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Prolonged deprivation of sleep-like rest raises metabolic rate in the Pacific beetle cockroach, *Diploptera punctata* (Eschscholtz)

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Summary

Rats respond to sustained sleep deprivation with increased mortality preceded by a rise in resting metabolic rate that may or may not be attributed to dysfunction of the thermoregulatory system. The present study was designed to test the hypothesis that deprivation of sleep-like rest will lead to increased metabolic rate in an ectothermic insect, the Pacific beetle cockroach. A mild alerting stimulus consisting of a brief <1% pulse of CO_2 and simultaneous 2 s rotation (1 cm motion) of the animal chamber consistently prevented the adoption of a sleep-like resting posture in cockroaches. Two groups of 15 male adult cockroaches were studied; a group targeted for deprivation of sleep-like rest (SD) was presented with one stimulus per minute continuously, and a group of stimulus controls (SC) was given the same number of stimuli per day but scheduled such that the animals received a 3 h interval without stimuli four times per day. This protocol led to significantly increased mortality in the SD group beginning on day 17 (averaging 0.57 deaths per day thereafter), but not in the SC group (averaging 0.17 deaths per day throughout). Oxygen consumption ($\dot{V}_{O_2}$) increased significantly after 4 weeks in the SD group but not the SC group. $\dot{V}_{O_2}$ was 82% above pre-deprivation baseline after 35 days in the SD group (P=0.009). Body mass was unchanged throughout. We conclude that sleep-like rest is essential for long-term viability in insects and that prolonged vigilance leads to an increase in whole-animal metabolic rate in this ectothermic species.

Key words: oxygen consumption, mortality, sleep, cockroach.

Introduction

Several studies have revealed behavioural evidence for a sleep-like resting state in Arthropoda, including examples from the classes Insecta, Arachnida and Malacostraca [e.g. moth (Andersen, 1968); honey bee (Kaiser, 1988); cockroach (Tobler and Neuner-Jehle, 1992); fruit fly (Shaw et al., 2000); scorpion (Tobler and Stalder, 1988); crayfish (Ramón et al., 2004)]. Periods of immobility in arthropods are considered to be 'sleep-like' when (a) they occur spontaneously with a circadian rhythm, (b) they are associated with reduced (but not zero) sensitivity to various sensory stimuli (i.e. increased response thresholds), (c) they are reversible either spontaneously or in response to the presentation of a natural stimulus of sufficient intensity and (d) when immobility exhibits a so-called 'homeostatic' mechanism of regulation such that a delay in its onset will induce a subsequent increase in the propensity for rest (Hendricks et al., 2000b).

The most convincing data supporting the hypothesis that invertebrates have a resting state that is analogous to mammalian sleep were obtained from the honey bee (*Apis mellifera*) by Kaiser and colleagues (Kaiser and Steiner-Kaiser, 1983; Kaiser, 1988; Kaiser, 2002; Sauer et al., 2003; Sauer et al., 2004). Those elegant studies included videotaped measurements of the behaviour of individual bees in isolation and in an observation hive, including quantitative analysis of head and antenna posture, and careful physiological measurements, including thermographic demonstration of reduced body temperature (indirect index of reduced metabolic rate), electromyographic (EMG) evidence of atonia in neck muscle and increased threshold of activation of neurons in the visual system. It was shown that muscle tone and the posture of the limbs and antennae together constitute a reliable and easily observed sign of the onset of a sleep-like resting state, a conclusion also reached for the cockroach *Blaberus giganteus* (Tobler and Neuner-Jehle, 1992). Indeed, Tobler and Neuner-Jehle were able to distinguish nine categories of behavioural arousal in the cockroach mainly on the basis of these two characteristics (Tobler and Neuner-Jehle, 1992), and these behavioural categories were also observed in the present study in the cockroach *Diploptera punctata*.

Recently, some of the above results have been replicated in the fruit fly *Drosophila melanogaster*. Unfortunately, the most reliable indicators of sleep-like rest, the antennae, are not easily monitored in this small insect, and in *Drosophila* a sleep-like resting state is generally defined as the absence of locomotor activity for intervals exceeding a specified duration, usually

5 min (Shaw et al., 2000; Hendricks et al., 2000a; Andretic and Shaw, 2005). The advantage of the fruit fly as an animal model of sleep-like rest is that a wide range of molecular and genetic techniques can be applied to test hypotheses about the regulation and functional mechanisms of sleep-like states (Hendricks et al., 2000b). Actographic studies in various *Drosophila* strains have shown that, during inactive periods, *Drosophila* exhibit some similarities to sleeping mammals at the genetic, biochemical, neurochemical and pharmacological levels (Shaw et al., 2000; Shaw et al., 2002; Hendricks et al., 2000a; Hendricks et al., 2001; Andretic et al., 2005; Kume et al., 2005; Ganguly-Fitzgerald et al., 2006; Koh et al., 2006).

