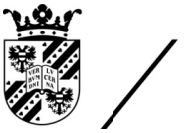
Interpreting the consequences of acute sleep deprivation

Stress physiology as mediator

Wieteke Beerling



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Wieteke Beerling

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Promotor

Prof. dr. J.M. Koolhaas

Copromotores

Dr. W.H.I.M. Drinkenburg

Dr. P. Meerlo

Beoordelingscommissie

Prof. dr. D.G.M. Beersma

Prof. dr. G. van Dijk

Prof. dr. D. Kalsbeek

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Chapter 1

General Introduction

SLEEP AND SLEEP SHORTAGE

Even though the exact function of sleep is unknown, the fact that humans spend around one third of their entire life sleeping suggests that sleep has important functions. In today's society, with our modern lifestyle of around the clock work and increased social pressures, more people deal with regular sleep deprivation (Bonnet and Arand, 1995; Hublin et al., 2001; Rajaratnam and Arendt, 2001). Not only the average time per day spent asleep has decreased, also disrupted normal sleep has become common in today's society (Bonnet and Arand, 1995; Hublin et al., 2001; Rajaratnam and Arendt, 2001). Sleep deprivation is therefore considered a serious problem in today's society. Animals also show cycles of behavioural rest and activity. Animals with complex nervous structures show sleep-like conditions during their resting phase, which are comparable to human sleep (Horne, 2002; Vassalli and Dijk, 2009). Sleep imposes a substantial risk to animals, therefore animals should not sleep unless sleep has an important function (Horne, 2002). Therefore, sleep is thought to be important for humans but also for many animals. This thesis aims at further understanding the consequences of sleep deprivation and focused on rats.

Sleep is a complex non-homogenous phenomenon with electrophysiological and neurochemical changes that follow a cyclic pattern. This makes it complex to determine the exact function of sleep. Sleep consists of two main states; non-rapid-eye-movement (NREM) sleep and REM (REM) sleep that alternate. In humans, NREM sleep can be subdivided into 3 stages that correspond to increasingly deeper stages of sleep (Brown et al., 2012). During sleep, most humans will cycle though the sleep stages a number of times. In rats, sleep is more fragmented than in humans, with shorter sleep durations but with in total around 12 h of sleep each day, and it is hard to distinguish the 3 NREM sleep stages (Borbely and Neuhaus, 1979; Brown et al., 2012). The different sleep stages are thought to have specific functions (Benington and Heller, 1995; Siegel, 1995; Walker and Stickgold, 2004; Brown et al., 2012).

One of the main hypotheses states that sleep serves a homeostatic function. The maintenance of brain homeostatic processes like energy balance, temperature regulation and detoxification have been suggested as a function of sleep (Walker and Berger, 1980; McGinty and Szymusiak, 1990; Inoue et al., 1995; Maquet, 1995;

Brown et al., 2012). Sleep is also thought to contribute to neuronal and synaptic plasticity, which is crucial for learning and memory processes (Maquet, 2001; Graves et al., 2001; Ellenbogen et al., 2006; Walker and Stickgold, 2006; Vassalli and Dijk, 2009; Brown et al., 2012). Existing literature suggests that NREM sleep is involved in consolidation of explicit, episodic memory whereas REM sleep is thought to be important for the consolidation of procedural memory and some types of emotional information processing and storage (Karni et al., 1994; Plihal and Born, 1999; Graves et al., 2001; Peigneux et al., 2001; Benington and Frank, 2003; Walker and Stickgold, 2004; Brown et al., 2012). Sleep deprivation studies have also shown that sleep is important for higher executive functions of the brain compromising mood, cognition and motor control (Bonnet, 1985; Maquet, 2001; Durmer and Dinges, 2005; Frank and Benington, 2006; Brown et al., 2012). According to the hypothesis of Tononi and Cirelli, NREM sleep primarily serves synaptic homeostasis. High neuronal activity present during waking induces synaptic potentation. The waking-induced synaptic potentation results in an increase in the strength, size and number of synapses and their connections (Tononi and Cirelli, 2006). This waking-induced synaptic potentation reduces the available space and energy reserves in the brain. NREM sleep serves to downscale the synaptic strength to lower energy levels and to weaken the synaptic connections. Disturbances in sleep-related synaptic homeostasis may affect learning, memory performance and mood. In addition, sleep deprivation studies indicate that sleep is involved in peripheral functions thereby affecting metabolic, endocrine and immunological systems (Spiegel et al., 1999; Vgontzas et al., 1999; Vgontzas et al., 2004; Gangwisch et al., 2006; Brown et al., 2012). For example, NREM sleep is important in the replenishment of glycogen stores (Benington and Heller, 1995) and growth hormone-releasing hormone and corticotrophin-releasing hormone regulate NREM sleep (Schussler et al., 2006).

Despite the fact that the exact function of sleep remains hypothetical, sleep loss studies have shown that sleep is important for proper physical and mental functioning. Epidemiological studies show correlations of habitual short sleep and sleep disorders, such as insomnia, with cardiovascular disorders (Schwartz et al., 1999; Ayas et al., 2003; Gangwisch et al., 2006) and psychiatric diseases (Ford and Kamerow, 1989; Chang et al., 1997; Riemann et al., 2001). In humans, chronic sleep shortage is most strongly associated with impairments of mood, cognitive functioning

and motor performance (Pilcher and Huffcutt, 1996; Dinges et al., 1997; Van Dongen et al., 2003).

Not only the function of sleep needs further clarification, also many of the mechanisms underlying the adverse effects of sleep loss remain speculative. Several hypotheses have been proposed. One of such hypotheses suggests involvement of the stress systems. Therefore, the focus of this thesis is to further explore the hypothesis that the stress systems are the core mediators of the adverse effects of sleep loss in rats.

SLEEP DEPRIVATION AND THE STRESS SYSTEMS

The sympathetic-adrenomedullary (SAM) system and the hypothalamic-pituitary-adrenocortical (HPA) axis are considered to be the two key players in the stress response and are both affected by sleep deprivation.

Sleep deprivation and the SAM system

Higher catecholamine levels, indicative of SAM system activation, have been found during sleep deprivation (Lusardi et al., 1996; Tochikubo et al., 1996; Irwin et al., 1999). The SAM system acts via two different pathways. One pathway consists of neurons that trigger the release of adrenalin from the adrenal medulla into the blood (Axelrod and Reisine, 1984; Johnson et al., 1992). The other pathway is the sympathetic nervous system, which innervates many organs in the body through the release of noradrenalin (Axelrod and Reisine, 1984; Johnson et al., 1992). The noradrenergic cell clusters in the brain stem are the basis for the sympathetic outflow. Activation of these noradrenergic neurons during stress is partly dependent on Corticotrophin-Releasing-Hormones (CRH) that are released during HPA axis activation (Koob, 1999). The released adrenalin and noradrenalin mobilize the energy needed to cope with the stressor. They achieve this by increasing the output of the heart and by increasing blood pressure. This results in an increased blood flow to the muscles and an increased metabolic rate (Leal-Cerro et al., 2003). Moreover, the released adrenalin affects the glucose metabolism thereby causing nutrients

stored in the muscles to become available to provide energy (Axelrod and Reisine, 1984; Johnson et al., 1992; Leal-Cerro et al., 2003).

One would expect sleep deprivation to be associated with increases in catecholamine levels as adrenalin and noradrenalin levels vary with the time of the day with lower levels during the circadian sleep phase (Akerstedt and Froberg, 1979; De Boer and van der Gugten, 1987). Sleep onset is associated with a rapid decline in catecholamines that are maintained low during sleep (Akerstedt and Froberg, 1979; Irwin et al., 1999). Most studies showed that sleep deprivation is associated with an increase in catecholamine levels towards levels seen during wakefulness. This is accompanied by increases in heart rate and blood pressure (Lusardi et al., 1996; Tochikubo et al., 1996; Irwin et al., 1999). Even brief awakenings from sleep have shown to be associated with sympathetic activation and with a temporary rise in heart rate and blood pressure (Sforza et al., 2004). Despite the fact that most studies show sympathetic activation in response to sleep deprivation, some studies report no significant sympathetic activation after sleep deprivation (Akerstedt and Froberg, 1979; Chen, 1991).

These differences can be explained by the fact that the extent of sympathetic activation is highly dependent on the method of sleep deprivation. Existing literature suggests that the extent of sympathetic activation is mainly related to the disruption and discontinuity of sleep rather than to the duration of sleep deprivation (Chen, 1991; Irwin et al., 1999; Tiemeier et al., 2002). Prolonged total sleep deprivation will result in a state of fatigue with decreased arousal, alertness and mental activity. Consequently, also sympathetic activity is reduced (Meerlo et al., 2008). In contrast, chronically restricted or disrupted sleep not associated with the same degree of fatigue may lead to increased sympathetic activation. Studies have shown that when recovery after acute sleep deprivation is insufficient, heart rate and blood pressure stay elevated (Lusardi et al., 1996; Tochikubo et al., 1996). It is suggested that initiating and maintaining wakefulness in a state of sleep debt requires higher sympathetic activation (Bergmann et al., 1989; Everson et al., 1989a). This is also in agreement with a study showing that chronic sleep restriction is an increased risk for hypertension and cardiovascular diseases (Gangwisch et al., 2006).

The activities during sleep deprivation also highly affect the degree of sympathetic activation. Sympathetic activation is dependent on the amount of

physical activity, emotional arousal and cognitive demand (Meerlo et al., 2008). Studies that report no or low increases in sympathetic activity during sleep deprivation often concern studies where organisms are in a relaxed position and do not require much emotional or cognitive demand. In contrast, sleep deprivation studies where organisms have to maintain alertness and have to perform during the sleep deprivation period showed higher sympathetic activity (Zhong et al., 2005; Meerlo et al., 2008).

Sleep deprivation and the HPA axis

Several studies in healthy subjects showed that sleep deprivation is also associated with mildly elevated plasma levels of the adrenocortical hormone cortisol (Akerstedt et al., 1980; von Treuer et al., 1996; Spiegel et al., 1999; Chapotot et al., 2001). In rodents, sleep deprivation is associated with a mild increase in corticosterone (Tobler et al., 1983; Suchecki et al., 1998; Meerlo et al., 2002; Hipolide et al., 2006; Sgoifo et al., 2006). These increases are considered as mild when compared to levels reached when exposed to a stressor. The elevations are indicative of hypothalamic-pituitary-adrenal (HPA) axis activation (von Treuer et al., 1996; Suchecki et al., 1998; Spiegel et al., 1999; Chapotot et al., 2001; Hipolide et al., 2006; Sgoifo et al., 2006; Meerlo et al., 2008).

HPA axis activation is regulated by the paraventricular nucleus (PVN) of the hypothalamus that secretes the Corticotrophin-Releasing-Hormone (CRH) and Vasopressin upon stimulation. These peptides stimulate the pituitary gland which results in the release of the adrenocorticotropic hormone (ACTH) into the bloodstream. In turn, ACTH stimulates the adrenal cortex to release glucocorticoids in the blood; cortisol in humans and corticosterone in rodents (Axelrod and Reisine, 1984; Johnson et al., 1992). One of the main effects of the released glucocorticoids is the supply of energy by converting proteins to glucose. The glucocorticoids are known to regulate a variety of other processes as well (Axelrod and Reisine, 1984; Johnson et al., 1992; Sapolsky et al., 2000; Leal-Cerro et al., 2003). The HPA axis system is well regulated. A negative feedback system consisting of glucocorticoids that act back on the hypothalamus and pituitary gland to suppress CRH and ACTH production, prevents chronically elevated concentrations of glucocorticoids in the

blood (Jacobson and Sapolsky, 1991; Johnson et al., 1992; Herman and Cullinan, 1997). The HPA axis receives regulatory inputs from the hippocampus, amygdala, the brain stem, the serotonergic system and the bed nucleus of the stria terminalis (Dinan, 1996; Ulrich-Lai and Herman, 2009). Furthermore, the CRH system and the brain stem noradrenergic system together form a feed-forward system whereby CRH activates noradrenergic activity, which in turn activates forebrain CRH activity (Koob, 1999).

HPA axis activity normally displays a daily rhythm that is controlled by the suprachiasmatic nucleus of the hypothalamus and is characterized by parallel changes in the levels of corticosterone and ACTH (Moore and Eichler, 1972). Glucocorticoid levels are the lowest early in the resting phase and show a peak just before the end of the sleeping phase. This peak prepares the body for awakening by mobilizing the energy substrates (Weitzman et al., 1971). The increase in glucocorticoids during sleep deprivation is therefore not surprising as glucocorticoid levels are suppressed during sleep as compared to during wakefulness. Studies in humans also showed elevated cortisol levels during arousal from sleep and during sleep fragmentation (Spath-Schwalbe et al., 1991; Ekstedt et al., 2004). The increased glucocorticoid levels during sleep deprivation disappear rapidly during subsequent recovery sleep (Meerlo et al., 2002; Sgoifo et al., 2006). When recovery sleep is not sufficient, chronic activation of the HPA axis is thought to occur. This has mainly been suggested in light of the development of mood disorders as both prolonged sleep disruption and prolonged elevated cortisol levels are hallmarks of depression (Buckley and Schatzberg, 2005). Some human studies also showed, that despite a rapid decline in cortisol levels during recovery in healthy subjects, elevated levels were present in the evening following sleep deprivation (Leproult et al., 1997; Spiegel et al., 1999). The mechanisms behind this recurrent activation are unknown.

Despite most studies showing mild increases in glucocorticoid levels in response to acute sleep deprivation, some studies showed no changes or even a decrease in glucocorticoid levels during acute sleep deprivation (Akerstedt et al., 1980; Rechtschaffen et al., 1983; Follenius et al., 1992). These differences may be due to the methods used to induce sleep deprivation. After all, the extend of HPA axis activation is highly dependent on the amount of physical activity, emotional arousal and cognitive demand during the period of sleep deprivation (Meerlo et al.,

2008). Higher HPA axis activation is observed when organisms engage in physical activity or face a cognitive challenge (Radomski et al., 1992; Chapotot et al., 1998; Rechtschaffen et al., 1999; Chapotot et al., 2001). In contrast, low corticosterone levels may be observed during sleep deprivation if organisms are not physiologically or psychologically active and are highly fatigued.

Adaptive and maladaptive consequences

The acute stress response is generally considered to be highly adaptive and allowing the organism to deal with a situation. The released stress hormones provide not only energy to the body (Axelrod and Reisine, 1984; Johnson et al., 1992; Leal-Cerro et al., 2003), they also act on the hippocampus and amygdala thereby causing enhancement of learning, enhancement of memory for emotional stimuli and an increase in behavioural responsiveness and alertness (McEwen and Sapolsky, 1995; Quirarte et al., 1997; Arnsten, 1998; Arnsten and Goldman-Rakic, 1998; McEwen and Seeman, 1999; McEwen, 1999a; McEwen, 1999b). Also, feelings of apprehension and fear are initiated by stress hormones (Lazarus, 1993). In addition, the immune system is affected; immune cells redistribute and the immune system prepares for possible infection or injury (Khansari et al., 1990; McEwen and Seeman, 1999; Heinz et al., 2003). Furthermore, the secretion of sex steroid hormones is reduced (Heinz et al., 2003). Regular sleep is thought to contribute to normal HPA axis and SAM system function and to maintain stress hormones at concentrations that are in a range that is protective for the brain (Abraham et al., 1997; Abraham et al., 2001).

Despite the adaptive functions of the stress response, chronic activation of the stress systems is not considered to be adaptive. Frequent exposure to high concentrations of stress hormones promotes neuronal damage and cell loss, particularly in the hippocampus (Sapolsky and Pulsinelli, 1985; Sousa et al., 1999). Also, conditions with high glucocorticoid levels are associated with smaller hippocampal volume and cognitive impairments (Sapolsky, 2000; Huang et al., 2009). Moreover, chronic stress system activation is considered to contribute to a wide range of stress related disorders such as depression, anxiety disorders, immunodeficiency and cardiovascular disorders (McEwen, 1998; McEwen and

Seeman, 1999; Korte et al., 2005). In this thesis we hypothesise that sleep deprivation causes chronic or altered stress system activation that in turn is responsible for some of the adverse effects associated with sleep deprivation.

Sleep deprivation as a stressor

The studies reviewed in the previous sections indicate that disturbance, restriction or complete deprivation of sleep is associated with at least a mild activation of the classical neuroendocrine stress systems. An important question is whether this implies that sleep deprivation constitutes a stressor that mediates the adverse consequences of sleep deprivation. This is not a trivial issue since it touches upon a serious conceptual problem in stress research. Hans Selye defined stress in 1950 as the non-specific response of the body to a noxious stimulus (Selye, 1950). This concept was later refined by distinguishing between a stressor and the stress response. Currently, a stressor is considered a stimulus that is a threat to homeostasis and the stress response is considered a reaction of the organism to regain homeostasis (Chrousos, 2009). This concept of stress has led to many discussions as many activities of an organism relate to maintenance of homeostasis but are not stressful (Levine, 1991; McEwen, 1998; Levine, 2005; Romero et al., 2009; Koolhaas et al., 2011). Levine and Ursine emphasized the view that stress should be considered as a process that includes the stimulus, the perceptual processing of the stimulus and the behavioural and physiological response(Levine, 1991; Levine, 2005). This has led to the general view that occurrence of a stress response is an indicator of a stressful situation (Armario, 2006). Therefore, a stimulus is in general considered stressful when the stress systems are activated.

However, stress should not only be interpreted as the physiological and hormonal activation of the stress systems. It should also be interpreted as a cognitive experience of aversion (Koolhaas et al., 2011). The problem here is that activation of the classical neuroendocrine stress systems not only occurs in response to aversive stimuli but sometimes also in response to positive and rewarding stimuli. In fact, these systems are crucial for mobilization of energy and are thus activated in any condition that requires or causes behavioural activation. Consequently, a considerable part of the physiological and hormonal responses to a certain situation

can be a direct reflection of the metabolic requirements for normal ongoing behavioural activity, rather than a reflection of the aversive nature of a condition (Koolhaas et al., 2011). Hennessy and Levine already recognized the stress system as a component of the general arousal system (Hennessy and Levine, 1979). They discussed these concepts of stress and arousal in terms of a psychoendocrine hypothesis; they suggest that the HPA axis can be considered as a component of a unified system of arousal and that HPA axis responses are a reflection of changes in arousal level (Hennessy and Levine, 1979). This is confirmed by studies showing SAM system and HPA axis activation in response to aversive, uncontrollable situations but also to rewarding, controllable situations. Studies showed that food or water reward, sexual behaviour and winning a social interaction elicit HPA axis responses that are similar in magnitude as highly aversive situations like social defeat (Schuurman, 1980; Bronson and Desjardins, 1982; De Boer et al., 1990a; Bonilla-Jaime et al., 2006; Koolhaas et al., 2011; Buwalda et al., 2012). These rewarding and controllable situations activate the SAM and HPA systems but should not be considered an aversive stressful situation. In line with these observations are the studies that show that uncontrollability is one of the main properties of a true aversive stressor (Schuurman, 1980; Muir and Pfister, 1986; De Boer et al., 1990a). Weiss already showed in 1972 that it is not the physiological nature of an aversive stimulus that induces stress pathology but the degree in which it can be predicted and/or controlled (Weiss, 1972).

In order to determine to what degree sleep deprivation constitutes a stressor and in order to interpret the consequences of sleep deprivation correctly, controlled rodent studies are needed that should study the stress system activation in concert with behaviour and should reduce stress confounding effects. The activation of stress systems in many studies may be the result of artefacts of the experimental procedures. We hypothesize that sleep deprivation can be considered a stressor when stress system activation dissociates from the behavioural response and when the activity of the stress systems reaches levels beyond those seen during relaxed wakefulness.

SLEEP DEPRIVATION AND STRESS REACTIVITY

Sleep deprivation may not only affect the basal activity of the stress systems but may also affect the responsivity of these systems to challenges. This will not be evident under strictly controlled stimulus-poor conditions but will become noticeable when subjects are facing new challenges or stressors on top of the deprived sleep (Meerlo et al., 2008). A few controlled rodent studies have been done. These suggest that acute sleep deprivation for less than a day does not affect the HPA axis response to a subsequent stressor (Meerlo et al., 2002; Novati et al., 2008). However, prolonged sleep deprivation (i.e., 48 h of total sleep deprivation) or chronic sleep restriction (20 h of sleep deprivation per day for a week or more) alters the HPA axis response to brief restraint or inescapable foot shock in rats (Meerlo et al., 2002; Sgoifo et al., 2006; Novati et al., 2008). Particularly, while the pituitary ACTH response was significantly dampened, the adrenal corticosterone response remained unaltered. These findings together suggest that the effect of sleep loss on stress reactivity may accumulate over time (Meerlo et al., 2002; Novati et al., 2008). Another study showed a slightly different effect of chronic REM sleep deprivation on stress HPA axis responsivity. Suchecki and co-workers showed that 96 h of REM sleep deprivation induced higher corticosterone levels after exposure to a mild stressor without affecting the ACTH response (Suchecki et al., 2002). The similarity with the chronic sleep restriction studies is that in both cases the adrenal corticosterone response is increased relative to the pituitary ACTH response.

There is also a limited number of controlled human studies. Some studies measured the physiological responses to mild and high intensity exercise after sleep deprivation (Martin and Chen, 1984; McMurray and Brown, 1984; Martin et al., 1986; Symons et al., 1988; Martin, 1988). Most of those studies showed no serious effect of sleep deprivation on the acute physiological and hormonal responses to a physical challenge. The cognitive and emotional perception of this challenge may however have been affected since a reduced perceived exertion and tolerance of physical exercise after sleep deprivation has been reported (Martin, 1981; Martin and Chen, 1984; Martin et al., 1986). Another study showed reduced cognitive and emotional responsiveness to work-related challenges after sleep deprivation (Zohar et al., 2005). Meerlo and co-workers suggested that even if sleep deprivation does not

affect the responsiveness of the neuroendocrine systems directly, it may do so indirectly by altering the perception of certain stimuli on a cognitive level and by changing the afferent inputs to the neuroendocrine control regions (Meerlo et al., 2008).

In this thesis we aimed to study in more detail and under non-confounded conditions whether sleep deprivation, by acting on the stress systems, affects the stress response to a challenging situation.

SLEEP DEPRIVATION AND ATTENTIONAL FUNCTIONING

In humans, reductions in sustained and selective attention are among the first signs of sleep loss and sleepiness. Studies showed that a single night of sleep loss leads to impairments in sustained attention tasks that require attention to auditory or visual stimuli (Jewett et al., 1999; Johnsen et al., 2002; Van Dongen et al., 2003) but also to stimuli with a higher sustained and selective attentional demand (Drummond et al., 2001). Sleepiness is commonly observed among nurses, air traffic controllers and truck drivers and has shown to lead to attention problems that can have serious consequences (Haraldsson et al., 1990; Dinges, 1995; Caldwell, 2001; Rogers, 2008).

While many attempts have been made to unravel the underlying mechanisms, these have found to be complex. The fact that attention is not a unitary phenomenon but more a term for several different varieties of attentional processes makes it complex to determine the underlying mechanisms. Studies suggest that the attentional deficits resulting from sleep loss reflect a dysregulation of behavioural control processes that rely on the prefrontal cortex (Norton, 1970; Durmer and Dinges, 2005), such as attention to relevant cues (Norton, 1970), flexible thinking (Harrison and Horne, 1999) and cognitive perseveration (Horne, 1988; Wimmer et al., 1992), without affecting aspects of executive function. This indicates that sleep deprivation has different effects on the underlying mechanisms of attention (Cordova et al., 2006). Studies also suggest involvement of the stress systems. Stress hormones do not only support metabolic processes and physical activity but also affect brain functioning, attentional processes, cognition and mood (McEwen and Sapolsky, 1995; Quirarte et al., 1997; Arnsten, 1998; Arnsten and Goldman-Rakic,

1998; McEwen and Seeman, 1999; McEwen, 1999a; McEwen, 1999b). Moreover, sleep loss and stress system activation are both thought to affect mechanisms that are involved in attentional processes (Johnson et al., 1992; Brown et al., 2012). Therefore, we hypothesize that stress system activation is also involved in the attentional deficits commonly observed after sleep deprivation.

There are only few controlled rat studies that assessed sleep-deprived rats in a behavioural assessment and separated the different attentional measures (Godoi et al., 2005; Cordova et al., 2006). These two studies showed that sleep deprivation affects selective and sustained attention without affecting behavioural control. In this thesis we aimed to show in a non-confounded way how acute sleep deprivation affects different attentional processes and the possible involvement of stress system activation.

AIM AND OUTLINE OF THE THESIS

Sleep deprivation is a problem in today's society and is associated with mental and physical impairments. The mechanisms underlying the adverse effects of sleep loss remain speculative. This thesis describes a series of studies that examined the effects of acute sleep deprivation on the basal stress response, on stress responsivity and on attentional functioning. The background of these studies is our hypothesis that sleep deprivation, by acting on the stress systems, may affect stress responsivity and attentional performance. This thesis focused on 4-6 h sleep deprivation. Four to six hours of sleep deprivation in rats has shown to lead to an acute sleep loss with significant increases in sleepiness for up to 3 h after its termination (McKenna et al., 2008). In order to interpret the outcome of our studies correctly, this thesis aimed to reduce stress confounding effects and to study stress system activation in concert with behaviour. We hypothesize that a considerable part of the stress system activation in response to a certain situation is a direct reflection of the metabolic requirements for ongoing behavioural activity, rather than a reflection of the stressfulness nature of the situation.

To study effects of sleep deprivation on stress responsivity, two different types of challenges were used; novelty exposure and frustrative non-reward stress. These

challenges were chosen because they have good ecological validity. This means that they have a relationship with the biology of the species and thus with the environmental challenges that the animal may meet in everyday life in its natural habitat (Koolhaas et al., 2006). Stressors with good ecological validity are thought to be more relevant for human mental and psychological stress and in addition have higher translational value to human studies (Koolhaas et al., 2006). They should not push the animals towards a stress-physiological ceiling, as is the case with many of the laboratory stressors used in experimental rodent stress and sleep research to date. The frustrative non-reward paradigm also allows for behavioural assessment of performance, impulsivity and motivation. Together with the 5-choice serial reaction time task (5-CSRTT) that was used in this thesis, this allowed us to assess how acute sleep deprivation affects different attentional measures.

The added value and novelty of the studies in this thesis is that confounding stress effects were minimized while using optimized measurements with neuroendocrine, physiological, behavioural and EEG sampling methodology and procedures. First of all, we applied gentle handling sleep deprivation in all our experiments to minimize stress confounding effects as much as possible. Animals were habituated to this process and physical and psychological stimulation was kept as low as possible to reduce stress system activation by other factors as sleep loss per se. Habituation to handling will reduce the extent of HPA axis and adrenalin activation caused by the gentle handling used to keep the animals awake (Dobrakovova et al., 1993). In addition, in many rodent studies, the used sampling procedures can be a serious confounding factor. The studies in this thesis used radiotelemetry and a small implantable transmitter to measure several physiological, behavioural and EEG parameters (Guiol et al., 1992; Van den, 1994; Leon et al., 2004; Greene et al., 2007; Tang et al., 2007), and an automated blood sampling methodology with catheterized animals for taking blood samples (Steffens, 1969; Thrivikraman et al., 2002; Royo et al., 2004; Abelson et al., 2005). Using such advanced sampling methods, the animals were able to freely move in their home cages and contact with the animals by the experimenter or entering the experimental room was not necessary.

In addition, the used sampling methods allow for multiple stress read-outs to be measured simultaneously, frequently and for several hours, in the same freely-

moving subject. Many experimental rodent studies lack the possibility to measure various relevant stress parameters in parallel and if they do have this possibility, confounding stress effects have often hampered the interpretation. In most rodent studies combing sleep deprivation with the stress response, only the corticosterone response has been measured. In this thesis, both the SAM system and HPA axis activation, but also the EEG and behavioural activity responses were measured, to provide an as complete as possible picture. Another added-value is the long-term and frequent assessment of these measurements to see their dynamics and recovery. This is important since each activated process has its own time course and recovery time (Koolhaas et al., 1997).

The parallel measurements of physiological, neuroendocrine, behavioural en EEG read-outs in this thesis also allowed analysis of the stress system activation in concert with behaviour to further study whether the changes in behavioural activity correlate with the stress system activation. These correlational analyses will further strengthen the interpretation and characterisation of the consequence of sleep deprivation and our concept of stress.

In **chapter 2** we aimed to validate the sampling methods by measuring behavioural, physiological (blood pressure, heart rate, body temperature and plasma corticosterone) and EEG responses during undisturbed control conditions. We investigated whether stress confounding effects were reduced with our sampling methods. Secondly, this study aimed to further evaluate the concept of stress. To address this, the behavioural, physiological, plasma corticosterone and EEG responses were studied before, during and after novel cage exposure. Forced confrontation with a novel cage is considered a psychological and aversive stressor. However, this interpretation is simply based on the occurrence of a stress response. We examined whether the neuroendocrine and physiological activation during novelty exposure can be explained by increased behavioural activity rather than a presumed cognitive state of aversion. To study this, detailed analyses of the time course of the novelty-induced physiological and hormonal responses in concert with the behavioural responses were used. This approach allowed for further clarification of the hormonal and physiological responses to novel cage exposure as a direct reflection of the metabolic requirements for the normal ongoing behavioural activity or of the stressful nature.

Chapter 3 aimed to assess how acute 4-h gentle handling sleep deprivation affects the basal activity of the stress systems. Besides reducing the stress confounding effects of the sampling procedures by using the same experimental methods as in chapter 2, also possible stress confounding effects of the sleep deprivation were reduced. Detailed time and correlational analysis were used to determine the relationship between changes in behavioural, physiological and hormonal stress parameters. These parameters were not only measured before and during sleep deprivation but also after sleep deprivation in order to study the recovery of responses. This study also examined whether 4 h of gentle handling sleep deprivation should be interpreted as stressful.

In **chapter 4** the findings and methods of chapter 2 and 3 were combined to investigate how 4 and 6 h of acute sleep deprivation affect the stress responsivity to novelty exposure and how stress physiology is involved. To study this, rats were sleep deprived for 4 or 6 h using the gentle handling sleep deprivation method. After a brief recovery of the sleep deprivation period, rats were exposed to novelty. The locomotor activity, heart rate, blood pressure, body temperature and plasma corticosterone as well as sleep-wake distribution and NREM delta power responses were examined from before sleep deprivation until recovery from novelty exposure. In this chapter the gentle handling sleep deprivation method was also further validated by studying the sleep-wake distribution and NREM delta power during and after 6 h of sleep deprivation.

Chapter 5 continued on the findings of chapter 4. It is suggested that a truly stressful condition needs to contain uncontrollability and unpredictability elements and should involve an environmental challenge which the animal may meet in everyday life in its natural habitat. Therefore chapter 5 focused on an operant-conditioning task in which rats are trained to lever press for food reinforcements. Next, when lever pressing is suddenly no longer rewarded, the animal faces absence of the expected behavioural consequence and supposedly experiences loss of control. Such loss of control has been associated with a strong activation of the stress systems and has been described as frustrative non-reward stress. In chapter 5 we used the same sampling and sleep deprivation methods as in chapter 4 but now investigated how the stress response to rewarded and non-rewarded operant lever press behaviour is affected by 6 h of sleep deprivation. We also studied the

responses to frustrative non-reward in concert with behaviour to assess whether the stress system activation was related to behavioural activity. To examine this, the responses were not only analysed during operant task exposure but also during the recovery from this task. Another aim of this study was to assess how acute sleep deprivation affects the performance, motivation and impulsivity of the animals during the operant task.

Chapter 6 continued on the findings of chapter 4 and 5 and focused in more detail on attentional functioning after 6-h gentle handling sleep deprivation. The same sampling and sleep deprivation methods were used as in chapter 4 and 5 but now animals were assessed in the 5-CSRTT. In the 5-CSRTT, rats detect and respond to a light stimulus presented randomly in one of 5 holes in order to get a food reward. The 5-CSRTT allows close observation of different component processes of attention, such as selective attention, sustained attention and behavioural control. Besides assessing these attentional aspects, gross locomotor activity, body temperature and polysomnographic parameters were also measured to control for the used procedures.

In c**hapter 7** the results of the experiments are summarized and discussed.

Chapter 2

Physiological and hormonal responses to novelty exposure in rats are mainly related to ongoing behavioural activity

W. Beerling[1, 2], J.M. Koolhaas[2], A. Ahnaou[1], J.A. Bouwknecht[1], S.F. de Boer[2], P. Meerlo[2] and W.H.I.M. Drinkenburg[1]

[1] Department of Neuroscience, Janssen Research & Development, a division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, 2340 Beerse, Belgium;
[2] Department of Behavioural Physiology, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

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ABSTRACT

Stress research has been dominated by a circular type of reasoning that occurrence of a stress response is bad. Consequently, the stimulus is often interpreted as stressful in terms of cognitive experience of aversiveness involving uncontrollability and unpredictability, which may have maladaptive and pathological consequences. However, the sympathico-adrenomedullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) axis are not only activated in response of the organism to challenges, but also prepare and support the body for behaviour. Therefore, a considerable part of the classical stress system activation to a certain situation can be a direct reflection of the metabolic requirements for normal ongoing behavioural activity, rather than a reflection of the aversive nature of the situation. In order to clarify this, behavioural, physiological, hormonal and electroencephalographic (EEG) responses to novel cage exposure were studied in adult male Sprague-Dawley rats. Forced confrontation with a novel cage has been interpreted as a psychological and aversive stressor. However, this interpretation is simply based on the occurrence of a stress response. The present study aimed at detailed analysis of the time course of the novelty-induced responses. Different parameters were measured simultaneously in freely moving rats, which allowed correlational comparisons. Hereto, radiotelemetry using a small implantable transmitter combined with permanent catheters and an automated blood sampling system were used. A camera was positioned above the cage to make recordings for further behavioural observational analysis. The results show that novelty exposure induced significant increases in locomotor activity, heart rate, blood pressure and plasma corticosterone together with a complete lack of sleep as compared to the control situation. The latency to reach significance and the duration of responses varied across parameters but all had recovered within 30 min after termination of novelty. The behavioural activity (locomotor activity and EEG wakefulness duration) response pattern was significantly correlated with that of heart rate, blood pressure and plasma corticosterone. Behavioural observations showed mainly explorative behaviour in response to novelty. Therefore, the present results indicate that the novelty-induced physiological and hormonal responses are closely related to the ongoing, mainly explorative behavioural activity induced by novelty. An interpretation in terms of metabolic support of ongoing behaviour seems to be more appropriate than the frequently used stress interpretation. The present study also emphasizes the added value of simultaneous assessment of behavioural, physiological and hormonal parameters under controlled, non-confounding conditions.

INTRODUCTION

Organisms are constantly subjected to demanding internal and external stimuli to which they have to respond adequately in order to survive. The sympathico-adrenomedullary (SAM) system and hypothalamic-pituitary-adrenal (HPA) axis are the two most important neuroendocrine pathways that are activated in response to such challenges. Activation of these pathways results in release of catecholamines (adrenalin and noradrenalin) and glucocorticoids (corticosterone or cortisol) into the bloodstream, which have a variety of effects that enable organisms to respond and adapt adequately to the demanding internal and external stimuli (Axelrod and Reisine, 1984; Johnson et al., 1992). A stimulus is considered stressful when it activates these so-called stress systems. However, there is a serious conceptual problem in stress research. Stress is not only interpreted as the physiological and hormonal activation of the two main stress systems. It is also often interpreted as a cognitive experience of aversiveness, involving uncontrollability and unpredictability, which may have maladaptive and pathological consequences. The stress response has however not only a maladaptive nature, but has also adaptive aspects. Even though this adaptive nature of the stress response has been emphasized by many authors (Selye, 1950; Selye, 1976; McEwen and Wingfield, 2003; Korte et al., 2005; De Kloet et al., 2005), stress research is still dominated by a circular type of reasoning that occurrence of a stress response is bad. Consequently, the stimulus is often interpreted as stressful in terms of aversiveness. However, SAM system and HPA axis activation may not be a sufficient indicator for such stress. The SAM system and HPA axis also have a main role in energy homeostasis and redistribution of blood to active organs and tissues, and are therefore known to prepare and support the body for behaviour (Axelrod and Reisine, 1984; Johnson et al., 1992; Leal-Cerro et al., 2003). Therefore, a considerable part of the classical stress system activation to a certain situation is a direct reflection of the metabolic requirements for normal ongoing behavioural activity, rather than a reflection of the aversive nature of the situation (Koolhaas et al., 2011). In agreement with this, studies showed that activation of the classical neuroendocrine stress systems not only occurs in response to aversive, uncontrollable situations but sometimes also in response to highly rewarding, controllable situations like sexual behaviour, winning a social interaction,

and food/water intake (Schuurman, 1980; Bronson and Desjardins, 1982; Shiraishi et al., 1984; De Boer et al., 1990a; Arnhold et al., 2009; Koolhaas et al., 2011; Buwalda et al., 2012).Hennessy and Levine discussed these concepts of stress and arousal in terms of a psychoendocrine hypothesis; they suggest that the HPA axis can be considered as a component of a unified system of arousal and that HPA axis responses are a reflection of changes in arousal level (Hennessy and Levine, 1979). In our view, when activation of the stress systems is mainly related to the ongoing behavioural activity induced by the situation, clear associations between behaviour and physiology should occur (Koolhaas et al., 2011). For a situation to be considered truly stressful in terms of uncontrollable and unpredictable, a dissociation between the behavioural activity levels and the physiological/hormonal responses should occur (Koolhaas et al., 2011). Analysis of the literature suggests that this may be reflected in the recovery of responses rather than the initiation (Schuurman, 1980; De Boer et al., 1990a; Garcia et al., 2000; Fish et al., 2005; Arnhold et al., 2009; Koolhaas et al., 2011).

In order to clarify this, we studied the behavioural, physiological and hormonal responses to novelty exposure, a stimulus that has a long history in experimental stress research in rodents. The implicit theory is that this may represent a mild stressor because it is an unknown and potentially dangerous situation (Gagliano et al., 2008). Also, forced exposure to an unfamiliar area is considered more stressful than when the animal is given the opportunity to freely move between a familiar and novel area (Misslin et al., 1982). In 1968, Adel and Friedman reported that brief forced exposure to a novel cage results in a rapid rise of corticosterone in rats (Ader and Friedman, 1968). Many studies followed, showing activation of the stress systems in response to novelty, and more specifically for rats to novel cage exposure, and using it as an acute, aversive and psychological stressor (Bassett et al., 1973; Pfister and King, 1976; Hennessy et al., 1979; Pfister, 1979; Brett et al., 1983; Armario et al., 1986; Flaherty et al., 1986; Muir and Pfister, 1986; De Boer et al., 1989; De Boer et al., 1990b; Buwalda et al., 1993; Meerlo et al., 1999; Tang et al., 2005; De Groote and Linthorst, 2007; Whyte and Johnson, 2007). Close observation of this literature shows that the interpretation of novel cage exposure as an aversive stressor is simply based on the occurrence of a stress response. However, because laboratory rats experience such a novel cage challenge once or

twice a week as part of the regular cleaning regime, habituation processes may have taken place (Stein, 1966; Groves and Thompson, 1970; Thompson, 2009). Weiss already showed in 1972, it is not the physiological nature of a stimulus that induces stress pathology but the degree in which it can be predicted and/or controlled (Weiss, 1972). Therefore, exposure to novelty may be only minimally stressful in terms of a cognitive state of aversion and unpredictability.

The present study aimed at frequent recording of behavioural, physiological, hormonal and electroencephalographic (EEG) parameters over time in response to 15-min novel cage exposure in rats. This allowed detailed analysis of the time course of the novelty-induced responses. Simultaneous registration of behavioural activity levels together with behavioural observations allowed correlational comparisons of the physiological and hormonal response patterns with ongoing behaviour. It is important to notice that measurements were taken while maintaining strictly controlled conditions with minimized confounding factors due to sampling procedures. This was achieved and controlled for by radiotelemetry using a small implantable transmitter to measure physiological and EEG parameters (Guiol et al., 1992; Van den Buuse, 1994; Leon et al., 2004; Greene et al., 2007; Tang et al., 2007), combined with an automated blood sampling system connected to permanently catheterized, freely-moving animals(Steffens, 1969; Thrivikraman et al., 2002; Royo et al., 2004; Abelson et al., 2005).Furthermore, a camera was positioned above the cage to make recordings for further behavioural observational analysis. The following parameters were measured: locomotor activity, heart rate, blood pressure, body temperature, EEG and plasma corticosterone concentrations. The EEG recordings were additionally analysed for vigilance states and together with the behavioural observations and locomotor activity measurements these gave detailed information about the ongoing behavioural activity of the animals. The primary goal of this study was to determine whether the novelty-induced physiological and hormonal responses are related to the ongoing behavioural activity induced by novelty and whether the frequently used stress interpretation is correct.

METHODS

Animals and housing

In a series of 2 experiments, two batches with in total 9 adult male Sprague-Dawley rats (Harlan, Horst, The Netherlands), weighing 310-330 g at the time of surgery, were used. For at least 2 weeks prior to surgery, animals were individually housed in polycarbonate cages (43x27x39 cm) containing bedding and cage enrichment. Immediately after surgery, each animal was placed within its home cage in the experimental set-up. This set-up consisted of a radiotelemetry set-up (DataSciences International, St. Paul, MN, USA) and an automated blood sampling system (AccuSampler Micro, DiLab, Lund, Sweden). Furthermore, a camera was positioned above the cage. The rats were housed in a room with constant temperature (21 ± 1°C), controlled humidity (50 ± 10%) and a fixed 12 : 12 h light/dark regime using a 30-min rise and dim period with lights on at 06.00h. The animals were daily handled for weighing purposes and standard rat chow and water were provided *ad libitum*. All experimental protocols were approved by the local ethical committee.

