. On the day of sampling the soldiers rose at 5:30 AM as usual and avoided any exercise before blood was drawn from a forearm vein by experienced phlebotomists. The collected blood was allowed to clot for 30 minutes at room temperature. The samples were then centrifuged at 1 200×g for 15 minutes at 4 °C and serum samples were stored in aliquots at –80 °C. Serum cortisol was quantified by the Elecsys Cortisol Assay (Roche Diagnostics, USA) and IL-6, TNF-α, and IL-10 were measured using a commercial enzyme-linked immunosorbent assays (R&D Systems, USA). Inter- and intra-assay variation was below 8%.

S100B measurement

Serum S100B levels were measured using the fully-automated electro-chemo-luminometric immunoassay (Elecsys S100 Assay #03175243, Roche Diagnostics, Germany) by the Cobas e411 analyzer according to

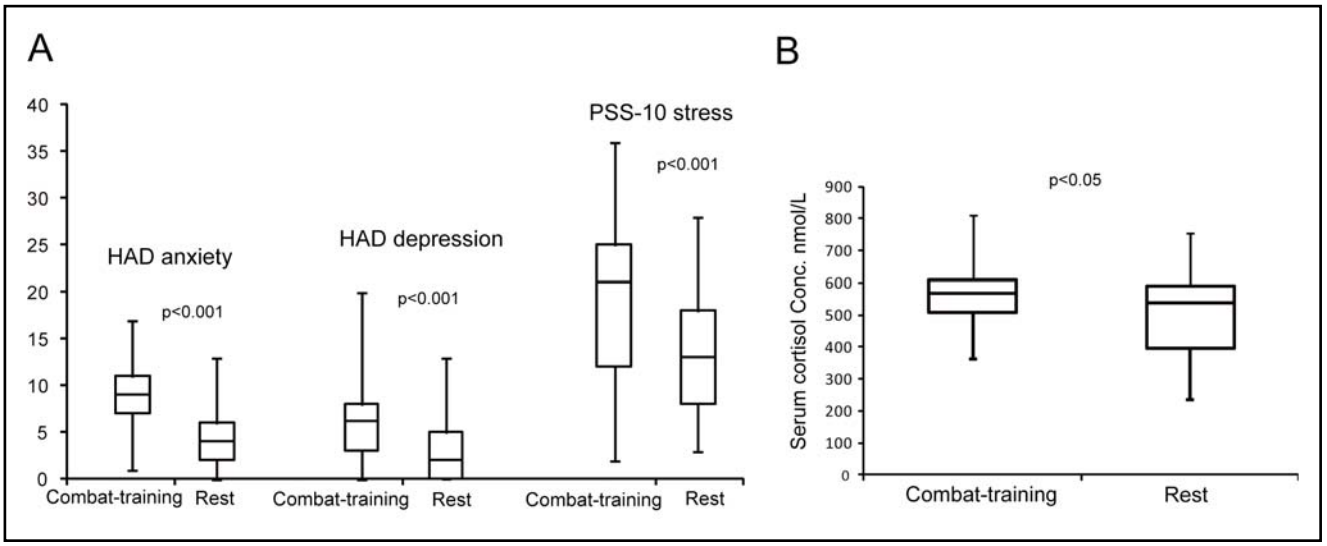

Fig. 1. Anxiety, depression and stress scores (A) and serum cortisol concentrations (B) in soldiers during combat training and at rest. Medians, interquartile ranges and absolute ranges are shown.

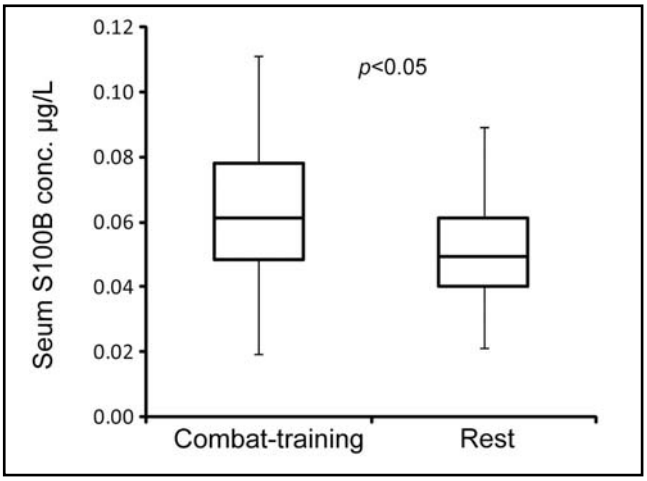

Fig. 2. Serum S100B levels during combat training and at rest. Medians, interquartile ranges and absolute ranges are shown.

the manufacturer's instructions. Inter- and intra-assay variation was below 5%.

