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Can sleep behaviour be used as an indicator of stress in group-housed rats (Rattus norvegicus)?

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Abstract

We assessed the value of sleep behaviour as a novel measure of stress in group-housed animals. We observed, non-invasively, sleep behaviour in 144 group-housed rats, and related it to other physiological and physical indicators of stress and welfare. Sleep frequency and duration correlated negatively with adrenal weight, and positively with bodyweight gain, and final bodyweight. Thus, low frequencies of sleep behaviour and low sleep duration correlate with some indicators of elevated physiological and physical stress, raising the possibility that sleep behaviour may provide an under-utilised, but potentially important, non-invasive indicator of stress and welfare for animals in groups.

Keywords: *adrenal glands, animal welfare, bodyweight, group-housing, laboratory rats, sleep behaviour*

Introduction

Sleep is defined, behaviourally, as a motionless state in which an animal lies, is not alert and has both eyes shut (eg Hurst *et al* 1999). Research on humans and non-human animals has attached many functions to sleep, including facilitating recovery from fatigue (Hobson 1968), aiding thermoregulation (Schmidek *et al* 1972) and protecting against excessive wear and tear (Everson *et al* 1989). Moreover, for non-human animals sleep plays an important role in the establishment and development of normal social behaviour at maturity (Watson & Henry 1977). The vital roles that sleep plays for normal body functions and well-being are highlighted by the results of sleep deprivation studies. For example, in rats sleep deprivation can have dire physiological and pathological consequences that may lead to death within a period of 2-3 weeks (Rechtschaffen *et al* 1983; Everson *et al* 1989).

The effects of chronic stress on sleep have been extensively studied. Studies in humans indicate that activity of the hypothalamic-pituitary-adrenal (HPA) axis can influence sleep patterns. This may be due to increased levels of corticotropin releasing factor, adrenocorticotrophic hormone and cortisol. HPA hyperactivity (as in chronic stress, ageing and depression) reduces sleep quality and causes sleep disturbances (Bradbury *et al* 1998). For example, depressive patients generally exhibit reductions in sleep efficiency, shortening of rapid-eye-movement sleep (and latency), and increases in the number of awakenings (Appelboom-Fondu *et al* 1988). Moreover, patients with abnormally high levels of cortisol (eg Cushing's disease) sleep less and have more nocturnal awakening before therapeutic reductions in adrenal steroid production (Krieger & Glick 1974). Research on laboratory rats has also shown that HPA hyperactivity can affect both sleep quantity and quality. Typically, in a chronic stressful situation, total sleeping frequency and duration decrease, with sleep liable to have more interruptions (Knat *et al* 1995; Cheeta *et al* 1997; Dugovic *et al* 1999). Physical stress reduces both sleep quality and quantity (Cheeta *et al* 1997; Bradbury *et al* 1998), and chronic psychological stress (eg subordination) reduces sleep quantity (Hurst *et al* 1999).

Electroencephalogram (EEG) is the most commonly used method for recording sleep in animals, particularly in laboratory species. However, as EEG is an invasive procedure, and usually requires stressful single housing of the subject (Gamallo *et al* 1986), alternative techniques are required. Despite the clear relationship between stress and sleep, to our knowledge, little work has been done on whether non-invasive measures of sleep behaviour correlate with other commonly used measures of animal stress, and thus whether or not they could be used as stress or welfare indicators. Our aim was to investigate this issue.

Materials and methods

This study was carried out as a part of a larger project investigating the effect of different housing conditions on the welfare of laboratory rats. We housed 348 newly weaned male Wistar rats (Harlan-UK) in three standard laboratory housing conditions (13 rats in a 48 × 33 cm cage [length × breadth]; 20 rats in a 62.5 × 40 cm cage [length × breadth]; 25 rats in a 62.5 × 40 cm cage

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Table 1 Ethogram for behavioural elements recorded.

Behavioural element	Definition
Sleep	Motionless state in which the animal lies, not alert, with both eyes shut, apparently asleep (duration/frequency).
Sleep disruption	The disruption of a sleeping bout by a cage mate, eg by movement (frequency).
Aggression	Various forms of aggressive interaction, (eg pouncing, wrestling), referring to the rat that initiates the aggressive act.

[length × breadth]; six replicates of each cage type). These cages initially provided either 125 (the first two) or 100 cm^2 of space allowance per each individual rat (the latter). The space allowance was then increased up to 150 and 125, and 175 and 150 cm^2, respectively by removing randomly selected non-focal individuals as the rats grew. Although this removal may have had some effect on the social stability of the remaining rats, the experience was similar for all treatments. Rats were housed in these conditions immediately after weaning, at four weeks of age, and remained so for five consecutive weeks, until nine weeks of age. Food and water were provided *ad libitum*, and all cages were supplied with bedding and nesting materials and maintained on a 12:12 h light: dark schedule with continuous dim, red lighting, and white light on between 0200 and 1400h.