Surgery

Surgery was performed under O_2-N_2O-isoflurane anaesthesia (5% induction; 2% maintenance). The analgesic Piritramide (dipidolor, 0.025 mg/kg, 0.1 ml/100 g body weight) was administered subcutaneously at the start of surgery. A transmitter to record gross locomotor activity, heart rate, blood pressure, body temperature and cortical EEG (TL11M2-C50-PXT, DataSciences International) was implanted intraperitoneally and attached to the abdominal wall to ensure stabilization (Greene et al., 2007). The catheter of the transmitter was inserted in the femoral artery to measure blood pressure. To place the two biopotential leads for cortical EEG measurements, animals were placed in a stereotaxic apparatus. The leads were led subcutaneously to the skull and the bare ends were placed in contact with the dura through holes in the skull. These electrodes were placed 3 mm on either side lateral to the midline and 6 mm posterior to bregma and were anchored to the skull with screws and dental cement. In addition, a heart catheter (polyurethane; ID: 0.6, OD: 0.84, DiLab) enabling blood samples to be collected in freely moving rats was

implanted (Steffens, 1969; Thrivikraman et al., 2002). The catheter was inserted through the right jugular vein with the end reaching the entrance of the right atrium. The other end was led subcutaneously through a Dacron button that was attached to the skin in the dorsal region of the neck. The catheter was then connected through a spiral to a swivel and connected either to the infusion pump or the automated blood sampling machine. After surgery, the animals were immediately connected to an infusion pump giving a continuous infusion (saline + heparin: 10 IU/ml; 0.24 ml/h) to ensure the catheter to stay open. The animals were allowed to recover for at least 2 weeks before start of the experiments.

Experimental design

Two experiments, with two batches of animals, were done with 4 weeks in between and using the exact same procedures. Statistical analysis showed no significant difference between the two experiments and therefore the data of all groups was taken together. After recovery from surgery, rats were subjected to either control or novelty-exposed conditions using a balanced and cross-over Williams design with 3-5 days in between sessions and using a standard circadian time window. Using this design, all animals were exposed to both conditions. Seven set-ups were used in parallel to reduce variability among animals and behavioural, physiological and EEG parameters were measured continuously and automatically. Concomitantly, blood was sampled automatically and repeatedly. Furthermore, recordings were made for further behavioural observational analysis. The data of a number of subjects could not be analysed further due to insufficient quality of their telemetry signals and/or blocked blood sampling catheters. As a result, the same animals were not always present in both groups resulting in n=7 for the control condition and n=6 for the novelty-exposed condition of which data of four animals is presented in both groups. All experimental procedures were performed during the first half of the light phase (i.e. the resting phase) and potentially disturbing stimuli were carefully avoided. The experimental day started with weighing the animals, activating their transmitters and connecting them to the automated blood sampling machines. Telemetric recordings and blood sampling started simultaneously at least 1 h later with a 30-min baseline

period, followed by a 15-min novelty exposure or control period with subsequent a 2-h post-novelty period.

Novelty exposure

Exposure to novelty was performed according to the protocol described by de Boer and co-workers (De Boer et al., 1990b). The experimenter entered the room at *t* = -2 min to gently pick up the rat from its home cage at *t* = -1 min and place it in a new, clean, empty cage comparable to its home cage. Novelty exposure therefore included entering the experimental room, brief handling and forced novel cage exposure. After collecting the *t* = 15 min blood sample, rats were returned to their home cages. Under control conditions, animals were left undisturbed in their home cages in the same experimental room.

Biotelemetric data acquisition and analysis

Gross locomotor activity, heart rate, blood pressure, body temperature and cortical EEG were monitored telemetrically using the Dataquest A.R.T. System (DataSciences International). The transmitter emitted temperature, blood pressure and EEG dependent frequency modulated signals. For locomotor activity, data was obtained by counting the number of changes in signal strength of the transmitter. Because the transmitter was implanted in the abdomen, vertical changes of the animal were hardly detected by the receiver, but horizontal changes were. All signals were received by an antenna board placed under each animal's cage and processed by a computer using Dataquest Labpro software. Heart rate values were calculated from blood pressure measurements. Locomotor activity, heart rate, blood pressure and body temperature data was sampled in 10-s epochs and averaged into 5-min bins. EEG bio-potential recordings were imported into Somnologica 3.2.0 software (MedcareFlaga, Reykjavik, Iceland) with a lower cut off at 0.7 Hz and a higher cut off at 40 Hz. The vigilance states were determined off-line in 4-s epochs. A concurrent EEG power spectrum for each epoch was used to aid in discriminating between states. Non-Rapid Eye Movement (NREM) sleep was scored based on the presence of high amplitude EEG waves and predominant EEG power in the delta range (0.5-4

Hz) and lack of body movement. Rapid Eye Movement (REM) sleep was characterized by highly regular low amplitude EEG with a dominance of theta activity (4.5-8 Hz) with reduced lower frequencies and general lack of body movements with occasional twitches. Wakefulness was scored based on irregular low-amplitude, fast EEG with a lack of visible theta dominance, and frequent body movements. The amount of time spent in wakefulness, NREM sleep and REM sleep was calculated in 15-min blocks.

Blood sampling and assays

Blood was sampled using the jugular vein catheter connected to the automated blood sampling machine. Animals were habituated to the blood sampling procedures to minimize possible stress-confounding effects of it. 30-µl samples were taken every 5 min and were stored inside the machine at 4 °C until centrifuged for 5 min at 3210 rpm. Subsequently, 12 µl of plasma was stored at –80 °C until assayed. Samples were analysed for corticosterone, in duplicate, using a double antibody radio immunoassay (Mouse/Rat Corticosterone^{125}I RIA Kit, MP Biomedicals, Solon, OH, USA) with a sensitivity of 3.0 ng/ml.

Behaviour

Behaviour was scored blindly and off line by one experimenter using The Observer 5.0 (Noldus, Wageningen, The Netherlands). Three periods were scored; a 20-min baseline period (t = -25 until t = -5 min), the 15-min novelty exposure period starting immediately after the animal was placed in the novel cage, and a 20-min post-novelty period starting immediately after the animal was returned to its home cage. For the control animals, the same circadian time periods were scored. The following behavioural classes were defined: 1) immobility; 2) consumption (eating and drinking); 3) grooming (including scratching); 4) rearing; 5) sniffing; 6) other behaviours (e.g. dig/move bedding, stretch and yawn). The 6th behavioural class was not affected by novelty exposure and was present in a low percentage and was therefore not included in the graphs. The relative duration (time spent on the

behaviour expressed as percentage of the observation time) was calculated for all behavioural classes for the three periods.

Statistics

To assess the effect of novelty on the behavioural, physiological, hormonal and EEG responses, a repeated measures ANOVA with Greenhouse-Geisser correction epsilon (ε) for violation of the homogeneity in variance assumption was used. Treatment (novelty exposure vs. control) was used as between-subjects factor (two levels) and the different time points or periods as within-subject factor. Even though a cross-over design was initially used, the data of the same animals was not always present in both groups. Therefore, treatment was used as between-subjects factor rather than as within-subject factor. When appropriate, *post-hoc* *t*-tests were performed to determine the specific time points at which novelty-exposed and control conditions differed. Paired *t*-tests were used to determine the differences between the baseline, novelty exposure and post-novelty exposure period. To get more insight into the dynamics of the telemetric and corticosterone measurements, the following calculations were performed. The maximum value (V_{MAX}) reached during the whole recording period and the time point at which this maximum value was achieved (T_{MAX}) were determined for each individual separately and a group mean was calculated across these values for each parameter. For NREM and REM sleep duration a reduction occurred and therefore the minimum value (V_{MIN}) was calculated together with the time point at which this minimum was achieved (T_{MIN}). An one-way ANOVA with treatment as within-subject variable was used to assess the effect of novelty exposure on V_{MAX} / V_{MIN} and T_{MAX} / T_{MIN}. For the novelty-exposed condition, a paired *t*-test was used to assess whether the T_{MAX} / T_{MIN} values of the different parameters were significantly different in order to see whether these parameters reached their V_{MAX} / V_{MIN} around the same time points. The Pearson's Bivariate correlation coefficient test was used to compute correlations for the novelty-exposed response patterns over time between the locomotor activity, physiological, hormonal and EEG wakefulness duration responses. The main focus was on correlations between the locomotor activity, EEG wakefulness duration response pattern and all other responses, as this will show to what extent behavioural activity levels are

reflected in the physiological and hormonal responses. For these correlations, the mean value for each parameter at a certain time point, as displayed in the graphs, was set together with the value of a different parameter at corresponding time point. Only values of which both measurements had a corresponding time point measurement were included therefore using bin widths of 5, 10 or 15 min depending on the parameter collected. All values, over the different time points, were used to compute a correlation coefficient. All analyses were performed using SPSS 15.0 software (SPSS inc., Chicago, IL, USA). The level of significance was accepted at *p*-values <0.05 and a trend was accepted at $p<0.10$. Data are presented as mean + SEM.

RESULTS

Dynamics of the behavioural, physiological and hormonal responses to novelty exposure

Locomotor activity

ANOVA revealed for the locomotor activity response a significant time*treatment interaction ($F_{32,352}=19.6$, $\varepsilon=0.07$, $p<0.001$), a significant treatment effect ($F_{1,11}=47.5$, $p<0.001$) and a significant time effect ($F_{32,352}= 20.8$, $\varepsilon=0.07$, $p<0.001$). Locomotor activity increased immediately after initiation of novelty and was short-lasting (Figure 1 panel A). Two peaks occurred, one when the animal was placed in the novel cage (t=2.5 min) and another one when the animal was placed back in its home cage (t=17.5 min). As Table 1 shows, the highest peak was achieved at $t=5.8 \pm 2.5$ min. The second peak was immediately followed by low activity levels; stable baseline and control levels were reached already 5 - 10 min after termination of novelty. Under control conditions, rats showed no significant variation in locomotor activity levels over the sampling period, with their activity levels fluctuating around 0.4 counts/min, indicating overall inactivity of these animals.

Heart rate

For the heart rate response, ANOVA revealed a significant time*treatment interaction ($F_{32,352}$=13.8, ε=0.2, p<0.001), a significant treatment effect ($F_{1,11}$=10.2, p=0.009) and a significant time effect ($F_{32,352}$= 20.2, ε=0.2, p<0.001). Heart rate showed a fast increase in response to novelty, starting immediately after transfer to the novel cage (Figure 1 panel B). The significantly higher maximum value as compared to the controls, as shown in Table 1, was reached at t=11.7 ± 3.5 min. Stable baseline and control levels were re-established 30 - 35 min after termination of novelty. Under control conditions, heart rate did not vary significantly over the sampling period and fluctuated closely around 340 beats per min (bpm).

Blood pressure

The blood pressure response to novelty showed a significant time*treatment interaction ($F_{32,352}$=3.2, ε=0.1, p=0.018), no significant treatment effect ($F_{1,11}$=1.4, p=0.26) and a significant time effect ($F_{32,352}$=9.7, ε=0.1, p<0.001). Exposure to novelty induced a fast, short-lasting increase in blood pressure, starting 0 - 5 min after the animals were placed in the novel cage (Figure 1 panel C). As Table 1 shows, a maximum increase was reached at t=12.5 ± 3.2 min. Stable baseline and control levels were reached 15 - 20 min after the animal was returned to its home cage. Under control conditions, rats did not show significant variations in blood pressure over the sampling period. Their blood pressure fluctuated around 110 Millimetres of Mercury (mm Hg).

Body Temperature

For the body temperature response, ANOVA revealed no time*treatment interaction ($F_{32,352}$=1.6, ε=0.1, p=0.20), no significant treatment effect ($F_{1,11}$=1.5, p=0.245) and a significant time effect ($F_{32,352}$= 10.0, ε=0.1, p<0.001). Also, no time*treatment interaction effect or treatment effect was found when only the recovery period was analysed. Even though body temperature is not increased significantly, it shows a slight increase for around 30 min starting 0 – 5 min after the animals were already

returned to their home cages (Figure 1 panel D). The maximum value reached, as shown in Table 1, was at t=32.5 ± 3.7 min. Under control conditions, body temperature seems to increase slightly, but not-significantly, during novelty exposure experienced by the experimental animals present in the same room. Over the sampling period, body temperature levels fluctuated around 37.7 Degree Celsius (°C).

Plasma Corticosterone

The plasma corticosterone response to novelty showed a significant time*treatment interaction ($F_{16,176}$=3.2, ε=0.2, p=0.033), a significant treatment effect ($F_{1,11}$=6.3, p=0.029) and a significant time effect ($F_{16,176}$=4.7, ε=0.2, p=0.006). The corticosterone response had a slow increase, reaching significance 5 - 10 min after novelty exposure was terminated (Figure 1 panel E). As Table 1 shows, a significant higher maximum increase, as compared to the controls, was achieved at t=19.2 ± 4.2 min. Plasma corticosterone levels reached baseline and control levels 20 - 25 min after termination of novelty exposure. At 75 – 85 min after termination of novelty, corticosterone levels fluctuated slightly but non-significantly as compared to the control condition (t=92.5 and t=102.5 min). Under control conditions, there was some variation in plasma corticosterone, however not significant. Their levels fluctuated around 36 ng/ml.

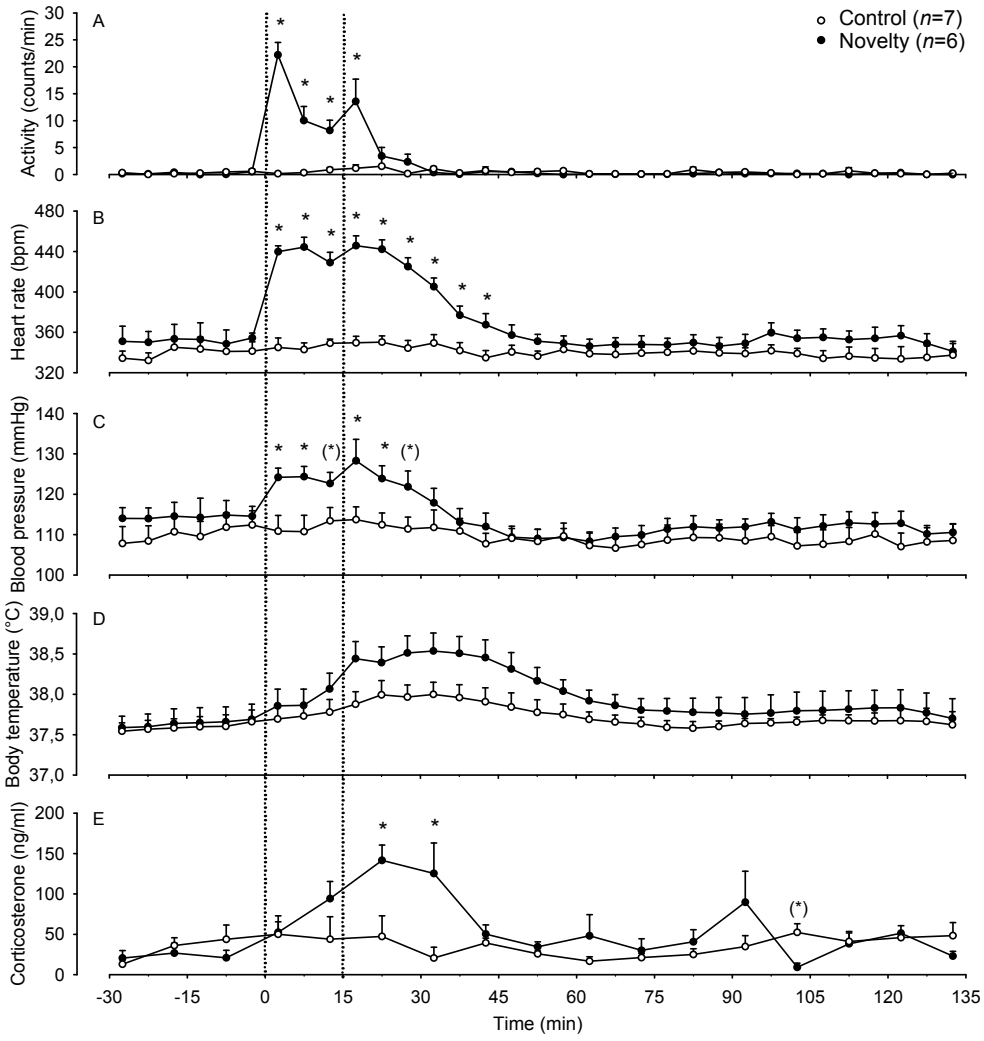

Figure 1. Time course of changes in locomotor activity (panel A), heart rate (panel B), blood pressure (panel C), body temperature (panel D) and plasma corticosterone (panel E) in response to novelty exposure. Data are expressed as 5-min averages + SEM for the physiological measurements and as 10-min mean values + SEM for the corticosterone measurements of the control (open symbols, *n*=7) and novelty-exposed condition (closed symbols, *n*=6). The responses are shown for the 30-min baseline period, the 15 min exposure to novelty or non-novelty control period (indicated by the area between the lines) and the 2-h post-novelty period. Significance: novelty-exposed versus control **p*<0.05; (*)*p*<0.10.

Changes in sleep-wake distribution in response to novelty exposure

The time course of changes in the amount of wakefulness, NREM and REM sleep under both control and novelty-exposed conditions is illustrated in Figure 2 (panel A-C respectively).

For wakefulness, ANOVA revealed a significant time*treatment interaction ($F_{10,110}$=7.4, ε=0.4, p<0.001), a significant treatment ($F_{1,11}$=16.7, p=0.002) and time effect ($F_{10,110}$=11.0, ε=0.4, p=<0.001). Also for NREM sleep, ANOVA showed a significant time*treatment interaction ($F_{10,110}$=6.5, ε=0.4, p=0.001), a significant treatment ($F_{1,11}$=12.5, p=0.005) and time effect ($F_{10,110}$=9.0, ε=0.4, p<0.001). For REM sleep, an almost significant time*treatment interaction ($F_{10,110}$=2.5, ε=0.4, p=0.059) was present and no significant time and treatment effects were found.

At baseline, there were no differences in time spent on the three vigilance states between the control condition and the novelty-exposed condition. During this baseline period, animals spent most of the 15-min intervals in NREM sleep (60 %), and less in wakefulness (33.3 %) and REM sleep (6.7 %).

During the 15 min of novelty exposure, there was a significant increase in time spent in wakefulness together with a significant reduction in time spent in NREM and REM sleep. This lasted until the first 15 min (t=22.5 min) after novelty exposure. Thus, the changes in response to novelty were fast with the maximum value for wakefulness reached at t=10.0 ± 2.5 min and the minimum value for NREM and REM sleep at t=5.0 ± 2.5 and t=0.0 ± 5.2 min respectively as shown in Table 1. Control and baseline levels were reached again for all three vigilance states between 15 – 30 min after termination of novelty. At 60 - 75 min after termination of novelty (t=82.5 min), wakefulness was slightly but significantly reduced for the novelty-exposed condition together with a significant slight increase in NREM sleep duration and with no effect on REM sleep. This lasted only for this 15-min interval. The animals under control conditions did not show any significant variation in vigilance states over the sampling period.

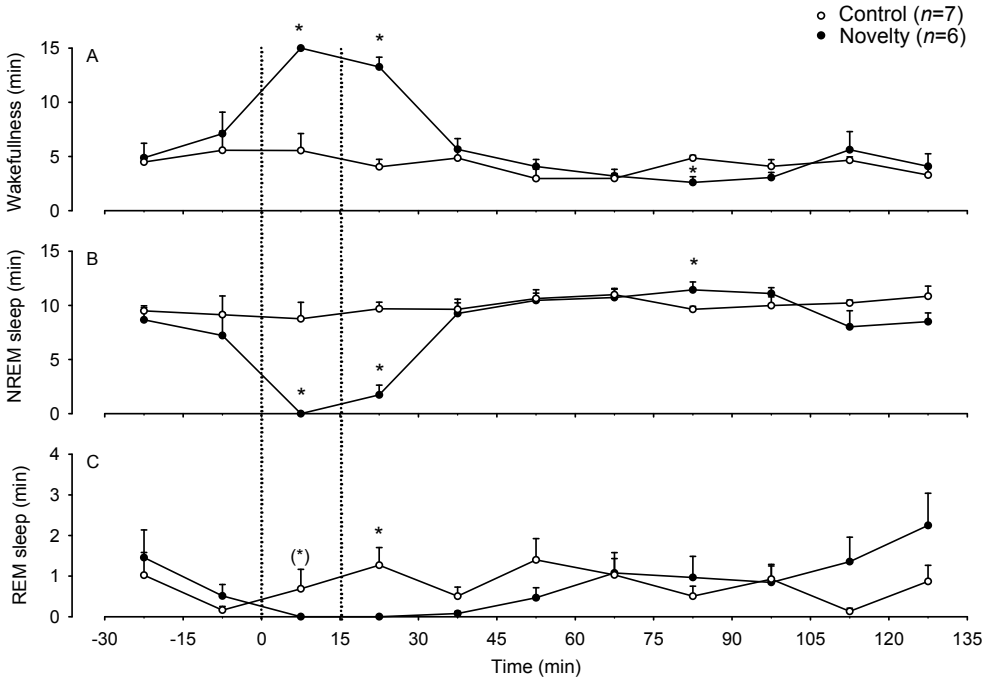

Figure 2. Time course of changes in the amount of wakefulness (panel A), NREM sleep (panel B) and REM sleep (panel C) in response to novelty exposure. Data are expressed as 15-min averages + SEM for the control condition (open symbols, *n*=7) and for the novelty-exposed condition (closed symbols, *n*=6). The responses are shown for the baseline period, the 15 min exposure to novelty or non-novelty control period (indicated by the area between the lines) and for the 2-h post-novelty period. Significance: novelty-exposed versus control **p*<0.05; (*)*p*<0.10.

Associations between the behavioural, physiological, hormonal and EEG responses

Maximum/minimum values reached with corresponding time points

The maximum value reached (V_{MAX}) and the time point at which this maximum was reached (T_{MAX}) for locomotor activity, heart rate, blood pressure, body temperature, plasma corticosterone and wakefulness duration were determined for each individual separately and a group mean was calculated across these values and is shown in Table 1.

Table 1. Maximum value reached (V_{MAX}) and time point at which this maximum was reached in minutes (T_{MAX}) for Act, HR, BP, BT, Cort and Wake. For NREM and REM the V_{MIN} and T_{MIN} are shown as a reduction occurred for these measures. The V_{MAX} / V_{MIN} and T_{MAX} / T_{MIN} were determined for each individual separately and a group mean was calculated across these values. The V_{MAX} / V_{MIN} is shown for the control condition (n=7) and for the novelty-exposed condition (n=6). The T_{MAX} / T_{MIN} is only shown for the novelty condition (n=6). Values are presented as mean ± SEM.

Parameter	Response	V_{MAX} / V_{MIN}			T_{MAX} / T_{MIN}	
		Control	Novelty		Novelty	
Act	Max	3.7 ± 1.2	22.5 ± 2.5*	counts/ min	5.8 ± 2.5	min
HR	Max	373.6 ± 7.6	454.8 ± 9.2*	bpm	11.7 ± 3.5	min
BP	Max	117.6 ± 3.3	129.3± 5.2(*)	mmHg	12.5 ± 3.2	min
BT	Max	38.1 ± 0.2	38.7± 0.2(*)	°C	32.5 ± 3.7	min
Cort	Max	88.8 ± 12.6	156.9± 25.2*	ng/ml	19.2 ± 4.2	min
Wake	Max	8.1 ± 1.2	15.0 ± 0.0*	min	10.0 ± 2.5	min
NREM	Min	6.2 ± 1.1	0.0 ± 0.0*	min	5.0 ± 2.5	min
REM	Min	0.0 ± 0.0	0.0 ± 0.0	min	0.0 ± 5.2	min

Act: locomotor activity; HR: heart rate; BP: blood pressure; BT: body temperature; Cort: plasma corticosterone; Wake: wakefulness duration; NREM: NREM sleep duration; REM: REM sleep duration. Significance: novelty-exposed versus control *p<0.05; (*)p<0.10.

For NREM sleep duration and REM sleep duration the V_{MIN} and T_{MIN} are shown as a reduction occurred for these measures. Significant differences were found between the novelty-exposed and control condition for the maximum values of locomotor activity ($F_{1,11}$=49.6, p<0.001), heart rate ($F_{1,11}$=47.1, p<0.001), plasma corticosterone ($F_{1,11}$=6.4, p=0.028) and wakefulness duration ($F_{1,11}$=26.7, p<0.001) and for the minimum value of NREM sleep duration ($F_{1,11}$=25.8, p<0.001). A trend to significance was found for the maximum values of blood pressure ($F_{1,11}$=3.7, p=0.079) and body temperature ($F_{1,11}$=4.3, p=0.062).

For the novelty-exposed condition, it was furthermore analysed whether the T_{MAX} / T_{MIN} values differed across the measured parameters. Locomotor activity, heart rate, blood pressure, wakefulness duration, NREM sleep duration and REM sleep duration

had a similar T_{MAX}/T_{MIN} meaning that these responses to novelty reached their maximum or minimum value around the same time points, making it likely that their responses were initiated together. Body temperature had a significantly higher T_{MAX} compared to all other responses and was thus a slower response than the others. The T_{MAX} of corticosterone did not differ significantly from the T_{MAX} of locomotor activity, heart rate and blood pressure but was significantly higher than that of wakefulness duration, NREM sleep duration and REM sleep duration but lower than the T_{MAX} of body temperature.

Correlations between responses

The correlation coefficients for the novelty-exposed response patterns over time of locomotor activity, heart rate, blood pressure, body temperature, plasma corticosterone and wakefulness duration are shown in Table 2. The main focus was on correlations between locomotor activity, EEG wakefulness duration responses and all other physiological responses, as this will show to what extent behavioural activity levels are reflected in the physiological and hormonal responses.

The correlation coefficient for the response pattern over time between locomotor activity and heart rate, and between locomotor activity and blood pressure was significant and was $r_{28}=0.77$ ($p<0.001$) and $r_{28}=0.79$ ($p<0.001$) respectively. The correlation coefficient with body temperature was not significant with $r_{28}=0.11$ ($p=0.57$). Between plasma corticosterone and locomotor activity also no significant correlation coefficient was found with $r_{14}=0.14$ ($p=0.64$). The locomotor activity pattern was significantly correlated with the pattern of wakefulness duration and was $r_9=0.89$ ($p=0.001$). Thus, the locomotor activity response pattern over time was significantly correlated with that of heart rate, blood pressure and wakefulness duration indicating that their responses over time to novelty exposure had similar temporal characteristics.

The pattern of wakefulness duration was also significantly correlated with that of heart rate and blood pressure with $r_9=0.97$ ($p<0.001$) and $r_9=0.96$ ($p<0.001$) respectively. Between wakefulness duration and body temperature, no significant correlation coefficient was found with $r_9=0.33$ ($p=0.39$). The correlation coefficient with plasma corticosterone was significant with $r_4=0.96$ ($p<0.001$). Thus, the EEG

wakefulness duration response pattern over time was significantly correlated with that of locomotor activity, heart rate, blood pressure and plasma corticosterone, indicating that these responses to novelty exposure had similar temporal characteristics.

Table 2. Correlation coefficient for the novelty-exposed response patterns over time of Act, HR, BP, BT, CORT and Wake (*n*=6).

	Act	HR	BP	BT	Cort	Wake
Act	1.00					
HR	0.77*	1. 00				
BP	0.79*	0.97*	1.00			
BT	0.11	0.52*	0.41*	1.00		
Cort	0.14	0.70*	0.68*	0.64*	1.00	
Wake	0.89*	0.97*	0.96*	0.33	0.96*	1.00

Act: locomotor activity; HR: heart rate; BP: blood pressure; BT: body temperature; Cort: plasma corticosterone; Wake: wakefulness duration. Significance: *p<0.05

Behavioural observations

The relative duration of five different behaviours for the baseline period, the novelty exposure period and the post-novelty period under both control and novelty-exposed conditions is shown in Figure 3.

ANOVA revealed for immobility a significant time*treatment interaction ($F_{2,22}$=27.3, ε=0.6, p<0.001), a significant treatment effect ($F_{1,11}$=25.2, p<0.001) and a significant time effect ($F_{2,22}$=31.0, ε=0.6, p<0.001). For consumption, only a significant time*treatment interaction ($F_{2,22}$=4.4, ε=0.8, p=0.036) was found. Also for grooming a significant time*treatment interaction was found ($F_{2,22}$=4.7, ε=0.8, p=0.03) with a close to significant treatment effect ($F_{1,11}$=3.6, p=0.085). Rearing had a significant time*treatment interaction ($F_{2,22}$=24.8, ε=0.6, p<0.001), a significant treatment effect ($F_{1,11}$=39.7, p<0.001) and a significant time effect ($F_{2,22}$=29.8, ε=0.6, p<0.001). For sniffing, ANOVA showed a significant time*treatment interaction ($F_{2,22}$=107.2, ε=0.6, p<0.001), a significant treatment effect ($F_{1,11}$=116.0, p<0.001) and a significant time effect ($F_{2,22}$=103.7, ε=0.6, p<0.001). *Post-hoc* analysis showed that the behaviours

during the baseline period did not differ between the two conditions. Since the experiment was performed during the resting phase (light period) of the animals, immobile behaviour was primarily seen during this baseline period for 89% of the time. Other behaviours that were present were grooming for around 7% and sniffing for 1.3% of the time. Under control conditions, no significant difference between the periods was found, indicating that these animals were immobile during most of the observation period.

Novelty exposure induced, as compared to the control condition, a significant increase in sniffing (66.3 ± 6.3% vs. 1.5 ± 1.4%), in rearing (9.2 ± 1.6% vs. 0.4 ± 0.3%) and in grooming (18.2 ± 6.2% vs. 2.6 ± 1.4%), together with a significant reduction in immobility (0.4 ± 0.4% vs. 84.3 ± 11.8%). This indicates that the animals were behaviourally active during exposure to novelty.

During the first 20 min after this exposure to novelty, as compared to the control condition, an increase in sniffing (11.7 ± 1.6% vs. 0.5 ± 0.2%), rearing (1.5 ± 0.7% vs. 0.0 ± 0.0%) and grooming (18.0 ± 2.5% vs. 4.4 ± 2.0%) together with a reduction in immobility (41.0 ± 10.2 % vs. 93.3 ± 2.5%) was still visible even though levels did go towards baseline levels again. Consumption occurred specifically after termination of novelty as compared to the control condition (19.0 ± 9.2% vs. 0 ± 0%). The animals were still behaviourally active during the first 20 min after termination of novelty.

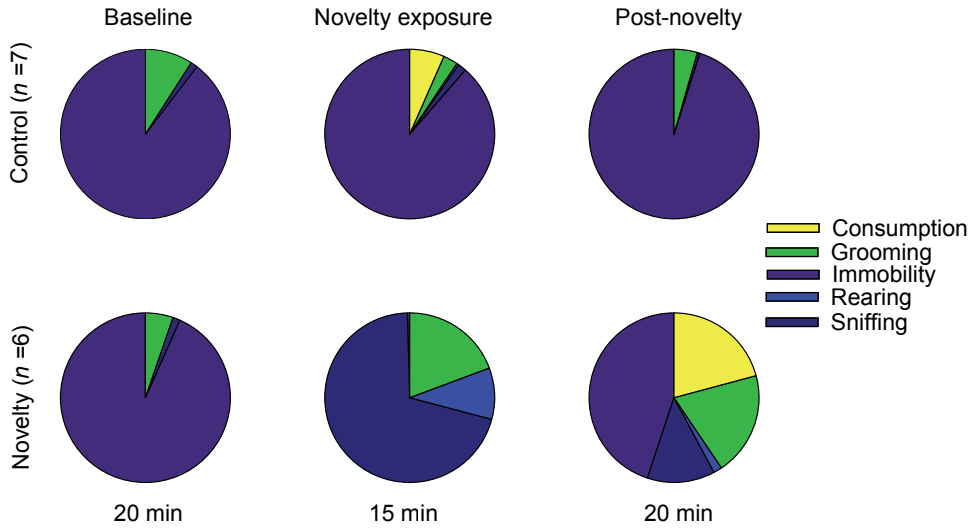

Figure 3. Relative duration (time spent on the behavior expressed as percentage of the observation time) for consumption, grooming, immobility, rearing and sniffing of the control (*n*=7) and novelty-exposed (*n*=6) condition. Data are shown for the baseline period (20 min; *t*=-25 until *t*=-5 min), the novelty exposure or non-novelty control period (15 min; starting immediately when the animal is placed in the novel cage) and the post-novelty period (20 min; starting immediately when the animal is returned to its home cage). Data are presented as means.

DISCUSSION

In the present study, behavioural, physiological, hormonal and EEG parameters were simultaneously and continuously measured in freely moving rats both under undisturbed home cage conditions and after brief novel cage exposure. The measurements under undisturbed home cage conditions showed normal and stable physiological values together with low corticosterone levels throughout the entire observation period. Only body temperature seemed to increase slightly, but not-significantly, after novelty exposure experienced by the other animals in the same room. It is unclear whether this is caused by the presence of the novelty-exposed animals in the same room because it is not reflected in any of the other measures. In addition, EEG vigilance states and behavioural observations showed that animals under control conditions were mainly inactive and asleep during the experimental period. Altogether, this indicates that under control conditions, animals were neither affected by the sampling procedures, nor significantly by the novelty exposure

experienced by the other animals in the same room. This shows that the obtained data are of high quality and are without stress-confounding effects.

Novelty exposure was associated with a complete lack of sleep and an increase in behavioural activity. The telemetry recordings showed a rapid increase in locomotor activity and the behavioural observations reveal an increase in explorative activities such as sniffing and rearing, but also in grooming. These findings are in accordance with other studies showing increased arousal during exposure of rats to a novel environment (Feenstra and Botterblom, 1996; De Groote and Linthorst, 2007; Gagliano et al., 2008). When the animals were returned to their home cages, locomotor activity rapidly declined towards low levels within 5 min, however, no presence of sleep initiation was observed during the first 15 min after termination of novelty. Behavioural observations showed that the animals reduced their time spent exploring and increased time spent immobile. Moreover, the animals still groomed and started to consume food and water. Since grooming and eating/drinking often do not cause total horizontal body movement, the radiotelemetry set-up did not detect this behaviour explaining the observed low levels of locomotor activity. Overall, the animals were behaviourally active until 15 - 20 min after termination of novelty exposure and from then on displayed normal levels of sleep.

Concomitant with these behavioural activity levels were the physiological and hormonal responses to novelty. Novel cage exposure induced temporal increases in heart rate, blood pressure and plasma corticosterone, indicating both SAM system and HPA axis activation. On average body temperature showed an increase in response to novelty exposure as well but this did not reach statistical significance. The latter is partly related to the fact that body temperature showed a slight non-significantly increase under control conditions as well. This may have prevented the increase under novelty-exposed conditions to reach significance. The various parameters showed different time courses in response to novelty. Both heart rate and blood pressure showed an acute and rapid increase in response to novelty, closely following the increase in behavioural activity. This is in agreement with the knowledge that SAM system activation results in the rapid release of catecholamines, which have immediate effects on the cardiovascular system thereby increasing the output of the heart and inducing vasodilatation (Axelrod and Reisine, 1984; Johnson et al., 1992). The corticosterone response showed a slower onset, reaching significance

when the animals were already returned to their home cages and followed the increase in behavioural activity with a delay. Corticosterone secretion is the final result of a neuroendocrine cascade of HPA axis activation, and is not a fast response (Axelrod and Reisine, 1984; Johnson et al., 1992). Such a delay is also visible for the observed slight increase in body temperature. Body temperature increases as a result of an increase in metabolism, in muscle activity and from peripheral vasoconstriction. The calculated time points at which the maximum change was achieved for the novelty-exposed condition confirm that body temperature and corticosterone were delayed. Furthermore, the locomotor activity, heart rate, blood pressure, wakefulness duration, NREM sleep duration and REM sleep duration responses were most likely initiated together. Upon termination of novel cage exposure, the time courses of the physiological and hormonal responses differed but all measures reached stable baseline and control levels within 30 min. The results show that the recovery of blood pressure closely followed the behavioural activity levels, whereas heart rate, body temperature and plasma corticosterone followed with a delay. Because all measurements were taken simultaneously in the same animal, reliable correlation analysis of the response patterns over time was possible. The main focus was on correlations between locomotor activity, EEG wakefulness duration and all other physiological and hormonal responses, as this will show to what extent behavioural activity levels are reflected in the physiological and hormonal responses. Significant correlations between the locomotor activity response pattern and the heart rate, blood pressure and wakefulness duration patterns were found indicating that these had comparable responses over time. The pattern of wakefulness duration was also significantly correlated with the heart rate and blood pressure pattern, and also with the plasma corticosterone pattern. The body temperature response was not correlated significantly with ongoing behavioural activity, which may have been caused by the non-significant increase found upon novelty exposure. As has been discussed, the duration of wakefulness is a more reliable indicator of ongoing behavioural activity during the recovery phase than the locomotor activity response. Therefore, we conclude that the heart rate, blood pressure and also plasma corticosterone responses to novelty exposure are closely related to ongoing behavioural activity and can thus be considered the normal support for the behaviour that was induced by novelty exposure (Koolhaas et al.,

2011). Indeed, the physiological and hormonal values during novelty exposure do neither exceed the average values observed during the active phase of circadian cycle under home cage conditions (Buttner and Wollnik, 1982; De Boer and van der Gugten, 1987), nor do they reach values related to voluntary wheel running (Boersma et al., 2011).

Gagliano and co-workers suggest that the behaviour of the animals in a novel environment is the result of the interaction of fear generated by the unknown space and the motivation to explore it (Gagliano et al., 2008).In our study, animals mainly explored the novel cage as reflected in high levels of sniffing and some rearing, present for around 75% of the time. Animals still showed some explorative behaviour when returned to their home cages (around 13% of the time) but were immobile almost half of the observed time. Also some grooming was present during and after novel cage exposure and is thought to be a stress-reducing behaviour. Van Erp and co-workers studied the grooming response to novel cage exposure and suggest this can have another function as well (van Erp et al., 1994). They found that rats placed in a novel cage steadily increased their grooming behaviour during the observation period, suggesting that grooming is not only an immediate response necessary to reduce arousal following stressors but may have a delayed restorative function as well (van Erp et al., 1994). Since our experiment involved brief handling, grooming may also have had a main function for the animal to clean and correct its fur. Some consumption of food and water specifically occurred after novelty exposure. It can be interpreted as stress-induced feeding, as has been reported after stressful situations (Morley et al., 1983; Dallman et al., 2006). However, the increased behavioural activity during novelty exposure may have induced some feeding to balance metabolic requirements as well.

In conclusion, the present results show that the novelty-induced physiological and hormonal responses in rats are closely related to the ongoing, mainly explorative behavioural activity of the animals. An interpretation in terms of metabolic support of ongoing behaviour seems to be more appropriate than the frequently used stress interpretation. The present study also emphasizes the added value of simultaneous assessment of behavioural, physiological and hormonal parameters under controlled, non-confounding conditions. It shows the importance of studying the time course of different stress variables in association with behavioural activity measurements.

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Chapter 3

Simultaneous assessment of behavioural, physiological and hormonal responses to acute gentle handling sleep deprivation in rats

W. Beerling[1, 2], J.M. Koolhaas[2], A. Ahnaou[1], P. Meerlo[2] and W.H.I.M. Drinkenburg[1]

[1] Department of Neuroscience, Janssen Research & Development, a division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, 2340 Beerse, Belgium;

[2] Department of Behavioural Physiology, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

ABSTRACT

Even though literature exists that supports the general hypothesis that sleep deprivation activates both the sympathico-adrenomedullary (SAM) system and the hypothalamic-pituitary (HPA) axis, there are still shortcomings in the present state of knowledge. Rodent sleep deprivation studies are often limited in the possibility to take multiple read-outs of stress in parallel. Moreover, we feel that the analysis of the activation of the stress systems in concert with behaviour may strengthen the interpretation of the consequences of sleep deprivation. Another shortcoming in rodent sleep deprivation studies is the use of stress-confounding sampling or sleep deprivation methods. Taking into account these shortcomings, the present study aimed to take physiological and hormonal measurements, in parallel, indicative of sympathetic and HPA axis activity, during and after 4-h gentle handling sleep deprivation in rats. Also, locomotor activity levels were measured. Radiotelemetry in combination with an automated blood sampling system for frequent and simultaneous measurement of multiple stress parameters in freely moving rats under controlled, non-confounding conditions was used. Detailed time and correlational analysis were used to determine the relationship between changes in behavioural (locomotor activity), physiological (heart rate, blood pressure, body temperature) and hormonal (corticosterone) stress parameters. The results showed that during sleep deprivation mild temporal increases in locomotor activity, body temperature and plasma corticosterone were induced with a tendency for an increase in heart rate and blood pressure as compared to the non-deprived control condition. Except for corticosterone, levels comparable to or slightly below normal waking levels during the last 30 min of the dark period preceding sleep deprivation were maintained. Upon termination of the sleep deprivation procedure, the responses showed different time courses but all returned to control values within 40 min. Correlational analysis showed that all physiological and locomotor activity response patterns over time were correlated among each other. The corticosterone response was only correlated with locomotor activity and heart rate. Altogether, we conclude that short sleep deprivation mildly affects the basal activity of the neuroendocrine and physiological stress systems. This stress system activation was closely related to the physical activity that was induced by our gentle-handling sleep deprivation method. Since a close dissociation between the physiological/hormonal and behavioural activity response was visible, and since all measurements stayed within the normal range, we conclude that 4-h sleep deprivation per se, as induced by our gentle handling procedure, has a minor impact on the animal.