Statistics

SPSS Statistics 17.0 software (SPSS Inc, Chicago, US) was used for statistical analysis. Data were expressed as medians and interquaritle ranges and comparisons between the combat-training and rest periods were performed using the paired sample t-test. Simple correlations between parameters were evaluated by the Pearson's rank correlation coefficient. A two-tailed p-value of <0.05 was considered statistically significant. Intra- and inter-assay variation coefficients were below 8% for all variables.

RESULTS

Thirty-nine non-smoker Asian male soldiers aged 19–23 years (mean: 20.7years) from the same MRF troop were included in the study. Two soldiers were excluded because of fever on the day of blood sampling. No soldiers had clinical evidence of infections or acute illnesses during the course of the study and there were no further study drop-outs.

Stress, anxiety and depression levels

Combat training significantly increased anxiety, depression and stress scores in soldiers compared to the rest period (all p<0.001) (Figure 1A). Serum cortisol was also significantly increased during combat training compared to at rest (p<0.05) (Figure 1B).

S100B, and inflammatory markers

Serum S100B was significantly greater during combat training than at rest (p<0.05)(Figure 2). Inflammatory cytokine serum levels were significantly higher during the combat-training period than at rest (p<0.01) (Figure 3). IL-10 concentrations were similar in both periods (4.12, 95%CI: 3.10–5.13, vs. 4.84, 95%CI: 2.68–6.99 pg/ml, p=0.56).

Correlations between mediators

There was a negative correlation between S100B and cortisol concentrations (r=–0.387, p<0.05) during stress, but not in the rest period (Figure 4). There were no correlations between the concentrations of immune mediators and S100B.

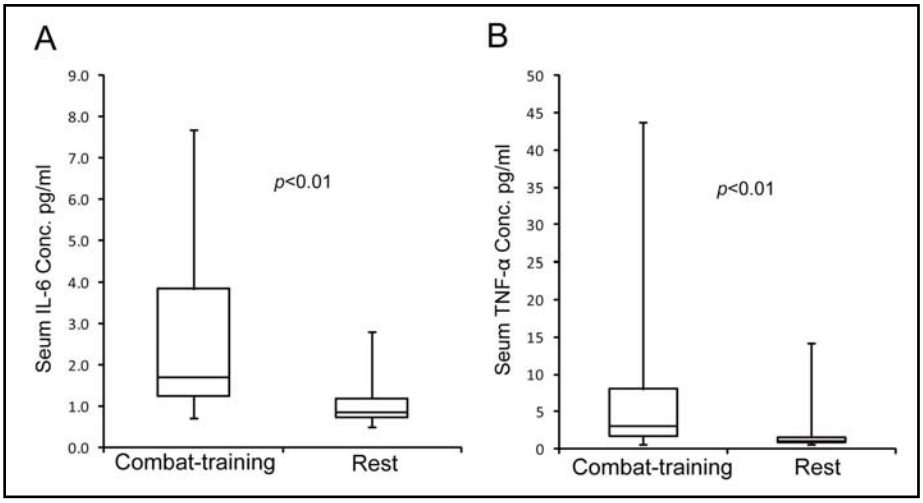

Fig. 3. Inflammatory cytokine serum concentrations (IL-6, A and TFN-a, B) during combat training and at rest. Medians, interquartile ranges and absolute ranges are shown.

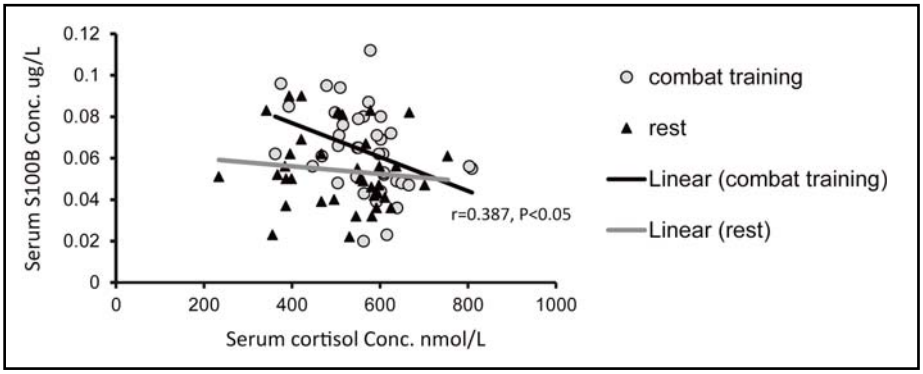

Fig. 4. Correlation between serum cortisol and S100B concentrations.