The sleeping and waking states of mammals are conventionally identified by interpretation of the electroencephalogram (EEG) and EMG, which show distinct and reproducible patterns in association with different behavioural vigilance states. By contrast, only a few attempts have been made to record state-related central nervous system (CNS) electrical activity patterns in invertebrates, and characteristic state-related electrophysiological patterns have not yet been established. Kaiser and Steiner-Kaiser described circadian and vigilance state effects on spontaneous and stimulus-evoked activity of optomotor interneurons in the optic lobes of the honey bee central nervous system (Kaiser and Steiner-Kaiser, 1983). Schuppe observed spike activity in the mushroom bodies of the honey bee that was correlated with antennal activity during sleep-like rest (Schuppe, 1995). Similarly, local field potentials in the region of the mushroom bodies were found to be correlated with behavioural activity and inactivity in *Drosophila* (Nitz et al., 2002; van Swinderen et al., 2004), and neural activity patterns in the median protocerebrum could be correlated with behavioural state in the crayfish, *Procambarus clarkii* (Ramón et al., 2004). Hence, invertebrate sleep-like resting behaviour appears to be associated with changes in the function of the CNS.

The somatic functions, if any, of mammalian sleep are unknown (Rechtschaffen, 1998). In an effort to address this, considerable research has been directed towards elucidating the functional consequences of sleep deprivation. In rats, prolonged sleep deprivation has many effects but two of the most obvious and reproducible are that metabolic rate increases progressively during the deprivation period and unrelenting deprivation is eventually lethal (Rechtschaffen et al., 1983; Eversen et al., 1989). Although the proximate cause of death has not yet been identified, these results suggest that sleep may be vital for life in mammals and that sleep serves a function linked directly or indirectly to regulation of cellular energy metabolism. It has been suggested that hypermetabolism results from a dysfunctional thermoregulatory system during sleep deprivation in rats (Rechtschaffen and Bergmann, 2002), but the mechanisms mediating the increased thermogenesis are not understood. Specifically, it remains unclear whether the increased heat production is a regulated or unregulated response of thermoeffector organs or a more general increase in tissue basal metabolic rate.

There is evidence that deprivation of sleep-like rest is also lethal in *Drosophila* and that death is preceded by induction of molecular chaperone proteins, of which HSP83 appears to play a key role (Shaw et al., 2002). Thus, we speculate that sleep deprivation may induce an as yet unidentified cellular stress that may in turn have the effect of raising basal cellular metabolism. Reasoning that studies of a non-endothermic species may help shed light on this issue, we hypothesized that prolonged deprivation of sleep-like rest would lead to hypermetabolism in an ectothermic insect, implying an underlying mechanism of thermogenesis that is independent of regulatory mechanisms associated with endothermy.

Materials and methods

Experiments were performed on adult male Pacific beetle cockroaches, *Diploptera punctata* Eschscholtz. Females were not used to avoid possible variations in metabolic rate due to vivipary. Cockroaches were taken at random from a colony of approximately 100 maintained in an acrylic container (50×30×14 cm) and supplied *ad libitum* with food (dry rodent chow) and drinking water (supplied *via* a soaked 'wick' protruding from a sealed water chamber). Animals were held at normal room temperature (21–23°C) on a 12 h:12 h light:dark cycle (lights on at 09.00 h EST) throughout the study, thereby circumventing any confounds associated with behavioural mechanisms of thermoregulation (Stevenson, 1985). In experiments where cockroaches were studied individually, they were tagged by application of a small spot of white liquid correction fluid on the elytra and then marker pens were used to render the white spot a distinctive colour on each cockroach. Application of the correction fluid and coloured ink was achieved by holding the cockroaches gently between two cotton wool balls, which immobilized them atraumatically without the need for anaesthesia. The coloured marks were found to remain visible for several months after one application and did not affect the behaviour or longevity of the insects.

