Brain, Behavior, and Immunity xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Sleep disruption and its effect on lymphocyte redeployment following an acute bout of exercise

Lesley A. Ingram [a,*], Richard J. Simpson [b], Eva Malone [a], Geraint D. Florida-James [a]

[a] Biomedicine and Sport and Exercise Research Group, School of Life, Sport & Social Science, Edinburgh Napier University, Sighthill Campus, Edinburgh, Scotland EH11 4BN, United Kingdom
[b] Laboratory of Integrated Physiology, Department of Health and Human Performance, University of Houston, 3855 Holman Street, Houston, TX 77204, USA

ARTICLE INFO

Article history:
Received 18 September 2014
Received in revised form 8 December 2014
Accepted 15 December 2014
Available online xxxx

Keywords:
Sleep
Exercise
Cytotoxic lymphocytes
Adrenaline
Noradrenaline
Cortisol
Immune system

ABSTRACT

Sleep disruption and deprivation are common in contemporary society and have been linked with poor health, decreased job performance and increased life-stress. The rapid redeployment of lymphocytes between the blood and tissues is an archetypal feature of the acute stress response, but it is not known if short-term perturbations in sleep architecture affect lymphocyte redeployment. We examined the effects of a disrupted night sleep on the exercise-induced redeployment of lymphocytes and their subtypes. 10 healthy male cyclists performed 1 h of cycling at a fixed power output on an indoor cycle ergometer, following a night of undisrupted sleep (US) or a night of disrupted sleep (DS). Blood was collected before, immediately after and 1 h after exercise completion. Lymphocytes and their subtypes were enumerated using direct immunofluorescence assays and 4-colour flow cytometry. DS was associated with elevated concentrations of total lymphocytes and $CD3^-/CD56^+$ NK-cells. Although not affecting baseline levels, DS augmented the exercise-induced redeployment of $CD8^+$ T-cells, with the naïve/early differentiated subtypes ($KLRG1^-/CD45RA^+$) being affected most. While the mobilisation of cytotoxic lymphocyte subsets (NK cells, $CD8^+$ T-cells γδ T-cells), tended to be larger in response to exercise following DS, their enhanced egress at 1 h post-exercise was more marked. This occurred despite similar serum cortisol and catecholamine levels between the US and DS trials. NK-cells redeployed with exercise after DS retained their expression of perforin and Granzyme-B indicating that DS did not affect NK-cell 'arming'. Our findings indicate that short-term changes in sleep architecture may 'prime' the immune system and cause minor enhancements in lymphocyte trafficking in response to acute dynamic exercise.

1. Introduction

Sleep disruption and deprivation are common in contemporary society and have been linked with poor health, decreased job performance and increased life-stress (Mosendane et al., 2008; Coffey et al., 2006). This problem is exacerbated by the demands placed on many professions that require employees to carry out shift work, and to travel across numerous time zones that often result in circadian disruption and further alterations to sleep architecture. Increasing evidence has associated sleep impairments with disruptions in the normal functioning of the immune system (Bryant et al., 2004) and elevated disease risk (Gottlieb et al., 2005; Meier-Ewert et al., 2004). Furthermore, circadian disruption is known to cause profound changes in hypothalamic–pituitary–adrenal axis (HPA) and sympathetic nervous system (SNS) activity and the resulting secretion of cortisol and catecholamines, which, in turn, are known to have marked effects on the immune system (Anane et al., 2009; Atanackovic et al., 2006).

A single bout of dynamic exercise causes a rapid increase in the blood lymphocyte count (lymphocytosis) (Gleeson, 2007; Simpson et al., 2007). Increased SNS activity and the release of catecholamines that bind to high affinity β_2 adrenergic-receptors on lymphocytes are largely responsible for this exercise-induced lymphocytosis (Anane et al., 2009; Atanackovic et al., 2006). Those lymphocyte subsets that exhibit phenotypes associated with enhanced cytotoxic and tissue-migrating potential (i.e. NK^{dim} cells, $CD8^+$ T-cells and γδ T-cells) are preferentially mobilised to the peripheral blood with exercise (Anane et al., 2009; Krüger and Mooren, 2007). Moreover, antigen experienced highly differentiated subsets of $CD8^+$ T-cells (i.e. $KLRG1^+$) are redeployed in relatively greater numbers to stress and exercise compared to naïve

* Corresponding author. Tel.: +44 1314553316.
E-mail addresses: Le.ingram@napier.ac.uk (L.A. Ingram), rjsimpson@uh.edu (R.J. Simpson), e.malone@napier.ac.uk (E. Malone), g.florida-james@napier.ac.uk (G.D. Florida-James).

http://dx.doi.org/10.1016/j.bbi.2014.12.018
0889-1591/

Please cite this article in press as: Ingram, L.A., et al. Sleep disruption and its effect on lymphocyte redeployment following an acute bout of exercise. Brain Behav. Immun. (2015), http://dx.doi.org/10.1016/j.bbi.2014.12.018

and "early" differentiated $CD8^+$ T-cells (Simpson et al., 2007; Campbell et al., 2009; Turner et al., 2010). Upon exercise cessation, there is a rapid egress of these same lymphocyte subsets resulting in a transient lymphocytopenia (Walsh et al., 2011; Simpson et al., 2007, 2006). During this time, lymphocytes may traffic to the spleen, lung, bone marrow and lymph nodes, which is important during times of immune activation and ensures regulation of the immune response (Dhabhar et al., 2012). This rapid redeployment of cytotoxic cells with exercise is believed to be an evolutionary conserved mechanism to protect the host during periods of acute stress when injury/infection is more likely to occur (Dhabhar et al., 2012).