DISCUSSION

In the current human study serum S100B as a marker of BBB permeability, inflammatory mediator concentrations, as well as stress indices, were increased significantly in soldiers during combat training compared to rest, suggesting that stress modulates the immune milieu and BBB permeability. The underlying causes of the frequently observed stress-related cognitive dysfunction in soldiers are not clear, but increased permeability of the BBB secondary to immune activation may be one of the underlying mechanisms (Garvey Wilson *et al.* 2009; Hoge *et al.* 2004; Lieberman *et al.* 2005b; Lieberman *et al.* 2005a; Vasterling *et al.* 2010). The BBB is a dynamically-controlled and crucial element in actively regulating brain ingress and egress of a wide range of mediators (Weiss *et al.* 2009). Immune activation and stress of various origins in animals have been shown to modulate BBB function and permeability (Hawkins & Davis 2005).

Chronic stress can affect cognitive functions directly via stress hormones such as cortisol or by activating the secretion of proinflammatory and type-2 cytokines such as IL-6 and by promoting the immune responses driven by these cytokines (Anisman 2009; Kiecolt-Glaser *et al.* 2003; Li *et al.* 2008; Lutgendorf *et al.* 1999; Miller *et al.* 2002; O'Brien *et al.* 2004). Furthermore, inflammatory mediators, including IL-6 and TNFα, affect the integrity of the BBB in vitro, predominantly by increasing degradation and decreasing synthesis of tight junction (TJ) proteins (Argaw *et al.* 2006; Claudio *et al.* 1994; de Vries *et al.* 1996; Diebel *et al.* 2005). We recently reported increased intestinal permeability in the same soldiers under stress, very likely driven by the effect of immune activation on the very similarly structured intestinal barrier (Li *et al.* 2013). Increased brain access of pro-inflammatory mediators, especially IL-1, has in animal studies resulted in decreased cognitive performance (Banks *et al.* 2001). Increased BBB permeability as a cause for cognitive change has been implicated in a wide range of clinical conditions with proven immune activation, including pain, ageing, peripheral inflammation, closed head injury, early diabetes, multiple sclerosis, lacunar stroke, cardiac surgery and central nervous system tumours as well as athletic exercise in high ambient temperatures (Farrall & Wardlaw 2009; Hawkins & Davis 2005; Trojano *et al.* 1992; Weiss *et al.* 2009). These changes in cognition affect memory, concentration, learning, associative tasks and mood (Farrall & Wardlaw 2009; Yadavalli *et al.* 2008; Bell & Zlokovic 2009). The increased S100B concentrations demonstrated during combat training stress may therefore reflect increased BBB permeability due to greater stress-induced levels of the pro-inflammatory mediators IL-6 and TFNα. In the current study no direct correlations between the inflammatory mediator and S100b concentrations were confirmed, but this may be due to the choice of mediators as well as the sampling frequency and needs to be investigated further. The relationship between BBB changes, immune activation during stress and cognitive changes also need to be studied in a larger group of soldiers and using specific cognitive paradigms. The resting measurements were performed 12 days after cessation of the combat exercises. Although acute stressors generally have a physiologic effect lasting several hours, it is currently not known if the effects of a more prolonged

and intense stressor would have reverted to baseline levels after a period of 12 days and future studies should include several measurement time-points extending to a longer observation period.

Stress mediators have a dual action and can also protect against potential damage by proinflammatory cytokines by suppressing inflammatory-mediated activation (Elenkov & Chrousos 2002). Glucocorticoids have, for example, been reported to prevent BBB breakdown in response to TNF-α through tightening of the endothelial barrier and maintenance of adequate TJ protein levels (Forster *et al.* 2008). The negative correlation between serum S100B cortisol levels could therefore be explained by a counterregulatory protective effect of cortisol on BBB integrity. Serial determinations of cortisol concentrations in saliva would have yielded a more accurate estimation of the HPA-axis activation than serum levels, as up to 80–90% of total serum cortisol is bound to cortisol-binding globulin (CBG) or serum albumin (Gozansky *et al.* 2005).