Deprivation of sleep-like rest

In the present study, a sleep-like resting state was defined as immobility of the limbs and antennae, with body posture parallel (within <10°) to the substrate. This definition corresponds to States 1–3 described by Tobler and Neuner-Jehle (Tobler and Neuner-Jehle, 1992) in the cockroach *Blaberus giganteus*. Preliminary studies were conducted to develop an effective yet benign arousal stimulus and to determine the appropriate inter-stimulus interval that would ensure long-term deprivation of sleep-like rest. In all experiments, the stimuli were presented to groups of cockroaches confined within horizontal acrylic cylinders (34 cm long, 5 cm i.d.). The cylinders were attached to a motor and driven at a rate of 3 revs min^{-1} when activated (Fig. 1A). The cylinders were ventilated with humidified air (relative humidity >90%) at a flow rate of approximately 10 l min^{-1}. Mortality was recorded once each morning and dead animals were removed from the chamber.

The stimulus used to maintain vigilance consisted of a combination of a brief pulse of CO_2 and simultaneous brief (2 s, moving a distance of approximately 1 cm) rotation of the chamber (see Results). Two groups of adult male cockroaches (starting with N=15 per group) were used to determine the effects of deprivation of sleep-like rest on metabolic rate. The deprived group (SD) were subjected to one stimulus per minute (1440 stimuli per day) continuously until the study was terminated upon the death of the last animal in the SD group on day 44. The

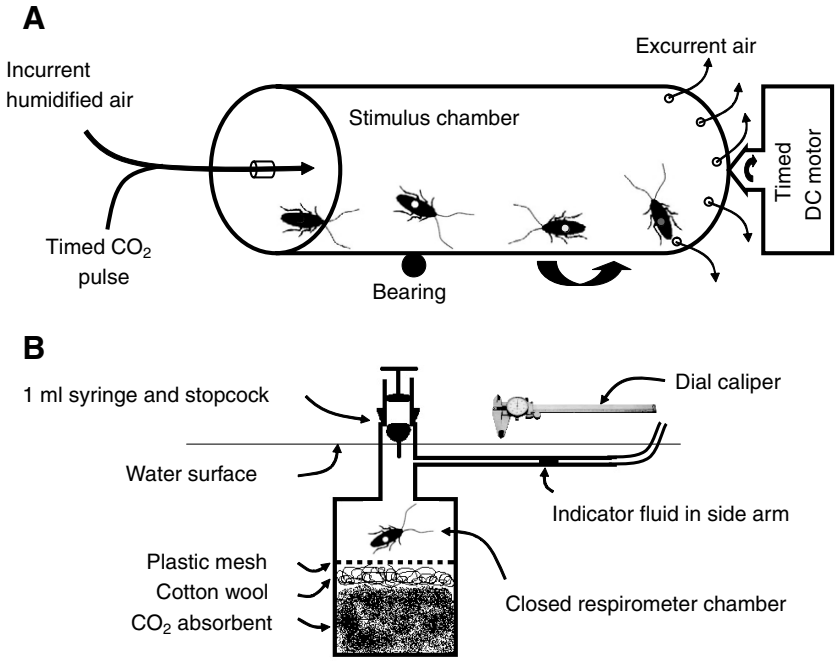

Fig. 1. Schematic diagram of the apparatus (not drawn to scale). (A) The chamber used for repetitive stimulation of the cockroaches. Stimuli consisted of 2 s rotation (1 cm motion) of the chamber and simultaneous pulse of CO_2 (peak concentration <1%). One chamber was scheduled to present stimuli continuously at the rate of 1 min^{-1} (SD group) and another chamber ran with a schedule of 6 h cycles with 3 h of stimuli at 2 min^{-1} and 3 h without stimuli (SC group). The study started with 15 animals per chamber. (B) Closed respirometry was used to measure oxygen consumption of individual cockroaches. Respiratory CO_2 was absorbed by Ascarite™, and the change in chamber gas volume due to consumption of O_2 was recorded every 10–20 min by measuring the position of a fluid indicator in the side arm. Ten animal chambers and four thermobarometers were all submerged to the level of the side arm in a constant-temperature water bath and studied simultaneously.

stimulus control group (SC) was subjected to the same total number of stimuli per day but they were scheduled such that stimuli were presented every 30 s for 3 h in each 6 h period, allowing a 3 h interval for consolidated sleep-like rest four times each day. On the first day of the study, the cockroaches were weighed and then resting oxygen consumption was measured as described below (day 0 baseline value). The cockroaches were immediately transferred to the motorized cylinders (15 cockroaches per cylinder) and subjected to deprivation (SD) or control (SC) protocols. They were provided with powdered rat food on the walls of the cylinder and a small piece of moist cotton wool, which precluded an accurate measurement of daily food consumption. The cylinders were cleaned during weekly measurements of oxygen consumption (i.e. days 7, 14, 21, 28, 35). The final metabolic rate measurement was conducted at 35 days because by that time mortality had depleted the number of animals in the SD group to 5.