INTRODUCTION

The neuroendocrine stress systems are thought to play an important role in sleep homeostasis. Activation of the two most important neuroendocrine stress systems, the sympathico-adrenomedullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) axis, has been found during wakefulness as compared to sleep (Akerstedt and Froberg, 1979; von Treuer et al., 1996; Suchecki et al., 1998; Irwin et al., 1999; Spiegel et al., 1999; Chapotot et al., 2001; Hipolide et al., 2006; Sgoifo et al., 2006; Meerlo et al., 2008). Moreover, during sleep deprivation, most human and animal studies found increased cortisol and corticosterone levels respectively, indicative of HPA axis activation (von Treuer et al., 1996; Suchecki et al., 1998; Spiegel et al., 1999; Chapotot et al., 2001; Hipolide et al., 2006; Sgoifo et al., 2006; Meerlo et al., 2008). Furthermore, higher catecholamine levels, indicative of SAM system activation, have been found during wakefulness as compared to sleep (Akerstedt and Froberg, 1979; Irwin et al., 1999). Stress hormones have a variety of effects on a behavioural, emotional, cognitive, physiological, hormonal, brain functioning and metabolic function. For these reasons, it is thought that some of the adverse consequences of sleep deprivation are mediated by changes in the activity of the stress systems.

While literature exists that supports the general hypothesis that sleep deprivation activates stress systems, there are still some shortcomings in the present state of knowledge (Meerlo et al., 2008). Sleep deprivation studies in animals are often limited in the possibility to take multiple read-outs of stress in parallel. As reviewed by Meerlo and co-workers, most studies just focused on how sleep deprivation affects either the SAM system or the HPA axis instead of looking at both systems simultaneously (Meerlo et al., 2008). Also, most studies only take hormonal read-outs whereas additional physiological read-outs are also indicative of sympathetic and HPA axis activity. For example, catecholamines are known to have an immediate effect on the cardiovascular system thereby affecting the output of the heart and inducing vasodilatation resulting in an increase in heart rate and blood pressure (Axelrod and Reisine, 1984; Johnson et al., 1992). Moreover, body temperature levels can increase indirectly as a result of both SAM system and HPA axis activation as well. Body temperature levels change upon changes in e.g.

metabolism, muscle activity and peripheral vasoconstriction that may result from sympathetic and HPA axis activity (Axelrod and Reisine, 1984) Even though all of this is known, studies thus far hardly measure different parameters simultaneously. Therefore, a shortcoming in the present state of knowledge exists on how multiple behavioural, physiological and hormonal stress read-outs are affected by sleep deprivation within the same animal and how these are correlated. Moreover, the analysis of the activation of the stress systems in concert with behaviour can facilitate the interpretation of the consequences of sleep deprivation. As discussed by Koolhaas and co-workers (Koolhaas et al., 2011), neuroendocrine stress system activation may closely reflect the metabolic and physiological demands required for behavioural activity rather than the stressfulness of a situation. They suggest that a situation can be considered truly stressful when dissociation between the physiological/hormonal and behavioural activity response occurs.

Another shortcoming in rodent sleep deprivation studies is the use of confounding sampling methods. The methods used to take measurements are often stressful in themselves. E.g. often the experimenter has to enter the experimental room and handle the animals or the animal is transferred to a different cage in order to take measurements. Moreover, also the sleep deprivation method used is stressful in itself. In order to keep animals awake, some sort of stimulation and forced wakefulness is required, which may activate the stress systems. Even though it is impossible to exclude the activation of the stress systems by other factors as sleep loss per se in rodent sleep deprivation studies, these factors should be minimized to reduce confounding outcomes.

Taking into account these shortcomings, the present study aimed to show in detail how 4-h gentle handling sleep deprivation affects the basal activity of the stress systems. Four to six hours of sleep deprivation in rats have shown to lead to an acute sleep loss with significant increases in sleepiness for up to 3 h after its termination (McKenna et al., 2008). Possible stress-confounding effects of the sampling and sleep deprivation procedure were significantly reduced in this study. Hereto, the gentle handling sleep deprivation method was used. This method has shown to significantly reduce sleep times and is considered a mild procedure when previous habituation to handling is included (Grassi-Zucconi et al., 1993; Grassi-Zucconi et al., 2006; Van der Borght et al., 2006; Hagewoud et al., 2010). Habituation to handling

will reduce the extent of HPA axis and adrenalin activation caused by the gentle handling used to keep the animals awake (Dobrakovova et al., 1993). Furthermore, we used radiotelemetry (Guiol et al., 1992; Van den Buuse, 1994; Leon et al., 2004; Greene et al., 2007; Tang et al., 2007) in combination with an automated blood sampling system (Steffens, 1969; Thrivikraman et al., 2002; Royo et al., 2004; Abelson et al., 2005) for frequent and simultaneous measurement of multiple stress parameters in freely moving rats under controlled, non-confounding conditions. Using these methods, the effect of short-term, 4-h sleep deprivation in rats on behavioural (locomotor activity), physiological (heart rate, blood pressure, body temperature) and hormonal (corticosterone) stress parameters was studied, in parallel, together with the recovery thereof. Detailed time and correlational analysis was used to further investigate the activity of the classical neuroendocrine and physiological stress system in response to acute sleep deprivation and to interpret the stressfulness.

METHODS

Animals and housing

At the beginning of the experiment, 18 adult male Sprague-Dawley rats (Harlan, Horst, The Netherlands), weighing 310-330 g at the time of surgery were used. They were individually housed for at least two weeks prior to surgery in polycarbonate cages (43x27x39 cm) containing bedding, cage enrichment and standard rat chow and water *ad libitum*. Immediately after surgery, each animal was placed within its home cage in the experimental set-up. This set-up consisted of a radiotelemetry set-up (DataSciences International, St. Paul, MN, USA) and an automated blood sampling system (AccuSampler Micro, DiLab, Lund, Sweden). Animals were then also daily handled for weighing purposes. The room was kept at constant temperature (21 ± 1°C), controlled humidity (50 ± 10%) and a fixed 12 : 12 h light/dark regime using a 30-min rise and dim period with lights on at 06.00h. All experimental protocols were approved by the local ethical committee.

Surgery

Surgery was performed under O_2-N_2O-isoflurane anaesthesia. The analgesic Piritramide (dipidolor, 0.025 mg/kg, 0.1 ml/100 g body weight) was administered subcutaneously at the start of surgery. A transmitter to record gross locomotor activity, heart rate, blood pressure, body temperature and cortical EEG (TL11M2-C50-PXT, DataSciences International) was implanted intraperitoneally and attached to the abdominal wall to ensure stabilization (Greene et al., 2007). The blood pressure catheter of the transmitter was inserted in the femoral artery. Furthermore, two biopotential leads for cortical EEG measurements were led subcutaneously to the skull and the bare ends of these electrodes were placed in contact with the dura at 3 mm on either side lateral to the midline and 6 mm posterior to bregma. They were anchored to the skull with screws and dental cement. In addition, a dual tubing jugular vein catheter (polyurethane; ID: 0.36, OD: 0.84, DiLab) allowing blood samples to be collected in freely moving rats was implanted (Steffens, 1969; Thrivikraman et al., 2002). The catheter was inserted through the right jugular vein with the tip of the catheter reaching the entrance of the right atrium. The catheter was led subcutaneously through a Dacron button that was attached to the skin in the dorsal region of the neck. The catheter was connected through a spiral to a swivel and connected either to the infusion pumps or to the automated blood sampling machine (AccuSampler Micro; DiLab). After surgery, animals were immediately connected to a continuous infusion of saline + heparin (10 IU/ml; 0.24 ml/h) to keep the catheter open. The animals were allowed to recover for at least two weeks before start of the experiments.

Experimental design

In total 12 animals survived surgery. After recovery from surgery, rats were subjected to either control or sleep deprivation conditions using a balanced and cross-over Williams design with 3 days in between sessions and using a standard circadian time window. Due to blocked blood sampling catheters, some animals were not subjected to both conditions. This resulted in n=7 for the control condition and n=7 for the sleep-deprived condition but not with the same animals present in both groups.

Seven set-ups were used in parallel to reduce variability among animals and behavioural, physiological and electroencephalogram (EEG) parameters were measured continuously and automatically. Concomitantly, blood was sampled automatically and repeatedly. All experimental procedures were performed during the first half of the light phase, i.e. the resting phase and potentially disturbing stimuli were carefully avoided. The experimental day started with weighing the animals, connecting them to the automated blood sampling system and activating their transmitters. Telemetric recordings and blood sampling started at least 1 h later with a 30-min and 20-min baseline period respectively. These baseline periods were followed for all measurements by a 4-h and 5-min sleep deprivation or non-deprived control period starting at the lights-on stimulus. These were followed by a 3.5-h post-deprivation recovery phase.

Sleep deprivation

Total sleep deprivation was started at lights-on by using the gentle handling method according to published methods (Grassi-Zucconi et al., 1993; Grassi-Zucconi et al., 2006). The animal was stroked on its back or moved whenever it showed behavioural signs of sleep. Only when this did not give visual activation anymore, the animal was lifted from its cage for a few seconds. This method has shown to effectively induce wakefulness for at least 80% of the sleep deprivation period (Grassi-Zucconi et al., 1993). Sleep deprivation was terminated after collecting the t= 245 min blood sample. Therefore, sleep deprivation actually lasted 4 h and 5 min but for simplicity reasons is further referred to as 4 h. Immediately following sleep deprivation, animals were left undisturbed. In order to habituate the animals to handling, they were handled for at least 10 min on the two days preceding the experimental day. Under control conditions, animals were left undisturbed and non-deprived in their home cages in the same experimental room.

Biotelemetric data acquisition and analysis

The implanted transmitter emitted blood pressure, body temperature and EEG dependent frequency modulated signals. For gross locomotor activity, data was obtained by counting the number of changes in signal strength of the transmitter. Because the transmitter was implanted in the abdomen, vertical changes of the animal were hardly detected by the receiver, but horizontal changes were. Heart rate values were calculated from the blood pressure registrations. All signals were received by an antenna board placed under each animal's cage and processed by a computer using Dataquest Labpro software (DataSciences International). Heart rate, blood pressure, body temperature and locomotor activity data were sampled from all seven animals in 10-s epochs and averaged and stored into 5-min bins. Due to too many artefacts in the EEG recordings, these signals could not be analysed.

Blood sampling and assays

Blood was sampled using the jugular vein catheter connected to the automated blood sampling machine. Animals were habituated to the blood sampling procedures to minimize possible stress-confounding effects of it. Small sample volumes of 30 µl were collected every 10 – 60 min. Samples were taken at higher frequency during recovery of sleep deprivation than during sleep deprivation to be able to monitor the recovery closely. The samples were stored inside the machine at 4°C until centrifuged for 5 min at 3210 rpm. Subsequently, plasma was stored at –80°C until assayed in duplicate for corticosterone with a double antibody radio immunoassay (Mouse/Rat Corticosterone ^{125}I RIA Kit, for research only, MP Biomedicals, LLC, Solon, OH, USA) with a sensitivity of 3.0 ng/ml.

Statistics

To assess the effect of sleep deprivation on the behavioural, physiological and hormonal responses, a repeated measures ANOVA with Greenhouse-Geisser correction epsilon (ε) for violation of the homogeneity in variance assumption was used. Even though the aim initially was to use a cross-over design, the data of the

same animals was not present in both groups. Therefore, treatment was used as a between-subjects factor (two levels; control and sleep deprivation) rather than as a within-subject factor. The different time points were used as within-subject factor. This analysis was performed separately for the sleep deprivation period and for the recovery of sleep deprivation period. When appropriate, *post-hoc t*-tests were performed to determine the specific time points at which the values under sleep-deprived and non-deprived control conditions differed. Paired *t*-tests were used to determine the differences between the 30-min dark, sleep deprivation and consecutive recovery period. The Pearson's Bivariate correlation coefficient test was used to compute correlations for the sleep-deprived response patterns over time between the locomotor activity, physiological and hormonal responses. For these correlations, the mean value for each parameter at a certain time point, as displayed in the graphs, was set together with the value of a different parameter at corresponding time point. Only values of which both measurements had a corresponding time point measurement were included therefore using different bin widths depending on the parameter collected. All values, over the different time points, were used to compute a correlation coefficient. All analyses were performed using SPSS 15.0 software (SPSS inc., Chicago, IL, USA). The level of significance was accepted at p-values <0.05 and a trend was accepted at $p<0.10$. Data are presented as mean + SEM.

RESULTS

Dynamics of the behavioural, physiological and hormonal responses to 4 h of sleep deprivation

Locomotor activity

Under non-deprived control conditions, locomotor activity levels significantly declined rapidly at the start of the light period ($p=0.02$), reaching stable values around 0.8 counts/min (Figure 1 panel A). During sleep deprivation a significant increase in locomotor activity compared to non-deprived control conditions was induced, with no significant time*treatment interaction, but with a significant treatment effect

($F_{1,12}$=13.4, p=0.003) and no significant time effect. Locomotor activity values stayed elevated during the whole period of sleep deprivation compared to the control situation but were lower than levels reached during the last 30 min of the dark phase immediately preceding sleep deprivation (p=0.005). When sleep deprivation was terminated, stable control levels were re-established within 5 min and reached levels significantly below those reached before the beginning of the light phase, i.e., circadian resting phase (p=0.001) and during sleep deprivation (p=0.008). ANOVA for the recovery of sleep deprivation period confirmed that no significant differences between sleep-deprived and control conditions were present during this period.

Heart rate

Under non-deprived control conditions, heart rate levels gradually declined at the start of the light period, reaching stable values around 361 bpm (Figure 1 panel B). Paired *t*-tests confirmed that the heart rate under non-deprived control conditions shows a tendency to decrease after the lights-on stimulus (p=0.09). Sleep deprivation induced a close to significant increase compared to the control condition, with no significant time*treatment interaction, but a close to significant treatment effect ($F_{1,12}$=4.04, p=0.06) and a significant time effect ($F_{48,576}$=4.07, ε=0.13 p=0.001). Heart rate values tended to stay elevated throughout the whole sleep deprivation period. In comparison to the last 30 min of the dark phase immediately preceding sleep deprivation, lower levels were reached during the 4-h sleep deprivation. At termination of the sleep deprivation procedure, heart rate values returned to stable control values within 5 min and ANOVA for the recovery period showed no significant difference between the sleep-deprived and non-deprived control condition. The heart rate values during the recovery of sleep deprivation period were significantly lower as those reached during sleep deprivation (p=0.004).

Blood pressure

Under non-deprived control conditions, blood pressure was not significantly affected by the lights-on stimulus and values fluctuated around 105 mmHg (Figure 1 panel C). Exposure to 4-h sleep deprivation did not affect blood pressure compared to baseline levels and to levels under non-deprived control conditions. Also during recovery of sleep deprivation, blood pressure did not differ from the control animals. However, the blood pressure values for the sleep-deprived condition were significantly below those reached during sleep deprivation ($p<0.001$) indicating that blood pressure declined after sleep deprivation.

Body temperature

After the lights-on stimulus, body temperature decreased slowly for the controls reaching stable values around 38 °C and reaching significantly lower values as compared to the baseline period, i.e. 30 min before the beginning of the light phase, i.e., circadian resting phase ($p=0.03$) (Figure 1 panel D). During 4-h sleep deprivation a significant increase in body temperature as compared to the control situation was induced with a close to significant time*treatment interaction ($F_{48,576}=2.07$, $\varepsilon=0.09$, $p=0.09$), a significant treatment effect ($F_{1,12}=5.05$, $p=0.04$) and a significant time effect ($F_{48,576}=8.99$, $\varepsilon=0.09$, $p<0.001$). Body temperature stayed elevated for the whole 4 h of sleep deprivation as compared to the non-deprived control condition but did not exceed baseline values indicating that levels comparable to before the beginning of the light phase, i.e., circadian resting phase, were maintained. ANOVA for the recovery of sleep deprivation period showed a significant time*treatment interaction ($F_{42,504}=5.49$, $\varepsilon=0.081$, $p=0.002$), no significant treatment effect, and a significant time effect ($F_{42,504}=5.82$, $\varepsilon=0.081$, $p=0.001$). After termination of the sleep deprivation procedure, body temperature decreased slowly to control levels within 40 min. The values during this recovery of sleep deprivation period were significantly below those reached during the sleep deprivation period ($p<0.001$).

Corticosterone

For the non-deprived control condition, plasma corticosterone levels increased slightly but significantly by the lights-on stimulus (p=0.004) and levels fluctuated around 27 ng/ml (Figure 1 panel E). The 4-h sleep deprivation induced a significant increase in corticosterone levels compared to the non-deprived control condition, with a significant time*treatment interaction ($F_{3,36}$=3.40, ε=0.82, p=0.04), a significant treatment effect ($F_{1,12}$=7.48, p=0.02) and a significant time effect ($F_{3,36}$=6.28, ε=0.82, p=0.003). The levels were also significantly increased compared to the last 30 min of the dark period (p<0.001). Corticosterone was mostly elevated at the beginning of the 4-h sleep deprivation period (maximum average of 76 ng/ml). During the 3-h 30-min recovery period immediately following sleep deprivation, the corticosterone response showed a close to significant time*treatment interaction ($F_{9,108}$=2.889, ε=0.318, p=0.052) and no significant sleep deprivation and time effects. Corticosterone stayed significantly elevated for another 15-min until reaching control levels again. Thirty minutes later, corticosterone levels significantly dropped below control levels for 60 min after which control levels were reached again. This resulted in significantly lower corticosterone levels in the recovery of sleep deprivation period compared to during sleep deprivation (p=0.03).

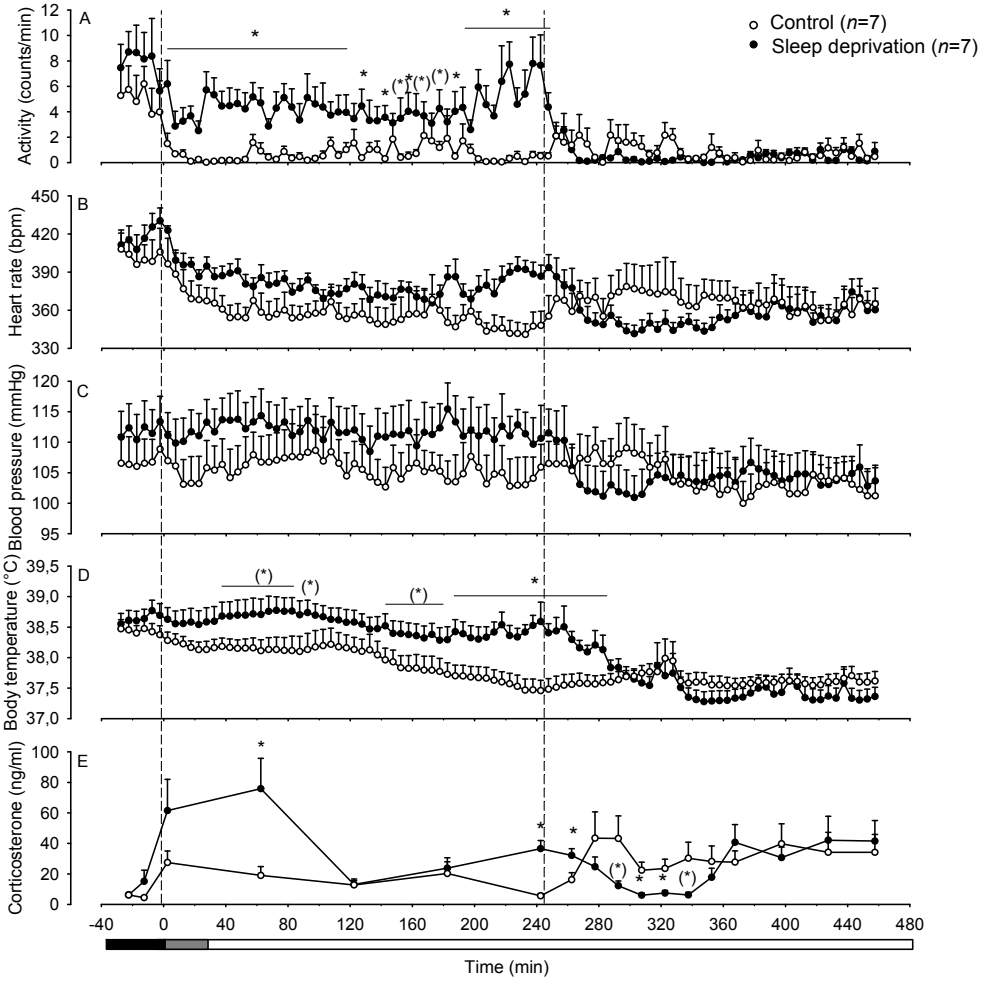

Figure 1. Time course of changes in locomotor activity (panel A), heart rate (panel B), blood pressure (panel C), body temperature (panel D) and plasma corticosterone (panel E) for the 4-h sleep-deprived condition (closed symbols, *n*=7) and for the control condition (open symbols, *n*=7). Data are expressed as 5-min averages + SEM for the physiological measurements and for every 10 - 60 min + SEM for the corticosterone measurements. The responses are shown for the last 30 min of the dark period, the 4-h (+ 5 min) sleep deprivation or non-deprived control period (indicated by area between the lines) and for the 3-h 30-min post-sleep deprivation period. The bars underneath the graph indicate the dark (black), the 30-min rise (grey) and light (white) period. Significance: sleep deprivation versus control *p<0.05; (*)p<0.10.

Associations between the behavioural, physiological and hormonal responses

Table 1 shows the correlation coefficient for the sleep-deprived response patterns over time of locomotor activity, heart rate, blood pressure, body temperature and plasma corticosterone. The pattern of locomotor activity showed a significant correlation with the pattern of heart rate (r=0.86, p<0.001), blood pressure (r=0.81, p<0.001), body temperature (r=0.81, p<0.001) and plasma corticosterone (r=0.53, p=0.04). The heart rate response pattern over time was also significantly correlated with the blood pressure (r=0.82, p<0.001), body temperature (r=0.78, p<0.001) and plasma corticosterone (r=0.61, p=0.01) response patterns over time. The blood pressure pattern was significantly correlated with the body temperature (r=0.78, p<0.001) pattern and tended to be correlated with that of plasma corticosterone (r=0.46, p=0.08). The body temperature response was not significantly correlated with the plasma corticosterone response but was with all other responses. Thus, all physiological response patterns over time and the behavioural locomotor activity pattern were correlated among each other during and after sleep deprivation. For the plasma corticosterone pattern, a significant correlation coefficient for the group mean was found with the pattern of locomotor activity, heart rate and a tendency for blood pressure.

Table 1. Correlation coefficient for the sleep-deprived response patterns over time of Act, HR, BP, BT and Cort (n=7).

	Act	HR	BP	BT	Cort
Act	1.00				
HR	0.86*	1. 00			
BP	0.81*	0.82*	1.00		
BT	0.81*	0.78*	0.78*	1.00	
Cort	0.53*	0.61*	0.46(*)	0.39	1.00

Act: locomotor activity; HR: heart rate; BP: blood pressure; BT: body temperature; Cort: plasma corticosterone. Significance:*p<0.05; (*)p<0.10.

DISCUSSION

One of the shortcomings in the present state of knowledge in sleep research is how multiple behavioural, physiological and hormonal read-outs of stress are affected by sleep deprivation within the same animal. Therefore, the present study investigated how behavioural, physiological and hormonal stress parameters were affected by 4-h gentle handling sleep deprivation. Acute sleep deprivation induced mild, temporal increases in gross locomotor activity, body temperature and plasma corticosterone and a tendency for an increase in heart rate and blood pressure compared to the non-deprived control condition. Locomotor activity and body temperature were increased at most time points during the sleep deprivation period, and also heart rate tended to be increased during most time points, whereas corticosterone was mostly increased during the first hour of sleep deprivation. The observed tendency for an increase in heart rate and blood pressure in response to sleep deprivation is indicative of sympathetic activity, whereas the increase in plasma corticosterone reflects HPA axis activity (Axelrod and Reisine, 1984; Johnson et al., 1992). Moreover, the increase in body temperature levels is most likely also indirectly related to both HPA axis and SAM activation. Body temperature levels will change as the result of e.g. increased metabolism, muscle activity and peripheral vasoconstriction that can be the results of sympathetic and HPA axis activation. Therefore we conclude that both the SAM system and HPA axis system were activated in response to 4-h sleep deprivation. This is in agreement with most other human and animal studies showing increased cortisol and corticosterone levels respectively during sleep deprivation, confirming HPA axis activity (von Treuer et al., 1996; Suchecki et al., 1998; Spiegel et al., 1999; Chapotot et al., 2001; Hipolide et al., 2006; Sgoifo et al., 2006; Meerlo et al., 2008). Other studies also showed higher catecholamine levels, indicating SAM system activation, during wakefulness as compared to during sleep (Akerstedt and Froberg, 1979; Irwin et al., 1999).

The activation of the SAM system and HPA axis in the present study should however be considered mild. Sleep deprivation prevented the normal decline in heart rate and body temperature that was observed in the control situation at the beginning of the light phase, i.e. circadian resting phase. Levels comparable to, or slightly below, normal waking levels during the last 30 min of the dark period preceding sleep

deprivation were maintained. This is in accordance with other studies showing an increase in heart rate and blood pressure towards levels reached during normal wakefulness (Tochikubo et al., 1996; Sforza et al., 2004). Only plasma corticosterone reached higher levels than during relaxed wakefulness but these levels can still be considered low as they were below levels seen during mild stress (Buwalda et al., 1993; Van den Buuse et al., 2001; Suchecki et al., 2002). Upon termination of the sleep deprivation procedure, the responses showed different time courses but all returned to control values within 40 min. Whereas heart rate had a rapid decline to control values upon termination of the sleep deprivation procedure, body temperature and plasma corticosterone showed a slower decline. These different time patterns are in agreement with the knowledge that SAM activation results in a rapid release of catecholamines, which in turn have immediate effects on the cardiovascular system (Axelrod and Reisine, 1984; Johnson et al., 1992) and the knowledge that corticosterone secretion is a final result of a neuroendocrine cascade activated by the HPA axis and is therefore not a fast response (Axelrod and Reisine, 1984; Johnson et al., 1992). Also, body temperature responses are in general delayed as this will change due to changes in metabolism, muscle activity and peripheral vasoconstriction.

The present study also reduced stress-confounding effects of the sampling procedures. The measurements under non-deprived control conditions confirm that this was achieved. Normal and stable physiological values together with low plasma corticosterone values were maintained throughout the entire observation period. Moreover, the low locomotor activity levels indicate that the control animals were inactive during most of the observation period. This was expected since the measurements were taken during the light phase, i.e. the resting phase. Altogether, this indicates that the animals under non-deprived control conditions were not affected by the sampling procedures which confirms that the used approach with automatical measurements under controlled, home cage and freely moving conditions, indeed allowed high quality un-confounded measurements of behavioural, physiological and hormonal stress parameters in parallel.

A point of discussion is to what degree sleep deprivation constitutes a stressor that mediates the adverse consequences of sleep deprivation. Another point of discussion is whether stress system activation in response to sleep deprivation is the

result of sleep loss per se or a consequence of the stressfulness of the used sleep deprivation procedure. Also, one should determine to what extent the stress system activation is due to physical activity induced to keep the animals awake. It is hypothesized that in order to consider sleep deprivation as stressful, it should increase the activity of the stress systems beyond those seen during relaxed wakefulness (Meerlo et al., 2008). As our combined measures showed, only corticosterone reached higher levels than during relaxed wakefulness and only during the first hour of sleep deprivation, but these levels were still below those seen during mild stress (Buwalda et al., 1993; Van den Buuse et al., 2001; Suchecki et al., 2002). We believe that the analysis of the activation of the stress systems in concert with behaviour may facilitate the interpretation of the consequences of sleep deprivation. The classical stress systems are not only activated in response to stressful situations, but are also crucial for mobilization of energy and are therefore activated in any condition that requires or causes behavioural activation (Leal-Cerro et al., 2003). Animals were forced to be awake by stroking them or moving them within their cages, which induced mild physical activity. Correlational analysis showed that the locomotor activity pattern during and after sleep deprivation was significantly correlated with the patterns of the physiological and hormonal responses. Therefore, a considerable part of the classical stress system activation in response to sleep deprivation is a direct reflection of the metabolic requirements for normal ongoing behavioural activity that was induced by our gentle handling sleep deprivation procedure. This supports the idea that mild stress system activation during sleep deprivation may have been necessary to support the increased activity and wakefulness associated with sleep deprivation. In line with the stress theory of Koolhaas and co-workers, this association of the physiological/hormonal responses with the behavioural response also indicates that sleep deprivation may not constitute a stressor (Koolhaas et al., 2011). The correlational analysis furthermore showed that the different read-outs indicative of sympathetic activity had a highly similar response pattern over time whereas the sympathetic response was to a lesser degree correlated with the HPA axis response.

Overall, we conclude that short sleep deprivation mildly affects the basal activity of the neuroendocrine and physiological stress systems. This stress system activation was closely related to the physical activity displayed by the animals during

the sleep deprivation session. This physical activity was induced by our gentle-handling procedure in order to provoke wakefulness. Since a close dissociation between the physiological/hormonal and behavioural activity response was visible, and since all measurements stayed within the normal range, we conclude that 4-h sleep deprivation per se, as induced by our gentle handling procedure, has a minor impact on the animal.

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Chapter 4

Short sleep deprivation by gentle handling is a mild activator of the stress systems but does not affect the subsequent response to novelty exposure

W. Beerling[1, 2], J.M. Koolhaas[2], A. Ahnaou[1], P. Meerlo[2] and W.H.I.M. Drinkenburg[1]

[1] Department of Neuroscience, Janssen Research & Development, a division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, 2340 Beerse, Belgium;
[2] Department of Behavioural Physiology, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

ABSTRACT

Sleep deprivation does not only affect the basal activity of the stress systems but may also affect the reactivity of these systems to challenges. Therefore, this study aimed to show how acute sleep deprivation affects the behavioural, physiological and hormonal responses to novelty exposure. Novelty exposure is known to induce mild activation of the stress systems and the physiological and hormonal responses are closely related to the ongoing, mainly explorative behavioural activity. We also analysed how acute gentle handling sleep deprivation affects physiological, hormonal and locomotor activity levels and sleep-wake distribution with NREM delta power to further validate the gentle handling sleep deprivation method. To study this, adult male Sprague-Dawley rats were exposed to either 4 or 6 h of total sleep deprivation by gentle handling and subsequently to 15-min novelty exposure by placing the rat in a clean, empty cage. Under control conditions, animals were left undisturbed. A set-up combining radiotelemetry and automated blood sampling was used for continuous monitoring of gross locomotor activity, heart rate, blood pressure, body temperature and plasma corticosterone levels as well as sleep-wake distribution and Non-Rapid Eye Movement (NREM) delta power. To do so, rats were implanted a small transmitter together with a permanent heart catheter. Measurements were taken during sleep deprivation followed by a recovery period with subsequent novelty exposure and a post-novelty phase. Sleep deprivation was associated with a near complete reduction of sleep and a concomitant increase in time spent awake. A tendency for a rebound in NREM sleep and a significant increase in delta power during NREM sleep was present upon termination of the sleep deprivation procedure. The increased delta power during NREM sleep was still present during the post-novelty exposure phase. Sleep deprivation also caused a mild, temporary increase in locomotor activity, heart rate, blood pressure and body temperature without affecting plasma corticosterone levels. Exposure to novelty, starting 1.5 h after sleep deprivation termination, induced an increase in locomotor activity, heart rate, blood pressure, body temperature and plasma corticosterone with a concomitant lack of NREM and REM sleep. Prior sleep deprivation did not affect these responses. Also the recovery of the responses after novelty exposure was unaffected by prior sleep deprivation. In conclusion, 6 h of sleep deprivation by our gentle handling procedure is sufficient to deprive rats from sleep and to cause effects that are still detectable after novelty exposure. Moreover, 4 and 6 h of sleep deprivation is a mild activator of the autonomic stress systems. We furthermore conclude that acute sleep deprivation does not affect the subsequent neuroendocrine and physiological stress response to novelty exposure as measured 1.5 h after sleep deprivation termination.

INTRODUCTION

The exact function of sleep is still unknown, yet it is common knowledge that sleep deprivation is detrimental to good health (Ford and Kamerow, 1989; Pilcher and Huffcutt, 1996). The underlying mechanisms are still poorly understood, but several hypotheses have been formulated. One of the hypotheses states that some of the adverse consequences of sleep deprivation are mediated by changes in activity of the neuroendocrine stress systems (Meerlo et al., 2008). The sympathico-adrenomedullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) axis are the two most important neuroendocrine stress systems. SAM system activation results in the release of the catecholamines adrenaline and noradrenalin into the bloodstream (Axelrod and Reisine, 1984; Johnson et al., 1992). Activation of the HPA axis results in the release of the pituitary adrenocorticotropic hormone (ACTH) which subsequently stimulates the release of the glucocorticoid cortisol in humans and corticosterone in rodents from the adrenal gland. These hormones in turn influence behavioural, emotional, cognitive, physiological, hormonal, brain functioning as well as metabolic functions to enable the individual to deal with the situation (Axelrod and Reisine, 1984; Johnson et al., 1992; Buckley and Schatzberg, 2005; Meerlo et al., 2008). As reviewed by Meerlo and co-workers, controlled human and rodent studies report mild activation of the stress systems, in particular of the HPA axis, in response to sleep deprivation (Akerstedt and Froberg, 1979; von Treuer et al., 1996; Suchecki et al., 1998; Irwin et al., 1999; Spiegel et al., 1999; Chapotot et al., 2001; Hipolide et al., 2006; Sgoifo et al., 2006; Meerlo et al., 2008). Moreover, existing literature suggests that chronic or frequent restriction of sleep may gradually alter the regulation of these stress systems, which may contribute to the symptomatology of stress-related disorders such as depression (Buckley and Schatzberg, 2005; Roman et al., 2005; Novati et al., 2008).

Sleep deprivation may not only affect the basal activity of the stress systems but may also affect the reactivity of these systems to challenges. Controlled rodent studies suggest that acute sleep deprivation (20 h of total sleep deprivation) does not affect the HPA axis response to a subsequent stressor whereas prolonged sleep deprivation (i.e. 48 h of total sleep deprivation) or 8 days of sleep restriction (repeated 20 h sleep deprivation per day) alters the HPA axis response to brief

restraint or inescapable foot shock stress in rats (Meerlo et al., 2002; Sgoifo et al., 2006; Novati et al., 2008). Particularly, the ACTH response was reduced; yet, the adrenal corticosterone response remained unaffected, perhaps indicating increased adrenal sensitivity to ACTH. Also the cardiac autonomic stress reactivity was altered by prolonged sleep curtailment (Sgoifo et al., 2006). Furthermore, in another study Suchecki and co-workers subjected rats to 96 h of paradoxical sleep deprivation and showed that subsequent exposure to a mild stressor was associated with higher corticosterone levels despite an unaltered ACTH response (Suchecki et al., 2002). This may again suggest hypersensitivity of the adrenal gland to ACTH input. Altogether, only a few controlled rat studies that largely focussed on prolonged sleep deprivation or sleep restriction and hormonal read-outs of HPA axis reactivity have been done. Therefore, the present state of knowledge on how acute sleep deprivation affects the activated stress response is limited.

The aim of the present study was to assess how short sleep deprivation affects behavioural, physiological and hormonal measures and how sleep deprivation affects the subsequent reactivity to novelty exposure. For this purpose, in two subsequent studies, rats were exposed to either 4 or 6-h sleep deprivation followed by a brief recovery of sleep deprivation and subsequent exposure to a novel cage. Four to six hours of sleep deprivation in rats leads to significant increases in sleepiness for up to 3 h after termination of sleep deprivation (McKenna et al., 2008). A 1.5-h recovery period after sleep deprivation was included to make sure that the stress systems were back to control levels before exposure to the novel cage (Meerlo et al., 2002). Novel cage exposure has a long history in experimental rodent stress research and is considered a mild psychological stressor with good ecological validity (Ader and Friedman, 1968; Hennessy et al., 1979; Pfister, 1979; Armario et al., 1986). In an earlier study we reported that novel cage exposure induces mild activation of the stress systems and that the novelty-induced physiological and hormonal responses are closely related to the ongoing, mainly explorative behavioural activity (Beerling et al., 2011) (Chapter 2). The present study reduced stress-confounding factors as much as possible by using a gentle handling sleep deprivation method. This method effectively reduces sleep time and causes only a mild activation of the stress systems (Grassi-Zucconi et al., 1993; Grassi-Zucconi et al., 2006; Van der Borght et al., 2006; Hagewoud et al., 2010). Furthermore,

radiotelemetry (Guiol et al., 1992; Van den Buuse, 1994; Leon et al., 2004; Greene et al., 2007; Tang et al., 2007) in combination with an automated blood sampling system (Steffens, 1969; Thrivikraman et al., 2002; Royo et al., 2004; Abelson et al., 2005) was used, which allowed frequent and simultaneous measurement of multiple stress parameters in freely moving rats under controlled, non-confounding conditions. Physiological (heart rate, blood pressure, body temperature) and hormonal (corticosterone) stress parameters, indicative of sympathetic and HPA axis activity, in response to sleep deprivation and novelty exposure were measured. In addition, changes in sleep-wake distribution (electroencephalographic EEG, electromyographic EMG), locomotor activity levels and Non-Rapid Eye Movement (NREM) delta power were determined to validate the gentle handling sleep deprivation procedure. Technological reasons prevented all measurements to be taken in one experiment, therefore two experiments using either 4 or 6 h of sleep deprivation were done. Some of the measures in both studies were the same, allowing for a comparison between the effects of 4 and 6 h of sleep deprivation. The primary goal of the present study was to determine how both the basal activity and reactivity of the neuroendocrine and physiological stress systems are affected by acute sleep deprivation in a non-confounded way.

METHODS

Animals, housing and experimental set-up

In a series of 2 experiments, we used 18 adult male Sprague-Dawley rats (Harlan, Horst, The Netherlands) for experiment 1 and 23 adult male Sprague-Dawley rats (Harlan, Horst, The Netherlands) for experiment 2, all weighing 310-330 g at the time of surgery, as subjects. For experiment 1, the same rats were used as for the study described in Chapter 3. All rats were individually housed for at least two weeks prior to surgery in polycarbonate cages (43x27x39 cm) containing bedding, cage enrichment and standard rat chow and water *ad libitum*. The home cage could be positioned in the experimental set-up. This set-up consisted of a radiotelemetry set-up (DataSciences International, St. Paul, MN, USA) and an automated blood sampling system (AccuSampler Micro, DiLab, Lund, Sweden). With 8 set-ups, not all

animals could be housed in this set-up simultaneously. Rats were therefore, after surgery, repeatedly placed within their home cages in this set-up and were housed for at least 24 h in the set-up prior to the experimental day. After surgery, animals were handled daily for weighing purposes. The room was kept at constant temperature (21 ± 1°C), controlled humidity (50 ± 10%) and a fixed 12 : 12 h light/dark regime using a 30-min dim/rise period with lights on at 06.00 a.m. The local ethical committee approved all experimental protocols.

Surgery

Surgery was performed under O_2-N_2O-isoflurane anaesthesia. The analgesic Piritramide (dipidolor, 0.025 mg/kg, 0.1 ml/100 g body weight) was administered subcutaneously at start of surgery. For experiment 1, a transmitter to record gross locomotor activity, heart rate, blood pressure, body temperature and cortical EEG (TL11M2-C50-PXT, DataSciences International) was implanted intraperitoneally and attached to the abdominal wall to ensure stabilization (Greene et al., 2007). The blood pressure catheter of the transmitter was guided into the femoral artery. Furthermore, two biopotential leads for cortical EEG measurements were led subcutaneously to the skull and the bare ends were placed in contact with the dura at 3 mm on either side lateral to the midline and 6 mm posterior to bregma and anchored to the skull with screws and dental cement (Tang et al., 2007). For experiment 2, a transmitter was implanted for measurement of gross locomotor activity, body temperature, EEG and EMG (TL11M2-F20-EET, DataSciences International). The leads for EEG registration were implanted as described above. The other two leads were bilaterally attached to the deeper neck muscles (cervical trapezius) for EMG registration. In addition, animals of both experiment 1 and 2 received a jugular vein catheter for blood sampling (Steffens, 1969; Thrivikraman et al., 2002). The catheter (polyurethane; ID: 0.36, OD: 0.84, DiLab) was inserted through the right jugular vein with the tip of the catheter reaching the entrance of the right atrium. The catheter was led subcutaneously through a Dacron button that was attached to the skin in the dorsal region of the neck. The Dacron button was connected through a spiral to the swivel and connected either to the infusion pumps or to the automated blood-sampling machine. After surgery, animals were connected

to a continuous infusion of saline + heparin (10 IU/ml; 0.12 ml/h each side of the dual catheter) to ensure the catheter to stay open. The animals were allowed to recover for at least two weeks before start of the experiments.

Experimental design

After recovery from surgery, two experiments were performed with 2 different groups of animals. The methods and experimental design used in these studies were largely the same. Experiment 1 includes a 4-h gentle handling sleep deprivation period and its effects on gross locomotor activity, heart rate, blood pressure, body temperature and plasma corticosterone. Experiment 2 includes a 6-h gentle handling sleep deprivation period and its effects on gross locomotor activity, body temperature, sleep-wake distribution, NREM delta power and plasma corticosterone.

For experiment 1, in total 12 animals survived the surgery. After some of them had completed the experiment as described in chapter 3, the rats were subjected to either non-deprived control conditions without novelty exposure, non-deprived control conditions with novelty exposure, sleep deprivation without novelty exposure or sleep deprivation with novelty exposure. This was done using a balanced and cross-over Williams design with 3-5 days in between sessions. Using this design, the animals were supposed to be exposed to all conditions with 3-5 days in between. However, due to blood clotting in the catheters, some rats did not experience all four conditions and therefore the same animals were not always present in all groups resulting in n=8 for the control condition without novelty exposure, n=6 for the control with novelty exposure condition, n=6 for sleep deprivation without novelty exposure and n=8 for sleep deprivation with novelty exposure. Eight set-ups were used in parallel to reduce variability among animals and behavioural, physiological and EEG parameters were measured continuously and automatically. Concomitantly, blood was sampled automatically and repeatedly. Statistical analysis showed no significant effect of prior testing as described in chapter 3 or significant effect of testing day, therefore the data of all testing days was taken together.