S100B, being predominantly of astrocytic origin, has been widely used as a marker for neurotrauma, neuroinflammation and of increased BBB permeability (Marchi *et al.* 2004; van Munster *et al.* 2009; Vos *et al.* 2004). Validation of S100B as a marker of BBB opening has been performed with imaging techniques (Kanner *et al.* 2003). Although peripheral origins of S100B such as fat, muscle, and marrow, have been reported a recent study has demonstrated that extracranial sources of S100B do not significantly affect serum levels in humans without traumatic brain or bodily injury from accident or surgery (Anderson *et al.* 2001; Pham *et al.* 2010). Animal research has also suggested the brain to be the main source of the increased release of S100B in BBB opening accompanying endotoxemia (Lipcsey *et al.* 2010). Nonetheless, a comparison between S100B and more specific quantitative measures of early BBB disruption would be of interest in future studies.

CONCLUSIONS

In this prospective study, we found that real-life, prolonged and intense combat-training stress increased systemic levels of major pro-inflammatory mediators and of S100B as a marker of BBB permeability. Both of these changes may represent mechanisms underlying the cognitive compromise seen in soldiers under operational stress. These mechanisms require further study in larger cohorts, and with additional markers of BBB opening and immune activation. If confirmed, pre-emption of these changes may provide opportunities for preventing the cognitive compromise commonly seen during and following severe combat and operational action.

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REFERENCES

1 Abbott NJ (2000). Inflammatory mediators and modulation of blood-brain barrier permeability. Cell Mol. Neurobiol. **20**: 131–147.
2 Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, and Begley DJ (2010). Structure and function of the blood-brain barrier. Neurobiol. Dis. **37**: 13–25.
3 Anderson RE, Hansson LO, Nilsson O, Liska J, Settergren G, and Vaage J (2001). Increase in serum S100A1-B and S100BB during cardiac surgery arises from extracerebral sources. Ann. Thorac. Surg. **71**: 1512–1517.
4 Anisman H (2009). Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. J. Psychiatry Neurosci. **34**: 4–20.
5 Argaw AT, Zhang Y, Snyder BJ, Zhao ML, Kopp N, Lee SC, *et al.* (2006). IL-1beta regulates blood-brain barrier permeability via reactivation of the hypoxia-angiogenesis program. J. Immunol. **177**: 5574–5584.
6 Banks WA, Farr SA, La Scola ME, and Morley JE (2001). Intravenous human interleukin-1alpha impairs memory processing in mice: dependence on blood-brain barrier transport into posterior division of the septum. J. Pharmacol. Exp. Ther. **299**: 536–541.
7 Baumgart DC and Dignass AU (2002). Intestinal barrier function. Curr. Opin. Clin. Nutr. Metab Care **5**: 685–694.
8 Bower JE, Ganz PA, Tao ML, Hu W, Belin TR, Sepah S, *et al.* (2009). Inflammatory biomarkers and fatigue during radiation therapy for breast and prostate cancer. Clin. Cancer Res. **15**: 5534–5540.
9 Claudio L, Martiney JA, and Brosnan CF (1994). Ultrastructural studies of the blood-retina barrier after exposure to interleukin-1 beta or tumor necrosis factor-alpha. Lab Invest **70**: 850–861.
10 Cohen S, Kamarck T, and Mermelstein R (1983). A global measure of perceived stress. J. Health Soc. Behav. **24**: 385–396.
11 Davies DC and Hardy JA (1988). Blood brain barrier in ageing and Alzheimer's disease. Neurobiol. Aging **9**: 46–48.
12 de Vries HE, Blom-Roosemalen MC, van OM, de Boer AG, van Berkel TJ, Breimer DD, *et al.* (1996). The influence of cytokines on the integrity of the blood-brain barrier in vitro. J. Neuroimmunol. **64**: 37–43.
13 Diebel LN, Liberati DM, Baylor AE 3rd, Brown WJ, and Diglio CA (2005). The pivotal role of tumor necrosis factor-alpha in signaling apoptosis in intestinal epithelial cells under shock conditions. J. Trauma **58**: 995–1001.
14 Elenkov IJ and Chrousos GP (2002). Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. Ann. N. Y. Acad. Sci. **966**: 290–303.
15 Esposito P, Chandler N, KandereK, Basu S, Jacobson S, Connolly R, *et al.* (2002). Corticotropin-releasing hormone and brain mast cells regulate blood-brain-barrier permeability induced by acute stress. J. Pharmacol. Exp. Ther. **303**: 1061–1066.
16 Esposito P, Gheorghe D, Kandere K, Pang X, Connolly R, Jacobson S, *et al.* (2001). Acute stress increases permeability of the blood-brain-barrier through activation of brain mast cells. Brain Res. **888**: 117–127.