Latency to sleep-like rest

A 'sleep latency test' was used to confirm that the stimulus protocol increased the propensity for sleep-like rest and to determine the minimum amount of time taken by the cockroaches to enter this state. In separate experiments, two groups of cockroaches (five animals that had been subjected to the stimulus protocol for 5 weeks and a second non-deprived control group of 10 animals) were observed undisturbed, and after they had all remained motionless for over two minutes the group were given a standard stimulus (simultaneous pulse of CO_2 and brief rotation as described above). The time taken to resume a sleep-like state was recorded for each animal. This was repeated 10 times and a median latency for each animal obtained. These median values were then combined into a sample mean (± s.e.m.) for each group.

Metabolic rate

Oxygen consumption ($\dot{V}_{O_2}$; μl O_2 g^{-1} h^{-1} STPD) of individual cockroaches was measured using a closed respirometry technique. Animals were weighed then placed in a metabolism chamber (25 ml glass bottle) as shown in Fig. 1B. The chamber was fitted with a T-connector and a stopcock connected in series for attachment of a horizontal side tube (vinyl tubing, 0.71 mm i.d.; Becton Dickinson Co., Bridgeport, NJ, USA) and a 1 ml syringe, respectively. The chamber contained approximately 15 ml of CO_2 absorbent [Ascarite (II) pellets, 8–20 mesh; Arcos Organics, Morris Plains, NJ, USA] covered with a thin layer of cotton wool and plastic mesh to prevent direct contact with the animal. When closed, changes in gas volume were measured by recording changes in the position of a black liquid indicator in the side tube. The liquid indicator was a mixture of distilled water, black ink and liquid soap (7:2.5:0.5 mixture ratio by volume). The soap decreased the surface tension, ensuring smooth movement of the fluid through the vinyl side tube. The position of the advance edge of the indicator was measured to the nearest 0.05 mm relative to a fixed mark on the tube using a hand-held dial caliper (model 15-100-500; Manostat Corp., Geneva, Switzerland). The relationship between linear dimension and internal volume of the side tube (α) was calibrated in two ways: measuring the length of a known mass of distilled water and measuring the length of a known volume of indicator solution injected using an Eppendorf pipette. Several such measurements were made and an average value (0.38 μl mm^{-1}) used in all calculations.

Each cockroach was placed on the plastic mesh within a chamber, then the three-way tap and side tube were assembled and the chamber was immersed to the level of the three-way tap in a large-volume water bath (at 23°C) and allowed to equilibrate for approximately 60 min with the side indicator tube open to the atmosphere. Once equilibrated, the respirometer was closed by injection of 40 μl of indicator fluid into the side tube. The syringe was used to adjust the initial position of the indicator fluid and then the three-way tap was closed and measurements of indicator position were made at

intervals of 10–20 min for approximately 2 h. Ten animal chambers and four thermobarometer chambers were studied simultaneously. Thermobarometers were the same as the animal chambers in every respect except that they did not contain a cockroach. An average of the four thermobarometer readings was taken and used in calculations of animal $\dot{V}_{O_2}$:

$$\dot{V}_{O_2} = (\Delta l_a - \Delta l_t) \times \alpha \times (60/\Delta t) \times (1000/M_b) \times (P_B/P_{Bstd}) \times (T_{Astd}/T_A) , \quad (1)$$

where Δl_a is the distance moved by the volume indicator (mm) in animal chamber a, Δl_t is the mean distance (mm) moved by the volume indicators of the four thermobarometer chambers, α is the length–volume proportionality constant of the indicator tube (μl mm^{-1}), Δt is the time interval (min) between two consecutive measurements, M_b is the cockroach body mass (mg), P_B is the barometric pressure (mmHg), P_{Bstd} is the standard barometric pressure (760 mmHg=101 325 Pa), T_{Astd} is the standard ambient temperature (273 K) and T_A is the chamber ambient temperature (K).

The behaviour of the animals was monitored by direct visual observation throughout the experiment and any locomotor activity was noted. In the majority of cases, the animals became inactive within approximately 30 min after being placed in the chamber but did not enter a sleep-like state, as judged by intermittent movements of the antennae and occasional changes in body position and limb postural tone. Any readings taken following a bout of locomotor activity were excluded from analysis so that all measurements of $\dot{V}_{O_2}$ represent animals in a vigilant resting state.