For experiment 2, in total 16 animals survived surgery. Because only eight animals could be tested simultaneously in the operant boxes, the 16 animals were divided into two groups of n=8 that had their experimental day on a different day in

the same week using a standard circadian time window. The animals of group 1 were exposed to sleep deprivation, sleep fragmentation and control conditions, all without novelty exposure. This was done using a balanced and cross-over Williams design with 7 days in between sessions. The animals of group 2 were also exposed to sleep deprivation, sleep fragmentation and control conditions but were all exposed to novelty exposure. This was also done using a balanced and cross-over Williams design with 7 days in between sessions. Eight set-ups were used in parallel to reduce variability among animals and behavioural, physiological and EEG parameters were measured continuously and automatically. Concomitantly, blood was sampled automatically and repeatedly. During sleep fragmentation, interruptions in sleep were induced 8 times per hour, with 1.5 min of interruption and 6 min of sleeping possibility. The purpose was to reduce NREM sleep episode duration and the amount of REM sleep. Since sleep fragmentation was not successfully induced, the sleep fragmentation protocol and outcomes will not be further described. The data of a number of subjects could not be analysed further due to insufficient quality of their telemetry signals and/or blocked blood sampling. This resulted in: n=4 for the control condition without novelty, n=5 for sleep deprivation without novelty, n=5 for the control condition with novelty and n=5 for sleep deprivation with novelty, for the locomotor activity, physiological and corticosterone measurements, and in n=7, n=8, n=6 and n=5 respectively for the sleep-wake responses and NREM delta power.

The experimental sleep deprivation and exposure to novelty were performed during the light phase, i.e. the circadian resting phase. The experimental day started with weighing the animals, connecting them to the automated blood sampling system and activating their transmitters. At least 1 h later, telemetric recordings and blood sampling started simultaneously for a 15-min baseline period. This baseline period was followed by either a 4-h sleep deprivation (experiment 1) or a 6-h sleep deprivation (experiment 2) period, or in either case by a non-deprived control period, all starting at lights-on. These were followed for both studies by a 1.5-h post-sleep deprivation recovery phase, based on unpublished pilot data, and subsequent 15-min novelty exposure or non-novelty control condition. These were followed by a 75-min post-novelty phase.

Sleep deprivation

Sleep deprivation started at lights-on using the gentle handling procedure according to published methods (Grassi-Zucconi et al., 1993; Grassi-Zucconi et al., 2006). In short, whenever the animal showed behavioural signs of sleep, it was stroked on its back or gently moved. When this did not result in visual activation of the animal, it was lifted from its cage for a few seconds. This method has shown to effectively induce wakefulness for at least 80% of the sleep deprivation period (Grassi-Zucconi et al., 1993). For experiment 1, sleep deprivation was terminated after collecting the *t*= 245 min blood sample. Therefore, sleep deprivation actually lasted 4 h and 5 min but for simplicity reasons is further referred to as 4 h. For experiment 2, sleep deprivation was terminated after collecting the *t*= 365 min blood sample, which made sleep deprivation last 6 h and 5 min, which is further described as 6 h. Immediately following sleep deprivation, animals were left undisturbed. In order to reduce stress caused by the sleep deprivation method, animals were handled for at least 10 min on the two days preceding the experimental day. For the control procedure in both studies, animals were left undisturbed and non-deprived in their home cages in the same experimental room.

Novelty exposure

Exposure to novelty was performed according to the protocol previously reported(De Boer et al., 1990b; Beerling et al., 2011). The experimenter entered the room at *t* = -2 min to gently pick up the rat from its home cage at *t* = -1 min and place it in a new, clean, empty cage otherwise comparable to its home cage. Novelty exposure therefore included entering the experimental room, brief handling and forced novel cage exposure. After collecting the *t* = 15 min blood sample, rats were returned to their home cages. Under non-novelty control conditions, animals were left undisturbed in their home cages while remaining in the same experimental room.

Biotelemetric data acquisition and analysis

Using the implanted transmitter and the Dataquest A.R.T. System (DataSciences International) behavioural, physiological and polysomnographic signals were telemetrically monitored. The transmitters emitted blood pressure, body temperature, EMG and EEG dependent frequency modulated signals. The gross locomotor activity signals were obtained by monitoring changes in the received signal strength that resulted from horizontal movement of the animals. Heart rate values were calculated from the blood pressure registrations. The signals were received by an antenna board placed underneath each animal's cage and were processed by a computer using Dataquest Labpro software. Heart rate, blood pressure, body temperature and locomotor activity data were sampled from all eight animals in 10-s epochs and were averaged and stored into 5-min bins. The polysomnographic bio-potential recordings were imported into NeuroScore Version 2.0.0 (DataSciences International). The vigilance states were determined off-line in 10-s epochs. A concurrent EEG power spectrum for each epoch was used to aid in discriminating between states. NREM sleep was scored based on the presence of high amplitude EEG waves and predominant EEG power in the delta range (0.5-4 Hz) and low muscle tone and lack of body movement. Rapid Eye Movement (REM) sleep was characterized by highly regular low amplitude EEG with a dominance of theta activity (4.5-8 Hz) with reduced lower frequencies and general lack of muscle tone with occasional twitches. Wakefulness was scored based on irregular low-amplitude, fast EEG, higher muscle tone, and frequent body movements. The amount of time spent in each vigilance state was calculated in 15-min blocks. The delta power during NREM sleep in the range of 0.5-4 Hz was analysed by Fast Fourrier Transformation (FFT) at a resolution of 0.25 Hz for 4 s NREM sleep epochs and was expressed as percentage of the total power (0-40 Hz). This was analysed only for the 1.5-h post-deprivation recovery phase and the 75-min post-novelty phase. Due to artefacts in the EEG signals for experiment 1, these were not further analysed.

Blood sampling and assays

Blood was sampled using the automated blood sampling system and the dual jugular vein catheter in both studies. Animals were habituated to the blood sampling procedures to minimize possible stress-confounding effects of it. Blood was sampled every 10 – 120 min in small sample volumes of 30-µl. Samples were taken at higher frequency during the recovery of sleep deprivation, novelty exposure and post-novelty to be able to monitor the response to novelty closely. The samples were stored inside the machine at 4°C until centrifuged for 5 min at 3210 rpm. Subsequently, plasma was stored at –80°C until assayed in duplicate for corticosterone with a double antibody radioimmunoassay (Mouse/Rat Corticosterone ^{125}I RIA Kit, MP Biomedicals, Solon, OH, USA) with a sensitivity of 3.0 ng/ml.

Statistics

For all measurements, the effect of sleep deprivation and novelty exposure were evaluated with two-way analysis of variance (ANOVA) with repeated measures and the Greenhouse-Geisser correction epsilon (ε) for putative lack of homogeneity in variance. Even though a cross-over design was initially used, the data of the same animals was not always present in both groups. Therefore, treatment was used as a between-subjects factor rather than as a within-subject factor. Analyses were done for the sleep deprivation period with subsequent 1.5-h post-deprivation phase and for the novelty exposure period with subsequent 75-min post-novelty phase in order to study the effect of sleep deprivation itself and then for the effect of sleep deprivation on the responses to novelty during the different time periods. If these ANOVA analyses did not show significant effects, the analyses were also done for the sleep deprivation and post-deprivation phase separately, and for the novelty and post-novelty phase separately, in order to check for an effect in the recovery. Since this did not give a difference in significance, the results are only shown and discussed for the analyses done for the sleep deprivation period with subsequent post-deprivation phase and for the novelty exposure period with subsequent post-novelty phase. Since there was no significant difference between the novelty and non-novelty control

condition prior to the actual novelty exposure, these groups were taken together for that time frame and analysed by *post-hoc t*-tests to determine at what time points control and sleep-deprived differed. Also, since no effect of sleep deprivation was found for the novelty exposure period and the post-novelty exposure phase, both novelty-exposed (sleep-deprived and control) and non-novelty exposed groups were taken together during this period and analysed by *post-hoc t*-tests to determine at what time points novelty and non-novelty differed. For NREM delta power only the post-deprivation and post-novelty exposure periods are shown and since no difference between novelty and the non-novelty control condition was found, these were taken together. All analyses were performed using SPSS 15.0 software (SPSS inc., Chicago, IL, USA). All tests were performed at a significance level of 0.05. Data are presented as mean + SEM.

RESULTS

Experiment 1: Dynamics of the behavioural, physiological and hormonal responses to 4 h of sleep deprivation with subsequent novelty exposure

Sleep deprivation and consecutive recovery

Data on locomotor activity, heart rate, blood pressure, body temperature and corticosterone during 4-h sleep deprivation or the non-deprived control condition and the 1.5-h post-deprivation recovery phase are illustrated in Figure 1 (panels A-E, respectively). Under non-deprived control conditions, after lights-on, stable values were reached for all measurements

For locomotor activity, repeated measures ANOVA revealed a significant time*sleep deprivation interaction ($F_{67,1608}$=10.2, ε=0.1, p<0.001) and a significant overall sleep deprivation effect ($F_{1,24}$=40.0 p<0.001). *Post-hoc* analysis showed that locomotor activity levels were increased during the whole sleep deprivation period until the first 5 min after termination of the sleep deprivation procedure as compared to the control condition. Twenty min later, locomotor activity in the sleep-deprived animals dropped significantly below control levels for 20 min.

Also for heart rate, ANOVA revealed a significant time*sleep deprivation interaction ($F_{67,1608}$=4.9, ε=0.1, p<0.001) and a significant sleep deprivation effect ($F_{1,24}$=8.5, p=0.008). Sleep deprivation induced a significant increase in heart rate as compared to the non-deprived control condition during most time points of the sleep deprivation period. Upon termination of the sleep deprivation procedure, heart rate levels decreased to control levels within 5 min.

For blood pressure, ANOVA showed a significant time*sleep deprivation interaction ($F_{67,1608}$=2.9, ε=0.2, p=0.002). *Post-hoc* analysis however showed no time points at which the sleep-deprived and non-deprived control condition significantly differed.

For body temperature ANOVA showed a significant time*sleep deprivation interaction ($F_{67,1608}$=2.8, ε=0.1, p=0.015) and a significant sleep deprivation effect ($F_{1,24}$=5.4, p=0.029). Body temperature reached significantly higher levels after 100 min of sleep deprivation and gradually returned to control levels 20 min after termination of the sleep deprivation procedure.

Plasma corticosterone levels were not significantly affected by sleep deprivation.

Novelty exposure and consecutive recovery

Figure 1 also shows the locomotor activity, heart rate, blood pressure, body temperature and corticosterone responses to 15-min novelty exposure or non-novelty control conditions starting 1.5 h after the termination of sleep deprivation and for the 75-min post-novelty phase (panels A-E, respectively). Under the non-novelty control conditions, a slight but non-significant increase in all measurements is visible during or briefly after the novelty exposure experienced by the other animals in the same room.

ANOVA showed that novelty exposure induced a significant time*novelty interaction ($F_{18,432}$=13.9, ε=0.2, p<0.001) and a significant novelty effect ($F_{1,24}$=25.9, p<0.001) but no effect of prior sleep deprivation on locomotor activity levels. Locomotor activity levels stayed elevated during exposure to the novel cage until 15 min after termination of novelty.

For heart rate levels a significant time*novelty interaction ($F_{18,432}$=12.4, ε=0.3, p<0.001) and a significant novelty effect ($F_{1,24}$=20.7, p<0.001) were found. The heart rate response to novelty was not affected by prior sleep deprivation. Heart rate

values were increased during exposure to novelty and gradually reached levels comparable to the non-novelty condition 30 min after termination of novelty.

For blood pressure, ANOVA showed a significant time*novelty interaction ($F_{18,432}$=9.6, ε=0.2, p<0.001) but no effects of the sleep deprivation condition. *Post-hoc* analysis only showed a significant difference between the novelty and non-novelty control condition 5 min after termination of novelty.

For body temperature a significant time*novelty effect ($F_{18,432}$=4.8, ε=0.2, p=0.003) was found. Body temperature slowly increased during novelty exposure and reached significantly higher levels 10 min after termination of novelty when the animals had already returned to their home cages. Levels comparable to the non-novelty condition were reached again after 20 min. Prior sleep deprivation had no effects on this temperature response.

Finally, for plasma corticosterone levels, ANOVA revealed a significant novelty effect ($F_{1,24}$=20.3, p<0.001) but no effects of prior sleep deprivation. Plasma corticosterone levels increased slowly during novelty, reaching significantly elevated levels at the end of the novelty exposure, which then remained elevated for 20 min.

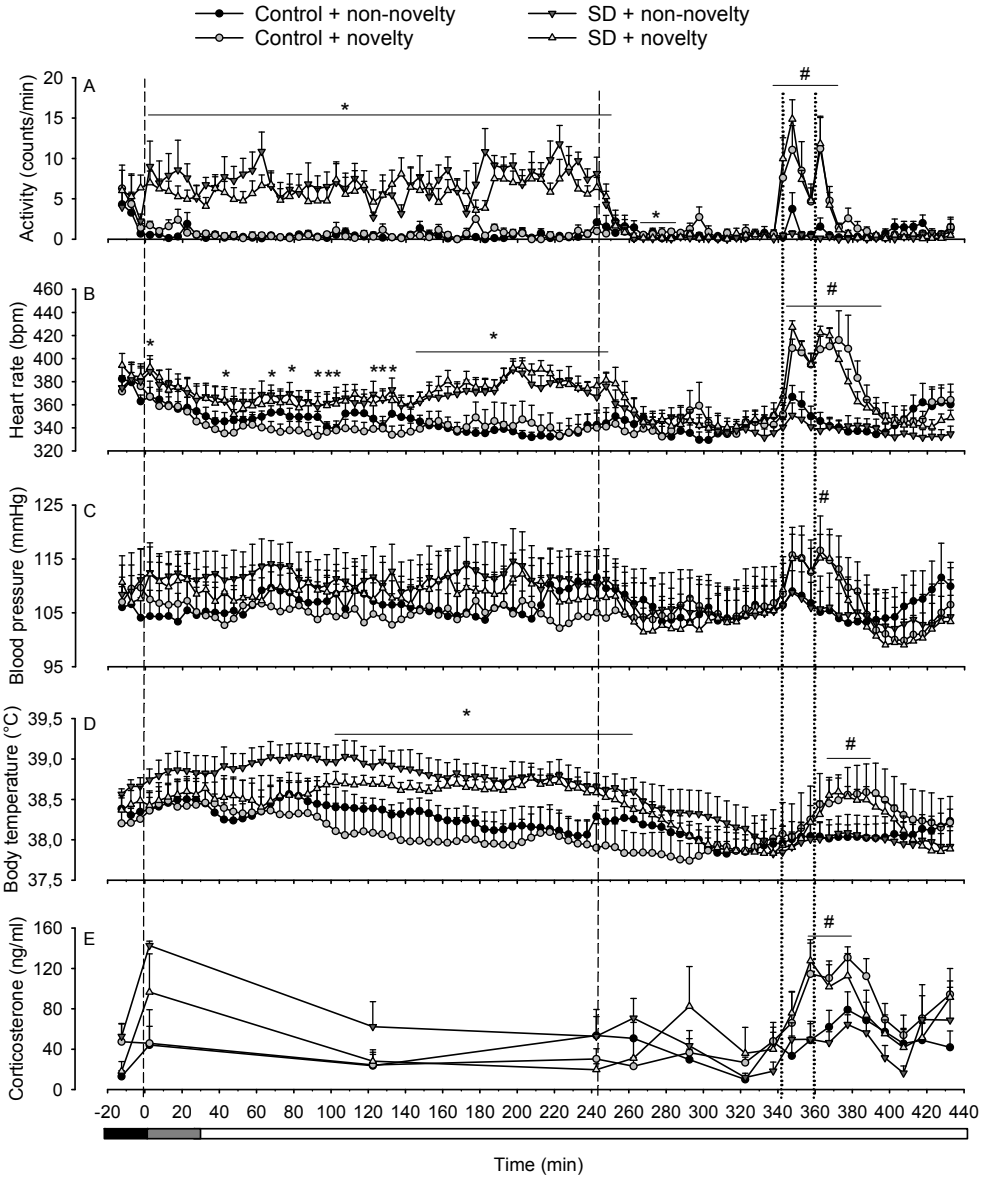

Figure 1. Time course of changes in locomotor activity (panel A), heart rate (panel B), blood pressure (panel C), body temperature (panel D) and plasma corticosterone (panel E) during the 15-min baseline period (i.e. last 15 min of dark period), the 4-h sleep deprivation or non-deprived control period (indicated by the area between the dashed lines), the 1.5-h recovery period and subsequent 15 min exposure to novelty or non-novelty (indicated by the area between the dotted lines) and the 75-min post-novelty period. Data are expressed as 5-min averages + SEM for the physiological measurements and as 10-120 min mean values + SEM for the corticosterone measurements. These are shown for the non-deprived control condition without novelty exposure (n=8), sleep deprivation without novelty exposure (n=6), non-deprived control condition with novelty exposure (n=6) and sleep deprivation with novelty exposure (n=8). The bars underneath the graph indicate the dark (black); the 30-min rise (grey) and light (white) period. Significance: sleep deprivation versus non-deprived control *p<0.05, novelty versus non-novelty, #p<0.05.

Experiment 2: Dynamics of the behavioural, physiological and hormonal responses to 6-h sleep deprivation with subsequent novelty exposure

Sleep deprivation and consecutive recovery

Locomotor activity, body temperature, and plasma corticosterone responses to 6 h of sleep deprivation or the non-deprived control condition and subsequent 1.5-h post-deprivation recovery are illustrated in Figure 2 (panel A-C, respectively). Under non-deprived control conditions, after the lights-on cue, stable values were reached for all measurements

Sleep deprivation affected the locomotor activity response significantly ($F_{1,15}$=17.1, p=0.001) and induced a significant time*sleep deprivation interaction ($F_{91,1365}$=2.2, ε=0.1, p=0.04). *Post-hoc* analysis showed that the sleep-deprived rats had increased locomotor activity levels throughout most of the sleep deprivation period. Upon termination of the sleep deprivation procedure, control activity levels were reached within 5 min.

For body temperature, ANOVA showed a significant time*sleep deprivation interaction ($F_{91,1365}$=3.1, ε=0.1, p=0.007) and a close to significant overall sleep deprivation effect ($F_{1,15}$=3.4, p=0.087). Body temperature reached significantly higher levels after 40 min of sleep deprivation and stayed elevated at several time points during the remainder of the sleep deprivation. Upon termination of the sleep deprivation procedure, control temperature levels were reached within 5 min.

Plasma corticosterone levels were unaffected by sleep deprivation.

Novelty exposure and consecutive recovery

Figure 2 also shows the locomotor activity, body temperature and plasma corticosterone responses to 15-min novelty exposure or non-novelty control conditions starting 1.5 h after the termination of the sleep deprivation, and the 75-min post-novelty phase (panel A-C, respectively). Under non-novelty control conditions, locomotor activity, body temperature and plasma corticosterone levels were not significantly affected by the novelty exposure experienced by the other animals in the same room.

Novelty exposure induced a significant time*novelty interaction ($F_{18,270}$=26.6, ε=0.3, p<0.001) and a significant overall effect ($F_{1,15}$=97.3, p<0.001) but no effect of prior sleep deprivation on locomotor activity levels. Locomotor activity levels were increased throughout the exposure to novelty and returned to non-novelty control levels 15 min after termination of novelty.

For body temperature a significant time*novelty interaction ($F_{18,270}$=12.7, ε=0.3, p<0.001) and a significant novelty effect ($F_{1,15}$=10.4, p=0.006) were found. In this case, a significant sleep deprivation effect was also found ($F_{1,15}$=9.0, p=0.009) but no interaction with novelty effects. *Post-hoc* analysis showed that body temperature gradually increased during novelty, reaching significantly elevated levels when the animals returned to their home cages. Non-novelty control levels were reached again 45 min later. Furthermore, the sleep-deprived animals, regardless of exposure to novelty, had lower body temperature levels at several time points.

For plasma corticosterone a significant time*novelty effect ($F_{8,120}$=4.6, ε=0.4, p=0.006) and a significant novelty effect ($F_{1,15}$=7.7, p=0.014) without effects of prior sleep deprivation were found. Plasma corticosterone levels were increased during novelty until 30 min afterwards.

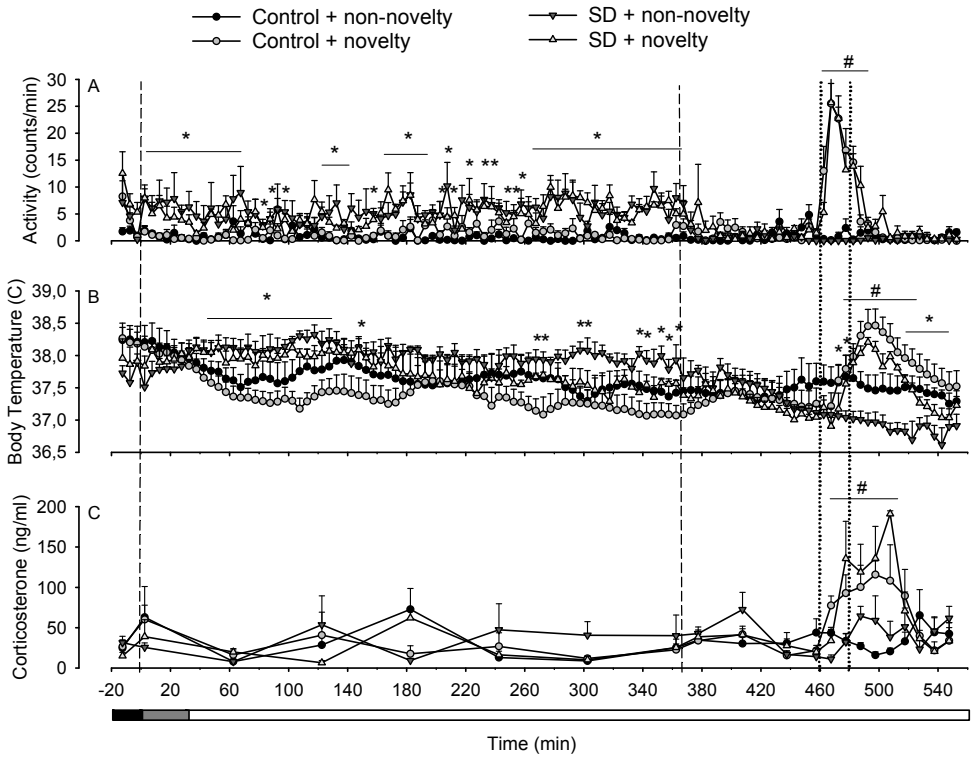

Figure 2. Time course of changes in locomotor activity (panel A), body temperature (panel B) and plasma corticosterone (panel C) during the 15-min baseline period (i.e. last 15 min of dark period), the 6-h sleep deprivation or non-deprived control period (indicated by the area between the dashed lines), the 1.5-h recovery period and subsequent 15 min exposure to novelty or non-novelty exposure (indicated by the area between the dotted lines) and the 75-min post-novelty period. Data are expressed as 5-min averages + SEM for the physiological measurements and as 10-60 min mean values + SEM for the corticosterone measurements. These are shown for the non-deprived control condition without novelty exposure (n=4), sleep deprivation without novelty exposure (n=5), non-deprived control condition with novelty exposure (n=5) and sleep deprivation with novelty exposure (n=5). The bars underneath the graph indicate the dark (black); the 30-min rise (grey) and light (white) period. Significance: sleep deprivation versus non-deprived control *p<0.05, novelty versus non-novelty, #p<0.05.

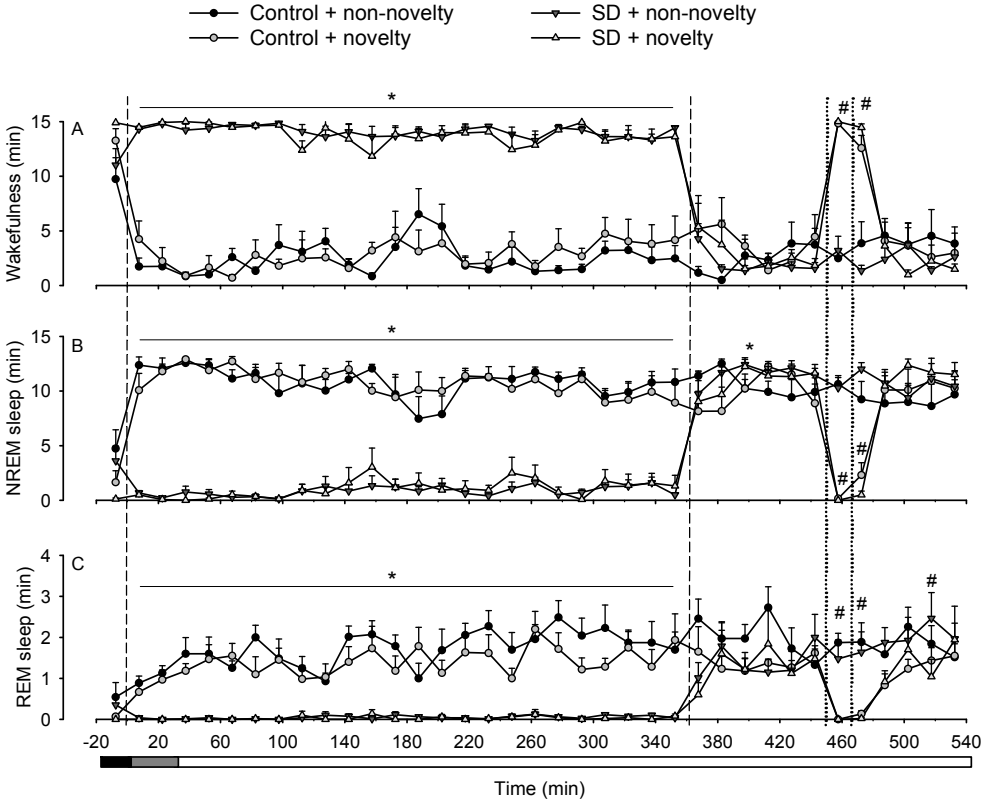

Figure 3. Time course of changes in the amount of wakefulness (panel A), NREM sleep (panel B) and REM sleep (panel C) during the 15-min baseline period (i.e. last 15 min of dark period), the 6-h sleep deprivation or non-deprived control period (indicated by the area between the dashed lines), the 1.5-h recovery period and subsequent 15 min exposure to novelty or non-novelty (indicated by the area between the dotted lines) and the 75-min post-novelty period. Data are expressed as 15-min averages + SEM and are shown for the non-deprived control condition without novelty exposure (n=7), sleep deprivation without novelty exposure (n=8), non-deprived control condition with novelty exposure (n=6) and sleep deprivation with novelty exposure (n=5). The bars underneath the graph indicate the dark (black); the 30-min rise (grey) and light (white) period. Significance: sleep deprivation versus non-deprived control *p<0.05, novelty versus non-novelty, #p<0.05.

Experiment 2. Changes in sleep-wake distribution and NREM delta power in response to 6 h of sleep deprivation with subsequent novelty exposure

Sleep deprivation and consecutive recovery

The amount of wakefulness, NREM sleep and REM sleep during and after 6-h sleep deprivation or non-deprived control conditions is illustrated in Figure 3 (panel A-C, respectively). Under non-deprived control conditions, animals spent most of their time asleep.

Sleep deprivation significantly increased the time spent in wakefulness with a concomitant reduction in time spent in NREM and REM sleep. For wakefulness, ANOVA showed a significant time*sleep deprivation interaction ($F_{29,638}$=23.0, ε=0.3, p<0.001) and a significant sleep deprivation effect ($F_{1,22}$=416.4, p<0.001). For NREM sleep a significant time*sleep deprivation interaction was found ($F_{29,638}$=23.6, ε=0.3, p<0.001) together with an overall significant sleep deprivation effect ($F_{1,22}$=332.4, p<0.001). For REM sleep also a significant time*sleep deprivation interaction ($F_{29,638}$=5.6, ε=0.3, p<0.001) and a significant sleep deprivation effect ($F_{1,22}$=140.3, p<0.001) were found. The time spent in wakefulness and NREM sleep was only affected during the sleep deprivation period and returned to stable control levels within 15 min upon termination of the sleep deprivation procedure. NREM sleep was slightly but significantly increased at 30 - 45 min after sleep deprivation. For REM sleep stable control levels were reached within 30 min upon termination of the sleep deprivation procedure.

Novelty exposure and consecutive recovery

Figure 3 also shows the amount of wakefulness, NREM sleep and REM sleep during 15-min novelty exposure or non-novelty control conditions starting 1.5 h after the termination of the sleep deprivation, and the 75-min post-novelty phase.

Exposure to novelty significantly increased the time spent awake with a concomitant decrease in time spent in NREM and REM sleep. For time spent in wakefulness, ANOVA showed a significant time*novelty interaction ($F_{5,110}$=22.5, ε=0.7, p<0.001) and a significant novelty effect ($F_{1,22}$=15.1, p=0.001) but no effect of the preceding sleep deprivation treatment. For NREM sleep, a significant time*novelty interaction

($F_{5,110}$=22.6, ε=0.7, p<0.001) together with a significant novelty effect ($F_{1,22}$=9.4, p=0.006) were found but again no effect of prior sleep deprivation. For REM sleep, also a significant time*novelty interaction ($F_{5,110}$=2.7, ε=0.8, p=0.039) with a significant novelty effect ($F_{1,22}$=17.9, p<0.001) but without effect of prior sleep deprivation was found. *Post-hoc* analysis showed that the time spent awake was significantly increased throughout the novelty exposure until 15 min afterwards. This increase was paralleled by a significant reduction in NREM and REM sleep that returned to levels comparable to the non-novelty control condition within 30 min upon termination of novelty. Furthermore, for REM sleep, a significant reduction as compared to the non-novelty condition was found 45 - 60 min upon termination of novelty.

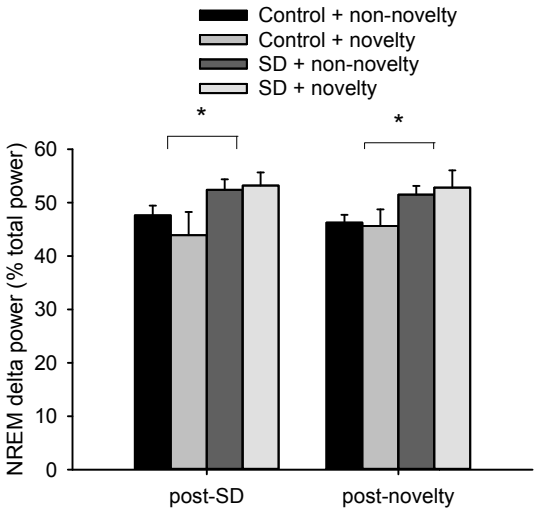

Figure 4. The NREM delta power expressed as percentage of the total power is shown for the 1.5-h post-sleep deprivation recovery period and for the 75-min post-novelty period. These are shown for the non-deprived control condition without novelty exposure (n=7), non-deprived control condition with novelty exposure (n=6), sleep deprivation without novelty (n=8) and sleep deprivation with novelty (n=5). Data are expressed as means + SEM. *type effect: sleep deprivation versus non-deprived control, p<0.05

The delta power during NREM sleep expressed as percentage of the total power during the 1.5-h post-deprivation recovery phase and during the 75-min post-novelty phase is illustrated in Figure 4. ANOVA showed a significant sleep deprivation effect ($F_{1,22}$=7.4, p=0.012) but there was no additional effect of the novelty exposure. *Post-hoc* analysis showed a significant increase in NREM delta power by sleep deprivation during the post-deprivation and during the post-novelty exposure phase.

DISCUSSION

The aim of the present study was to assess how 4 and 6 h of sleep deprivation affect physiological and hormonal measures, indicative of sympathetic and HPA axis

activity, and how sleep deprivation affects the subsequent reactivity of these stress systems to novelty exposure. The parameters were measured simultaneously and at high-frequency in freely moving rats, while reducing stress-confounding effects of the sampling and sleep deprivation procedure as much as possible. All measurements were taken during the light phase; the circadian resting phase in rats. Under non-deprived control conditions the animals indeed were inactive and asleep most of the observation period. Furthermore, normal and stable levels of heart rate, blood pressure and body temperature and also of plasma corticosterone were maintained throughout most of the control condition (Buttner and Wollnik, 1982; De Boer and van der Gugten, 1987). Taken together, this suggests that the animals under non-deprived control conditions were not affected by the sampling procedures.

The polysomnographic recordings confirm that 6-h gentle handling sleep deprivation effectively prevented rats from sleeping. During sleep deprivation, only short and scattered NREM microsleeps were observed whereas REM sleep was completely suppressed The short NREM microsleeps that were seen are practically unavoidable during sleep deprivation and are an indication for increasing sleep pressure during deprivation(Friedman et al., 1979). The efficacy of our sleep deprivation procedure is also confirmed by the occurrence of a compensatory rebound afterwards. Furthermore, 30 – 45 min after sleep deprivation, a mild but significant increase in NREM sleep was visible as compared to the non-deprived control condition. Also, the delta power during NREM sleep was elevated during the post-sleep deprivation recovery period, which is considered a marker for homeostatic sleep drive and an indicator of sleep intensity (Borbely and Neuhaus, 1979; Franken et al., 1991; Borbely, 1998). This increased NREM delta power was also observed during the post-novelty exposure phase. This compensatory response is similar to that reported in previous studies using short sleep deprivation in rats (Borbely and Neuhaus, 1979; McKenna et al., 2008). Thus, 6 h of sleep deprivation by our gentle handling procedure was sufficient to deprive rats from sleep and to cause effects that are still detectable after novelty exposure.

Sleep deprivation induced mild, temporal increases in gross locomotor activity, heart rate, blood pressure and body temperature. The increase in heart rate and blood pressure in response to sleep deprivation are indicative of sympathetic activity (Axelrod and Reisine, 1984; Johnson et al., 1992), which may also be responsible for

the mild but gradual increase in body temperature. Such an increase in sympathetic activity is necessary to support the wakefulness and activity associated with the sleep deprivation (Leal-Cerro et al., 2003). Furthermore, the observed sympathetic activation is in agreement with other studies showing higher catecholamine levels and also higher heart rate and blood pressure levels during wakefulness as compared to during sleep in both animals and humans (Akerstedt and Froberg, 1979; Tochikubo et al., 1996; Irwin et al., 1999; Sforza et al., 2004). Plasma corticosterone levels were not affected by short sleep deprivation. While several human and animal studies showed increased glucocorticoid levels during sleep deprivation (von Treuer et al., 1996; Suchecki et al., 1998; Spiegel et al., 1999; Chapotot et al., 2001; Hipolide et al., 2006; Sgoifo et al., 2006; Meerlo et al., 2008), in most cases these increases were moderate and various other studies found no significant changes in cortisol or corticosterone levels at all (Rechtschaffen et al., 1983; Follenius et al., 1992). Also the SAM activation in response to sleep deprivation in our study should be considered mild. In fact, sleep deprivation appeared to prevent the normal decline in locomotor activity, heart rate and body temperature that was observed in the control situation at the beginning of the light phase, i.e., circadian resting phase. In addition, it maintained levels comparable to the normal waking levels as measured during the last 15 min of the dark period preceding sleep deprivation. Indeed, these levels did not exceed the average values observed during the active phase of the circadian cycle under home cage conditions as reported in previous studies (Buttner and Wollnik, 1982). Upon termination of the sleep deprivation procedure, the various responses showed different time courses but all returned to resting control values within 20 min. In general, heart rate, blood pressure and locomotor activity showed a rapid decline to control values upon termination, whereas body temperature showed a slower decline. The rapid decline in heart rate and blood pressure is in agreement with the knowledge that SAM activation results in a rapid release of catecholamines, which have immediate effects on the cardiovascular system (Axelrod and Reisine, 1984; Johnson et al., 1992). For body temperature a delay was expected, as body temperature will change due to changes in metabolism, muscle activity and peripheral vasoconstriction. Altogether, sleep deprivation affected the SAM system mildly and recovery occurred within 20 min upon termination. Moreover, no

differences between 4 and 6 h of sleep deprivation on gross locomotor activity, body temperature and plasma corticosterone levels were observed.

A common issue of discussion regarding sleep deprivation studies in laboratory animals is that the observed effects may not only be a consequence of sleep loss per se, but fully or partly due to the stressful components of the procedure used to sleep deprive the animals (Meerlo et al., 2008). As discussed, our sleep deprivation method just caused a mild activation of the SAM system with levels not above those reached during normal wakefulness. Moreover, the HPA axis, at least in terms of glucocorticoid release as only plasma corticosterone was measured, was not affected. In that sense, our method was not associated with a high degree of stress. On the other hand, some rats appeared to be in an agitated state towards the end of the sleep deprivation session. Even though published studies do not mention agitation specifically, hyperactivity and increased aggressiveness have been reported after sleep deprivation (Hicks et al., 1979; Gessa et al., 1995; Tartar et al., 2008). Whether this is a direct consequence of the method used to induce wakefulness or of sleep loss per se remained unclear. Interestingly, this agitation was only observed at the end of the sleep deprivation session and was not reflected in a change in stress system activation. This suggests that this agitation may be the result of accumulating sleep debt and tiredness, rather than a consequence of stress induced by the sleep deprivation method but future studies are needed to confirm this.

In order to study effects of prior sleep deprivation on subsequent responses to a new challenge, an undisturbed recovery period following sleep deprivation was included before rats were exposed to novelty (Meerlo et al., 2002; Sgoifo et al., 2006). As the results show, all physiological and behavioural measurements in response to sleep deprivation had reached control levels within this brief recovery period and therefore the subsequent novelty responses were not confounded by altered basal levels. The animals under sleep-deprived conditions had a sleep deficit but were not in an agitated state anymore when exposed to novelty exposure. Novelty exposure induced an increase in locomotor activity, heart rate, blood pressure, body temperature and plasma corticosterone. Locomotor activity, heart rate and blood pressure increased immediately upon novelty exposure whereas the increases in body temperature and plasma corticosterone were much slower, reaching significance only by the end of the novelty exposure or even when the

animals were already returned to their home cages. Levels comparable to non-novelty control conditions were reached within 30 min upon termination of novelty exposure for all measurements. These patterns of responses to novelty exposure were comparable with those reported in our previous study (Beerling et al., 2011) (Chapter 2). Clearly, physical activity was induced by novelty and the SAM system and HPA axis were activated, yet, previous 4 or 6 h of sleep deprivation with subsequent 1.5 h recovery did not affect these responses. This finding is in agreement with previous studies reporting that 20 h of total sleep deprivation did not affect the corticosterone and ACTH response to physical stressors like restraint and inescapable foot shock stress (Meerlo et al., 2002; Novati et al., 2008). Whether the SAM system was also unaffected was not addressed in those studies. Those studies also showed that a longer duration of total sleep deprivation or repeated sleep restriction gradually lead to an attenuated ACTH response (Meerlo et al., 2002; Novati et al., 2008). It was therefore suggested that the effects of sleep deprivation on stress reactivity may accumulate over time. Whether the behavioural, physiological and hormonal responses to novelty exposure will be affected by prolonged sleep deprivation remains to be studied.

Overall, 6 h of sleep deprivation by our gentle handling procedure is sufficient to deprive rats from sleep and to cause effects that are still detectable after novelty exposure. The present study also shows that short sleep deprivation induces mild behavioural activity and is a mild activator of the SAM system while the HPA axis is unaffected. We furthermore conclude that acute sleep deprivation does not affect the subsequent neuroendocrine and physiological stress response to novelty exposure as measured 1.5 h after sleep deprivation termination. Thus, acute sleep deprivation does not affect stress system reactivity to novelty exposure, despite a significant sleep loss.

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Chapter 5

Sleep-deprived rats have normal behavioural and physiological responses to frustrative non-reward stress

W. Beerling[1, 2], J.M. Koolhaas[2], A. Ahnaou[1], P. Meerlo[2] and W.H.I.M. Drinkenburg[1]

[1] Department of Neuroscience, Janssen Research & Development, a division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, 2340 Beerse, Belgium;
[2] Department of Behavioural Physiology, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

ABSTRACT

The outcomes and the interpretation of the outcomes of studies combining sleep deprivation with exposure to a challenging situation do not only depend on the sleep deprivation duration but also on the nature of the challenging situation. We here hypothesize that a situation, in order to be truly stressful, needs to contain uncontrollability and unpredictability elements and should involve an environmental challenge which the animal meets in everyday life in its natural habitat. Therefore, the present study used an operant-conditioning task in which rats are trained to lever press for food or water reinforcements. Next, when lever pressing is suddenly no longer rewarded, the animal faces absence of the expected behavioural consequence and supposedly experiences loss of control. Such loss of control has been associated with a strong activation of the stress systems and has been described as frustrative non-reward stress. This study aimed to determine in a non-confounded way how the reactivity of the sympathico-adrenomedullary (SAM) system and hypothalamic-pituitary-adrenal (HPA) axis to rewarded and non-rewarded operant lever press behaviour in rats are affected by prior acute sleep deprivation. The second aim of this study was to assess how acute sleep deprivation affects the animal's behaviour associated with the operant task. A set-up combining radiotelemetry and automated blood sampling was used for continuous monitoring of gross locomotor activity, heart rate, blood pressure, body temperature and plasma corticosterone. To do so, rats were implanted a small transmitter together with a permanent heart catheter. Sleep deprivation caused a mild, temporary increase in locomotor activity, body temperature and plasma corticosterone. Subsequent anticipation to the operant lever press task induced increases in all measurements, therefore activating the SAM system, the HPA axis and inducing behavioural activity. Animals which were subsequently not rewarded showed a reduced elevation in locomotor activity, heart rate, blood pressure and body temperature together with a slightly stronger elevation in plasma corticosterone as compared to the rewarded animals. The non-rewarded condition was thus associated with a reduced SAM system activation and reduced behavioural activity but with an elevated HPA axis activation as compared to reward. Upon recovery of the operant task, the rewarded groups reached levels comparable to before exposure to the operant task but the non-rewarded groups maintained an increased HPA axis activation that was still present during the last 30 min of the post-operant recovery period. Prior sleep deprivation did not affect the stress response to the rewarded and non-rewarded condition. Overall, we can conclude that reward induced stress system activation that mainly served as physiological support of behaviour, but that non-reward, which is uncontrollable and unpredictable, caused a delayed HPA axis recovery. This study also shows that acute sleep deprivation did not affect the neuroendocrine and physiological stress response in anticipation of the operant task or to either the rewarded or non-rewarded condition, as measured 1.5 h after sleep deprivation termination. Moreover, the performance, impulsivity and motivation of the animals during the operant task, either during reward or non-reward, were not affected by prior acute sleep deprivation.