17 Farrall AJ and Wardlaw JM (2009). Blood-brain barrier: ageing and microvascular disease--systematic review and meta-analysis. Neurobiol. Aging **30**: 337–352.
18 Forster C, Burek M, Romero IA, Weksler B, Couraud PO, and Drenckhahn D (2008). Differential effects of hydrocortisone and TNFalpha on tight junction proteins in an in vitro model of the human blood-brain barrier. J. Physiol **586**: 1937–1949.
19 Garvey Wilson AL, Messer SC, and Hoge CW (2009). U.S. military mental health care utilization and attrition prior to the wars in Iraq and Afghanistan. Soc. Psychiatry Psychiatr. Epidemiol. **44**: 473–481.
20 Gerlach R, Demel G, Konig HG, Gross U, Prehn JH, Raabe A, *et al.* (2006). Active secretion of S100B from astrocytes during metabolic stress. Neuroscience **141**: 1697–1701.
21 Hawkins BT and Davis TP (2005). The blood-brain barrier/neurovascular unit in health and disease. Pharmacol. Rev. **57**: 173–185.
22 Hoge CW, Castro CA, Messer SC, McGurk D, Cotting DI, and Koffman RL (2004). Combat duty in Iraq and Afghanistan, mental health problems, and barriers to care. N. Engl. J. Med. **351**: 13–22.
23 Huber JD (2008). Diabetes, cognitive function, and the blood-brain barrier. Curr. Pharm. Des **14**: 1594–1600.
24 Huber JD, Hau VS, Borg L, Campos CR, Egleton RD, and Davis TP (2002). Blood-brain barrier tight junctions are altered during a 72-h exposure to lambda-carrageenan-induced inflammatory pain. Am. J. Physiol Heart Circ. Physiol **283**: H1531–H1537.
25 Huber JD, Witt KA, Hom S, Egleton RD, Mark KS, and Davis TP (2001). Inflammatory pain alters blood-brain barrier permeability and tight junctional protein expression. Am. J. Physiol Heart Circ. Physiol **280**: H1241–H1248.
26 Jehn CF, Kuehnhardt D, Bartholomae A, Pfeiffer S, Krebs M, Regierer AC, *et al.* (2006). Biomarkers of depression in cancer patients. Cancer **107**: 2723–2729.
27 Kanner AA, Marchi N, Fazio V, Mayberg MR, Koltz MT, Siomin V, *et al.* (2003). Serum S100beta: a noninvasive marker of blood-brain barrier function and brain lesions. Cancer **97**: 2806–2813.
28 Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, Atkinson C, Malarkey WB, and Glaser R (2003). Chronic stress and age-related increases in the proinflammatory cytokine IL-6. Proc. Natl. Acad. Sci. U. S. A **100**: 9090–9095.
29 Kleindienst A, Hesse F, Bullock MR, and Buchfelder M (2007). The neurotrophic protein S100B: value as a marker of brain damage and possible therapeutic implications. Prog. Brain Res. **161**: 317–325.
30 Leonard BE (2005). The HPA and immune axes in stress: the involvement of the serotonergic system. Eur. Psychiatry 20 Suppl **3**: S302–S306.
31 Li S, Wang C, Wang W, Dong H, Hou P, and Tang Y (2008). Chronic mild stress impairs cognition in mice: from brain homeostasis to behavior. Life Sci. **82**: 934–942.
32 Li X, Kan EM, Lu J, Cao Y, Wong RK, Keshavarzian A, and Wilder-Smith CH (2013). Combat-training increases intestinal permeability, immune activation and gastrointestinal symptoms in soldiers. Aliment. Pharmacol. Ther. **37**: 799–809.
33 Lieberman HR, Bathalon GP, Falco CM, Kramer FM, Morgan CA 3rd, and Niro P (2005a). Severe decrements in cognition function and mood induced by sleep loss, heat, dehydration, and undernutrition during simulated combat. Biol. Psychiatry **57**: 422–429.
34 Lieberman HR, Bathalon GP, Falco CM, Morgan CA 3rd, Niro PJ, and Tharion WJ (2005b). The fog of war: decrements in cognitive performance and mood associated with combat-like stress. Aviat. Space Environ. Med. **76**: C7–14.
35 Lipcsey M, Olovsson M, Larsson E, Einarsson R, Qadhr GA, Sjolin J, *et al.* (2010). The brain is a source of S100B increase during endotoxemia in the pig. Anesth. Analg. **110**: 174–180.