Statistics

Mortality data were compared using Kaplan-Meier survival curves:

$$S(ti) = [(ri - di)/ri] \times S(ti - 1) , \quad (2)$$

where $S(ti)$ is the proportion of animals alive on day ti, $S(ti–1)$ is the proportion of animals alive one day before day ti, ri is the number of animals alive at the start of day ti, and di is the number of animals that died on day ti. Paired t-test was used for within-group comparison of the change of $\dot{V}_{O_2}$ between pre-deprivation baseline and end-deprivation (day 35). Unpaired t-test was used for between-group comparisons of body mass and sleep latency. In all tests the null hypothesis was rejected when $P<0.05$.

Results

Arousal stimulus

A combination of a brief pulse of CO_2 and simultaneous brief rotation (moving a distance of approximately 1 cm) presented at 1 min intervals was effective in maintaining vigilance. That is, visual observations of the insects showed that they were aroused by every stimulus, with distinct movement of the antennae and mild locomotor activity (usually only a few steps). During each stimulus, the CO_2 concentration peaked rapidly to a maximum concentration not exceeding 1% and then decreased in an approximately exponential washout curve to baseline within 20 s. The CO_2 concentration was above 0.5% for less than 6 s per stimulus. Presentations of the CO_2 pulse alone or brief rotation alone were found to be ineffective in maintaining wakefulness for more than a few days, whereas the cockroaches did not habituate to the combined stimulus (CO_2 + rotation) for 72 consecutive days in a pilot study in which the stimuli were presented at 6 min intervals (for 17 days), 5 min intervals (8 days), 4 min intervals (7 days), 3 min intervals (7 days), 2 min intervals (7 days), 1 min intervals (26 days). A longer rotation (quarter turn of the chamber without a concurrent CO_2 pulse) was found to successfully prevent sleep-like rest but it also resulted in premature death of the animals, usually in less than 14 days. It was also found that presentation of the combined stimulus at a rate greater than two per minute, even when scheduled with four daily 2 h intervals without stimuli, caused an increase in mortality.

Latency to sleep-like rest

This was recorded as the time taken to resume a sleep-like resting posture after a single arousing stimulus during the normal resting phase of the light:dark cycle. Control cockroaches had a mean latency of 356±46 s (N=10), and cockroaches that had been deprived of sleep-like rest for 5 weeks (SD group) had a mean latency of 55±6 s (N=5). The sleep latency in the SD group was statistically significantly shorter than controls ($P<0.0002$, unpaired t-test).

Metabolic rate

A statistically significant difference was found between the SD and SC groups after 4 weeks. Metabolic rate of the SC group did not change significantly over the experiment, whereas metabolic rate of the SD group increased significantly. A paired comparison of $\dot{V}_{O_2}$ on day 0 (baseline) *versus* day 35 for those animals that survived to the end of the study (Fig. 2A) indicated

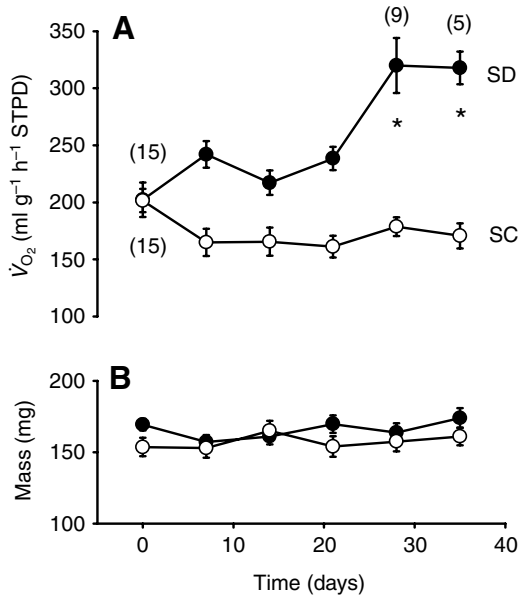

Fig. 2. Rate of oxygen consumption (A) and body mass (B) of two groups of cockroaches measured weekly during prolonged deprivation of sleep-like rest (SD; filled circles) or control protocol (SC; open circles). Symbols indicate means ± s.e.m., sample size=10, except where indicated by numbers in parentheses. Asterisks indicate values significantly different from baseline (day 0) and significantly different from corresponding values in SC group.

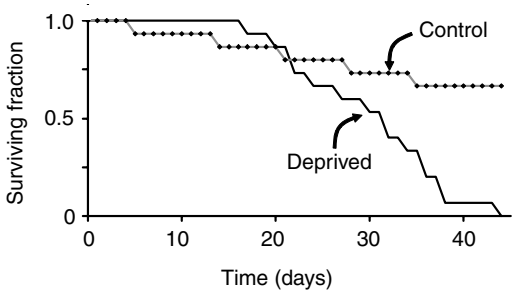

Fig. 3. Kaplan-Meier survival curves for SC (control, dotted line) and SD (deprived, solid line) cockroaches over the 44-day study.

that $\dot{V}_{O_2}$ of the SD group changed by +81.8% (P=0.009) whereas that of the SC group did not change significantly (–2.3%, P=0.755). Body mass of the cockroaches did not differ between SD (166±19 mg) and SC (156±22 mg) groups and did not vary significantly over the course of the experiment (Fig. 2B).