INTRODUCTION

Existing literature suggests that sleep plays an important role in stress regulation (Akerstedt and Froberg, 1979; von Treuer et al., 1996; Suchecki et al., 1998; Irwin et al., 1999; Spiegel et al., 1999; Chapotot et al., 2001; Hipolide et al., 2006; Sgoifo et al., 2006; Meerlo et al., 2008). Both controlled human and rodent studies showed that deprivation of sleep is associated with increases in the activity of the two major neuroendocrine stress system; the sympathico-adrenomedullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) axis. Sleep deprivation may not only affect the basal activity of the stress systems, but may also affect the reactivity of these systems to challenges (Meerlo et al., 2008). The few controlled studies reported in rats suggest that short sleep deprivation (20 h of total sleep deprivation) does not affect the HPA axis response to stressful situations (Meerlo et al., 2002; Novati et al., 2008) whereas prolonged sleep deprivation (i.e. 48 h of total sleep deprivation) or 8 days of sleep restriction (repeated 20 h of sleep deprivation per day) alters the HPA axis response to brief restraint or inescapable foot shock stress in rats (Meerlo et al., 2002; Sgoifo et al., 2006; Novati et al., 2008).

The outcomes and the interpretation of the outcomes of studies combining sleep deprivation with exposure to a challenging situation do not only depend on the sleep deprivation duration but also on the nature of the challenging situation. In an earlier study we showed that acute sleep deprivation does not affect the stress response to novelty exposure (Chapter 4). A close relationship has been reported between the physiological and hormonal responses and the normal ongoing behaviour induced by this novelty exposure (Beerling et al., 2011). Consequently, the responses to novelty exposure were most adequately interpreted in terms of metabolic support of ongoing behaviour rather than the frequently used stress interpretation. In light of these findings, the next step would be to study whether acute sleep deprivation affects the responses to a truly stressful situation. As was reviewed by Koolhaas and co-workers, a truly stressful situation should consist of uncontrollability and unpredictability (Koolhaas et al., 2011). Moreover, a stressor should have good ecological validity and involve an environmental challenge, which the animal meets in everyday life in its natural habitat (Koolhaas et al., 2006). Even though short sleep deprivation has been shown not to affect the stress response to

the aversive and unpredictable situation of restraint or foot shock stress (Meerlo et al., 2008; Novati et al., 2008), this does not exclude the possibility that acute sleep deprivation affects the stress response to a more psychological stressor. Restraint and foot shock stress should be considered as physical stressors that push the animal towards a stress-physiological ceiling (Koolhaas et al., 2006).

Therefore, the present study used an operant-conditioning task in which rats are trained to lever press for food or water reinforcements. When lever pressing is suddenly no longer rewarded (extinction), the animal faces absence of the expected behavioural consequence and supposedly experiences loss of control. Such loss of control has been associated with a strong activation of the stress systems and has been described as a psychological stressor, i.e. frustrative non-reward stress. This stressor has good ecological validity as uncontrollability and unpredictability of food are part of everyday life in nature. In order to study how the stress response to frustrative non-reward is affected by sleep deprivation, physiological, hormonal and behavioural responses were studied. To achieve simultaneous and continuous measurement of these responses while reducing stress-confounding effects, radiotelemetry (Guiol et al., 1992; Van den Buuse, 1994; Leon et al., 2004; Greene et al., 2007; Tang et al., 2007) and an automated blood sampling system were used (Steffens, 1969; Thrivikraman et al., 2002; Royo et al., 2004; Abelson et al., 2005) . This study also reduced the possibility that the stress systems were activated by other factors as sleep loss per se. Therefore, gentle handling sleep deprivation was applied in this experiment. Earlier studies in our lab showed that this method induced only a mild activation of the stress systems but nonetheless did reduce sleep times significantly (Chapter 3 and 4). A 1.5-h recovery period after sleep deprivation was included to ensure that the stress systems were back to control levels before exposure to the operant task. Using these methods, the present study aimed to determine in a non-confounded way how the reactivity of the classical neuroendocrine and physiological stress systems to rewarded and non-rewarded operant lever press behaviour is affected by prior acute sleep deprivation. The operant-conditioning task additionally allows for behavioural assessment of performance, impulsivity and motivation that will give more information on the attentional performance of the animals. Sleep loss and stress system activation are both thought to affect mechanisms that are involved in attentional processes

(Johnson et al., 1992; Brown et al., 2012). Therefore, the second aim of the present study was to assess how acute sleep deprivation affects the animal's behaviour associated with an operant task.

METHODS

Animals, housing and experimental set-up

At the beginning of the experiment, 16 adult male Sprague-Dawley rats (Harlan, Horst, The Netherlands), weighing 305-340 g, were trained in the Skinner box (see experimental design). The animals were individually housed in polycarbonate cages containing bedding and cage enrichment. The home cage could be positioned in the experimental set-up. This set-up consisted of a radiotelemetry set-up (DataSciences International, St. Paul, MN, USA), an automated blood sampling system (AccuSampler Micro, DiLab, Lund, Sweden) and a skinner box (ERG, Radboud Universiteit Nijmegen, Nijmegen, the Netherlands). With 7 set-ups, not all animals could be housed in this set-up simultaneously. Rats were daily positioned within their home cages in this set-up during the training session and were housed for at least 24 h in the set-up prior to the experimental day. The room was kept at constant temperature (21 ± 1°C), controlled humidity (50 ± 10%) and a fixed 12 : 12 h light/dark regime using a 30-min dim/rise period with lights on at 06.00 a.m. The animals were daily handled for weighing purposes and water was provided *ad libitum*. Food access was restricted to 6 - 12 g of laboratory chow per day to maintain the animals at approximately 85% of their free-feeding body weights. During recovery of surgery, rats had full access to food prior to re-training. The local ethical committee approved all experimental protocols.

Experimental design

Animals were first trained in the Skinner box. When stable rates of responding were reached, the animals had their surgery. Upon surgery, two animal died, resulting in *n*=14 left to be re-trained. After reaching again a stable performance, animals were subjected to either control or sleep deprivation conditions using a balanced and cross-over Williams design, and all experienced the rewarded condition of the operant task. One week later at comparable circadian time points and with at least 3 subsequent normal training sessions in between, the animals were exposed to the same condition (control or sleep deprivation) but now all experienced the non-rewarded condition of the operant task. Another week later, animals that were sleep-deprived before became controls and the control animals were sleep-deprived, and all experienced the rewarded condition of the operant task. Another week later, the animals were exposed to the same condition (control or sleep deprivation) but experienced non-reward. Using this design, all animals were exposed to all conditions with 7 days in between and with at least 5 normal training sessions of which 3 subsequent normal training sessions in between. Seven set-ups were used in parallel to reduce variability among animals and behavioural, physiological and EEG parameters were measured continuously and automatically. Concomitantly, blood was sampled automatically and repeatedly. Because only seven animals could be tested simultaneously in the operant boxes, the animals were divided into two groups of *n*=7 that had their experimental day on a different day in the same week using a balanced design and standard circadian time window. Statistical analysis showed no significant effect of testing day therefore the data of all groups was taken together. The data of a number of subjects could not be analysed further due to insufficient quality of their telemetry signals and/or blocked blood sampling catheters. As a result, the same animals were not always present in all groups resulting in: control + reward *n*=13; control + non-reward *n*=12; sleep deprivation + reward *n*=14; sleep deprivation + non-reward *n*=13. The experimental procedures were performed during the light phase, i.e. the circadian resting phase and potentially disturbing stimuli were carefully avoided. The experimental day started with weighing the animals, connecting them to the automated blood sampling system and activating their transmitters. At least 1 h later, telemetric recordings and blood sampling started simultaneously for a 15-min baseline period. This baseline period was followed by a

6-h sleep deprivation period or by a non-deprived control period, both starting at lights-on. These were followed by a 1.0-h post-sleep deprivation recovery phase and subsequent 30-min anticipation to the operant task with subsequent 30 min of operant testing (reward or non-reward). This was followed by a 1.5-h post-operant recovery phase.

Operant task

Apparatus

The Skinner box (ERG, Radboud Universiteit Nijmegen) consisted of a retractable lever, mounted on the right of the food tray which was connected with a food pellet dispenser. A water bottle was mounted on the side of the cage to which animals had access continuously. A light stimulus was positioned inside the food tray to trigger food tray entrance when a pellet was delivered. Infrared beams detected food pellet collection. Because the animals were housed within the Skinner box set-up, a covering metal plate was placed in front of the food tray and retractable lever upon termination of training and testing.

Protocol

The protocol used was adapted from the protocol described by de Boer and co-workers (De Boer et al., 1990a). On the first day of food restriction, rats were additionally given, within their home cages, 15 highly palatable food pellets (Dustless precision pellets, 45 mg Rodent purified diet; Bio-Services, Uden, The Netherlands) to familiarize them to the taste of these reinforcers. During the following two days, animals were placed within their home cages in the experimental set-up and the covering metal plates were removed for 30 min and 20 precision pellets were placed in the food tray to habituate the animals to the place where the reinforcer is delivered. Also 10 pellets were placed on the lever to familiarize the animals with the lever. Subsequently, animals were trained to press the lever to obtain the food pellets. Every session was preceded by 15 minutes during which the animals were placed within their home cages in the experimental set-up, followed by a 30-min anticipation

period during which the covering metal plate was removed and the animal had access to the retracted lever and food tray. After 30 min, the lever would come out and the training session started. Training was done with 3 groups of animals that subsequently completed such a session. The order in which the 3 groups were trained was changed every day and every day a group was housed overnight in the experimental set-up. Animals were first given 5 sessions of continuous reinforcement with a session duration of 30 min. Thereafter, they received 7 times a 30-min session with reinforcements presented according to a variable interval (VI) 15-s schedule with a range of 2 to 32 s. Subsequently, the animals had their surgery and were allowed to recover for 9-13 days with full access to food. Upon recovery, animals were food restricted again and re-trained in two groups on one continuous reinforcement session followed by 8 days on the VI-15 reinforcement schedule. Stable performance was reached for all animals during this period. Every experimental day was preceded by at least 3 subsequent training sessions and the animals were housed for at least 24 h in the experimental set-up prior to the experimental day. During the experimental days, rats were exposed to 30 min of anticipation with subsequent 30-min reinforcement on the VI-15 schedule or 30-min extinction. During extinction, the lever was available but no food was delivered in the food tray. This is further described as the non-rewarded condition and is also referred to as the frustrative non-reward stress.

Analysis

For the training days, the number of lever presses, feeder visits and rewards was determined. Also the efficiency was determined by dividing the number of rewards by the number of lever presses. These were calculated in order to determine when stable performance was reached. For the experimental day, the number of trials that were completed, the number of lever presses and the number of visits to the feeder were determined for the 30-min rewarded or non-rewarded session.

Sleep deprivation

Sleep deprivation started at lights-on using the gentle handling procedure according to published methods (Grassi-Zucconi et al., 1993; Grassi-Zucconi et al., 2006). In short, whenever the animal showed behavioural signs of sleep, it was stroked on its back or gently moved. When this did not result in visual activation of the animal, it was lifted from its cage for a few seconds. In a previous study, this method has shown to effectively induce wakefulness during the sleep deprivation period (Chapter 4). Sleep deprivation was terminated after collecting the *t*= 365 min blood sample, which made sleep deprivation last 6 h and 5 min, which is further described as 6 h. Immediately following sleep deprivation, animals were left undisturbed. In order to reduce stress caused by the sleep deprivation method, animals were handled for at least 10 min on the two days preceding the experimental day. For the control procedure, animals were left undisturbed and non-deprived in their home cages in the same experimental room.

Surgery

After successfully finishing the Skinner Box training, animals were chronically implanted with a transmitter and a permanent heart catheter. Surgery was performed under O_2-N_2O-isoflurane anaesthesia. The analgesic Piritramide (dipidolor, 0.025 mg/kg, 0.1 ml/100 g body weight) was administered subcutaneously at start of surgery. A transmitter to record gross locomotor activity, heart rate, blood pressure, body temperature and cortical EEG (TL11M2-C50-PXT, DataSciences International) was implanted intraperitoneally and attached to the abdominal wall to ensure stabilization (Greene et al., 2007). The blood pressure catheter of the transmitter was guided into the femoral artery. Furthermore, two biopotential leads for cortical EEG measurements were led subcutaneously to the skull and the bare ends were placed in contact with the dura at 3 mm on either side lateral to the midline and 6 mm posterior to bregma and anchored to the skull with screws and dental cement (Tang et al., 2007). In addition, animals received a jugular vein catheter for blood sampling (Steffens, 1969; Thrivikraman et al., 2002). The catheter (CBAS heparin-coated polyurethane; Instech Solomon, Plymouth Meeting, USA) was inserted through the

right jugular vein with the tip of the catheter reaching the entrance of the right atrium. The catheter was led subcutaneously through a Dacron button that was attached to the skin in the dorsal region of the neck. The Dacron button was connected through a spiral to the swivel and connected either to the infusion pumps or to the automated blood-sampling machine. After surgery, animals were connected to a continuous infusion of saline + heparin (10 IU/ml; 0.24 ml/h) to ensure the catheter to stay open. The animals were allowed to recover for 9-13 days.

Biotelemetric data acquisition and analysis

Using the implanted transmitter and the Dataquest A.R.T. System (DataSciences International) behavioural, physiological and polysomnographic signals were telemetrically monitored. The transmitter emitted blood pressure, body temperature and EEG dependent frequency modulated signals. The gross locomotor activity signals were obtained by monitoring changes in the received signal strength that resulted from horizontal movement of the animals. Heart rate values were calculated from the blood pressure registrations. The signals were received by an antenna board placed underneath each animal's cage and were processed by a computer using Dataquest Labpro software (DataSciences International). Locomotor activity, heart rate, blood pressure and body temperature data were sampled from all eight animals in 10-s epochs and were averaged and stored into 30-min bins. Deviations as compared to the second half hour of the post-sleep deprivation period were also calculated for the anticipation period, exposure to the operant task and the recovery thereof. Due to artefacts in the EEG signals, these were not further analysed.

Blood sampling and assays

Blood was sampled using the automated blood sampling system and the jugular vein catheter. Animals were habituated to the blood sampling procedures to minimize possible stress-confounding effects of it. Blood was sampled every 10 – 180 min in sample volumes of 60-µl. Samples were taken every 180 min during sleep deprivation and every 30 min during recovery of sleep deprivation. Samples were taken every 10 min during the anticipation period, during the operant task and during

the 1.5-h recovery after the operant task. The samples were stored inside the machine at 4°C until centrifuged for 5 min at 3210 rpm. Subsequently, plasma was stored at –80°C until assayed in duplicate for corticosterone with a double antibody radioimmunoassay (Mouse/Rat Corticosterone^{125}I RIA Kit, MP Biomedicals, Solon, OH, USA) with a sensitivity of 3.0 ng/ml. Thirty-minute averages were calculated from the anticipation period until the end of the 1.5-h post-operant recovery period.

Statistics

To assess the effect of sleep deprivation and non-reward on all variables, a repeated measures ANOVA with Greenhouse-Geisser correction epsilon (ε) for violation of the homogeneity in variance assumption was used. Even though a cross-over design was initially used, the data of the same animals was not always present in all groups. Therefore, treatment was used as a between-subjects factor rather than as a within-subject factor. Sleep deprivation (sleep deprivation vs. control) and non-reward (non-reward vs. reward) were used as between-subject factors and the different time points or periods as within-subject factor. When appropriate, *post-hoc t*-tests were performed to determine the specific time points at which the groups differed. Analyses were done for two different time blocks: the period of sleep deprivation with 1.0 h post-sleep deprivation recovery and for the anticipation period, exposure to the operant task with 1.5-h post-operant recovery. If these ANOVA analyses did not show significant effects, the analyses were also done for the sleep deprivation and post-deprivation phase separately, and for the anticipation with exposure to the operant task and the post-operant phase separately, in order to check for an effect in the recovery. Since this did not give a difference in significance, the results are only shown and discussed for the analyses done for the period of sleep deprivation with post-deprivation recovery and for the anticipation period, exposure to the operant task with post-operant recovery.

For the operant task performance, *t*-tests were used to assess the effect of sleep deprivation or non-reward. The level of significance was accepted at *p*-values <0.05. Data are presented as mean + SEM.

RESULTS

Dynamics of the behavioural, physiological and hormonal responses to 6 h of sleep deprivation with subsequent frustrative non-reward stress

Sleep deprivation and consecutive recovery

Data on locomotor activity, heart rate, blood pressure, body temperature and corticosterone responses during 6-h sleep deprivation or the non-deprived control condition and the 1.0-h post-deprivation recovery phase are illustrated in Figure 1 (panels A-E, respectively).

ANOVA showed a significant time*sleep deprivation interaction for locomotor activity ($F_{13,624}$=10.4, ε=0.5, p<0.001), blood pressure ($F_{13,624}$=5.6, ε=0.6, p<0.001), body temperature ($F_{13,624}$=2.0, ε=0.5, p=0.009) and plasma corticosterone ($F_{4,192}$=3.9, ε=0.6, p=0.006). A significant effect of time was found for all measurements. An overall sleep deprivation effect was found for locomotor activity ($F_{1,48}$=84.8, p<0.001), heart rate ($F_{1,48}$=4.1, p=0.05) and plasma corticosterone ($F_{1,48}$=8.9, p=0.005). For blood pressure and plasma corticosterone, a significant time*sleep deprivation*non-reward effect was also found ($F_{13,624}$=2.6, ε=0.6, p=0.01; $F_{1,48}$=6.0, p=0.018 respectively). *Post-hoc* analysis showed increased activity levels during the sleep deprivation period that reached control levels immediately upon termination of the sleep deprivation procedure. Heart rate reached significantly higher levels at some time points during the last 3-h of the sleep deprivation period and mainly as compared to the control + reward group. For blood pressure, only one time point showed a significant increase for the sleep-deprived + reward group as compared to the control + reward group. In contrast, body temperature levels were significantly increased by sleep deprivation during most of the sleep deprivation period. For plasma corticosterone, at baseline, the control + reward group had significantly lower corticosterone levels as compared to the sleep-deprived + non-reward group. These lower levels were reached again during the last 5 min of the sleep deprivation period as compared to the sleep deprivation + reward group and during the first 30 min of recovery of sleep deprivation as compared to all groups. Only during the first 5 min of the sleep deprivation period, the sleep deprivation + reward group had significantly higher plasma corticosterone levels as compared to all groups.

Upon termination of the sleep deprivation procedure, all responses reached control levels within 1 h. The only exemption was the body temperature for the sleep-deprived + reward group, which stayed significantly elevated as compared to the other groups.

Frustrative non-reward stress and consecutive recovery

Data on the locomotor activity, heart rate, blood pressure, body temperature and corticosterone responses during the anticipation period starting 1 h after termination of the sleep deprivation, and during exposure to the operant task with subsequent 1.5-h post-operant recovery phase are illustrated in Figure 1 (panels A-E, respectively).

ANOVA showed a significant time*non-reward interaction for locomotor activity ($F_{4,192}$=3.6, ε=0.4, p=0.046), heart rate ($F_{4,192}$=10.6, ε=0.7, p<0.001), blood pressure ($F_{4,192}$=9.4, ε=0.5, p<0.001) and body temperature ($F_{4,192}$=4.9, ε=0.5, p=0.009). A significant effect of time was found for all measurements. An overall non-reward effect was found for plasma corticosterone ($F_{1,48}$=26.6, p<0.001) and a close to significant non-reward effect for body temperature ($F_{1,48}$=2.3, p=0.066). Prior sleep deprivation only affected heart rate and plasma corticosterone responses. For heart rate, a significant time*sleep deprivation interaction was found ($F_{4,192}$=2.8, ε=0.5, p=0.045) and for plasma corticosterone a significant time*sleep deprivation*non-reward interaction was found ($F_{4,192}$=3.3, ε=0.9, p=0.016).

To account for the unrecovered body temperature response for the sleep-deprived + reward group, the difference as compared to the last 30 min of the sleep deprivation recovery period was calculated for locomotor activity, heart rate, blood pressure and body temperature. These are shown in Figure 2 (panels A-D, respectively) for the anticipation period and exposure to the operant task and the 1.5-h post-operant recovery period. ANOVA showed a significant time*non-reward interaction for delta locomotor activity ($F_{4,192}$=3.6, ε=0.4, p=0.046), delta heart rate ($F_{4,192}$=10.6, ε=0.7, p=0<0.001), delta blood pressure ($F_{4,192}$=9.4, ε=0.5, p<0.001) and delta body temperature ($F_{4,192}$=4.9, ε=0.5, p=0.009). A significant time effect was also found for all responses. A significant time*sleep deprivation interaction was found for delta

heart rate ($F_{4,192}$=2.8, ε=0.7, *p*=0.045) and a significant sleep deprivation effect was found for delta body temperature ($F_{1,48}$=4.9, *p*=0.031).

In anticipation of the operant task, *post-hoc* analysis of the absolute values showed an elevated body temperature for the sleep deprivation + reward group and elevated plasma corticosterone levels for the control + non-reward group. For delta blood pressure, an increased change in blood pressure for the sleep deprivation + non-reward group as compared to the control + reward group was found.

During exposure to the operant task, a tendency for a decrease in absolute and delta locomotor activity was observed for the non-rewarded animals as compared to the rewarded animals. Furthermore, a decreased heart rate for the control + non-reward group as compared to both rewarded groups was found. For delta heart rate, this effect was only observed as compared to the control + reward group. For body temperature, higher absolute body temperature levels were found for the sleep deprivation + reward group as compared to all other groups. Whereas non-reward seems associated with lower locomotor activity, heart rate, blood pressure and body temperature levels, plasma corticosterone levels were increased during non-reward. The sleep deprivation + non-reward group had higher levels as compared to both rewarded groups.

During the first 30 min of the post-operant recovery period, the delta blood pressure was higher for both non-rewarded groups as compared to the rewarded groups. Moreover, delta body temperature was significantly decreased for the sleep deprivation + reward group as compared to both control groups. In addition, the control + non-reward group had higher plasma corticosterone levels as compared to the other groups. During the 30 – 60-min post-operant task recovery period, lower absolute activity levels were reached for the sleep deprivation + reward group as compared to the non-reward groups. This was not reflected in the delta locomotor activity and lasted for these 30 min only. During the same period, the sleep deprivation + non-reward group had a higher delta blood pressure than the control + reward group. The last 30 min of the post-operant recovery period, significantly higher corticosterone levels were reached for the non-rewarded groups as compared to the rewarded groups.

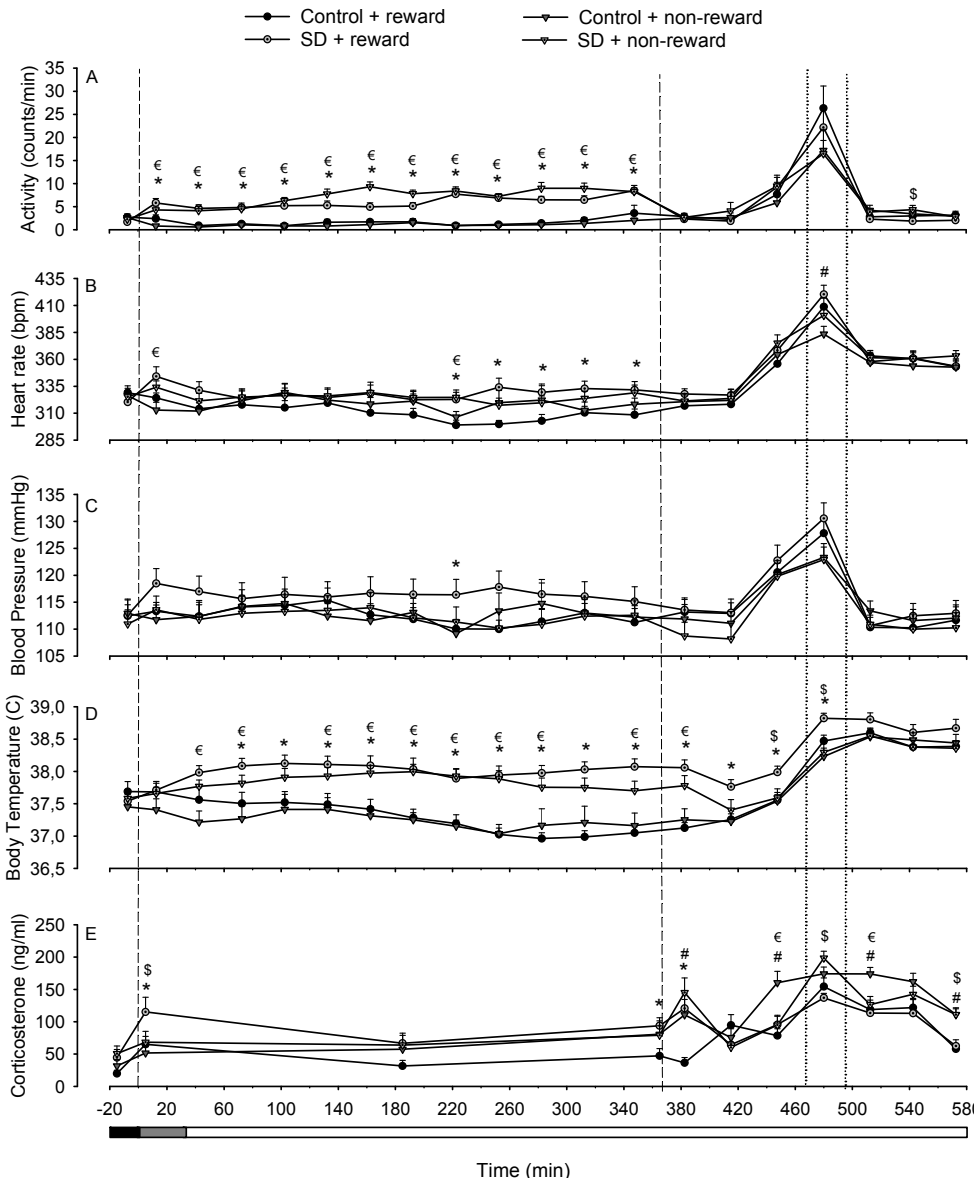

Figure 1. Time course of changes in locomotor activity (panel A), heart rate (panel B), blood pressure (panel C), body temperature (panel D) and plasma corticosterone (panel E) during the 15-min baseline period (i.e. last 15 min of dark period), the 6-h sleep deprivation or non-deprived control period (indicated by the area between the dashed lines) and the 1.0-h recovery period. It also shows the subsequent anticipation to the operant task (30 min), followed by exposure to the operant task (indicated by the area between the dotted lines) and the 1.5-h post-operant recovery period. Data are expressed as 30-min averages + SEM for the physiological measurements and as 30-180 min mean values + SEM for the corticosterone measurements. These are shown for the control condition with reward (n=13), sleep deprivation with reward (n=14), control condition with non-reward (n=12) and sleep deprivation with non-reward (n=13). The bars underneath the graph indicate the dark (black); the 30-min rise (grey) and light (white) period. Significance: control with reward versus sleep deprivation with reward *p<0.05, control with non-reward versus control with reward #p<0.05, sleep deprivation with reward versus sleep deprivation with non-reward \$$p$<0.05 and control with non-reward versus sleep deprivation with non-reward €p<0.05.

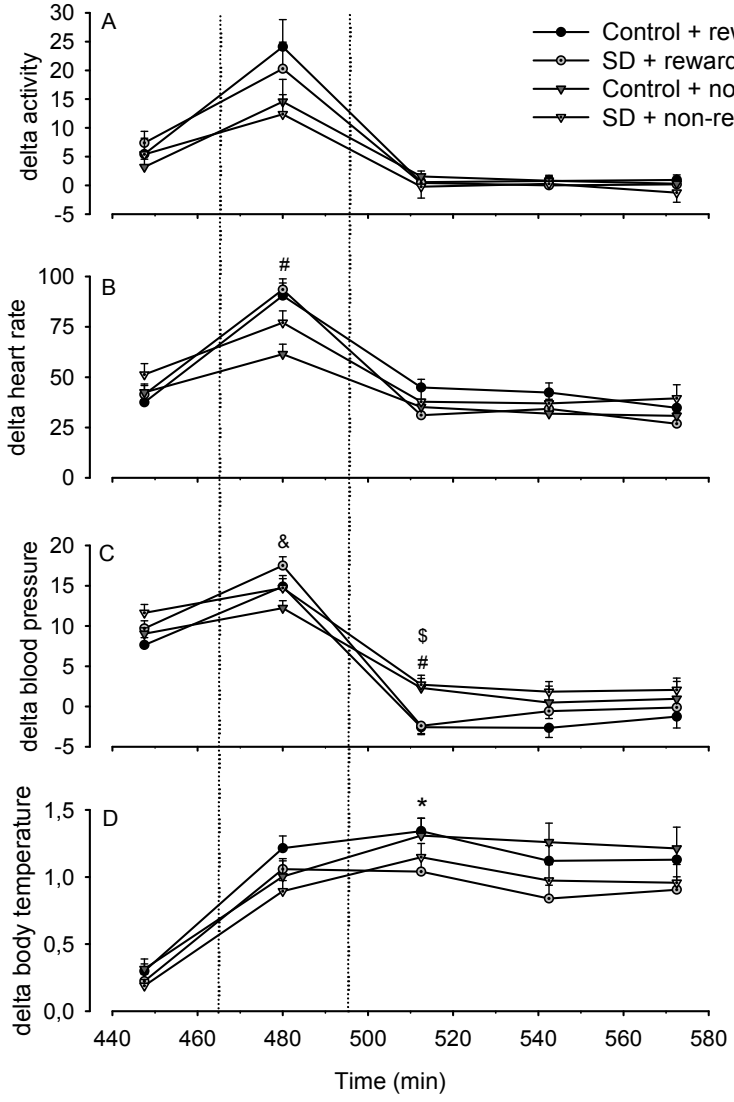

Figure 2. Time course of the changes in delta locomotor activity (panel A), heart rate (panel B), blood pressure (panel C) and body temperature (panel D) as compared to the last 30 min of the sleep deprivation recovery period. The deltas are shown in anticipation of the operant task (30 min), during exposure to the operant task (indicated by the area between the dotted lines) and the 1.5-h post-operant recovery period. Deltas are expressed as 30-min averages +/- SEM. These are shown for the control condition with reward (n=13), sleep deprivation with reward (n=14), control condition with non-reward (n=12) and sleep deprivation with non-reward (n=13). Significance: control with reward versus sleep deprivation with reward *p<0.05, control with non-reward versus control with reward #p<0.05, sleep deprivation with reward versus sleep deprivation with non-reward \$$p$<0.05 and control with non-reward versus sleep deprivation with non-reward €p<0.05.

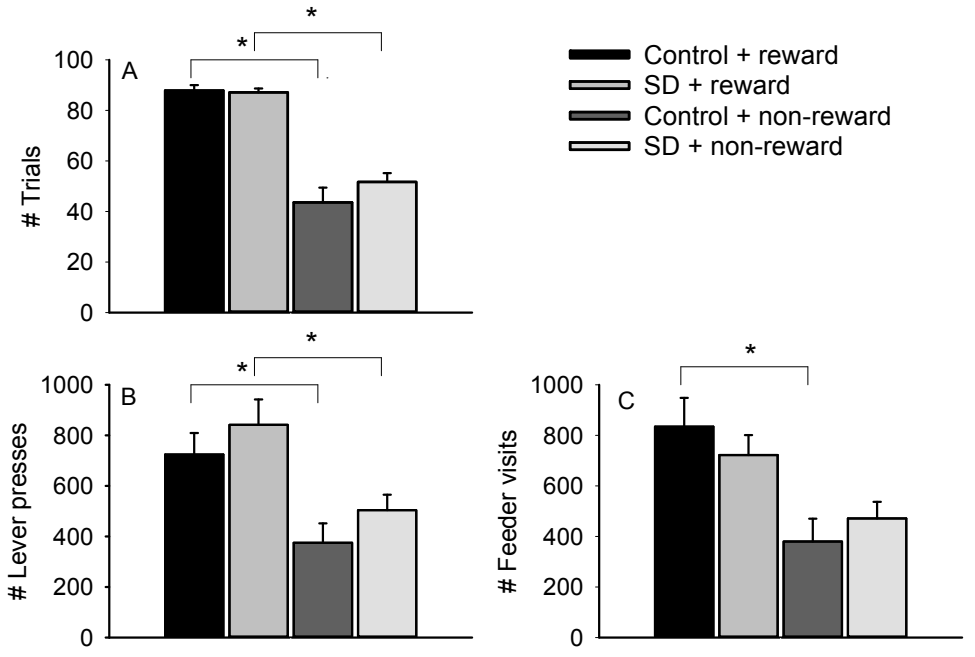

Figure 3. The operant task performance is shown for the number of trials (panel A), the number of lever presses (panel B) and the number of visits to the feeder (panel C) at 1.5 h after termination of sleep deprivation or non-deprived control conditions. Data are expressed as mean + SEM. These are shown for the control condition with reward (*n*=13), sleep deprivation with reward (*n*=14), control condition with non-reward (*n*=12) and sleep deprivation with non-reward (*n*=13). Significance: **p*<0.05

Operant task behaviour

Data on the operant task performance is illustrated in Figure 3. It shows the number of trials that was completed by the rats (panel A), the number of lever presses (panel B) and the number of visits to the feeder (panel C) at 1.5 h after termination of sleep deprivation or non-deprived control conditions. The number of trials that was initiated, the number of lever presses and the number of feeder visits were all significantly lower for those rats which did not receive a reward as compared to the ones which were rewarded. Prior sleep deprivation did not affect these behaviours.

DISCUSSION

The present study aimed to determine in a non-confounded way how acute sleep deprivation affects the reactivity of the classical neuroendocrine and physiological stress systems to rewarded and non-rewarded operant lever press behaviour. Secondly, this study aimed to assess how behaviour during the operant task is affected by acute sleep deprivation.

In anticipation of the operant lever press task, all physiological measures increased for all groups. This was not affected by prior sleep deprivation. Therefore, anticipation activated the SAM system and HPA axis and increased the activity levels which is in accordance with observations of de Boer and co-workers (De Boer et al., 1990a). The responses further increased during the operant task for all groups and a non-reward effect became visible. Animals which were not rewarded showed a reduced elevation in gross locomotor activity, heart rate, blood pressure and body temperature levels together with a slightly stronger elevation in plasma corticosterone as compared to the rewarded animals and this was not affected by sleep deprivation. Non-reward was thus associated with a reduced SAM system activation but an elevated HPA axis activation as compared to reward. Also, non-reward was associated with reduced locomotor activity levels that were reflected in reduced number of trials initiated, lever presses and feeder visits, reflecting an apparent loss of motivation. The reduced SAM system activation in the non-reward groups is in line with the view that SAM system activation is associated with physical activity (Axelrod and Reisine, 1984; Leal-Cerro et al., 2003). The findings are also in line with previous studies that found a reduced elevation in noradrenalin during non-reward (De Boer et al., 1990a) and higher HPA axis activation (Boulos and Terman, 1980; Coover et al., 1984; De Boer et al., 1990a). The increased HPA axis activation in response to non-reward is also in line with other studies showing stronger HPA axis activation when losing behavioural control (Coover et al., 1971a; Coover et al., 1971b; Davis et al., 1976; Hart and Coover, 1982; Coe et al., 1983; Osborne, 1986). De Boer and co-workers further showed that adrenalin dissociated from noradrenalin, and was increased during non-reward. They also showed a close association between SAM system activation and individual differences in behavioural lever press activity (De Boer et al., 1990a). In the present study, some individual differences in behavioural

lever press activity were found but these were not correlated with the behavioural, physiological and hormonal responses or with prior sleep deprivation.

The recovery of the physiological responses after exposure to an operant lever press task, with prior sleep deprivation, has not previously been studied. The present study shows no difference between reward and non-reward on SAM system activation during the last 30 min of the post-operant recovery period. The locomotor activity and blood pressure responses all decreased during the 1.5-h recovery period to levels comparable to before exposure to the operant task. Heart rate also decreased but maintained higher levels for all groups and body temperature did not decrease. Whereas the rewarded groups reached corticosterone levels comparable to the levels before exposure to the operant task, the non-rewarded groups maintained an increased HPA axis activation during the post-operant recovery period that was still present during the last 30 min of recovery. Prior sleep deprivation did not affect the recovery of the responses after reward and non-reward.

Our observations are in agreement with our view of the concept of stress. Activation of the SAM and HPA axis system alone is not enough to characterize stress as this activation sometimes also occurs in response to highly rewarding and controllable situations (Schuurman, 1980; Bronson and Desjardins, 1982; Shiraishi et al., 1984; De Boer et al., 1990a; Arnhold et al., 2009; Buwalda et al., 2012). This was also observed in our study, with activation of the SAM system and HPA axis during reward. In this rewarded condition, the stress system activation is thought to be crucial for mobilization of energy needed to complete the task. Indeed, our results show that the neuroendocrine and physiological stress response has similar temporal characteristics as the behavioural activity response. This suggests that the stress system activation during the rewarded condition is a reflection of the metabolic requirements for normal ongoing behavioural activity. As reviewed by Koolhaas and co-workers, a situation should be considered a truly stressful situation, when containing uncontrollability and unpredictability (Koolhaas et al., 2011). We hypothesized that a truly stressful situation causes dissociation between the behavioural activity response and the physiological/hormonal responses. In our study, the plasma corticosterone response had different temporal charecteristics than the behavioural activity response during non-reward, whereas SAM system activation showed similar temporal characteristics as the behavioural activity response. This

was also observed during the recovery of the responses, except for the body temperature response that showed a delayed recovery for all groups during the recovery and therefore also had different temporal characteristics as compared to the behavioural response. When comparing the SAM system and HPA axis activation under rewarded and non-rewarded conditions, the data suggests that the difference in SAM system activation between rewarded and non-rewarded conditions is due to differences in behavioural activity. In contrast, the plasma corticosterone difference between the rewarded and non-rewarded condition at the end of the post-operant recovery period was not due to a difference in behavioural activity as both groups displayed low behavioural activity. Therefore, we can conclude that non-reward, also referred to as frustrative non-reward stress, caused a delayed HPA axis recovery. This is in line with studies showing a delayed recovery of the corticosterone and/or SAM system response after an uncontrollable situation as compared to a controllable one, that was not due to differences in behavioural activity (Schuurman, 1980; Garcia et al., 2000; Fish et al., 2005; Arnhold et al., 2009; Koolhaas et al., 2011).

The operant task also allowed for behavioural observations. The results show that acute sleep deprivation did not affect the number of lever presses, feeder visits and trials initiated under rewarded and non-rewarded conditions. The number of initiated trials corresponds to the number of rewards received by the rewarded animals. This indicates that acute sleep deprivation did not affect the performance of the rewarded animals. Also the motivation and impulsivity of the rewarded animals was not affected by sleep deprivation. The non-rewarded animals did show a reduced motivation but this was not affected by a prior sleep deprivation. Therefore, acute sleep deprivation did not affect the attentional performance of the animals in the operant task. Sleep loss and stress system activation are both thought to affect mechanisms that are involved in attentional processes (Johnson et al., 1992; Brown et al., 2012). We suggest that more prolonged sleep deprivation may affect the behavioural measures in the operant tasked used in this study.

Under non-deprived control conditions, the animals showed normal levels of heart rate, blood pressure, body temperature and plasma corticosterone (Buttner and Wollnik, 1982; De Boer and van der Gugten, 1987) together with low activity levels. Therefore, the animals were most likely not affected by the sampling procedures. Other confounding factors may however have been present, as unexpected group

differences were observed. Baseline differences were found and also a non-reward effect was observed during the sleep deprivation period while the rats were not yet exposed to the operant task. Individual variation was not the cause of these group differences and other explanations could also not be found.

To minimize stress confounding effects of the sleep deprivation procedure as much as possible, the gentle handling sleep deprivation method was applied in this experiment. The efficacy of 6-h sleep deprivation by our gentle handling method was confirmed by our previous study (Chapter 4). Sleep deprivation enhances locomotor activity and body temperature throughout the sleep deprivation period whereas blood pressure and heart rate were only increased at some time points. These minor increases are indicative of SAM system activation that may be needed to provide metabolic support for the increased activity and wakefulness associated with sleep deprivation (Axelrod and Reisine, 1984; Johnson et al., 1992; Leal-Cerro et al., 2003). Also plasma corticosterone levels were only slightly affected by short sleep deprivation which is in accordance with other rodent studies showing moderate increases in plasma corticosterone during sleep deprivation (Suchecki et al., 1998; Sgoifo et al., 2006; Meerlo et al., 2008). For all responses, levels were not increased above those reached during the active phase under home cage conditions (Buttner and Wollnik, 1982). Therefore, acute sleep deprivation affected the stress systems mildly. Despite this mild activation of the stress systems, some animals appeared to be in an agitated state towards the end of the sleep deprivation period. This agitated state was reflected in the animals reacting more aggressive to our handling. Even though published results do not mention agitation specifically, hyperactivity and increased aggressiveness have been reported after sleep deprivation (Hicks et al., 1979; Gessa et al., 1995; Tartar et al., 2008). It is unknown whether this is a consequence of sleep loss per se or of the sleep deprivation method. We suggest that this is the result of accumulating sleep debt and tiredness, rather than a consequence of stress induced by the procedure, as agitation only occurred towards the end of the sleep deprivation session. Future studies are needed to confirm this To minimize confounding effects of this agitated state as much as possible in our experiment, we applied an undisturbed recovery period following sleep deprivation (Meerlo et al., 2002; Sgoifo et al., 2006). The animals appeared not to be in an agitated state anymore after this 1-h recovery of sleep deprivation period. We

previously showed that 6 h of sleep deprivation induced a sleep deficit that was still present after 1.5 h recovery of sleep deprivation (Chapter 4).