We recorded sleep and aggression over the five week experiment (see ethogram; Table 1). We collected sleep, physiological and pathological data from eight focal rats in each cage. Each rat was observed for a continuous eight minutes per session, for four sessions per week and for five observation weeks (160 min per rat, in total), allowing a maximum behavioural bout length of eight minutes. Each week, two sessions were executed during the light phase (1000 and 1200h respectively) and two during the dark phase (1400 and 1600h).

Behaviour was collected using the Psion (Work About ®). Weight gain was calculated from weekly weight measurements. Animals were euthanased at the end of the experiment, their bodyweights recorded, blood samples immediately collected by cardiac puncture, and adrenal and thymus glands removed, trimmed, and weighed.

Data analysis

The frequency and duration of the behavioural data (aggression, sleep and sleep interruption) over the five observation weeks and the four sessions were totalled to allow comparison with measures collected only once at the end of the experiment (eg adrenal and thymus gland weights, plasma corticosterone, final bodyweight). Sleep data were separated into light phase (combined data from the 1000 and 1200h sessions) and dark phase (combined data from the 1400 and 1600h sessions).

Data met the requirements for parametric tests following square-root transformation. Correlations between sleep data as total durations (seconds) and total frequency, total aggression frequency, weight gain and body weight (g), adrenal weight (mg), thymus weight (mg), and plasma corticosterone (ng ml^{-1}), were determined using a partial correlation technique, partialling out the effect of housing condition and replicate (SPSS version 12.0).

Results

Total sleep frequency and duration correlated negatively with adrenal weight and positively with bodyweight gain, and final bodyweight (Table 2). However, we found no significant correlations between sleep behaviour and aggression, plasma corticosterone or thymus weight. There was a strong positive correlation between total sleep duration and frequency, and between these measures and sleep disruption. All of these findings (except the correlations between sleep duration and adrenal weight and weight gain) were evident when light phase sleep was analysed on its own. However, there was no significant correlation between dark phase sleep frequency and duration and any of the stress measures.

Discussion

High sleep frequency and duration correlated with increased bodyweight and weight gain, and decreased adrenal weight suggesting that they may indicate good welfare. Bodyweight, weight gain, and adrenal weight have been shown to be useful indicators of animal welfare (eg Broom 1986; Manser 1992) as they are affected by chronic social stress. It is interesting to note that most of these correlations held for sleep during the light phase, but not during the dark phase. As well as reflecting the natural tendency of rats to sleep during the light phase, this may indicate the importance of adequate sleep being achieved during this phase.

We could not find any significant correlations between sleep behaviour and aggression, plasma corticosterone and thymus weight. It may be that post weaning aggression was insufficiently severe to induce changes in these indicators. Also, plasma corticosterone levels show a clear diurnal rhythm (Chang & Opp 2002) and may thus be a good measure of acute stress at the time of sample collection. However, a single sample may be a less reliable indicator of stress over a longer time and hence not expected to correlate strongly with longer-term sleep behaviour data.

Our interpretation that increased sleep frequency indicates low levels of stress could be criticised because stress is known to induce sleep fragmentation which should also lead to an increase in frequency of sleep bouts. However, the effect of stress in fragmenting sleep has generally been observed under single housing conditions (Dugovic *et al* 1999). It is quite possible that, under group-housing condi-

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Table 2 Partial correlation coefficients between sleep and other measures used.

	Sleep frequency		Sleep duration (s)	
	Partial *r*	df	Partial *r*	df
Sleep frequency			0.88***	141
Sleep duration	0.88***	141		
Sleep interruption	0.65***	141	0.32***	141
Aggression	ns	141	ns	141
Adrenal weight (mg)	-0.22**	141	-0.18*	141
Thymus weight (mg)	ns	141	ns	141
Final bodyweight (g)	0.25**	141	0.25**	141
Weight gain (m)	0.23**	141	0.20**	141
Corticosterone (ng ml^{-1})	ns	141	ns	141

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = non-significant.

tions, an animal can only achieve high sleep duration by increasing sleep frequency, due to frequent interruptions by cage mates. Our results confirm that it was those animals that slept for a long time that, indeed, had a higher sleep frequency, and more interruptions of sleeping bouts.

The correlations between sleep measures and bodyweight, weight gain and adrenal weight, although statistically significant, were not very high. Longer recording periods, especially during the light phase, may have led to stronger correlations and provided further information about sleep behaviour, particularly concerning bout length, but this was beyond the scope of this study. More work is needed before we can recommend recording sleep behaviour in place of (rather than in conjunction with) other commonly used measures of welfare. We are therefore currently studying the effects of environmental modification and husbandry procedures on sleep, alongside other behavioural, physiological and pathological indicators of stress and welfare, in the laboratory rat.

Animal welfare implications

Our results suggest that, in groups of rats housed in standard laboratory cages, low frequencies of sleep behaviour and low sleep duration correlate with some indicators of elevated physiological and physical stress. This raises the possibility that stress experienced under these conditions disrupts sleep patterns, and that sleep behaviour may provide an under-utilised, but potentially important, non-invasive indicator of stress and welfare; particularly when minimal disruption to the animals under observation is required.

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