Mortality

Kaplan-Meier survival curves for SD and SC groups are shown in Fig. 3. In the first 16 days of the study there was no mortality in the SD group. Starting on day 17, however, SD animals began to die and mortality rate remained relatively high and approximately constant for the remainder of the study, averaging 0.57 deaths per day, or 1 death every 1.75 days, in the SD group. Half of the SD group had died after 30 days and the last animal died on day 44, whereas 10 of the SC group remained alive at 44 days. Mortality rate was approximately constant throughout the study in the SC group (Fig. 3) at 0.13 deaths per day, or 1 death every 7.7 days. This was similar to control animals kept within an acrylic tube without stimulation, which had an average mortality rate of 0.17 deaths per day, or 1 death every 6 days (data not shown).

Discussion

This study has found that prolonged deprivation of a sleep-like resting state raises resting (vigilant) metabolic rate by 82% and leads eventually to death in the Pacific beetle cockroach, *Diploptera punctata*. Others have shown that prolonged (2–5 weeks) deprivation of sleep is lethal in rats (Rechtschaffen et al., 1983; Everson et al., 1989) and prolonged intermittent stimulation of locomotor activity (2–3 days) is lethal in *Drosophila* (Shaw et al., 2002). The present data therefore reinforce the suggestion that sleep-like rest serves basic functions that are essential for life in insects as well as mammals. The present study has also shown that the increase in resting metabolic rate during prolonged deprivation of sleep-like rest occurs in an ectothermic insect species and is therefore not confined to endothermic species, as would be predicted from the hypothesis that increased metabolic heat production is a consequence of a sleep-deprivation-induced perturbation of the temperature regulating system (Rechtschaffen and Bergmann, 2002; Rechtschaffen et al., 1989). However, as in previous studies in rats (Koban and Swinson, 2005; Rechtschaffen et al., 1989), the data do not resolve the question of whether hypermetabolism is causally linked to mortality.

Since the rate of mortality in stimulated control cockroaches (SC group) was similar to that of unstimulated insects, we can tentatively conclude that the elevated mortality in the deprived cockroaches (SD group) resulted from a lack (or severe fragmentation) of sleep-like rest. However, the data cannot fully rule out the possibility that the stimulus itself contributed to mortality. Long-term sleep deprivation studies in any species suffer from the drawback that they cannot entirely control for the non-specific effects of the stimulus used to maintain vigilance. Under ideal circumstances, control animals would receive exactly the same number, quality and schedule of stimuli. Unfortunately, it is impossible to achieve this while ensuring normal sleep in the control animals and maintained vigilance in the experimental group. The compromise adopted in the present study was to schedule unstimulated rest intervals of 3 h per 6 h period, with the assumption that the animals would exploit these to obtain a normal daily quota of sleep-like rest. We found that this ‘stimulus control’ (SC) protocol did not cause increased mortality compared with unstimulated cockroaches. A similar approach was used by Shaw et al. in *Drosophila* and no deaths were observed in their control flies (Shaw et al., 2002).

Rechtschaffen and colleagues developed the disk-over-water technique for rats, which features a ‘yoked control’ protocol (Rechtschaffen et al., 1989). In this technique, the yoked control animal receives the same number and timing of stimuli as the rat targeted for sleep deprivation but it is able to sleep whenever the sleep-deprived rat is spontaneously awake. This approach suffers from the disadvantage that the two rats do not receive the same quality of stimuli because the sleep-deprived rat is always in the early stages of sleep when stimulated whereas the control rat receives some stimuli when awake or after variable amounts of sleep. Hence, it is not possible to distinguish between lack of sleep and repetitive arousal as the causative factor in the differences in response between deprived and control rats. Furthermore, the frequent stimulation needed to maintain wakefulness in the deprived rat results in significant partial deprivation in the control rat also (Everson et al., 1989). Unfortunately, we were unable to record cockroach behaviour in the deprivation apparatus used in the present study so we could not determine whether the control animals were partially deprived of sleep-like rest. Nevertheless, daily visual observations indicated that both the SD and SC groups were aroused by the stimuli throughout the study and that the SC group spent time during the unstimulated intervals in a state of behavioural sleep-like rest. By contrast, the cockroaches in the SD group were rarely seen to adopt a sleep-like resting posture. Furthermore, the SC group maintained a high level of vigilance with intense escape responses during handling, whereas the SD group became lethargic and relatively unresponsive.