Overall, we can conclude that reward induced stress system activation that mainly served as physiological support of behaviour, but that non-reward, which is uncontrollable and unpredictable, caused a delayed HPA axis recovery. This study also shows that acute sleep deprivation did not affect the neuroendocrine and physiological stress response in anticipation of the operant task or to either the rewarded or non-rewarded condition, as measured 1.5 h after sleep deprivation termination. This finding is in agreement with studies showing that short sleep deprivation (20 h) does not affect the HPA axis response to physical stressors like restraint stress and inescapable footstock stress (Meerlo et al., 2002; Novati et al., 2008). Despite the use of a natural and psychological stressor containing unpredictability and uncontrollability, still no effect of prior sleep deprivation was found in our study. Whether the stress responses to frustrative non-reward stress will be affected by prolonged sleep deprivation remains to be studied. Also the performance, impulsivity and motivation of the animals during the operant task, either during reward or non-reward, were not affected by prior acute sleep deprivation.

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Chapter 6

Attentional and physiological aspects measured in the 5-choice serial reaction time task are unaffected by acute sleep deprivation in rats

W. Beerling[1,2], M.C. Schippers[2], J.M. Koolhaas[2], D. Pemberton[1], A. Ahnaou[1], P. Meerlo[2] and W.H.I.M. Drinkenburg[1]

[1] Department of Neuroscience, Janssen Research & Development, a division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, 2340 Beerse, Belgium;

[2] Department of Behavioural Physiology, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

ABSTRACT

Controlled human and rodent studies show that sleep deprivation reduces sustained and selective attention and can lead to impairments in attention tasks. This study aimed to investigate, in a non-confounded way, how acute, 6-h sleep deprivation affects different attentional processes in the 5-choice serial reaction time task (5-CSRTT) in rats. In the 5-CSRTT, rats detect and respond to a 0.5-slight stimulus presented randomly in one of 5 holes in order to get a food reward. The 5-CSRTT allows close observation of different component processes of attention, such as selective attention, sustained attention and behavioural control. Besides assessing these attentional aspects, gross locomotor activity, body temperature and polysomnographic parameters were measured by radiotelemetry using a small implantable transmitter. After training the animals to criteria, 5-CSRTT performance and telemetric measurements were assessed at comparable circadian time points, on the baseline day, on the experimental day starting 1.5 h after the termination of the sleep deprivation and after 24 h recovery of sleep deprivation. The results showed that during sleep deprivation a significant increase in locomotor activity and body temperature was induced with a concomitant lack of Rapid Eye Movement (REM) sleep and almost no Non-Rapid Eye Movement (NREM) sleep occurrence as compared to the non-deprived controls. The delta power during NREM sleep was unaffected during sleep deprivation. Upon termination of the sleep deprivation procedure, locomotor activity, body temperature and REM sleep duration reached levels at or below those of the controls. NREM sleep duration was significantly increased with a concomitant decrease in wakefulness duration. Thirty minutes after termination of the 5-CSRTT, NREM and REM sleep duration were increased for the sleep-deprived animals with a decreased delta power during NREM sleep. This indicates that 6-h sleep deprivation induced a significant sleep loss, which caused effects that are still detectable 30 min after termination of the 5-CSRTT. Sleep deprivation did not affect the response accuracy, correct responses, omissions, premature responses and correct response latency in the 5-CSRTT as measured 1.5 h after sleep deprivation termination. Also body temperature and locomotor activity levels were unaffected. Perseverative responses were slightly increased by sleep deprivation. This effect was mainly caused by a change in the control animals that decreased their amount of perseverative responses as compared to baseline, due to unknown reason. We therefore conclude that acute gentle handling sleep deprivation does not affect sustained and selective attention and behavioural control in the 5-CSRTT.

INTRODUCTION

Today's society is full of acute and chronic sleep disturbance, both in healthy subjects as a result of lifestyle (e.g. shift work, (social) jet lag and prolonged working hours) and in patients with sleep, medical or psychiatric disorders. Despite sleep shortage, most people are daily faced with complex situations, e.g. neurocognitive tasks or stressful challenges. In humans, reductions in sustained and selective attention are among the first consequences of sleep loss and sleepiness. Studies showed that a single night of sleep loss leads to impairments in sustained attention tasks that require attention to auditory or visual stimuli (Jewett et al., 1999; Johnsen et al., 2002; Van Dongen et al., 2003), but also to stimuli with a higher selective and sustained attentional demand (Drummond et al., 2001). While many attempts have been made to unravel the underlying mechanisms, these are still poorly understood. The fact that attention is not a unitary phenomenon but more a term for several different varieties of attentional processes makes it complex to determine the underlying mechanisms. One of the hypothesized mechanisms is involvement of the stress systems. Stress hormones do not only support metabolic processes and physical activity but also affect brain functioning, cognition and mood (McEwen and Sapolsky, 1995; Quirarte et al., 1997; Arnsten, 1998; Arnsten and Goldman-Rakic, 1998; McEwen and Seeman, 1999; McEwen, 1999a; McEwen, 1999b). The processes involved in attention mainly rely on the prefrontal cortex (Norton, 1970; Durmer and Dinges, 2005). Interesting is that chronic stress has shown to affect the prefrontal cortex and to result in deficits in prefrontal cortex -mediated behaviours. Therefore, stress system activation may be involved in the attentional deficits observed after sleep deprivation.

Earlier studies in our lab showed that acute sleep deprivation does not affect the stress responses to ecologically valid challenges like novelty exposure and frustrative non-reward stress (Chapter 4 and 5). This is in accordance with other studies showing no effect of acute sleep deprivation on the stress responses to physical stressors like restraint and inescapable foot shock stress (Meerlo et al., 2002; Novati et al., 2008). Moreover, studies in our lab also showed that acute sleep deprivation does not affect the animal's motivation, impulsivity and performance in an operant task that was used to induce frustrative non-reward stress (Chapter 5). This

was surprising as studies suggest that sleep deprivation has effects on the underlying mechanisms of attention (Norton, 1970; Horne, 1988; Wimmer et al., 1992; Harrison and Horne, 1999; Durmer and Dinges, 2005; Cordova et al., 2006). Therefore, the present study aimed to study in more detail whether acute sleep deprivation affects attentional and physiological responses in a neurocognitive task by using the same sleep deprivation method as in our previous studies (Chapter 3, 4 and 5) but by using a more complex and specified operant task that allows for close observation of different component processes of attention, such as selective attention, sustained attention and behavioural control. Previous studies suggest that attentional deficits resulting from sleep loss reflect a dysregulation of behavioural control processes that rely on the prefrontal cortex (Norton, 1970; Durmer and Dinges, 2005), such as attention to relevant cues (Norton, 1970), flexible thinking (Harrison and Horne, 1999) and cognitive perseveration (Horne, 1988; Wimmer et al., 1992) without affecting aspects of executive function. This indicates that sleep deprivation has different effects on the underlying mechanisms of attention.

For this purpose, the 5-choice serial reaction time task (5-CSRTT) was used. The 5-CSRTT is a task in which rats have to detect and respond to a light stimulus presented randomly in one of 5 holes in order to get a food reward. In this task, the animal must maintain attention to the 5 holes in order to detect the stimulus and respond correctly. Accurate responding thus requires sustained and selective attention in both the temporal and spatial domains. In addition, because responses prior to the presentation of the stimulus (premature responses) are counted as error and terminate the trial, the task also places demands on inhibitory control and allows for impulsivity measurement. The 5-CSRTT also allows for perseverative responding measurements that also give more information on the behavioural control. Also, the location, duration, and timing (pre-stimulus delay) of the visual stimulus can be varied across trials, enabling assessments of sustained and selective attention as well as impulsivity. The 5-CSRTT is comparable to the continuous performance test and psychomotor vigilance task in humans (Robbins, 2002; Bari et al., 2008). In humans, these tests are often used in combination with sleep disturbance, which makes the results of disturbed attentional performance in the 5-CSRTT translational to the human situation. An additional advantage is that neurobiological mechanisms of attention are extensively studied on the basis of the 5-CSRTT, by use of lesion

studies and pharmacological agents that influence one or more parameters of the task (Robbins, 2002; Dalley et al., 2004; Dalley et al., 2008; Bari et al., 2008). Two previous articles have investigated the effects of sleep disturbance on 5-CSRTT performance (Godoi et al., 2005; Cordova et al., 2006). Both studies found impairment on different attentional parameters after acute sleep deprivation and prolonged paradoxical sleep deprivation.

While the study of Cordova and co-workers already showed that acute sleep deprivation affects the 5-CSRTT performance (Cordova et al., 2006), the present study aimed to show the effect of acute sleep deprivation in a non-confounded way by reducing the possibility that stress systems are activated by other factors as sleep loss per se. Therefore, a 1.5-h recovery period after sleep deprivation was included to ensure that the stress systems were back to control levels before 5-CSRTT assessment. Moreover, the present study used a gentle handling sleep deprivation procedure. Studies in our lab showed that this method induced only a mild activation of the stress systems when animals were habituated but nonetheless did reduce sleep times significantly (Chapter 3 and 4). Habituation to handling will reduce the extent of HPA axis and adrenalin activation caused by the gentle handling used to keep the animals awake (Dobrakovova et al., 1993). To control for the sleep deprivation procedure, polysomnographic parameters were measured. Also, this study measured locomotor activity and body temperature levels to show how these were affected by acute sleep deprivation and subsequent 5-CSRTT assessment Stress-confounding effects of the sampling procedure were reduced, and controlled for, by radiotelemetry using a small implantable transmitter to measure simultaneously and continuously the parameters in freely-moving rats (Leon et al., 2004; Greene et al., 2007; Tang et al., 2007). Using these methods, this study aimed to determine in a non-confounded way how acute sleep deprivation affects different attentional processes in the 5-CSRTT.

METHODS

Animals and housing

At the beginning of the experiment 62 adult male Sprague-Dawley rats (Harlan, Horst, The Netherlands), weighing 385 g on average, were trained in the 5 CSRTT (see experimental design section below). The animals were individually housed in polycarbonate cages containing bedding and cage enrichment. The room was kept at constant temperature (21 ± 1°C), controlled humidity (50 ± 10%) and a fixed 12 : 12 h light/dark regime using a 30-min dim/rise period with lights on at 06.00 a.m. The animals were handled daily for weighing purposes and water was provided *ad libitum*. Food access was restricted to approximately 15 g of laboratory chow per day. During recovery of surgery, rats had full access to food prior to re-training. The local ethical committee approved all experimental protocols.

Experimental design

Animals were first trained in the 5-CSRTT, then received surgery, and were re-trained after a recovery period until reaching a stable performance. In total 32 animals survived surgery and fulfilled the criteria of stable performance with ≥50% response accuracy, ≥45 correct responses and ≤ 20% omissions on a stimulus duration of 0.5 s. Twenty-one rats were pseudo randomly assigned to the following two experimental groups: 6-h sleep deprivation (*n*=11) or non-deprived control conditions (*n*=10). The other 11 animals were used to validate a sleep fragmentation protocol that will not be further described here. The average response accuracy, correct responses and omissions were matched between groups. Eight 5-CSRTT set-ups were used in parallel to reduce variability among animals. Because only 8 animals could be tested in the 5-CSRTT simultaneously, the animals were divided into two groups, using a balanced design, that had their baseline day, experimental day and 24-h recovery on different days in the same week but at comparable circadian time points. These two groups were divided into two subgroups, which were sleep-deprived and assessed after each other in the 5-CSRTT with a delay of 45 min. Statistical analysis showed no significant effect of testing day, time or 45-min delay

on all measurements. Therefore the data of all groups was taken together. During baseline compared to their 5-CSRTT performance during training days, one rat had decreased commenced and completed trials and two rats showed excessive perseverative responses. These rats were excluded from all analyses and this resulted in a distribution of *n*=10 for 6-h sleep deprivation and *n*=8 for non-deprived control conditions. All experimental procedures were performed during the light phase, i.e. the resting phase and potentially disturbing stimuli were carefully avoided. On the baseline day, the animals were assessed in the 5-CSRTT at comparable circadian time points as for the experimental day. The experimental day started with 30 min baseline telemetric measurements, followed by 6-h sleep deprivation starting at lights-on or 45 min after lights-on or non-deprived control conditions. This was followed by a 1.5-h post-deprivation recovery phase during which all animals were left undisturbed in their home cages, followed by 30-min testing in the 5-CSRTT. Following the 5-CSRTT, rats were left undisturbed during a 24-h recovery period after which rats were re-tested in the 5-CSRTT. During the experimental day and 24-h recovery of sleep deprivation period, locomotor activity, body temperature, EEG and electromyographic (EMG) were continuously registered.

5-choice serial reaction time task

Apparatus

Training and testing for the 5-CSRTT were conducted in operant boxes, measuring 30.5 cm x 24.1 cm x 29.2 cm (Med Associates Inc, Georgia, USA) that were placed in sound-isolated and ventilated enclosures. Five evenly spaced apertures (2.5 cm x 2.5 cm x 2.2 cm) were located on the curved rear wall of the chamber, 2 cm from the grid floor. A light stimulus (LED, 6.4 mm diameter, 28 V) was positioned at the back of each hole. The opposite wall contained a food tray, connected to a pellet dispenser. Highly palatable food pellets (Dustless precision pellets, 45 mg Rodent purified diet, Bio-Serv, Frenchtown, USA) were automatically delivered in the food tray. A yellow house light (LED, 28 V, 100 mA) was positioned above the food tray. All holes including the food tray were equipped with infrared beams (1.0 cm from front) to detect nose pokes and food collection. The apparatus was controlled by the

computer program MED-PC IV (Med Associates Inc.). A camera was positioned above the 5-CSRTT box to make recordings during a session for further observational analysis. For this study, the recordings were not analysed.

Protocol

The protocol for 5-CSRTT training was adapted from the protocol described by Bari and co-workers (Bari et al., 2008). Training and testing in the 5-CSRTT were always done on weekdays. At the first day of food restriction, rats were given additionally 10-15 precision pellets in their home cages to familiarize them to the taste of the reinforcer. During the following two days, rats were placed for 20 min in the 5-CSRTT boxes with 15 precision pellets in the food tray and the house light were switched on to habituate them to the boxes and the place where the reinforcer is given. The next training phase consisted of four days of 30-min sessions with all lights in the holes switched on. Any nose poke in an arbitrary hole was rewarded with one precision pellet. After a nose poke, all lights were switched off until the rat poked in the food tray to start a new trial. After four training days the rats reached an average of 200 correct nose pokes within the 30-min session. Subsequently, rats were trained to respond to a single visual stimulus presented in one of the five holes. Every trial was initiated by a nose poke into the food tray. An initial precision pellet was delivered into the food tray at the beginning of each session to initiate the first trial. A nose poke into the food tray initiated an inter-trial interval (ITI), after which a light stimulus was presented in one of the five holes. A correct nose poke in this hole within a limited hold (LH) period was rewarded with a precision pellet. Incorrect responses, failures to respond (omissions) and responses before presentation of a light stimulus (premature responses) were punished with a 5 s period of darkness (time-out, TO) without delivery of a precision pellet. After a TO period, the house-light was switched on and the rat should poke in the food tray to start a new trial (Figure 1). Repeated responses in the five holes after the presentation of a stimulus were recorded as a perseverative response, but were not punished. Each session lasted 30 min or until 100 trials had been completed, whichever occurred first. Each rat was always tested in the same box throughout the training and testing phases. The stimulus duration was initially set at 30 s, with an ITI of 10 s, a LH period of 10 s. After five days, rats

reached an average response accuracy (as indicated by (#correct responses/(#correct responses + #incorrect responses))*100%) of more than 80% and less than 1% omissions. Then the stimulus duration was gradually decreased to 1 s. After 7-9 days on 1 s stimulus duration, rats underwent surgery. After surgery, rats had 7-11 days to recover with full access to food. After recovery from surgery, rats were re-trained, starting one day on 10-s stimulus duration and gradually decreasing to 0.25 s (Table 1). Since rats showed a stable performance on 0.5 s with ≥50% response accuracy, ≥45 correct responses and ≤ 20% omissions, final training and testing was done on 0.5-s stimulus duration, 5-s ITI, 5-s LH and 5-s TO protocol.

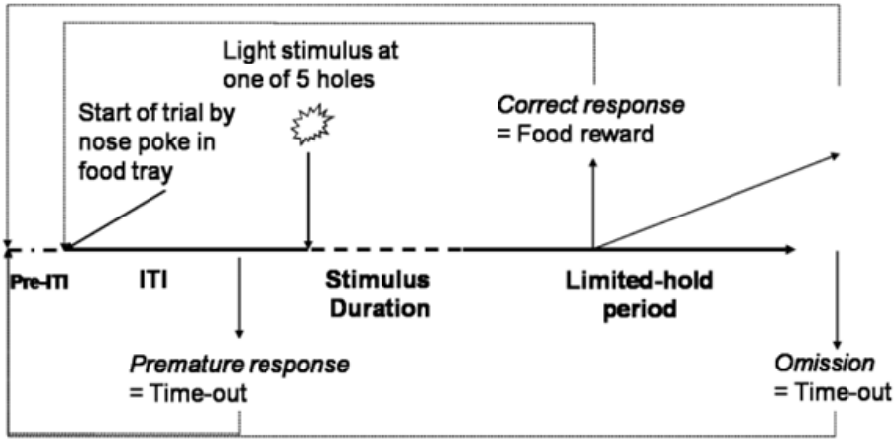

Figure 1. Schematic overview of a trial in the 5-CSRTT. A trial starts when the rats pokes in the food tray, initiating an inter-trial interval (ITI), after which a stimulus light is presented in one of the five holes. A correct nose poke in this hole within a limited hold (LH) period is rewarded with a precision pellet. Incorrect responses, failures to respond (omissions) and responses before presentation of a stimulus light (premature responses) are punished with a period of darkness (time-out) without delivery of a precision pellet. After a time-out period, the house-light was switched on and the rat is allowed to start a new trial. Repeated responses in the five holes after the presentation of a stimulus are recorded as a perseverative response, but are not punished.

Analysis

To assess 5-CSRTT performance the following variables were analysed:

- Commenced trials: number of correct + incorrect + premature + omissions
- Completed trials: number of correct + incorrect responses
- Response accuracy: number of correct responses/(completed trials)*100%

- Percentage correct responses: number of correct responses/(commenced trials)*100%
- Errors of omission, failing to respond to a stimulus within the prescribed time unit (=stimulus duration + limited hold period): number of omissions/(commenced trials)*100%
- Premature responses, response prior to the onset of the target stimulus in the ITI: number of premature responses/(commenced trials)*100%
- Perseverative responses, repeated responses at the holes after visual stimulus has occurred: number of perseverative responses/(commenced trials)*100%
- Correct response latency: the time between onset of light stimulus and a correct nose poke.

These measures were assessed for the baseline day, the experimental day starting 1.5 h after the termination of the sleep deprivation procedure and after 24 h recovery of sleep deprivation. For perseverative responses, the change as compared to baseline was also calculated for each individual and an average was calculated for the group mean.

Table 1. 5-CSRTT training schedule before and after surgery, in chronological order

Stimulus Duration (s)	Inter-trial interval (s)	Limited hold (s)	Time-out (s)	Days (nr)	Comments
30	10	10	5	5	
10	5	5	5	1	
2	5	5	5	5	
1	5	5	5	7-9	
1.5	5	5	5	1	
1.2	5	5	5	1	
1	5	5	5	7-9	
10	5	5	5	1	After surgery
2	5	5	5	1	
1.5	5	5	5	2	
1.2	5	5	5	1	
1	5	5	5	9	
0.5	5	5	5	6	
0.25	5	5	5	6	
0.5	5	5	5	5-8	

Sleep deprivation

Sleep deprivation was induced for 6 h starting at the beginning of the 30-min rise period for one subgroup and starting 45 min later for the other subgroup. Statistical analyses showed no effect of the 45-min delay on all measurements. Sleep was deprived by the gentle handling method. Whenever the animal showed behavioural signs of sleep, the lid of the cages was lifted or the animal was stroked on its back or moved. When this did not result in visual activation of the animal, it was lifted from its cage for a few seconds (Grassi-Zucconi et al., 2006). In order to habituate the animals to handling, they were handled for at least 10 min on the two days preceding the experimental day. Control rats were left undisturbed and non-deprived in their home cages in the same room.

Surgery

After successfully finishing 5-CSRTT training, animals were chronically implanted with electrodes to allow for EEG and EMG recording. Surgery was performed under O_2-N_2O-isoflurane anaesthesia. The analgesic Piritramide (dipidolor, 0.025 mg/kg, 0.1 ml/100 g body weight) was administered subcutaneously at start of surgery. A transmitter (TL11M2-F20-EET, DataSciences International, St. Paul, MN, USA) was implanted which allowed body temperature, gross locomotor activity, EEG and EMG to be measured. The body of the transmitter was placed intraperitoneally in the abdominal cavity. Two biopotential leads were bilaterally attached to the deeper neck muscles (cervical trapezius) for EMG registration. The other two biopotential leads for cortical EEG measurements were led subcutaneously to the skull and placed in contact with the dura at 3 mm on either side lateral to the midline and 6 mm posterior to bregma and anchored to the skull with screws and dental cement (Tang et al., 2007). After surgery, the animals were allowed to recover for 7-11 days.

Biotelemetric data acquisition and analysis

Gross locomotor activity, body temperature, cortical EEG and EMG were telemetrically measured. The transmitter emitted body temperature, EMG and EEG dependent frequency modulated signals. For gross locomotor activity, data was obtained by counting the number of changes in signal strength of the transmitter. Because the transmitter was implanted in the abdomen, vertical changes of the animal were hardly detected by the receiver, but horizontal changes were. Signals were received by an antenna board placed underneath the home cage and in an upright position at one side of the 5-CSRTT box and were processed by a computer using Dataquest Labpro software. Body temperature and locomotor activity data were sampled in 10-s epochs and averaged and stored into 5-min bins. Body temperature and locomotor activity data were calculated for the 15-min period preceding sleep deprivation, for the 6-h sleep deprivation period with subsequent 1.5-h recovery of sleep deprivation period and during the first 20 min of the 5-CSRTT. These were calculated for the same animals as those for which 5-CSRTT results are shown. The EMG and EEG recordings were sampled at a rate of 250 Hz. EEG and EMG analyses were done by visual scoring using Somnologica Version 3.2 (Medcare Flaga, Reykjavik, Iceland) and NeuroScore Version 2.0.0 (DataSciences International). The vigilance states were determined off-line in 10-s epochs. A concurrent EEG power spectrum for each epoch was used to aid in discriminating between states. Non-Rapid Eye Movement (NREM) sleep was scored based on the presence of high amplitude EEG waves and predominant EEG power in the delta range (0.5-4 Hz) and low muscle tone and lack of body movement. Rapid Eye Movement (REM) sleep was characterized by highly regular low amplitude EEG with a dominance of theta activity (4.5-8 Hz) with reduced lower frequencies and general lack of muscle tone with occasional twitches. Wakefulness was scored based on irregular low-amplitude, fast EEG with a lack of visible theta dominance, and higher muscle tone and frequent body movements. Due to artefacts in the EEG or EMG signals, the vigilance data of some rats could not be analysed, resulting in a distribution of control n=4 and sleep deprivation n=8. The amount of time spent in wakefulness, NREM and REM sleep was calculated in 15-min blocks. These were calculated for the 15 min preceding sleep deprivation and for the 6-h sleep deprivation with subsequent 1.5 h recovery of sleep deprivation. Due to too many

artefacts in the EEG and EMG recordings during 5-CSRTT assessment, these signals could not be analysed and were consequently only analysed for 1-h duration, starting 30 min after termination of the 5-CSRTT in order to see whether a sleep deficit was (still) present. The delta power during NREM sleep in the range of 0.5-4 Hz was analysed using Somnologica Version 3.2 (Medcare) by Fast Fourrier Transformation (FFT) at a resolution of 0.25 Hz for 10-s NREM sleep epochs.

Statistics

All data were statistically analysed by repeated measures ANOVA with Greenhouse-Geisser correction epsilon (ε) for violation of the homogeneity in variance assumption. Treatment was used as between-subjects factor (two levels; control and sleep deprivation) and the different time points as within-subject factor. In case of a significant difference, post hoc *t*-tests were performed to determine the specific time points at which sleep-deprived and control animals differed. For the sleep-wake distribution, NREM delta, body temperature and locomotor activity responses, this analysis was performed separately for the sleep deprivation with subsequent 1.5-h post-deprivation phase and for the 5-CSRTT assessment or subsequent recovery phase, in order to study the effect of sleep deprivation itself and then for the effect of sleep deprivation on the 5-CSRTT assessment or recovery. If these ANOVA analyses did not show a significant sleep deprivation effect, the analyses were also done for the sleep deprivation and post-deprivation phase separately, in order to check for an effect in recovery. Since this did not give a difference in significance, the results are only shown and discussed for the analyses done for the period of sleep deprivation with post-deprivation recovery.

The 5-CSRTT performance was also analysed per period (baseline, experimental day, 24 h recovery). Paired *t*-tests were used to determine the differences between the baseline, experimental day and 24-h recovery from sleep deprivation. All analyses were performed using SPSS 15.0 software (SPSS inc., Chicago, IL, USA). Differences were considered significant at $p<0.05$. Data are presented as mean + SEM.

RESULTS

Changes in sleep-wake distribution and NREM delta power in response to 6 h sleep deprivation with subsequent 5-CSRTT assessment

Sleep deprivation and consecutive recovery

Data on time spent in wakefulness, NREM and REM sleep and the NREM delta power during 6-h sleep deprivation or the non-deprived control condition and the 1.5-h post-deprivation recovery phase are illustrated in Figure 2 (panels A-D, respectively). During the 15 min preceding sleep deprivation, no significant differences in time spent in wakefulness, NREM and REM sleep were present. Under non-deprived control conditions, the time spent in wakefulness, NREM and REM sleep stayed fairly stable and animals were asleep most of the time.

During sleep deprivation a significant reduction in NREM and REM sleep duration with a concomitant increase in time spent awake was induced as compared to the non-deprived control group. For NREM sleep, ANOVA showed a significant time*treatment interaction ($F_{23,230}$=3.2, ε=0.2, p=0.023) and a treatment effect ($F_{1,10}$=242.6 p<0.001). NREM sleep duration was significantly decreased during the whole sleep deprivation period. For REM sleep, no significant time*treatment interaction was found but a significant treatment effect ($F_{1,10}$=64.3, p<0.001) was present. *Post-hoc* analysis showed that REM sleep duration was significantly decreased during most time points of the sleep deprivation period. During 6 h sleep deprivation, NREM sleep remained below 2 min per 15-min interval and REM sleep was completely abolished indicating that the rats were almost continuously deprived from sleep. These reductions in NREM and REM sleep were concomitant with increases in time spent awake, with a significant time*treatment interaction ($F_{2,230}$=2.6, ε=0.2, p=0.049) and a significant treatment effect ($F_{1,10}$=245.1, p<0.001). Wakefulness duration was increased for the sleep-deprived condition as compared to the non-deprived control condition throughout the sleep deprivation period.

Upon termination of the sleep deprivation procedure, REM sleep duration reached control levels immediately. NREM sleep duration was significantly increased for the sleep-deprived condition as compared to the control condition for 30 min starting 15 min upon termination of the sleep deprivation procedure, with a significant treatment

effect ($F_{1,10}$=13.6, p=0.004) but no interaction effect. Also wakefulness duration was still affected upon termination of the sleep deprivation procedure with a significant treatment effect ($F_{1,10}$=15.0, p=0.003). *Post-hoc* analysis showed that wakefulness duration was significantly decreased by sleep deprivation for 45 min starting from the first 15 min of the post-deprivation recovery period.

The delta power during NREM sleep remained fairly stable under non-deprived control conditions throughout the experimental period (Figure 2 panel D). Sleep deprivation did not affect NREM delta power during the 6-h sleep deprivation period and also not during the 1.5-h post-deprivation recovery period.

5-CSRTT consecutive recovery

In order to see whether a sleep deficit was still present upon termination of the 5-CSRTT, when rats were returned to their home cages, the sleep-wake distribution and NREM delta power were analysed for 1-h starting 30 min after termination of the 5-CSRTT. This is illustrated for the time spent in wakefulness, NREM and REM sleep and the NREM delta power in Figure 2 (panels A-D, respectively).

During this 1-h period, the sleep-deprived animals showed significantly reduced wakefulness duration with increased NREM and REM sleep duration. For wakefulness, ANOVA showed only a significant treatment effect ($F_{1,9}$=12.7, p=0.006) with reduced wakefulness duration during most of this hour. For NREM sleep also only a significant treatment effect was found ($F_{1,9}$=10.0, p=0.012) with increased levels during most of the 1-h recovery of 5-CSRTT period. Furthermore, REM sleep duration also showed a significant treatment effect ($F_{1,9}$=8.6, p=0.017) and tended to be significantly increased (p<0.01) for the last 45 min of this 1-h post-5-CSRTT recovery period.

During the hour starting 30 min after termination of the 5-CSRTT, a significant treatment effect was found ($F_{1,10}$=5.0, p=0.049) for the NREM delta power response. *Post-hoc* analysis showed only a trend to significance (p=0.059) with lower NREM delta power for the sleep-deprived animals for 15 min starting 1 h after termination of the 5-CSRTT.

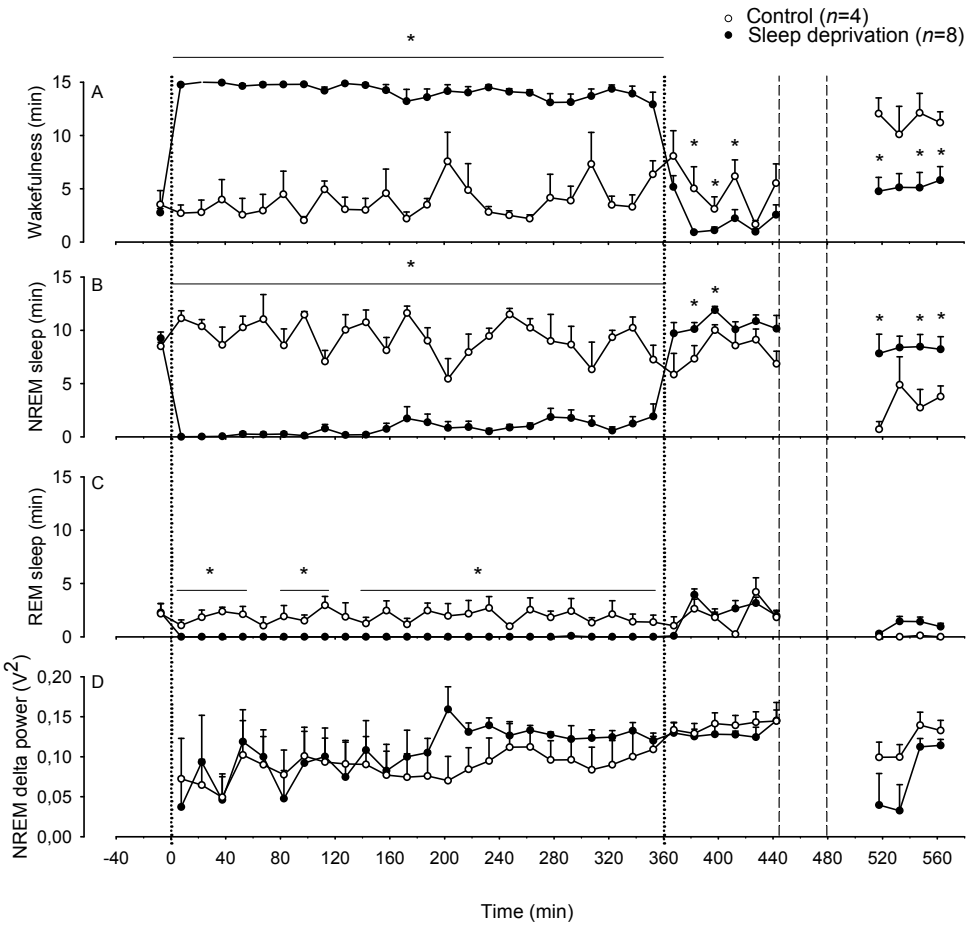

Figure 2. Time course of changes in the amount of wakefulness (panel A), NREM sleep (panel B), REM sleep (panel C) and NREM delta power (panel D) during the 15-min baseline period, the 6-h sleep deprivation or non-deprived control period (indicated by the area between the dotted lines) and the 1.5-h recovery period. Subsequent is the 30-min 5-CSRTT assessment (indicated by the area between the dashed lines) of which polysomnographic recordings were not analysed. 30 min upon termination of the 5-CSRTT, the parameters are shown for another hour. Data are expressed as 15-min averages + SEM and are shown for the control condition (n=4) and the sleep deprivation condition (n=8). Significance: sleep deprivation versus control *p<0.05.

Dynamics of the locomotor activity and body temperature response to 6 h sleep deprivation with subsequent 5-CSRTT assessment

Sleep deprivation and consecutive recovery

Data on locomotor activity and body temperature responses during 6-h sleep deprivation or the non-deprived control condition and the 1.5-h post-deprivation recovery phase are illustrated in Figure 3 (panels A-B, respectively).

Under non-deprived control conditions, locomotor activity levels were low before the beginning of the sleep deprivation period and remained stable and low during sleep deprivation, indicating overall inactivity of these animals. During sleep deprivation, an increase in locomotor activity levels above control levels was induced, with no significant time*treatment interaction and a significant treatment effect ($F_{1,16}$=124.9, p<0.001). Activity levels were increased almost at every time point during the 6-h sleep deprivation period. During the 1.5-h post-deprivation recovery period, no significant time*treatment interaction was found, but a significant treatment effect ($F_{1,16}$=10.6, p=0.005) was present. *Post-hoc* analysis showed that activity levels were only significantly decreased for 5 min at the end of this recovery period as compared to the control group.

Under non-deprived control conditions, body temperature was significantly decreased during the sleep deprivation period as compared to the 30 minutes preceding the sleep deprivation period (p=0.003). Stable values were eventually reached. During 6 h sleep deprivation, a significant time*treatment interaction was found ($F_{71,1136}$=1.8, ε=0.1, p=0.005), with a significant treatment effect ($F_{1,16}$=6.6, p=0.021). Body temperature increased gradually upon sleep deprivation and was significantly increased within 25 min and continued to be elevated during most time points of the sleep deprivation period. Upon termination of the sleep deprivation procedure, body temperature showed a significant time*treatment interaction ($F_{17,272}$=7.3, ε=0.2, p<0.001), with no treatment effect. Body temperature decreased slowly upon termination and took 20 min to reach control levels and then kept on decreasing, reaching levels significantly below control levels during the last 5 min of the post-deprivation recovery period

5-CSRTT

Data on the locomotor activity and body temperature responses during the first 20 min of 5-CSRTT assessment are also illustrated in Figure 3 (panels A-B, respectively).

During the first 20 min of the 5-CSRTT assessment starting 1.5 h after the termination of the sleep deprivation, the locomotor activity levels increased. No difference was found between the sleep-deprived and control animals.

Also body temperature increased during the first 20 min of the 5-CSRTT task. The control animals had a significantly lower body temperature during the first 15 min of the task as compared to the sleep-deprived animals. ANOVA showed a significant treatment effect ($F_{1,16}$=5.1, p=0.038) and a significant time effect ($F_{3,48}$=115.1, ε=0.7, p<0.001) as analysed during the first 20 min of the task. This difference was already present before the animals were exposed to the 5-CSRTT.

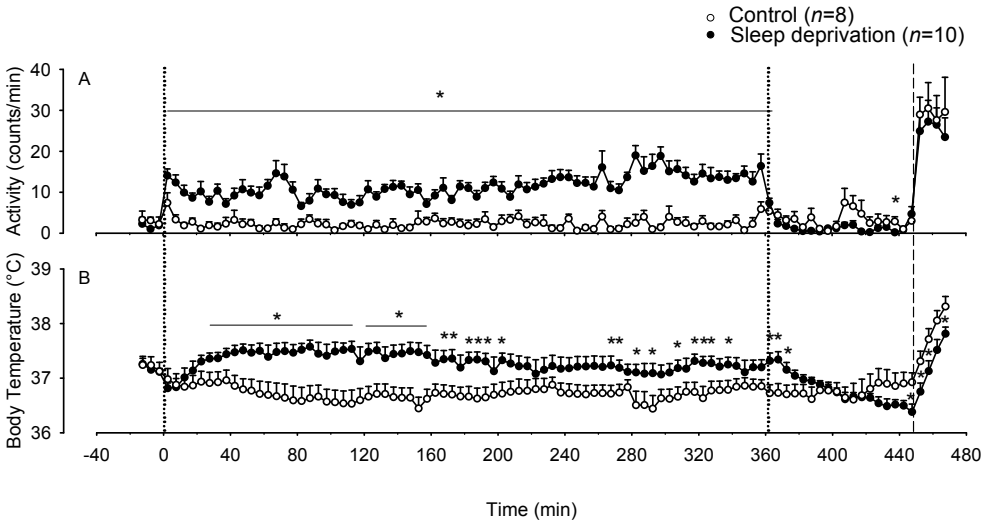

Figure 3. Time course of changes in locomotor activity (panel A) and body temperature (panel B) responses during the 30-min baseline period, the 6-h sleep deprivation or non-deprived control period (indicated by the area between the dotted lines) with subsequent 1.5-h post-deprivation recovery phase and the first 20 min of the 5-CSRTT assessment (indicated by the area between the dashed lines). Data are expressed as 5-min averages + SEM and are shown for the control condition (n=8) and the sleep deprivation condition (n=10). Significance: sleep deprivation versus control *p<0.05.

5-CSRTT performance

Table 2 shows the 5-CSRTT performance of the control and sleep-deprived animals on the baseline day, on the experimental day starting 1.5 h after termination of the sleep deprivation or non-deprived procedure and after 24 h recovery of sleep deprivation. For the response accuracy, correct responses, omissions, premature responses and correct response latency, ANOVA showed no significant time*treatment interactions, no time effects and also no treatment effects, indicating that these responses were not affected by sleep deprivation and did also not change over the days for both groups. For perseverative responses, ANOVA showed a significant time*treatment interaction ($F_{2,32}$=4.8, ε=0.8, p=0.021). *Post-hoc* analysis showed no significant difference between the sleep-deprived and non-deprived control condition either on the baseline day, on the experimental day or after 24 h of recovery. Paired *t*-tests however showed for the control animals a significant decrease in perseverative responses on the experimental day as compared to the baseline day (p=0.028) as is shown in Figure 4 panel A. For the sleep-deprived animals, a trend to a significant increase was found on the experimental day as compared to the baseline day (p=0.094). After 24 h recovery of sleep deprivation no differences were present anymore.

When expressing these perseverative responses as the change as compared to baseline, as shown in Figure 4 panel B, no significant time*treatment interaction was present but a significant treatment effect was found ($F_{1,16}$=7.1, p=0.017). *Post-hoc* analysis showed a significant increased change in perseverative responses for the sleep-deprived animals as compared to the control animals on the experimental day (p=0.019) and a close to significant increase after 24 h recovery of sleep deprivation (p=0.055).

Table 2. 5-CSRTT performance on the baseline day, on the experimental day starting 1.5 h after termination of sleep deprivation or non-deprived procedures (exp. day) and after 24 h recovery of sleep deprivation. The correct responses, omissions and premature responses are shown as percentage responses of total commenced trials, which were 100 in all cases. Data are shown for the control (*n*=8) and sleep-deprived (*n*=10) animals and are expressed as mean ± SEM.