36 Lutgendorf SK, Garand L, Buckwalter KC, Reimer TT, Hong SY, and Lubaroff DM (1999). Life stress, mood disturbance, and elevated interleukin-6 in healthy older women. J. Gerontol. A Biol. Sci. Med. Sci. **54**: M434–M439.
37 Lutgendorf SK, Weinrib AZ, Penedo F, Russell D, DeGeest K, Costanzo ES, *et al.* (2008). Interleukin-6, cortisol, and depressive symptoms in ovarian cancer patients. J. Clin. Oncol. **26**: 4820–4827.
38 Marchi N, Cavaglia M, Fazio V, Bhudia S, Hallene K, and Janigro D (2004). Peripheral markers of blood-brain barrier damage. Clin. Chim. Acta **342**: 1–12.
39 Meyers CA, Albitar M, and Estey E (2005). Cognitive impairment, fatigue, and cytokine levels in patients with acute myelogenous leukemia or myelodysplastic syndrome. Cancer **104**: 788–793.
40 Miller GE, Cohen S, and Ritchey AK (2002). Chronic psychological stress and the regulation of pro-inflammatory cytokines: a glucocorticoid-resistance model. Health Psychol. **21**: 531–541.
41 Musselman DL, Miller AH, Porter MR, Manatunga A, Gao F, Penna S, *et al.* (2001). Higher than normal plasma interleukin-6 concentrations in cancer patients with depression: preliminary findings. Am. J. Psychiatry **158**: 1252–1257.
42 O'Brien SM, Scott LV, and Dinan TG (2004). Cytokines: abnormalities in major depression and implications for pharmacological treatment. Hum. Psychopharmacol. **19**: 397–403.
43 Pham N, Fazio V, Cucullo L, Teng Q, Biberthaler P, Bazarian JJ, *et al.* (2010). Extracranial sources of S100B do not affect serum levels. PLoS. One. **5**: e12691
44 Scaccianoce S, Del BP, Pannitteri G, and Passarelli F (2004). Relationship between stress and circulating levels of S100B protein. Brain Res. **1004**: 208–211.
45 Tanaka Y, Marumo T, Omura T, and Yoshida S (2008). Early increases in serum S100B are associated with cerebral hemorrhage in a rat model of focal cerebral ischemia. Brain Res. **1227**: 248–254.
46 Teepker M, Munk K, Mylius V, Haag A, Moller JC, Oertel WH,*et al.* (2009). Serum concentrations of s100b and NSE in migraine. Headache **49**: 245–252.
47 Theoharides TC and Konstantinidou AD (2007). Corticotropin-releasing hormone and the blood-brain-barrier. Front Biosci. **12**: 1615–1628.
48 Trojano M, Manzari C, and Livrea P (1992). Blood-brain barrier changes in multiple sclerosis. Ital. J. Neurol. Sci. **13**: 55–64.
49 van Munster BC, Korse CM, de Rooij SE, Bonfrer JM, Zwinderman AH, and Korevaar JC (2009). Markers of cerebral damage during delirium in elderly patients with hip fracture. BMC. Neurol. **9**: 21.
50 Vasterling JJ, Proctor SP, Friedman MJ, Hoge CW, Heeren T, King LA, *et al.* (2010). PTSD symptom increases in Iraq-deployed soldiers: comparison with nondeployed soldiers and associations with baseline symptoms, deployment experiences, and postdeployment stress. J. Trauma Stress. **23**: 41–51.
51 Vos PE, Lamers KJ, Hendriks JC, van HM, Beems T, Zimmerman C, *et al.* (2004). Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. Neurology **62**: 1303–1310.
52 Watson P, Black KE, Clark SC, and Maughan RJ (2006). Exercise in the heat: effect of fluid ingestion on blood-brain barrier permeability. Med. Sci. Sports Exerc. **38**: 2118–2124.
53 Weiss N, Miller F, Cazaubon S, and Couraud PO (2009). The blood-brain barrier in brain homeostasis and neurological diseases. Biochim. Biophys. Acta **1788**: 842–857.
54 Wright JL and Merchant RE (1994). Blood-brain barrier changes following intracerebral injection of human recombinant tumor necrosis factor-alpha in the rat. J. Neurooncol. **20**: 17–25.
55 Zigmond AS and Snaith RP (1983). The hospital anxiety and depression scale. Acta Psychiatr. Scand. **67**: 361–370.