The latencies to onset of a sleep-like resting state were six times longer in control animals than in the SD animals at the end of the study, indicating a substantial difference between groups in the drive to enter sleep-like rest, which is consistent with the assumption that control animals received significant amounts of sleep-like rest under these conditions. Preliminary data from studies of locomotor activity using time-lapse video found no ‘rebound’ increase in immobility following one week of the SD protocol in *Diploptera* (N=20 in each group of SD and SC). This is inconsistent with the predictions based on a

homeostatic control mechanism for sleep (Hendricks et al., 2000b). However, these preliminary recordings were made in constant light, which was found to damp the circadian rhythms in locomotor activity and rest over 3–5 days. Activity tended toward the low level normally seen during the light phase of the LD cycle. Hence, it is unclear whether the lighting conditions may have masked a response to the deprivation protocol, and further work is needed to clarify this question. We therefore consider sleep latency to be a more convincing indicator of increased sleep drive following extended wakefulness.

Diploptera were found to be surprisingly susceptible to relatively mild intermittent enforced activity. Even a slow quarter turn of the chamber (5 s rotation once per minute) led to exhaustion and premature mortality in less than 14 days. Hence, we elected to use two very mild stimuli (a brief pulse of CO_2 and a simultaneous small shift in the animal chamber), which in combination were found to reliably arouse the animals without habituation. This choice of stimulus was based on our preliminary observation that the animals were alerted by exhaled air from human observers, especially when the latter was associated with a mild tactile stimulus. Hence, we were able to exploit a chemosensory mechanism involved in natural avoidance behaviour to evoke long-term vigilance. In order to match the total number of stimuli between groups, the SC group received two stimuli per minute during each 3 h stimulus period, a stimulus frequency double that of the SD group, which can be interpreted as a higher instantaneous stimulus intensity in the SC group. In preliminary studies, we noted that further increases in stimulus frequency in control animals had an appreciable effect on mortality, showing that the stimulus, when presented at great enough frequency, could have a detrimental effect on the animals. Nevertheless, it appears that the chosen control protocol was relatively benign because the SC group failed to exhibit changes in body mass, metabolic rate, mortality or general behaviour throughout the study. It is well established that insects are responsive to CO_2 and that the effects of CO_2 are highly variable, depending upon species and CO_2 concentration as well as many other factors such as temperature and humidity (Nicolas and Sillans, 1989). However, for the following reasons we consider it unlikely that CO_2 was a direct cause of mortality in the SD group. High concentrations of CO_2 (10–100%) have a narcotic effect and are used for insect immobilization or population control, but Gannon et al. found that prolonged continuous exposure to very high doses (e.g. 11.5–16.2 h in 60% CO_2 in air at 20°C) was required to kill cockroaches, *Blatta orientalis* (Gannon et al., 2001). By contrast, the animals in the present study were exposed only to low doses (<1% maximum) for very brief periods (above 0.5% for 6 s per min), and these stimuli activated rather than suppressed alert vigilance. Moreover, in a pilot study, the addition of 2% CO_2 to the air ventilating the home cage of the cockroaches had no obvious effect on behaviour or mortality. Also, since a high dose of CO_2 acts as an anaesthetic, it would be predicted to suppress metabolic rate, whereas in the present study low doses led eventually to an elevation of oxygen uptake in the SD group but not the SC group.

Many insect species, including *Drosophila* and mosquitoes (*Anopheles gambiae*), are capable of detecting very low concentrations of CO_2 using extremely sensitive olfactory sensory neurons (Jones et al., 2007; Suh et al., 2004). Stimulation of these receptors evokes innate chemotaxic responses such as host-seeking in mosquitoes or avoidance in *Drosophila* (Suh et al., 2004) and *Diploptera* (present study). These chemotaxic responses are alerting and, in those species that exhibit avoidance reactions, probably form part of an anti-predator response that, in the case of *Diploptera*, does not habituate readily, making it an effective stimulus for enforcing long-term maintenance of vigilance.