		Baseline day	Experimental day	After 24 h recovery
Response accuracy	*Control*	65.81±2.59	64.34±2.51	63.39±2.26
	SD	63.73±2.62	67.24±2.75	67.04±2.22
Correct responses	*Control*	56.13±2.91	56.25±2.74	54.75±2.31
	SD	54.10±2.87	56.90±2.66	57.70±2.83
Omissions (%)	*Control*	2.50±0.46	3.38±0.89	3.63±0.56
	SD	3.10±0.46	3.50±0.79	3.10±0.41
Premature responses	*Control*	12.50±1.43	9.38±1.28	10.13±1.09
	SD	12.30±1.76	12.00±1.45	11.20±1.90
Correct response	*Control*	0.76±0.06	0.78±0.04	0.82±0.06
	SD	0.77±0.04	0.73±0.04	0.71±0.05

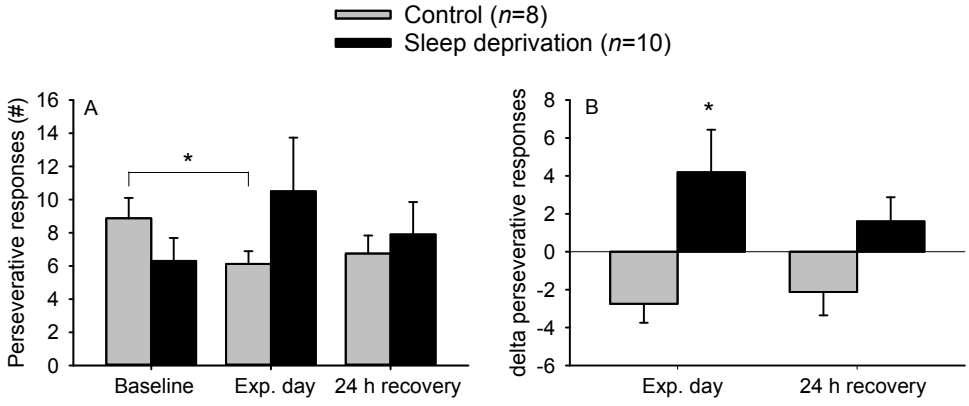

Figure 4. Panel A shows the number of perseverative responses in the 5-CSRTT on the baseline day, on the experimental day starting 1.5 h after termination of sleep deprivation or non-deprived procedures (exp. day) and after 24 h recovery of sleep deprivation. At the experimental day, animals were assessed in the 5-CSRTT after 6 h sleep deprivation or the non-deprived control condition and subsequent 1.5-h post-deprivation recovery. Panel B shows the mean change in number of perseverative responses for the experimental day and after 24 h recovery of sleep deprivation as compared to the baseline day. Data are expressed as mean +/- SEM and are shown for the control condition (n=8) and sleep deprivation condition (n=10). Significance: sleep deprivation versus control *p<0.05

DISCUSSION

The sleep-wake distributions confirm that 6 h gentle handling sleep deprivation significantly prevented rats from sleep. During sleep deprivation, REM sleep was completely absent. Only some NREM sleep was present with a duration below 2 min per 15-min interval. These so-called micro sleeps are practically unavoidable during sleep deprivation and indicate an increased sleep pressure during deprivation (Friedman et al., 1979). Upon termination of the sleep deprivation procedure, a rebound in NREM sleep duration was visible with a concomitant reduction in wakefulness duration. Thirty minutes upon termination of the 5-CSRTT, when animals were returned to their home cages, another increase in NREM duration with concomitant reduction in wakefulness duration was visible for the sleep-deprived animals. Interestingly, the control animals seem to increase their time spent awake, reduce their NREM duration and almost do not enter REM sleep upon termination of the 5-CSRTT as compared to before 5-CSRTT. The sleep-deprived animals slightly

increased their time spent awake as compared to before 5-CSRTT assessment but were still asleep half the time. This indicates that the sleep-deprived animals still had a sleep deficit when assessed in the 5-CSRTT and that this deficit was still present for more than 30 min after termination of the 5-CSRTT. These sleep-wake responses are in accordance with the measured gross locomotor activity levels. The sleep-deprived animals also had a significant lower body temperature during the recovery of sleep deprivation and during 5-CSRTT assessment. This decreased body temperature may also be a consequence of the sleep depth. The increased NREM sleep duration upon 6 h sleep deprivation is in accordance with a previous study in our lab (Chapter 4) and has also been shown in previous sleep deprivation studies (Borbely, 1998). It has been proposed to reflect an elevation in homeostatic sleep drive (Borbely and Neuhaus, 1979). Also, increased NREM delta power is considered a marker for homeostatic sleep drive, indicating sleep intensity (Borbely and Neuhaus, 1979; Franken et al., 1991; Borbely, 1998). However, in our study, the delta power during NREM sleep was not affected by prior sleep deprivation as measured during the 1.5-h post-deprivation period despite a NREM sleep rebound. Upon termination of the 5-CSRTT, the delta power during NREM sleep even seemed to be decreased for the sleep-deprived group. This may be explained by the hypothesis that sleep intensity and sleep duration are separately regulated (Borbely, 1982; Borbely, 1998). Such may imply that a quantitative compensation for sleep loss, i.e. an increase in NREM sleep duration, is a sufficient compensation after a short-term sleep deprivation of 6 h.

Despite a significant sleep deficit, the selective and sustained attentional performance of the sleep-deprived rats, as indicated by the response accuracy, percentage omissions, number of correct responses and correct response latency, in the 5-CSRTT was not affected. Because the errors of omission and correct response latencies were not impaired, it can be concluded that there was no decreased motivation for 5-CSRTT performance. Sleep deprivation did also not affect the impulsivity of the animals, as shown by a lack of change in premature responses, indicating that the inhibitory control was not affected. A slight increase in compulsive behaviour upon sleep deprivation was observed when the perseverative responses were expressed as change to baseline. This effect was mainly caused by a change in the control animals that decreased their amount of perseverative responses as

compared to baseline, due to unknown reason. The sleep-deprived rats did not increase the amount of perseveration when compared to baseline but did when compared to control conditions. Also, analysis of individual data of the sleep-deprived rats showed a strong increase in some rats, whereas others remained stable or even showed a small decrease in the amount of perseverative responses as compared to the non-deprived rats. Alterations in inter-individual variability in perseverative responses was also reported in a previous study on 5-CSRTT performance following 10 h sleep deprivation; but a significant overall effect of sleep deprivation on the perseverative responses was not found (Cordova et al., 2006). Perseverance has been attributed to a compulsive inability to disengage from responding once it has been initiated. Lesions studies have shown that selective lesions in the prefrontal cortex cause an increase in perseverative responses (Chudasama et al., 2003).

The two other rat studies combining sleep deprivation with the 5-CSRTT did show selective and sustained attentional deficits after sleep deprivation (Godoi et al., 2005; Cordova et al., 2006). Cordova and co-workers showed selective and sustained attentional impairments in response to 10 h gentle handling sleep deprivation without affecting motivation, impulsivity and compulsivity (Cordova et al., 2006). Godoi and co-workers exposed rats to 96 h sleep deprivation by the platform technique thereby inducing mainly paradoxical sleep deprivation (Godoi et al., 2005). They showed impaired attentional performance after 96 h deprivation of sleep together with a reduced motivation and no effects on impulsivity and compulsivity. There are several methodological aspects in the use of the 5-CSRTT that could account for the different outcomes in our study as compared to these two rodent studies.

Differences in sleep deprivation procedure and sleep deprivation duration play a role. Godoi and co-workers used 96 h of REM sleep deprivation whereas we used 6-h total sleep deprivation (Godoi et al., 2005). This essential difference may account for the different outcomes and make it hard to further compare that study with ours. The study of Cordova and co-workers used a sleep deprivation method and duration more comparable to ours (Cordova et al., 2006). They used the gentle handling sleep deprivation method, but their method used sensory, auditory and tactile stimulation whereas our procedure used only tactile stimulation with previous habituation (Cordova et al., 2006). Furthermore, we observed in previous studies and also in the

present study that some rats became agitated towards the end of the sleep deprivation session (Chapter 3, 4 and 5). This may imply that agitation is the result of accumulating sleep debt and tiredness, rather than a consequence of stress induced by the sleep deprivation procedure but future studies are needed to confirm this. Even though published studies do not mention agitation specifically, hyperactivity and increased aggressiveness have been previously reported after termination of the sleep deprivation procedure (Hicks et al., 1979; Gessa et al., 1995; Tartar et al., 2008). In order to eliminate possible confounding effects such as stress, agitation and exercise in our experiment, an undisturbed recovery period following sleep deprivation was included before rats were assessed in the 5-CSRTT. During this 1.5-h recovery of sleep deprivation period, body temperature and locomotor activity levels reached stable control levels and body temperature even decreased below control levels during the last 5 min of this recovery period. Previous studies in our lab confirm that the physiological and hormonal stress responses reach control levels during this post-deprivation recovery period but that a sleep deficit is still present (Chapter 3 and 4). The sleep-wake distribution data after 6 h of sleep deprivation in this study showed that, despite the 1.5-h recovery of sleep deprivation period, animals still had a sleep deficit when assessed in the 5-CSRTT. Therefore, the sleep-deprived rats still had a sleep deficit, but were not in an agitated state anymore at the moment the rats were assessed in the 5-CSRTT. This raises the question whether the selective and sustained attentional deficits in the 5-CSRTT after sleep deprivation found in the other rodent studies are the result of some animals being in an agitated state. Also, in most articles, detailed training protocols are missing, possibly leading to a difference in acquiring the task, differences in cognitive processes involved and maximum stable performance reached across different studies. In our study, animals did hardly reach a performance above 50% response accuracy and over 45% correct responses on 0.5-s stimulus duration, even though the performances were stable. In the study of Cordova and co-workers rats attained 70% response accuracy and 70% correct responses on 0.5-s stimulus duration (Cordova et al., 2006). In the study of Godoi and co-workers a performance of over 70% accuracy was reached on a 1-s stimulus duration (Godoi et al., 2005). The performance reached in our study is however still above the chance level of 20% and all animals had a stable performance before they were sleep-deprived.

The present study used the same experimental methods as in our previous studies that showed no effect of acute sleep deprivation on the stress responses to novelty exposure and frustrative non-reward stress and on the motivation, impulsivity and performance of animals during an operant task (Chapter 4 and 5). While using the same sleep deprivation and telemetric methods, the present study confirms that acute sleep deprivation does also not affect different component processes of attention. Selective and sustained attention, motivation and impulsivity as assessed in the 5-CSRTT, starting 1.5 h after sleep deprivation termination, were not affected by prior sleep deprivation despite a significant sleep deficit. Also body temperature and locomotor activity levels during the 5-CSRTT assessment were unaffected by prior 6-h sleep deprivation. We therefore conclude that acute gentle handling sleep deprivation does not affect sustained and selective attention and behavioural control in the 5-CSRTT.

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Chapter 7

General Discussion

STRESS SYSTEM ACTIVATION AS PHYSIOLOGICAL SUPPORT OF BEHAVIOUR?

In order to interpret the consequences of sleep deprivation and the outcomes of our studies correctly, this thesis aimed to study stress system activation in concert with behaviour. In this thesis we support the concept of stress that has been proposed by Koolhaas and co-workers (Koolhaas et al., 2011). They suggest that a considerable part of the classical stress system activation is a direct reflection of the metabolic requirements for normal ongoing behavioural activity, rather than of the stressful nature of the situation (Koolhaas et al., 2011). This hypothesis is supported by studies showing that physical activity results in stress system activation via some of the same brain mechanisms as stressors (Axelrod and Reisine, 1984; Johnson et al., 1992; Koob, 1999). This is described in more detail in the next paragraph. Stress system activation occurs in any condition that requires or causes behavioural activation. Studies confirm that the stress systems are not only activated in response to aversive stimuli but also in response to positive and rewarding stimuli (Schuurman, 1980; Bronson and Desjardins, 1982; De Boer et al., 1990a; Bonilla-Jaime et al., 2006; Koolhaas et al., 2011; Buwalda et al., 2012).

In chapter 2 we further studied the concept of stress by measuring physiological, hormonal, behavioural and electroencephalographic (EEG) responses before, during and after novel cage exposure in rats. Forced confrontation with a novel cage has been interpreted as a stressor but this interpretation is simply based on the occurrence of a stress response. As Weiss showed and as has also been discussed and hypothesized by Koolhaas and co-workers, a condition, in order to be considered truly stressful, needs to involve uncontrollability and unpredictability (Weiss, 1972; Koolhaas et al., 2011). Unpredictability can be characterized by the absence of an anticipatory response (Koolhaas et al., 2011). Uncontrollability is thought to be mainly characterized by a perceived reception of loss of control and a delayed recovery of the stress response (Koolhaas et al., 2011). From this point of view, we hypothesize that novel cage exposure is only mildly uncontrollable and unpredictable, or possibly even not at all, since cages are cleaned on a weekly basis. The results in chapter 2 show that novelty exposure induces temporal sympathico-adrenomedullary (SAM) system and hypothalamic-pituitary-adrenal (HPA) axis activation together with explorative activities and grooming. Correlational analysis

showed that the heart rate, blood pressure and plasma corticosterone responses to novelty exposure were closely related to the ongoing behavioural activity. This shows that stress system activation in response to novelty is closely related to the behavioural activity of the animals. The stress system activation was mild, with physiological and hormonal levels not exceeding the average values reached during the active phase of the circadian cycle under home cage conditions (Buttner and Wollnik, 1982; De Boer and van der Gugten, 1987) or values related to voluntary wheel running (Boersma et al., 2011). Moreover, recovery upon termination of novelty exposure was fast and these recovery responses were also closely correlated with the behavioural response. These observations further strengthen our hypothesis that the physiological and hormonal responses to novelty exposure are mandatory to support the behaviour induced. An interpretation in terms of metabolic support of ongoing behaviour therefore seems to me more appropriate than the frequently used stress interpretation.

Dissociation between the behavioural and physiological/hormonal responses is expected to occur in a truly stressful situation, containing uncontrollability and unpredictability. The hypothesis is that an uncontrollable and unpredictable situation can be distinguished from a controllable and predictable one by the recovery of the responses rather than the magnitude of the response (Koolhaas et al., 2011). This hypothesis is supported by several studies that showed a delayed recovery of the corticosterone and/or SAM system response after an uncontrollable situation as compared to a controllable one, that was not due to differences in behavioural activity (Schuurman, 1980; Garcia et al., 2000; Fish et al., 2005; Arnhold et al., 2009; Koolhaas et al., 2011). The findings of chapter 5 further support our hypothesis that a considerable part of stress system activation serves as physiological support of behaviour and that dissociation between the behavioural and physiological/hormonal response occurs in a uncontrollable and unpredictable situation. In chapter 5 animals were assessed in an operant-conditioning task in which rats are trained to lever press for food reinforcements. When lever pressing is suddenly no longer rewarded, the animal faces absence of the expected behavioural consequence and experiences loss of control. Such unexpected loss of control has been described as frustrative non-reward stress and is highly uncontrollable and unpredictable (De Boer et al., 1990a). We therefore hypothesized that dissociation between stress system

activation and behavioural activity would occur. Our results show that during the rewarded condition, which is highly controllable and predictable, both the SAM system and HPA axis were activated and that this stress system activation reduced during the recovery of the condition. This stress system activation had similar temporal characteristics as the behavioural activity response that was induced by the condition and that decreased during the recovery. Consequently, the stress system activation in response to reward can be adequately interpreted in terms of metabolic support of behaviour induced by the situation. In contrast, during the non-rewarded condition, dissociation occurred between the plasma corticosterone response and the behavioural activity response whereas SAM system activation and behavioural activity showed similar temporal characteristics. The animals under the non-rewarded condition had a decreased behavioural activity during the trial as compared to the rewarded animals. This indicates that these animals had a reduced motivation to complete the task. This reduced motivation was expected under non-rewarded conditions and was associated with a concomitant reduced SAM system activation but with increased corticosterone levels as compared to the rewarded condition. The increased HPA axis activation in response to non-reward is in line with other studies showing stronger HPA axis activation when losing behavioural control (Coover et al., 1971a; Coover et al., 1971b; Davis et al., 1976; Hart and Coover, 1982; Coe et al., 1983; Osborne, 1986). During recovery of the non-rewarded condition, the dissociation between SAM system activation and HPA axis activation was even more pronounced. Whereas the behavioural activity and SAM system activation decreased more and reached levels comparable to the rewarded animals, corticosterone levels remained elevated. Therefore, we conclude that reward induced stress system activation that mainly served as physiological support of behaviour, but that non-reward, also referred to as frustrative non-reward stress, caused a delayed HPA axis recovery. This is in line with studies showing a delayed recovery of the corticosterone and/or SAM system response after an uncontrollable situation as compared to a controllable one, that was not due to differences in behavioural activity (Schuurman, 1980; Garcia et al., 2000; Fish et al., 2005; Arnhold et al., 2009; Koolhaas et al., 2011).

In our experiment, we found a delayed recovery for the corticosterone response but not for the SAM system response after frustrative non-reward stress.

The mechanisms underlying the change in SAM system activation during recovery are largely unknown. For the HPA axis response, it is hypothesized that a delayed recovery after an uncontrollable situation is caused by a delayed onset of the rapid non-genomic corticosteroid receptor mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) feedback inhibition that is induced by the released glucocorticoids (De Kloet et al., 2008). This negative feedback inhibition system is thought to play an important role in the termination of the corticosterone response. A study showed temporal dynamics of the MR and GR distribution in brain areas involved in the feedback inhibition (Buwalda et al., 2001). They showed that GR binding in brain areas like the hypothalamus and hippocampus was decreased one week after social defeat and that MR binding was reduced by 40% in the septum and hippocampus when measured 3 weeks after social defeat (Buwalda et al., 2001). Another study showed no difference in the recovery of the HPA axis response after sexual behaviour or social defeat (Buwalda et al., 2012). The authors explained this by the temporal dynamics of the MR and GR distribution and hypothesized that a difference in HPA axis recovery will occur with a longer delay after the social defeat experience (Buwalda et al., 2012). Another study suggests involvement of the parasympathetic nervous system in the recovery of the HPA axis response (Arnhold et al., 2009). That study showed a rapid decline of plasma corticosterone levels in thirsty rats during drinking that could be prevented by subdiaphragmatic vagotomy. It is unsure what in our study caused the dissociation between the SAM system and the HPA axis response during non-reward. There may be a difference in the neural pathways for stress-induced stimulation of the SAM and HPA system. Also, the different SAM system and HPA axis response by non-reward may reflect or serve different physical and psychological processes involved in the adaptation to the demands. Furthermore, SAM system activation is considered closely associated with physical activity, and more specifically, noradrenalin has found to be closely associated with this (Christensen and Galbo, 1983; Axelrod and Reisine, 1984; Leal-Cerro et al., 2003). Studies have found a reduced elevation in noradrenalin during non-reward (De Boer et al., 1990a) with a higher HPA axis activation (Boulos and Terman, 1980; Coover et al., 1984; De Boer et al., 1990a). De Boer and co-workers further showed that the adrenalin response dissociated from noradrenalin, and that adrenalin levels were increased together with corticosterone levels during non-reward

whereas noradrenalin levels were reduced (De Boer et al., 1990a). This is in accordance with another study that suggests that there are different pathways that control the adrenalin and noradrenalin response and showed reduced corticosterone and adrenalin levels to repeated handling whereas noradrenalin dissociated and stayed elevated (Dobrakovova et al., 1993). In the studies described in this thesis, we did not study noradrenalin and adrenalin levels. Possibly, the dissociation observed between the HPA axis and SAM system response in our experiment is related to a dissociation occurring between the noradrenalin and adrenalin responses, where the noradrenalin reduction may be more pronounced than the adrenalin increase.

The results of chapter 2 and 5 confirm our hypothesis that a situation, in order to be considered truly stressful, needs to contain uncontrollability and unpredictability elements. Moreover, these studies confirm the hypothesis that an uncontrollable and unpredictable situation can be distinguished from a controllable and predictable one by dissociation between the behavioural and physiological/hormonal stress responses and that this is reflected in the recovery of the responses. These studies also confirm the need to study the stress responses in concert with behaviour in order to interpret the results correctly in terms of metabolic support of ongoing behaviour or in terms of stress.

SLEEP DEPRIVATION AND ACTIVATION OF THE BASAL STRESS SYSTEMS

This thesis also aimed to further investigate our hypothesis that stress system activation is responsible for some of the adverse consequences of sleep loss. To study this, we firstly focused on the effect of sleep deprivation on the basal stress systems.

The results of chapter 3, 4 and 5 show that acute 4 and 6 h of gentle handling sleep deprivation induced activation of the stress systems. Overall, this was reflected in increases in body temperature, heart rate, blood pressure and plasma corticosterone as compared to the non-deprived control condition. Also locomotor activity levels were increased. These increases were mild and were not always statistically significant during the whole sleep deprivation period. This observation is in line with other experimental rodent studies reporting mild activation of, in particular,

the HPA axis (Suchecki et al., 1998; Meerlo et al., 2002; Hipolide et al., 2006; Sgoifo et al., 2006).

Sleep deprivation prevented the normal decline in locomotor activity, heart rate and body temperature that was observed in the control situation at the beginning of the light phase, i.e., circadian resting phase. Levels comparable to or slightly below the normal waking levels as measured during the last 30 min of the dark period preceding sleep deprivation were maintained. This is in accordance with other studies showing that sleep deprivation induced an increase in heart rate and blood pressure towards levels reached during normal wakefulness (Tochikubo et al., 1996; Sforza et al., 2004). Only plasma corticosterone reached higher levels than during relaxed wakefulness but these levels can still be considered low as they were below levels seen during mild stress and were only reached during the first hour of sleep deprivation (Buwalda et al., 1993; Van den Buuse et al., 2001; Suchecki et al., 2002).

Whereas other rodent studies mainly focused on the stress response during sleep deprivation and have limited data on the recovery, this thesis aimed to show, in detail, the dynamics of the recovery of the stress response after sleep deprivation using frequent sampling (Suchecki et al., 1998; Meerlo et al., 2002; Hipolide et al., 2006; Sgoifo et al., 2006). Our studies show that the responses had different recovery times but all had returned to control levels within 45 min after termination of the sleep deprivation procedure. The data presented indicate that activation of the stress systems upon acute sleep deprivation has temporal effects. A study by Meerlo and co-workers also showed a mild HPA axis activation in response to 48 h of sleep deprivation that disappeared within a few hours after termination (Meerlo et al., 2002). Despite fast recovery of the HPA axis in that study, another study showed that two days were needed for the increased heart rate to recover after 48 h of sleep deprivation (Sgoifo et al., 2006).

Activation of the SAM system and the HPA axis by sleep deprivation is likely the result of complex, reciprocal interactions between different arousal systems in the brain (Meerlo et al., 2008). Sleep deprivation can induce SAM system and HPA axis activation simultaneously via the Corticotrophin-Releasing-Hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus and via noradrenergic cell clusters in the brain stem, that together have strong reciprocal stimulatory interactions and form a feed-forward system involved in arousal and stress (Axelrod and Reisine,

1984; Johnson et al., 1992; Koob, 1999; Brown et al., 2012). Activation of the noradrenergic cell clusters during stress, resulting in output of the autonomic nervous system, is thought to be, at least partly, dependent on CRH (Koob, 1999). CRH does not only act as neurotransmitter involved in arousal under stressful conditions, but is also a regulator of wakefulness and spontaneous behaviour in conditions without stress (Chang and Opp, 2001). By these mechanisms, any condition requiring behavioural activity, either stressful or unstressful, can result in stress system activation. The noradrenergic cell clusters are not only involved in arousal under stressful conditions, but also play a major role in forebrain activation and EEG arousal (Grzanna and Fritschy, 1991; Berridge and Waterhouse, 2003; Brown et al., 2012). During waking, increases in firing rates have been observed as compared to during sleep. These firing rates are associated with arousal and attention (ston-Jones et al., 1994). Higher SAM system and HPA axis activation, thought to be the result of higher firing rates of the noradrenergic cell clusters, is seen in sleep deprivation studies where subjects are involved in mental challenges (Radomski et al., 1992; Zhong et al., 2005). The CRH neurons and noradrenergic cell clusters also receive input from other systems that may contribute to HPA axis and SAM system activation during sleep deprivation. The orexin (also known as hypocretin) system, with orexins produced by neurons located in the lateral hypothalamus, is such a system. The orexin neurons send projections to the hypothalamus and the noradrenergic cell clusters in the brainstem where they can affect CRH release and autonomic function and by that way stimulate SAM system and HPA axis activity (Peyron et al., 1998; Samson and Resch, 2000; Sutcliffe and de, 2002; Ferguson and Samson, 2003; Brown et al., 2012). The orexin system has multiple functions and one important role is the regulation of sleep and arousal states (Sutcliffe and de, 2000; Brown et al., 2012). Orexin neurons show activity during active waking as compared to during sleep or relaxed wakefulness (Kiyashchenko et al., 2002; Mileykovskiy et al., 2005). Moreover, as reviewed by Meerlo and co-workers, when waking is associated with cognitive and physical arousal the orexin system may play a role in maintaining arousal and promoting SAM system and HPA axis activity needed to support this arousal and cognitive functioning (Meerlo et al., 2008) .

Altogether, our studies show that acute gentle handling sleep deprivation for 4 or 6 h affects the basal activity of the stress systems mildly and temporarily. Different

brain mechanisms are involved in stress system activation induced by sleep deprivation. The CRH neurons in the paraventricular nucleus of the hypothalamus and the noradrenergic cell clusters in the brain stem are thought to play a main role. Stress system activation caused by these pathways is associated with the amount of wakefulness, arousal, physical activity, mental activity and stressfulness induced. Therefore, in order to interpret the results correctly, it should be further discussed to what extent these factors may have contributed to stress system activation induced by acute sleep deprivation in our experiments.

CONSEQUENCE OF SLEEP LOSS OR SLEEP DEPRIVATION METHOD

Sleep deprivation is often interpreted as a stressor that mediates the adverse consequences of sleep deprivation but this interpretation is mainly based on the occurrence of the stress response. However, other factors may have contributed to stress system activation as well. A common point of discussion is whether stress system activation in response to sleep deprivation is the result of sleep loss per se or a consequence of the stressfulness of the used sleep deprivation procedure. Another point that should be evaluated is to what extent the stress system activation is due to physical and mental activity induced to keep the animals awake. All these factors, via mainly the same brain mechanisms, may contribute to stress system activation induced by sleep deprivation. While it is impossible to fully separate these factors, we were able to investigate their contribution in our experiments in more detail.

The results of chapter 4 and 6 show that our gentle handling sleep deprivation procedure completely suppressed REM sleep and also diminished NREM sleep to a large extend. Only short and scattered NREM microsleeps were still observed. In both chapters a rebound in NREM sleep duration was visible with a concomitant reduction in wakefulness duration upon termination of the 6-h sleep deprivation procedure. This rebound was still visible after 1.5 h of recovery of sleep deprivation that took place before the animals were exposed to the stressful or cognitive challenge. After termination of the sleep deprivation procedure also an elevated delta power during NREM sleep was observed, that was present during the 1.5 h recovery of sleep deprivation until after exposure to novelty. The NREM sleep EEG delta

power is considered a marker for homeostatic sleep drive (Borbely and Neuhaus, 1979; Borbely, 1998). This increased delta power during NREM sleep was not observed in the experiment described in chapter 6 even though the same sleep deprivation method and duration was used as in the experiment in chapter 4. An explanation for this difference is not obvious. Despite this difference, both studies do confirm that 4 and 6 h of sleep deprivation by gentle handling are efficiently inducing a sleep loss, which causes effects that are still detectable after 1.5 h of recovery sleep after termination of sleep deprivation. Even though a significant sleep deficit was induced, this does not mean that sleep loss per se is responsible for the mild stress system activation observed during sleep deprivation in our experiments. It may also be the consequence of the stressfulness induced by the sleep deprivation method.

To minimize stress confounding effects of the sleep deprivation procedures, we applied the so called gentle handling sleep deprivation procedure in all our experiments. This method has shown to significantly reduce sleep times and is considered a mild procedure when previous habituation to handling is included (Grassi-Zucconi et al., 1993; Grassi-Zucconi et al., 2006; Van der Borght et al., 2006; Hagewoud et al., 2010). In this thesis, rats were habituated to the same handling procedures that were used to keep the animals awake during sleep deprivation. Habituation to handling will reduce the extent of HPA axis and adrenalin activation caused by the gentle handling used to keep the animals awake (Dobrakovova et al., 1993). Moreover, animals were kept awake by stroking or lifting them up briefly and not by any rude or unexpected handling. Although our sleep deprivation procedure induced only a mild activation of the stress systems, it was noted that some animals became agitated towards the end of the sleep deprivation session. Even though published studies do not mention agitation specifically, hyperactivity and increased aggressiveness have been previously reported after sleep deprivation (Hicks et al., 1979; Gessa et al., 1995; Tartar et al., 2008). It remains unclear what causes this agitation and if this is related to the sleep deprivation procedure or to sleep loss per se. Interestingly, this agitation was only observed during the last stage of sleep deprivation and was not reflected in a change in stress system activation. We therefore hypothesize that the agitation is the result of accumulating sleep debt and tiredness, rather than a consequence of stress induced by the sleep deprivation

procedure but future studies are needed to confirm this. Disturbed synaptic homeostasis may account for this change in mood. During sleep deprivation, high neuronal activity is present that will induce synaptic potentation. Synaptic potentation results in synaptic strengthening (Tononi and Cirelli, 2006). This is thought to be an accumulative effect and sleep normally serves to downscale the synaptic strength (Tononi and Cirelli, 2006). Disturbances in sleep-related synaptic homeostasis may affect mood. We also hypothesized that sleep deprivation can be considered a stressor when stress system activation dissociates from the behavioural response and when the activity of the stress systems reaches levels beyond those seen during relaxed wakefulness. Our studies show that mild and temporary stress system activation was induced and that levels below those seen during relaxed wakefulness were reached. We also showed a close relationship between the behavioural activity induced to keep the animals awake and the stress system activation. In view of our concept of stress, this would imply that the stress system activation by sleep deprivation can be more appropriately interpreted in terms of metabolic support of behaviour. Therefore, sleep deprivation, as used in our experiments, can be considered minimally stressful. This is important as people that stay awake longer are not per definition stressed or experiencing stressful situations during this period of awakening.

The mild stress system activation observed during sleep deprivation may also be the result of physical activity that the animals displayed during sleep deprivation. Correlational analysis showed that the locomotor activity pattern during and after sleep deprivation was related with the patterns of the physiological and hormonal responses. The physical activity levels reached during sleep deprivation did not exceed levels reached during the last 30 - 60 min of the dark period immediately preceding sleep deprivation. Physical activity was induced by our sleep deprivation method. Therefore, a considerable part of the stress system activation during sleep deprivation was necessary to support the increased physical activity and wakefulness that was induced to keep the animals awake. Also mental activity may contribute to stress system activation. Our gentle handling sleep deprivation procedure did not involve a mental challenge therefore mental activity induced by our gentle handling sleep deprivation procedure can be considered minimal.

While it is impossible to completely separate the possible causes of stress system activation in response to sleep deprivation, we were able to study their contribution in our experiments in more detail. We showed that the stressfulness of the sleep deprivation procedure was minimized and that the mental activity induced can be considered minimal. We did show that a significant sleep deficit was induced that may account for the stress system activation. However, the behavioural activity and wakefulness induced to keep the animals awake accounts for the stress system activation as well. Normal activity levels as compared to wakefulness were reached. We therefore conclude that, in our experiments, by using the gentle handling sleep deprivation procedure, we successfully minimized stress and mental activity and induced normal physical activity. In controlled studies but also in real life, the activities done to prevent the organism to fall asleep will always induce some psychological or physical activity and hence induce activation of the physiological and neuroendocrine systems that support this activity. Therefore, these effects should be taking into account when interpreting outcomes of sleep deprivation studies. Also, differences in mental and physical activity induced by sleep deprivation will result in differences in stress system activation across sleep deprivation studies.

SLEEP DEPRIVATION AND STRESS RESPONSIVITY

Even though sleep deprivation only mildly affects the basal activity of the stress systems, this does not exclude the possibility that acute sleep deprivation does not affect stress system reactivity to challenges. This will not be evident under strictly controlled stimulus-poor conditions but will become noticeable when subjects are facing new challenges or stressors on top of the deprived sleep (Meerlo et al., 2008). The present study aimed to show whether sleep deprivation, by acting on the neuroendocrine stress systems, affects stress responsivity. In chapter 4 the rats were exposed to novelty and in chapter 5 to frustrative non-reward stress after acute sleep deprivation. Both situations were carefully chosen and have good ecological validity and translational value. As was discussed, stress response activation by novelty exposure should be interpreted in terms of metabolic support of behaviour rather than the commonly used stress interpretation, whereas for frustrative non-reward stress

the commonly used stress interpretation seems to be correct. Rats were exposed to novelty or frustrative non-reward stress after a brief 1.5 h recovery period. This recovery period after sleep deprivation was induced to make sure the animals were not in an agitated state anymore when exposed to the challenge. Results in both studies show that a sleep deficit was still present after this recovery period.

Both studies also show that acute sleep deprivation does not affect the stress responses to novelty exposure or frustrative non-reward stress based on measurements of plasma corticosterone, heart rate, blood pressure and body temperature. Also, locomotor activity levels were unaffected by prior sleep deprivation. Acute sleep deprivation did also not affect the stress response in anticipation of the operant task or to the rewarded condition of the operant task. Furthermore, the recovery of the responses after the challenges were terminated, were not affected by prior sleep deprivation. Altogether, sleep deprivation did not affect stress system reactivity to a controllable and predictable situation or to an uncontrollable and unpredictable one. Human studies suggest that sleep deprivation may not affect the activated stress response but can affect the perception of stressors (Martin, 1981; Martin and Chen, 1984; Martin et al., 1986; Zohar et al., 2005). We tried to assess this aspect by monitoring behaviour of the animals during novelty exposure and during the frustrative non-reward challenge after the animal had been exposed to sleep deprivation. The performance, impulsivity and motivation during the operant task was unaffected by prior sleep deprivation. Even though quantification of psychological perception in animals is not obvious, in the present studies no changes in the behaviour of the animals were found. We can therefore conclude that despite a sleep deficit, rats in our experiments were able to deal with a challenge when looking at our measurements of behaviour and stress system activation.

Studies exposing rodents to a stressful challenge after they have been sleep-deprived are limited in number. Moreover, the studies that have been done have used different sleep deprivation durations, different types of challenges and different sleep deprivation methods, thereby making it hard to fully compare their results (Meerlo et al., 2002; Suchecki et al., 2002; Sgoifo et al., 2006; Novati et al., 2008). Two studies showed that 20 h of sleep deprivation did not affect the HPA axis response to brief restraint stress whereas 48 h of sleep deprivation and 8 days of

sleep restriction did (Meerlo et al., 2002; Novati et al., 2008). Particularly, while the pituitary ACTH response was significantly dampened, the adrenal corticosterone response remained unaltered. These findings suggest that the effects of sleep deprivation on stress reactivity may accumulate over time (Meerlo et al., 2002; Sgoifo et al., 2006; Novati et al., 2008). Chronic sleep deprivation or restriction may cause gradual changes in the brain that may affect stress system responsivity and stress sensitivity (Roman et al., 2005; Roman et al., 2006; Novati et al., 2008). As has been discussed, the CRH neurons in the paraventricular nucleus of the hypothalamus and the noradrenergic cell clusters in the brain stem are thought to play a main role in the effect of acute sleep deprivation on the stress systems. It has been suggested that prolonged sleep deprivation can change the regulation of sympathetic activity but there is little and controversial evidence for this (Bergmann et al., 1989; Meerlo et al., 2008). It has also been suggested that chronic sleep deprivation can cause gradually developing alterations in CRH signalling, but only few studies have been done (Meerlo et al., 2008). Acute sleep deprivation is associated with increased CRH neuronal activity. Chronic sleep deprivation may result in chronic CRH over-stimulation that can cause reduced CRH sensitivity. A study showed that this occurred in the striatum and pituitary (Fadda and Fratta, 1997). Whether this also occurs in other brain areas is unknown but would be interesting to investigate further as the CRH system is not only involved in the regulation of the stress response but also in cognitive, emotional and behavioural arousal (Koob, 1999). Studies also showed that the serotonergic system is affected by chronic sleep deprivation and chronic sleep restriction. The serotonergic system is involved in sleep regulation, and particularly, in the brain stem's sleep generating system (Jouvet, 1999; Ursin, 2002; Brown et al., 2012). Serotonin is thought to prepare the organism for sleep by attenuating brain systems responsible for cortical activation and behavioural arousal (Cape and Jones, 1998; Brown et al., 2012). The serotonin system has more roles, and one is mediating the stress response and emotionality (Schwartz et al., 1999; Hensler, 2006). Studies showed that chronic sleep restriction, consisting of 8 days with repeated 20 h sleep deprivation per day, can cause a gradual reduction in the sensitivity of postsynaptic serotonin-1A receptors. This reduced sensitivity persisted for days, even after unrestricted recovery sleep, and was not mediated by forced activity or adrenal stress hormones (Roman et al., 2005; Roman et al., 2006). The

reduced CRH sensitivity and reduced sensitivity of the serotonin-1A receptor system may, at least partly, account for the altered HPA axis response to stress that has been observed in studies using chronic sleep restriction (Meerlo et al., 2008). Interestingly, depressed patients show blunted ACTH but normal cortisol responses to CRH injections, and they show blunted physiological responses to a serotonin-1A agonist (Holsboer et al., 1987; Lesch, 1991; Arborelius et al., 1999; Sobczak et al., 2002). This is similar to what has been observed in rats that were chronically restricted of sleep (Meerlo et al., 2002; Roman et al., 2005; Sgoifo et al., 2006; Roman et al., 2006; Novati et al., 2008). This suggests that chronic sleep deprivation or restriction can cause gradual changes in the CRH and serotonergic system, up to what is seen in depression. Chronic sleep deprivation may affect other neurotransmitter systems involved in stress system regulation as well.

Altogether, our studies show that 4 and 6 h of sleep deprivation do not affect stress responsivity to novelty exposure and frustrative non-reward stress. Despite a significant sleep deficit induced, animals were most likely able to completely recover from this sleep deficit. Since rats normally sleep approximately 12 h each day, the animals lost half of their sleep (Borbely and Neuhaus, 1979). We did not study the complete recovery in this thesis but studies suggest that after acute sleep deprivation, a complete recovery is initiated and animals make up for the NREM sleep that was lost. Chronic sleep deprivation also induces recovery but the sleep rebound observed does not contain as much NREM sleep as that was lost (Everson et al., 1989a; Everson et al., 1989b; Everson, 1995; Rechtschaffen et al., 1999). Therefore, animals may not completely recover from chronic sleep deprivation. Most likely, 4 and 6 h of sleep deprivation induced a significant sleep loss but animals were able to recover completely. We also hypothesize that brain systems involved in sleep and stress system regulation are affected, but that this is associated with the effects related to acute sleep deprivation, with acute and mild SAM system and HPA activation occurring during sleep deprivation itself, and not with the effects seen after chronic sleep deprivation. Changes in stress responsivity may not be evident immediately but may gradually develop under conditions of chronic restricted and deprived sleep (Meerlo et al., 2008). We therefore expect that longer sleep deprivation duration, using the same sleep deprivation method, will cause gradual

changes in the brain that may affect stress system responsivity to novelty exposure and frustrative non-reward stress.

SLEEP DEPRIVATION AND ATTENTIONAL FUNCTIONING

While our studies show that there is no effect of acute sleep deprivation on stress system reactivity to challenges, we were still interested to see if the ability to deal with an attentional challenge would be affected and whether this is mediated by the stress systems. Studies suggest that sleep deprivation has different effects on the underlying mechanisms of attention and affects different component processes of attention (Norton, 1970; Horne, 1988; Wimmer et al., 1992; Harrison and Horne, 1999; Durmer and Dinges, 2005; Cordova et al., 2006). There are only few controlled rat studies that assessed sleep-deprived rats in a behavioural assessment that allowed for separate different attentional measurements (Godoi et al., 2005; Cordova et al., 2006).

In chapter 5 we showed that 6 h of gentle handling sleep deprivation did not affect attentional functioning in an operant task as measured 1.5 h after sleep deprivation termination. This operant task involved a paradigm where animals had to press a lever to retrieve a food reward. During the rewarded condition, performance, motivation and impulsivity were not affected by prior sleep deprivation. Moreover, sleep deprivation did not affect the stress response during the operant task, as measured by body temperature, blood pressure, heart rate and plasma corticosterone levels and did also not affect the recovery of the stress response after termination of the task. Chapter 6 shows that acute sleep deprivation did also not affect selective and sustained attentional performance in the 5-choice serial reaction time task (5-CSRTT). In the 5-CSRTT, rats detect and respond to a light stimulus presented randomly in one of 5 holes in order to get a food reward. Also, motivation and impulsivity were not affected by prior sleep deprivation. An effect of prior sleep deprivation on perseveration was observed but this was mainly caused by a change in the control animals that decreased their amount of perseverative responses as compared to baseline, due to unknown reason. The sleep-deprived rats did not increase the amount of perseveration when compared to baseline but did when

compared to control conditions. Also, analysis of individual data of the sleep-deprived rats showed a strong increase in some rats, whereas others remained stable or even showed a small decrease in the amount of perseverative responses as compared to the non-deprived rats. Individual variability in perseverative responses was also reported in a previous study on 5-CSRTT performance following 10 h sleep deprivation; but a significant overall effect of sleep deprivation on the perseverative responses was not found in that study (Cordova et al., 2006). Perseverance has been attributed to a compulsive inability to disengage from responding once it has been initiated. We were unable to measure stress read-outs during the 5-CSRTT. It would be interesting to study these during a next study. The two other rat studies that have combined sleep deprivation with subsequent 5-CSRTT assessment showed selective and sustained attentional deficits after 10-h total sleep deprivation (Cordova et al., 2006) and 96 h of REM sleep deprivation (Godoi et al., 2005) but no effects on behavioural control.

There are several methodological aspects that could account for the different outcomes among our study and the two other studies that assessed animals in the 5-CSRTT after sleep deprivation. Differences in sleep deprivation procedure and sleep deprivation duration play a role. Godoi and co-workers used 96 h of REM sleep deprivation whereas we used 6-h total sleep deprivation (Godoi et al., 2005). This essential difference may account for the different outcomes and make it hard to further compare that study with ours. The study of Cordova and co-workers used a sleep deprivation method and duration more comparable to ours (Cordova et al., 2006). They used the gentle handling sleep deprivation method, but their method used sensory, auditory and tactile stimulation whereas our procedure used only tactile stimulation (Cordova et al., 2006). Also, we habituated the animals to the same handling procedures that were used to keep the animals awake during sleep deprivation. Habituation to handling will reduce the extent of HPA axis and adrenalin activation caused by the gentle handling used to keep the animals awake (Dobrakovova et al., 1993). This may also reduce the effect of sleep deprivation, mediated in part by the stress systems, on attention. Cordova and co-workers used 4, 7 and 10 h of total sleep deprivation and found an increase in response latency to correct responses during sleep deprivation and a positive interaction with the duration of sleep deprivation. In my opinion, there is a possibility that this interaction is also

caused by an accumulating sleep depth as the rats were exposed to 4, 7 and 10 h of sleep deprivation subsequently with only one day in between to recover. In our experiment we furthermore included a post-deprivation recovery period before rats were assessed in the attentional task. As discussed previously, rats became agitated towards the end of the sleep deprivation session. In chapter 6 we showed that, despite the 1.5-h recovery of sleep deprivation period, animals still had a sleep deficit for more than 30 min after termination of the 5-CSRTT but were not in an agitated state anymore when assessed in the 5-CSRTT. This raises the question whether the sustained and selective attentional deficits found in experimental rodent sleep deprivation studies are the result of animals being in a different emotional or behavioural state. A study showed that attentional deficits are highly correlated with increased agitation (Corrigan et al., 1992). Since it is unknown if this agitation is the result of sleep loss or of the sleep deprivation procedure, we suggest that studies should include a recovery time before exposing rats to an attentional task to minimize confounding effects. Also, future studies are needed to determine the cause of this agitation. Furthermore, a standardized training protocol for the 5-CSRTT should be developed and used among studies. In most articles, detailed training protocols are missing, possibly leading to a difference in acquiring the task, differences in cognitive processes involved and maximum stable performance reached across different studies. In our study, rats reached a 20% lower performance in the 5-CSRTT as compared to the other rat studies that have combined sleep deprivation with subsequent 5-CSRTT assessment. The performance reached in our study is however still above the chance level of 20% and all animals had a stable performance before they were sleep-deprived.