Studies that have examined the effects of sleep deprivation on the immune system are scarce and largely conflicting, perhaps due to disparate methodologies (partial sleep deprivation of a few hour to complete sleep deprivation for up to 48 h) and heterogeneous outcome measures. Blood leukocyte numbers have been reported to increase following chronic sleep restriction (4 h for 3 days) (Kerhofs et al., 2007; Boudjeltia et al., 2008) and 24 h (Born et al., 1997), whilst others report no effect (Ricardo et al., 2009). While sleep deprivation studies provide useful information on the effects of lack of sleep, in this contemporary society, it is perhaps disrupted sleep that is more commonly experienced. Indeed, the effects of sleep disruption on immunity could have important implications for the health of athletes and those working in professions that perturb the circadian system (i.e. the military, fire service and police force), especially when physically demanding tasks are performed. That is, infection susceptibility may be greater if sleep disruption is found to impinge normal lymphocyte redeployment and immuno-surveillance following acute bouts of physical stress. No study, to our knowledge, has examined the immune response to an acute stressor following a period of sleep disruption.

The aim of this study was to determine if a single night of sleep disruption alters blood lymphocyte redeployment following a single bout of strenuous exercise. We hypothesised that acute exercise performed the day after a night of disrupted sleep would amplify the redeployment of blood lymphocytes when compared to exercise performed after an undisrupted night of sleep. We also hypothesised that the effects of sleep disruption would be more prominent among the cytotoxic cells ($CD8^+$ T-cells, $\gamma\delta$ T-cells and NK-cells) that are typically redeployed in relatively greater numbers with exercise (Anane et al., 2009; Kruger et al., 2008).

2. Methodology

2.1. Participants

Ten healthy adult males (mean ± SD) age: 27 ± 8 years; height: 176 ± 7 centimetres (cm); mass: 73.9 ± 8 kilograms (kg) participated in the study. Participants were non-smokers who were taking no medication or supplementation, familiar with cycling time trials (TT), had abstained from alcohol and caffeine orientated beverages 24 h before participation and had been free from illness at least 2 weeks prior to the first trial. After receiving both oral and written information pertaining to the risks and demands associated with the study, each participant gave written informed consent. Ethical approval for the study was granted from the ethics committee at Edinburgh Napier University.

2.2. Experimental design

All participants completed a 40 km TT on a cycle ergometer (Kingcycle Trainer Tester Rig, Kingcycle, Buckinghamshire, UK). Heart rate (Polar S610, Finland) was monitored at 5 s intervals throughout the test. After completion of the TT all participants were provided with an actiwatch (AW, Cambridge Neurotechnology Ltd.), a small wristwatch unit used to measure behaviours associated with sleep. Prior to participation in the study, participants were screened for a 7 day period in order to monitor their regular sleep–wake cycle. If individuals consistently slept for less than 6 h a night they were excluded from the study.

2.3. Trial conditions

Participants were instructed to attend the laboratory on 2 further occasions interspersed by 1 week in a randomised counterbalanced order. The exercise protocol consisted of 1 h of exercise on a cycle ergometer (Kingcycle Trainer Tester Rig, Kingcycle, Buckinghamshire, UK) at a fixed wattage (90% mean wattage (W) ± 10 W obtained from the 40 km TT). Data from the 40 km TT (mean ± SD): 55:12 ± 2:18 min; average power (mean ± SD) 265 ± 27 watts (W). Heart rate (HR) was recorded at 5 s intervals during the trial (Polar, Finland, S610) and rate of perceived exertion (RPE, Borg, 1970) was recorded at 5 km intervals. Before each trial participants also completed the Epworth Sleepiness Scale (ESS, Johns, 1991). Experimental trials occurred after a night of undisrupted sleep (US; 8 h) and a night of disrupted sleep (DS; woken every hour during an 8 h period). For both trials participants were instructed to go to bed at 2300 h and remain there until 700 h. During the DS trial an alarm clock was set to go off 1 h after entering bed. Upon awakening from the alarm participants were required to open an envelope that contained a set of instructions (see below):

- Push the button on your actiwatch.
- Reset the alarm to go off 1 h from now.
- Stand up and switch on the light.
- Complete the arousal and alertness questionnaire and also the profile of mood states (BRUMS, Terry et al., 1999).
- Switch off the light.
- Return to bed.

2.4. Questionnaires

The ESS contains 8 situations which the participant has to score related to the chance of falling asleep at that precise moment if they were to partake in the situations. A Likert scale of 0–3 is use (0 = no chance of dozing, 3 = high chance of dozing). The total score of all situations is then used to calculate sleepiness. RPE compromises of a scale from 6 to 20 where participants rate how difficult they find the exercise bout (6 = no exertion at all, 20 = maximal exertion).

2.5. Sleep measures

An actiwatch was worn on the non-dominant wrist and used to measure sleep behaviours the night prior to each trial. The actiwatch records movement in any of 3-dimensions using an accelerometer that has a sensitivity of 0.01 g. Data was logged continuously in 1 min epochs. When participants entered bed at night and once they were awake in the morning they were instructed to push the button on the front of the actiwatch enabling sleep onset and awakening times to be determined. During the DS trial, each disruption during the night was recorded by the participant pushing the button on the actiwatch. This allowed recording and confirmation of the sleep disruption events. Adherence during the night DS was confirmed at 100% by the actiwatch trace, with each participant waking every hour of the night.

Q1 Please cite this article in press as: Ingram, L.A., et al. Sleep disruption and its effect on lymphocyte redeployment following an acute bout of exercise. Brain Behav. Immun. (2015), http://dx.doi.org/10.1016/j.bbi.2014.12.018