In wild-type (Canton-S) strains of *Drosophila*, mechanical stimuli applied automatically at 20–30 s intervals eventually led to death after 60–70 h (Shaw et al., 2002). Extensive control protocols failed to find any evidence that the stimuli were excessively stressful or traumatic, suggesting that fragmentation or lack of sleep-like rest was the proximate cause of death in *Drosophila*, as it appears to be in *Diploptera*. However, the survival times were much shorter in fruit flies, suggesting that *Drosophila* are more susceptible to deprivation of sleep-like rest than *Diploptera*, at least when measured on an absolute time scale. Interestingly, there is a cross-species correlation between deprivation survival time and normal longevity; both are approximately 10 times longer in the cockroach than in the fruit fly. Furthermore, in *Drosophila*, reduced sleep consolidation has been found to be correlated with reduced life span (Koh et al., 2006), and short-sleeping mutant flies were also found to have a reduced life span (Cirelli et al., 2005). The susceptibility of *Drosophila* and *Diploptera* to deprivation of sleep-like rest may therefore be similar when measured on a physiological time scale.

Koh et al. described a correlation between sleep consolidation and life span that is possibly mediated by temperature, metabolic rate and oxidative stress in *Drosophila* (Koh et al., 2006). It would be of interest to determine whether these factors also influence tolerance to sleep-like rest deprivation. Cellular oxidative stress does not appear to be increased in mammalian tissues during long-term sleep deprivation (Gopalakrishnan et al., 2004), but studies of molecular chaperone proteins suggest the presence of other forms of cellular stress in the brain (Cirelli, 2006; Naidoo et al., 2005). Furthermore, Shaw et al. found that the lethal effects of deprivation of sleep-like rest in *Drosophila* were delayed by induction of heat shock protein Hsp83, and sensitivity to deprivation was greatly increased in mutant flies lacking Hsp83 (the latter died in less than 25% of the survival time of wild-type flies) (Shaw et al., 2002).

Resting metabolic rates of adult male *Diploptera punctata* were within the range that has been observed in a variety of other cockroach species, after accounting for effects of body size and ambient temperature (Bartholomew and Lighton, 1985; Birchard and Arendse, 2001; Coelho and Moore, 1989). As expected, in the present study, there was some variation between animals and within animals during recordings. The latter may have arisen from variations in muscle tone and from a discontinuous breathing pattern (Marais and Chown, 2003). Unfortunately, the apparatus used in this study was not able to resolve discontinuous respiration due to the intermittent mode of data recording and the damping effect of the kinetics of CO_2 absorption in the chamber. However, any temporal variability

in oxygen uptake was negated by averaging multiple readings over a 2 h recording interval after deletion of any elevated values recorded during occasional periods of observed activity.

The mechanisms underlying the elevation of metabolic rate (i.e. increased metabolic thermogenesis) during sustained deprivation of sleep-like rest cannot be determined from the present study. In evaluating the results of their extensive studies of long-term sleep deprivation in rats, Rechtschaffen and colleagues suggested that the increase in metabolic rate is likely to be a consequence of two main factors; an elevation of hypothalamic temperature set point and reduced capacity for suppression of heat loss (Bergmann et al., 1989; Rechtschaffen et al., 1989; Rechtschaffen and Bergmann, 2002). This implies that sleep deprivation may have a primary effect on the integrative function of central neural and endocrine mechanisms involved in thermoregulation. Indirect evidence points to an important role for brown adipose tissue (BAT) as a thermoeffector organ generating at least some of the excess heat (Balzano et al., 1990; Koban and Swinson, 2005).

Cockroaches are ectothermic and, as such, do not raise metabolic rate in response to thermal stress. Maintenance of a constant thermal environment throughout this study prevented any influence of behavioural thermoregulation on *Diploptera* metabolic rate (Stevenson, 1985). The present data from cockroaches therefore suggest that long-term deprivation of sleep-like rest has an effect on metabolic heat production that is independent of thermoregulatory function. That is, the data suggest an effect of prolonged vigilance on basal metabolic rate, implying that sleep may have a more general role in the maintenance of energetic efficiency at the cellular level. Whether this is also the case in mammals is unclear, and this issue is deserving of further study. For example, it would be of interest to know whether the effect is tissue-specific. It is surprising that cerebral metabolic rate was minimally affected by prolonged sleep deprivation in rats (Everson et al., 1994), considering the widespread assumption that the CNS is a major functional target of sleep (Hobson, 2005). Alternatively, sleep-deprivation-induced dysfunction of central nervous function could have an indirect effect on whole-animal metabolism *via* alterations in the energy turnover of peripheral tissues under neural control. One promising candidate is skeletal muscle, for example, where a change in motor tone could conceivably lead to increased muscle metabolic rate. Given the logistical and ethical issues associated with long-term sleep deprivation studies in mammals, the present findings suggest that an insect model may be a productive alternative for the study of cellular mechanisms underlying the increase in metabolic heat production during prolonged wakefulness.

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