Studies suggest that the attentional deficits commonly observed after sleep loss reflect mainly a dysregulation of behavioural control processes that rely on the prefrontal cortex (Norton, 1970; Durmer and Dinges, 2005), and more specifically, of disruptions of the basal forebrain cholinergic function, that project to the prefrontal cortex (Muir et al., 1992; McGaughy et al., 2002; Brown et al., 2012). This effect of sleep deprivation is thought to be the result of increases in adenosine levels that occur during wakefulness and that inhibit the cholinergic neurons in the basal forebrain (Hawryluk et al., 2012; Brown et al., 2012). A study showed that adenosine administration in the basal forebrain results in sustained attention impairments that

were mimicking the effects seen after 24 h of sleep deprivation (Christie et al., 2008). Studies also showed that cholinergic disruptions in the medial frontal cortex can lead to an increase in perseverative responses (McGaughy et al., 2002; Chudasama et al., 2003). In this thesis we focused on the possible involvement of the stress systems. We did not find an effect of sleep deprivation on sustained and selective attentional functioning and on behavioural control, but studies do suggest that if attentional performance is affected by sleep deprivation, the stress systems may also be involved. Interestingly, the medial prefrontal cortex does not only play a role in attentional functioning, but also affects activity of the HPA axis (Diorio et al., 1993). The medial prefrontal cortex is a target for the negative feedback effects of glucocorticoids on HPA axis activity. Acute activation of the stress response may induce acetylcholine release in the hippocampus and forebrain that act on the HPA axis and on the cholinergic function of the prefrontal cortex (Gilad, 1987; Gilad et al., 1987; Brown et al., 2012). Chronic stress system activation affects the prefrontal cortex and causes a gradual decline of cholinergic functioning, resulting in deficits of prefrontal cortex mediated behaviours, such as attention (Mizoguchi et al., 2001; Srikumar et al., 2006). The noradrenergic cell clusters may also be involved. As was discussed, the noradrenergic cell clusters show increases in firing rates during waking as compared to during sleep (Grzanna and Fritschy, 1991; Berridge and Waterhouse, 2003; Brown et al., 2012) and this is thought to be associated with attentional performance (ston-Jones et al., 1994). Prolonged sleep deprivation may change the regulation of the noradrenergic system and by that way affect attentional performance, but there is little and controversial evidence for this (Bergmann et al., 1989; Meerlo et al., 2008). The prefrontal cortex is also very sensitive to catecholamines and too little or too much catecholamine release in the prefrontal cortex may weaken cognitive control of behaviour and attention (Brennan and Arnsten, 2008). Since sleep deprivation is in general associated with stress system activation and by that way results in catecholamine release, it may affect attentional performance by this mechanism as well. The CRH system may also be involved in the attentional deficits commonly observed after sleep deprivation. Chronic sleep deprivation results in reduced CRH sensitivity in the striatum and pituitary (Fadda and Fratta, 1997). This may also occur in the prefrontal cortex where the CRH receptors are, in part, involved in attentional processes (Jaferi and Bhatnagar, 2007). This has

been supported by a study that showed that transgenic mice overproducing CRH have a mild accuracy impairment in a version of the 5-CSRTT (van Gaalen et al., 2003). The noradrenergic cell clusters and the CRH system are also activated as part of the stress response.

Overall, the reduced attentional performance that is commonly observed after sleep deprivation may be the results of sleep loss per se, but also of stress system activation. Sleep loss and stress system activation both affect mechanisms that are involved in attentional processes. There is a considerable neurobiological overlap of attention, stress system and sleep processes. In our experiments, no effect of prior sleep deprivation on attentional performance was found while confounding effects were minimized. We expect that attentional deficits will become visible, using the same methods, after more prolonged sleep deprivation.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This thesis describes a series of experiments that applied an animal model of acute sleep deprivation to study the consequences of insufficient sleep on the basal stress response, on stress responsivity and on attentional functioning under controlled, non-confounded conditions. The background of these studies is the hypothesis that sleep deprivation, by acting on the stress systems, may affect stress responsivity and attentional performance. One concern about the effects of sleep deprivation on stress system activity in rodents is that this may not be a consequence of sleep loss per se but also a consequence of the procedures used. The experiments presented in this thesis confirm that our animal model allows for multiple stress parameters and different attentional parameters to be measured while confounding stress effects of the used procedures are minimized.

The data presented in this thesis demonstrate that acute sleep deprivation induces mild and temporary stress system activation that is closely associated with the behavioural activity levels induced to keep the animals awake. This thesis confirms the need to study the stress response in concert with behaviour in order to interpret the consequences of stress system activation correctly. Our data show that stress system activation in response to novelty exposure and acute gentle handling

sleep deprivation can be interpreted in terms of metabolic support of behaviour whereas the stress response to frustrative non-reward stress is a reflection of the stressfulness. The data presented in this thesis furthermore show no effect of acute sleep deprivation on subsequent stress responsivity to novelty exposure, frustrative non-reward stress and the rewarded condition in the operant-conditioning task. Also, different attentional parameters, as measured in the 5-CSRTT and operant-conditioning task, are not affected by prior sleep deprivation. These findings can be explained by the fact that the mild stress system activation during sleep deprivation recovers completely upon termination of sleep deprivation and the close association of the stress response with behavioural activity. The results therefore provide further support for the hypothesis that stress physiology is a mediator of the consequences of sleep deprivation.

This thesis has focused on the relationship between sleep loss and stress system activation and the consequences on stress responsivity and attentional functioning. The relationship between sleep loss and stress system activation is a complex and bidirectional relationship and an interesting area of research. For this thesis, we focused on one direction of this relationship: the effect of sleep loss on the stress systems. The experiments presented in this thesis further contribute to the knowledge of this relationship and its consequences on stress responsivity and attentional functioning. This thesis also discusses possible underlying mechanisms. However, important and interesting questions remain to be answered.

It is important to further establish the neurobiological mechanisms that are affected by sleep loss, stress system activation and attentional functioning. The mechanisms underlying the consequences of sleep loss are complex and remain speculative. A deeper knowledge of the neurobiological mechanisms will help with the interpretation of the relationship between sleep loss and the stress systems and its consequences on stress responsivity and attentional functioning

Another important question to be answered is how more prolonged sleep deprivation or chronic sleep restriction affects stress responsivity and attentional measures, using the same animal models as described in this thesis, and how this is mediated by stress physiology. Whereas rats and humans may recover from acute sleep deprivation, more prolonged sleep deprivation or chronic sleep restriction may gradually alter the regulation of the stress systems and affect attentional functioning

and stress responsivity. This knowledge is important considering the increased number of people in our society that are chronically sleep deprived and the serious consequences on health and wellbeing that have been associated with this.

Reference List

Abelson, K. S., Adem, B., Royo, F., Carlsson, H. E., Hau, J., 2005. High plasma corticosterone levels persist during frequent automatic blood sampling in rats. In Vivo. 19, 815-819.

Abraham, I., Veenema, A. H., Nyakas, C., Harkany, T., Bohus, B. G., Luiten, P. G., 1997. Effect of corticosterone and adrenalectomy on NMDA-induced cholinergic cell death in rat magnocellular nucleus basalis. J. Neuroendocrinol. 9, 713-720.

Abraham, I. M., Harkany, T., Horvath, K. M., Luiten, P. G., 2001. Action of glucocorticoids on survival of nerve cells: promoting neurodegeneration or neuroprotection? J. Neuroendocrinol. 13, 749-760.

Ader, R., Friedman, S. B., 1968. Plasma corticosterone response to environmental stimulation: effects of duration of stimulation and the 24-hour adrenocortical rhythm. Neuroendocrinology. 3, 378-386.

Akerstedt, T., Froberg, J. E., 1979. Sleep and stressor exposure in relation to circadian rhythms in catecholamine excretion. Biol. Psychol. 8, 69-80.

Akerstedt, T., Palmblad, J., de la, T. B., Marana, R., Gillberg, M., 1980. Adrenocortical and gonadal steroids during sleep deprivation. Sleep. 3, 23-30.

Arborelius, L., Owens, M. J., Plotsky, P. M., Nemeroff, C. B., 1999. The role of corticotropin-releasing factor in depression and anxiety disorders. J. Endocrinol. 160, 1-12.

Armario, A., 2006. The hypothalamic-pituitary-adrenal axis: what can it tell us about stressors? CNS. Neurol. Disord. Drug Targets. 5, 485-501.

Armario, A., Montero, J. L., Balasch, J., 1986. Sensitivity of corticosterone and some metabolic variables to graded levels of low intensity stresses in adult male rats. Physiol Behav. 37, 559-561.

Arnhold, M. M., Yoder, J. M., Engeland, W. C., 2009. Subdiaphragmatic vagotomy prevents drinking-induced reduction in plasma corticosterone in water-restricted rats. Endocrinology. 150, 2300-2307.

Arnsten, A. F., 1998. Catecholamine modulation of prefrontal cortical cognitive function. Trends Cogn Sci. 2, 436-447.

Arnsten, A. F., Goldman-Rakic, P. S., 1998. Noise stress impairs prefrontal cortical cognitive function in monkeys: evidence for a hyperdopaminergic mechanism. Arch. Gen. Psychiatry. 55, 362-368.

Axelrod, J., Reisine, T. D., 1984. Stress hormones: their interaction and regulation. Science. 224, 452-459.

Ayas, N. T., White, D. P., Manson, J. E., Stampfer, M. J., Speizer, F. E., Malhotra, A., Hu, F. B., 2003. A prospective study of sleep duration and coronary heart disease in women. Arch. Intern. Med. 163, 205-209.

Bari, A., Dalley, J. W., Robbins, T. W., 2008. The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. Nat. Protoc. 3, 759-767.

Bassett, J. R., Cairncross, K. D., King, M. G., 1973. Parameters of novelty, shock predictability and response contigency in corticosterone release in the rat. Physiol Behav. 10, 901-907.

Beerling, W., Koolhaas, J. M., Ahnaou, A., Bouwknecht, J. A., De Boer, S. F., Meerlo, P., Drinkenburg, W. H., 2011. Physiological and hormonal responses to novelty exposure in rats are mainly related to ongoing behavioral activity. Physiol Behav. 103, 412-420.

Benington, J. H., Frank, M. G., 2003. Cellular and molecular connections between sleep and synaptic plasticity. Prog. Neurobiol. 69, 71-101.

Benington, J. H., Heller, H. C., 1995. Restoration of brain energy metabolism as the function of sleep. Prog. Neurobiol. 45, 347-360.

Bergmann, B. M., Everson, C. A., Kushida, C. A., Fang, V. S., Leitch, C. A., Schoeller, D. A., Refetoff, S., Rechtschaffen, A., 1989. Sleep deprivation in the rat: V. Energy use and mediation. Sleep. 12, 31-41.

Berridge, C. W., Waterhouse, B. D., 2003. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res. Brain Res. Rev. 42, 33-84.

Boersma, G. J., Benthem, L., van, D. G., Scheurink, A. J., 2011. Individual variation in the (patho)physiology of energy balance. Physiol Behav. 103, 89-97.

Bonilla-Jaime, H., Vazquez-Palacios, G., rteaga-Silva, M., Retana-Marquez, S., 2006. Hormonal responses to different sexually related conditions in male rats. Horm. Behav. 49, 376-382.

Bonnet, M. H., 1985. Effect of sleep disruption on sleep, performance, and mood. Sleep. 8, 11-19.

Bonnet, M. H., Arand, D. L., 1995. We are chronically sleep deprived. Sleep. 18, 908-911.

Borbely, A. A., 1982. A two process model of sleep regulation. Hum. Neurobiol. 1, 195-204.

Borbely, A. A., 1998. Processes underlying sleep regulation. Horm. Res. 49, 114-117.

Borbely, A. A., Neuhaus, H. U., 1979. Sleep-Deprivation - Effects on Sleep and EEG in the Rat. Journal of Comparative Physiology. 133, 71-87.

Boulos, Z., Terman, M., 1980. Food availability and daily biological rhythms. Neurosci. Biobehav. Rev. 4, 119-131.

Brennan, A. R., Arnsten, A. F., 2008. Neuronal mechanisms underlying attention deficit hyperactivity disorder: the influence of arousal on prefrontal cortical function. Ann. N. Y. Acad. Sci. 1129, 236-245.

Brett, L. P., Chong, G. S., Coyle, S., Levine, S., 1983. The pituitary-adrenal response to novel stimulation and ether stress in young adult and aged rats. Neurobiol. Aging. 4, 133-138.

Bronson, F. H., Desjardins, C., 1982. Endocrine responses to sexual arousal in male mice. Endocrinology. 111, 1286-1291.

Brown, R. E., Basheer, R., McKenna, J. T., Strecker, R. E., McCarley, R. W., 2012. Control of sleep and wakefulness. Physiol Rev. 92, 1087-1187.

Buckley, T. M., Schatzberg, A. F., 2005. On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: normal HPA axis activity and circadian rhythm, exemplary sleep disorders. J. Clin. Endocrinol. Metab. 90, 3106-3114.

Buttner, D., Wollnik, F., 1982. Spontaneous short-term fluctuations in the daily pattern of heart rate, body temperature and locomotor activity in the laboratory rat. Lab Anim. 16, 319-326.

Buwalda, B., Felszeghy, K., Horvath, K. M., Nyakas, C., De Boer, S. F., Bohus, B., Koolhaas, J. M., 2001. Temporal and spatial dynamics of corticosteroid receptor down-regulation in rat brain following social defeat. Physiol Behav. 72, 349-354.

Buwalda, B., Nyakas, C., Koolhaas, J. M., Bohus, B., 1993. Neuroendocrine and behavioral effects of vasopressin in resting and mild stress conditions. Physiol Behav. 54, 947-953.

Buwalda, B., Scholte, J., De Boer, S. F., Coppens, C. M., Koolhaas, J. M., 2012. The acute glucocorticoid stress response does not differentiate between rewarding and aversive social stimuli in rats. Horm. Behav. 61, 218-226.

Caldwell, J. A., 2001. The impact of fatigue in air medical and other types of operations: a review of fatigue facts and potential countermeasures. Air Med. J. 20, 25-32.

Cape, E. G., Jones, B. E., 1998. Differential modulation of high-frequency gamma-electroencephalogram activity and sleep-wake state by noradrenaline and serotonin microinjections into the region of cholinergic basalis neurons. J. Neurosci. 18, 2653-2666.

Chang, F. C., Opp, M. R., 2001. Corticotropin-releasing hormone (CRH) as a regulator of waking. Neurosci. Biobehav. Rev. 25, 445-453.

Chang, P. P., Ford, D. E., Mead, L. A., Cooper-Patrick, L., Klag, M. J., 1997. Insomnia in young men and subsequent depression. The Johns Hopkins Precursors Study. Am. J. Epidemiol. 146, 105-114.

Chapotot, F., Buguet, A., Gronfier, C., Brandenberger, G., 2001. Hypothalamo-pituitary-adrenal axis activity is related to the level of central arousal: effect of sleep deprivation on the association of high-frequency waking electroencephalogram with cortisol release. Neuroendocrinology. 73, 312-321.

Chapotot, F., Gronfier, C., Jouny, C., Muzet, A., Brandenberger, G., 1998. Cortisol secretion is related to electroencephalographic alertness in human subjects during daytime wakefulness. J. Clin. Endocrinol. Metab. 83, 4263-4268.

Chen, H. I., 1991. Effects of 30-h sleep loss on cardiorespiratory functions at rest and in exercise. Med. Sci. Sports Exerc. 23, 193-198.

Christensen, N. J., Galbo, H., 1983. Sympathetic nervous activity during exercise. Annu. Rev. Physiol. 45, 139-153.

Christie, M. A., McKenna, J. T., Connolly, N. P., McCarley, R. W., Strecker, R. E., 2008. 24 hours of sleep deprivation in the rat increases sleepiness and decreases vigilance: introduction of the rat-psychomotor vigilance task. J. Sleep Res. 17, 376-384.

Chrousos, G. P., 2009. Stress and disorders of the stress system. Nat. Rev. Endocrinol. 5, 374-381.

Chudasama, Y., Passetti, F., Rhodes, S. E., Lopian, D., Desai, A., Robbins, T. W., 2003. Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity. Behav. Brain Res. 146, 105-119.

Coe, C. L., Stanton, M. E., Levine, S., 1983. Adrenal responses to reinforcement and extinction: role of expectancy versus instrumental responding. Behav. Neurosci. 97, 654-657.

Coover, G. D., Goldman, L., Levine, S., 1971a. Plasma corticosterone increases produced by extinction of operant behavior in rats. Physiol Behav. 6, 261-263.

Coover, G. D., Goldman, L., Levine, S., 1971b. Plasma corticosterone levels during extinction of a lever-press response in hippocampectomized rats. Physiol Behav. 7, 727-732.

Coover, G. D., Murison, R., Sundberg, H., Jellestad, F., Ursin, H., 1984. Plasma corticosterone and meal expectancy in rats: effects of low probability cues. Physiol Behav. 33, 179-184.

Cordova, C. A., Said, B. O., McCarley, R. W., Baxter, M. G., Chiba, A. A., Strecker, R. E., 2006. Sleep deprivation in rats produces attentional impairments on a 5-choice serial reaction time task. Sleep. 29, 69-76.

Corrigan, J. D., Mysiw, W. J., Gribble, M. W., Chock, S. K., 1992. Agitation, cognition and attention during post-traumatic amnesia. Brain Inj. 6, 155-160.

Dalley, J. W., Mar, A. C., Economidou, D., Robbins, T. W., 2008. Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. Pharmacol. Biochem. Behav. 90, 250-260.

Dalley, J. W., Theobald, D. E., Bouger, P., Chudasama, Y., Cardinal, R. N., Robbins, T. W., 2004. Cortical cholinergic function and deficits in visual attentional performance in rats following 192 IgG-saporin-induced lesions of the medial prefrontal cortex. Cereb. Cortex. 14, 922-932.

Dallman, M. F., Pecoraro, N. C., la Fleur, S. E., Warne, J. P., Ginsberg, A. B., Akana, S. F., Laugero, K. C., Houshyar, H., Strack, A. M., Bhatnagar, S., Bell, M. E., 2006. Glucocorticoids, chronic stress, and obesity. Prog. Brain Res. 153, 75-105.

Davis, H., Memmott, J., Macfadden, L., Levine, S., 1976. Pituitary-adrenal activity under different appetitive extinction procedures. Physiol Behav. 17, 687-690.

De Boer, S. F., de Beun, R., Slangen, J. L., van der Gugten, J., 1990a. Dynamics of plasma catecholamine and corticosterone concentrations during reinforced and extinguished operant behavior in rats. Physiol Behav. 47, 691-698.

De Boer, S. F., Koopmans, S. J., Slangen, J. L., van der Gugten, J., 1989. Effects of fasting on plasma catecholamine, corticosterone and glucose concentrations under basal and stress conditions in individual rats. Physiol Behav. 45, 989-994.

De Boer, S. F., Koopmans, S. J., Slangen, J. L., van der Gugten, J., 1990b. Plasma catecholamine, corticosterone and glucose responses to repeated stress in rats: effect of interstressor interval length. Physiol Behav. 47, 1117-1124.

De Boer, S. F., van der Gugten, J., 1987. Daily variations in plasma noradrenaline, adrenaline and corticosterone concentrations in rats. Physiol Behav. 40, 323-328.

De Groote, L., Linthorst, A. C., 2007. Exposure to novelty and forced swimming evoke stressor-dependent changes in extracellular GABA in the rat hippocampus. Neuroscience. 148, 794-805.

De Kloet, E. R., Joels, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 463-475.

De Kloet, E. R., Karst, H., Joels, M., 2008. Corticosteroid hormones in the central stress response: quick-and-slow. Front Neuroendocrinol. 29, 268-272.

Dinan, T. G., 1996. Serotonin and the regulation of hypothalamic-pituitary-adrenal axis function. Life Sci. 58, 1683-1694.

Dinges, D. F., 1995. An overview of sleepiness and accidents. J. Sleep Res. 4, 4-14.

Dinges, D. F., Pack, F., Williams, K., Gillen, K. A., Powell, J. W., Ott, G. E., Aptowicz, C., Pack, A. I., 1997. Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. Sleep. 20, 267-277.

Diorio, D., Viau, V., Meaney, M. J., 1993. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. J. Neurosci. 13, 3839-3847.

Dobrakovova, M., Kvetnansky, R., Oprsalova, Z., Jezova, D., 1993. Specificity of the effect of repeated handling on sympathetic-adrenomedullary and pituitary-adrenocortical activity in rats. Psychoneuroendocrinology. 18, 163-174.

Drummond, S. P., Gillin, J. C., Brown, G. G., 2001. Increased cerebral response during a divided attention task following sleep deprivation. J. Sleep Res. 10, 85-92.

Durmer, J. S., Dinges, D. F., 2005. Neurocognitive consequences of sleep deprivation. Semin. Neurol. 25, 117-129.

Ekstedt, M., Akerstedt, T., Soderstrom, M., 2004. Microarousals during sleep are associated with increased levels of lipids, cortisol, and blood pressure. Psychosom. Med. 66, 925-931.

Ellenbogen, J. M., Payne, J. D., Stickgold, R., 2006. The role of sleep in declarative memory consolidation: passive, permissive, active or none? Curr. Opin. Neurobiol. 16, 716-722.

Everson, C. A., 1995. Functional consequences of sustained sleep deprivation in the rat. Behav. Brain Res. 69, 43-54.

Everson, C. A., Bergmann, B. M., Rechtschaffen, A., 1989a. Sleep deprivation in the rat: III. Total sleep deprivation. Sleep. 12, 13-21.

Everson, C. A., Gilliland, M. A., Kushida, C. A., Pilcher, J. J., Fang, V. S., Refetoff, S., Bergmann, B. M., Rechtschaffen, A., 1989b. Sleep deprivation in the rat: IX. Recovery. Sleep. 12, 60-67.

Fadda, P., Fratta, W., 1997. Stress-induced sleep deprivation modifies corticotropin releasing factor (CRF) levels and CRF binding in rat brain and pituitary. Pharmacol. Res. 35, 443-446.

Feenstra, M. G., Botterblom, M. H., 1996. Rapid sampling of extracellular dopamine in the rat prefrontal cortex during food consumption, handling and exposure to novelty. Brain Res. 742, 17-24.

Ferguson, A. V., Samson, W. K., 2003. The orexin/hypocretin system: a critical regulator of neuroendocrine and autonomic function. Front Neuroendocrinol. 24, 141-150.

Fish, E. W., DeBold, J. F., Miczek, K. A., 2005. Escalated aggression as a reward: corticosterone and GABA(A) receptor positive modulators in mice. Psychopharmacology (Berl). 182, 116-127.

Flaherty, C. F., Rowan, G. A., Pohorecky, L. A., 1986. Corticosterone, novelty-induced hyperglycemia, and chlordiazepoxide. Physiol Behav. 37, 393-396.

Follenius, M., Brandenberger, G., Bandesapt, J. J., Libert, J. P., Ehrhart, J., 1992. Nocturnal cortisol release in relation to sleep structure. Sleep. 15, 21-27.

Ford, D. E., Kamerow, D. B., 1989. Epidemiologic study of sleep disturbances and psychiatric disorders. An opportunity for prevention? JAMA. 262, 1479-1484.

Frank, M. G., Benington, J. H., 2006. The role of sleep in memory consolidation and brain plasticity: dream or reality? Neuroscientist. 12, 477-488.

Franken, P., Tobler, I., Borbely, A. A., 1991. Sleep homeostasis in the rat: simulation of the time course of EEG slow-wave activity. Neurosci. Lett. 130, 141-144.

Friedman, L., Bergmann, B. M., Rechtschaffen, A., 1979. Effects of sleep deprivation on sleepiness, sleep intensity, and subsequent sleep in the rat. Sleep. 1, 369-391.

Gagliano, H., Fuentes, S., Nadal, R., Armario, A., 2008. Previous exposure to immobilisation and repeated exposure to a novel environment demonstrate a marked dissociation between behavioral and pituitary-adrenal responses. Behav. Brain Res. 187, 239-245.

Gangwisch, J. E., Heymsfield, S. B., Boden-Albala, B., Buijs, R. M., Kreier, F., Pickering, T. G., Rundle, A. G., Zammit, G. K., Malaspina, D., 2006. Short sleep duration as a risk factor for hypertension: analyses of the first National Health and Nutrition Examination Survey. Hypertension. 47, 833-839.

Garcia, A., Marti, O., Valles, A., Dal-Zotto, S., Armario, A., 2000. Recovery of the hypothalamic-pituitary-adrenal response to stress. Effect of stress intensity, stress duration and previous stress exposure. Neuroendocrinology. 72, 114-125.

Gessa, G. L., Pani, L., Fadda, P., Fratta, W., 1995. Sleep deprivation in the rat: an animal model of mania. Eur. Neuropsychopharmacol. 5 Suppl, 89-93.

Gilad, G. M., 1987. The stress-induced response of the septo-hippocampal cholinergic system. A vectorial outcome of psychoneuroendocrinological interactions. Psychoneuroendocrinology. 12, 167-184.

Gilad, G. M., Rabey, J. M., Gilad, V. H., 1987. Presynaptic effects of glucocorticoids on dopaminergic and cholinergic synaptosomes. Implications for rapid endocrine-neural interactions in stress. Life Sci. 40, 2401-2408.

Godoi, F. R., Oliveira, M. G., Tufik, S., 2005. Effects of paradoxical sleep deprivation on the performance of rats in a model of visual attention. Behav. Brain Res. 165, 138-145.

Grassi-Zucconi, G., Cipriani, S., Balgkouranidou, I., Scattoni, R., 2006. 'One night' sleep deprivation stimulates hippocampal neurogenesis. Brain Res. Bull. 69, 375-381.

Grassi-Zucconi, G., Menegazzi, M., De Prati, A. C., Bassetti, A., Montagnese, P., Mandile, P., Cosi, C., Bentivoglio, M., 1993. c-fos mRNA is spontaneously induced in the rat brain during the activity period of the circadian cycle. Eur. J. Neurosci. 5, 1071-1078.

Graves, L., Pack, A., Abel, T., 2001. Sleep and memory: a molecular perspective. Trends Neurosci. 24, 237-243.

Greene, A. N., Clapp, S. L., Alper, R. H., 2007. Timecourse of recovery after surgical intraperitoneal implantation of radiotelemetry transmitters in rats. J. Pharmacol. Toxicol. Methods. 56, 218-222.

Groves, P. M., Thompson, R. F., 1970. Habituation: a dual-process theory. Psychol. Rev. 77, 419-450.

Grzanna, R., Fritschy, J. M., 1991. Efferent projections of different subpopulations of central noradrenaline neurons. Prog. Brain Res. 88, 89-101.

Guiol, C., Ledoussal, C., Surge, J. M., 1992. A radiotelemetry system for chronic measurement of blood pressure and heart rate in the unrestrained rat validation of the method. J. Pharmacol. Toxicol. Methods. 28, 99-105.

Hagewoud, R., Havekes, R., Novati, A., Keijser, J. N., Van der Zee, E. A., Meerlo, P., 2010. Sleep deprivation impairs spatial working memory and reduces hippocampal AMPA receptor phosphorylation. J. Sleep Res. 19, 280-288.

Haraldsson, P. O., Carenfelt, C., Laurell, H., Tornros, J., 1990. Driving vigilance simulator test. Acta Otolaryngol. 110, 136-140.

Harrison, Y., Horne, J. A., 1999. One night of sleep loss impairs innovative thinking and flexible decision making. Organ Behav. Hum. Decis. Process. 78, 128-145.

Hart, R. P., Coover, G. D., 1982. Plasma corticosterone and free fatty acid responses and positive behavioral contrast. Physiol Behav. 29, 483-488.

Hawryluk, J. M., Ferrari, L. L., Keating, S. A., Arrigoni, E., 2012. Adenosine inhibits glutamatergic input to basal forebrain cholinergic neurons. J. Neurophysiol. 107, 2769-2781.

Heinz, A., Hermann, D., Smolka, M. N., Rieks, M., Graf, K. J., Pohlau, D., Kuhn, W., Bauer, M., 2003. Effects of acute psychological stress on adhesion molecules, interleukins and sex hormones: implications for coronary heart disease. Psychopharmacology (Berl). 165, 111-117.

Hennessy, J. W., Levine, S., 1979. Stress, arousal, and the pituitary-adrenal system: a psychoendocrine hypothesis. Progr Psychobiol Physiol Psychol. 8, 133-174.

Hennessy, M. B., Heybach, J. P., Vernikos, J., Levine, S., 1979. Plasma corticosterone concentrations sensitively reflect levels of stimulus intensity in the rat. Physiol Behav. 22, 821-825.

Hensler, J. G., 2006. Serotonergic modulation of the limbic system. Neurosci. Biobehav. Rev. 30, 203-214.

Herman, J. P., Cullinan, W. E., 1997. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci. 20, 78-84.

Hicks, R. A., Moore, J. D., Hayes, C., Phillips, N., Hawkins, J., 1979. REM sleep deprivation increases aggressiveness in male rats. Physiol Behav. 22, 1097-1100.

Hipolide, D. C., Suchecki, D., Pimentel de Carvalho, P. A., Chiconelli, F. E., Tufik, S., Luz, J., 2006. Paradoxical sleep deprivation and sleep recovery: effects on the hypothalamic-pituitary-adrenal axis activity, energy balance and body composition of rats. J. Neuroendocrinol. 18, 231-238.

Holsboer, F., von, B. U., Buller, R., Heuser, I., Steiger, A., 1987. Stimulation response to corticotropin-releasing hormone (CRH) in patients with depression, alcoholism and panic disorder. Horm. Metab Res. Suppl. 16, 80-88.

Horne, J., 2002. Why sleep? Biologist (London). 49, 213-216.

Horne, J. A., 1988. Sleep loss and "divergent" thinking ability. Sleep. 11, 528-536.

Huang, C. W., Lui, C. C., Chang, W. N., Lu, C. H., Wang, Y. L., Chang, C. C., 2009. Elevated basal cortisol level predicts lower hippocampal volume and cognitive decline in Alzheimer's disease. J. Clin. Neurosci. 16, 1283-1286.

Hublin, C., Kaprio, J., Partinen, M., Koskenvuo, M., 2001. Insufficient sleep--a population-based study in adults. Sleep. 24, 392-400.

Inoue, S., Honda, K., Komoda, Y., 1995. Sleep as neuronal detoxification and restitution. Behav. Brain Res. 69, 91-96.

Irwin, M., Thompson, J., Miller, C., Gillin, J. C., Ziegler, M., 1999. Effects of sleep and sleep deprivation on catecholamine and interleukin-2 levels in humans: clinical implications. J. Clin. Endocrinol. Metab. 84, 1979-1985.

Jacobson, L., Sapolsky, R., 1991. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocr. Rev. 12, 118-134.

Jaferi, A., Bhatnagar, S., 2007. Corticotropin-releasing hormone receptors in the medial prefrontal cortex regulate hypothalamic-pituitary-adrenal activity and anxiety-related behavior regardless of prior stress experience. Brain Res. 1186, 212-223.

Jewett, M. E., Dijk, D. J., Kronauer, R. E., Dinges, D. F., 1999. Dose-response relationship between sleep duration and human psychomotor vigilance and subjective alertness. Sleep. 22, 171-179.

Johnsen, B. H., Laberg, J. C., Eid, J., Hugdahl, K., 2002. Dichotic listening and sleep deprivation: vigilance effects. Scand. J. Psychol. 43, 413-417.

Johnson, E. O., Kamilaris, T. C., Chrousos, G. P., Gold, P. W., 1992. Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. Neurosci. Biobehav. Rev. 16, 115-130.

Jouvet, M., 1999. Sleep and serotonin: an unfinished story. Neuropsychopharmacology. 21, 24S-27S.

Karni, A., Tanne, D., Rubenstein, B. S., Askenasy, J. J., Sagi, D., 1994. Dependence on REM sleep of overnight improvement of a perceptual skill. Science. 265, 679-682.

Khansari, D. N., Murgo, A. J., Faith, R. E., 1990. Effects of stress on the immune system. Immunol. Today. 11, 170-175.

Kiyashchenko, L. I., Mileykovskiy, B. Y., Maidment, N., Lam, H. A., Wu, M. F., John, J., Peever, J., Siegel, J. M., 2002. Release of hypocretin (orexin) during waking and sleep states. J. Neurosci. 22, 5282-5286.

Koob, G. F., 1999. Corticotropin-releasing factor, norepinephrine, and stress. Biol. Psychiatry. 46, 1167-1180.

Koolhaas, J. M., Bartolomucci, A., Buwalda, B., De Boer, S. F., Flugge, G., Korte, S. M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van, D. G., Wohr, M., Fuchs, E., 2011. Stress revisited: a critical evaluation of the stress concept. Neurosci. Biobehav. Rev. 35, 1291-1301.

Koolhaas, J. M., De Boer, S. F., Buwalda, B., 2006. Stress and Adaptation; Toward Ecologically Relevant Animal Models. Current Directions in Psychological Science. 15, 109-112.

Koolhaas, J. M., Meerlo, P., De Boer, S. F., Strubbe, J. H., Bohus, B., 1997. The temporal dynamics of the stress response. Neurosci. Biobehav. Rev. 21, 775-782.

Korte, S. M., Koolhaas, J. M., Wingfield, J. C., McEwen, B. S., 2005. The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. Neurosci. Biobehav. Rev. 29, 3-38.

Lazarus, R. S., 1993. From psychological stress to the emotions: a history of changing outlooks. Annu. Rev. Psychol. 44, 1-21.

Leal-Cerro, A., Gippini, A., Amaya, M. J., Lage, M., Mato, J. A., Dieguez, C., Casanueva, F. F., 2003. Mechanisms underlying the neuroendocrine response to physical exercise. J. Endocrinol. Invest. 26, 879-885.

Leon, L. R., Walker, L. D., DuBose, D. A., Stephenson, L. A., 2004. Biotelemetry transmitter implantation in rodents: impact on growth and circadian rhythms. Am. J. Physiol Regul. Integr. Comp Physiol. 286, R967-R974.

Leproult, R., Copinschi, G., Buxton, O., Van, C. E., 1997. Sleep loss results in an elevation of cortisol levels the next evening. Sleep. 20, 865-870.

Lesch, K. P., 1991. 5-HT1A receptor responsivity in anxiety disorders and depression. Prog. Neuropsychopharmacol. Biol. Psychiatry. 15, 723-733.

Levine, S., 2005. Developmental determinants of sensitivity and resistance to stress. Psychoneuroendocrinology. 30, 939-946.

Levine, S. U. H., 1991. What is stress. Stress:Neurobiology and Neuroendocrinology.3-12.

Lusardi, P., Mugellini, A., Preti, P., Zoppi, A., Derosa, G., Fogari, R., 1996. Effects of a restricted sleep regimen on ambulatory blood pressure monitoring in normotensive subjects. Am. J. Hypertens. 9, 503-505.

Maquet, P., 1995. Sleep function(s) and cerebral metabolism. Behav. Brain Res. 69, 75-83.

Maquet, P., 2001. The role of sleep in learning and memory. Science. 294, 1048-1052.

Martin, B. J., 1981. Effect of sleep deprivation on tolerance of prolonged exercise. Eur. J. Appl. Physiol Occup. Physiol. 47, 345-354.

Martin, B. J., 1988. Sleep loss and subsequent exercise performance. Acta Physiol Scand. Suppl. 574, 28-32.

Martin, B. J., Bender, P. R., Chen, H., 1986. Stress hormonal response to exercise after sleep loss. Eur. J. Appl. Physiol Occup. Physiol. 55, 210-214.

Martin, B. J., Chen, H. I., 1984. Sleep loss and the sympathoadrenal response to exercise. Med. Sci. Sports Exerc. 16, 56-59.

McEwen, B. S., 1998. Stress, adaptation, and disease. Allostasis and allostatic load. Ann. N. Y. Acad. Sci. 840, 33-44.

McEwen, B. S., 1999a. Stress and hippocampal plasticity. Annu. Rev. Neurosci. 22, 105-122.

McEwen, B. S., 1999b. Stress and the aging hippocampus. Front Neuroendocrinol. 20, 49-70.

McEwen, B. S., Sapolsky, R. M., 1995. Stress and cognitive function. Curr. Opin. Neurobiol. 5, 205-216.

McEwen, B. S., Seeman, T., 1999. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. Ann. N. Y. Acad. Sci. 896, 30-47.

McEwen, B. S., Wingfield, J. C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2-15.

McGaughy, J., Dalley, J. W., Morrison, C. H., Everitt, B. J., Robbins, T. W., 2002. Selective behavioral and neurochemical effects of cholinergic lesions produced by intrabasalis infusions of 192 IgG-saporin on attentional performance in a five-choice serial reaction time task. J. Neurosci. 22, 1905-1913.

McGinty, D., Szymusiak, R., 1990. Keeping cool: a hypothesis about the mechanisms and functions of slow-wave sleep. Trends Neurosci. 13, 480-487.

McKenna, J. T., Cordeira, J. W., Christie, M. A., Tartar, J. L., McCoy, J. G., Lee, E., McCarley, R. W., Strecker, R. E., 2008. Assessing sleepiness in the rat: a multiple sleep latencies test compared to polysomnographic measures of sleepiness. J. Sleep Res. 17, 365-375.

McMurray, R. G., Brown, C. F., 1984. The effect of sleep loss on high intensity exercise and recovery. Aviat. Space Environ. Med. 55, 1031-1035.

Meerlo, P., Horvath, K. M., Nagy, G. M., Bohus, B., Koolhaas, J. M., 1999. The influence of postnatal handling on adult neuroendocrine and behavioural stress reactivity. J. Neuroendocrinol. 11, 925-933.

Meerlo, P., Koehl, M., Van der, B. K., Turek, F. W., 2002. Sleep restriction alters the hypothalamic-pituitary-adrenal response to stress. J Neuroendocrinol. 14, 397-402.

Meerlo, P., Sgoifo, A., Suchecki, D., 2008. Restricted and disrupted sleep: effects on autonomic function, neuroendocrine stress systems and stress responsivity. Sleep Med Rev. 12, 197-210.

Mileykovskiy, B. Y., Kiyashchenko, L. I., Siegel, J. M., 2005. Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron. 46, 787-798.

Misslin, R., Herzog, F., Koch, B., Ropartz, P., 1982. Effects of isolation, handling and novelty on the pituitary--adrenal response in the mouse. Psychoneuroendocrinology. 7, 217-221.

Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., Tabira, T., 2001. Effect of chronic stress on cholinergic transmission in rat hippocampus. Brain Res. 915, 108-111.

Moore, R. Y., Eichler, V. B., 1972. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res. 42, 201-206.

Morley, J. E., Levine, A. S., Rowland, N. E., 1983. Minireview. Stress induced eating. Life Sci. 32, 2169-2182.

Muir, J. L., Dunnett, S. B., Robbins, T. W., Everitt, B. J., 1992. Attentional functions of the forebrain cholinergic systems: effects of intraventricular hemicholinium, physostigmine, basal forebrain lesions and intracortical grafts on a multiple-choice serial reaction time task. Exp. Brain Res. 89, 611-622.

Muir, J. L., Pfister, H. P., 1986. Corticosterone and prolactin responses to predictable and unpredictable novelty stress in rats. Physiol Behav. 37, 285-288.

Norton, R., 1970. The effects of acute sleep deprivation on selective attention. Br. J. Psychol. 61, 157-161.

Novati, A., Roman, V., Cetin, T., Hagewoud, R., den Boer, J. A., Luiten, P. G., Meerlo, P., 2008. Chronically restricted sleep leads to depression-like changes in neurotransmitter receptor sensitivity and neuroendocrine stress reactivity in rats. Sleep. 31, 1579-1585.

Osborne, B., 1986. Behavioral and adrenal responses and meal expectancy in rats with fornix transection. Physiol Behav. 37, 499-502.

Peigneux, P., Laureys, S., Delbeuck, X., Maquet, P., 2001. Sleeping brain, learning brain. The role of sleep for memory systems. Neuroreport. 12, A111-A124.

Peyron, C., Tighe, D. K., Van Den Pol, A. N., de, L. L., Heller, H. C., Sutcliffe, J. G., Kilduff, T. S., 1998. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J. Neurosci. 18, 9996-10015.

Pfister, H. P., 1979. The glucocorticosterone response to novelty as a psychological stressor. Physiol Behav. 23, 649-652.

Pfister, H. P., King, M. G., 1976. Adaptation of the glucocorticosterone response to novelty. Physiol Behav. 17, 43-46.

Pilcher, J. J., Huffcutt, A. I., 1996. Effects of sleep deprivation on performance: a meta-analysis. Sleep. 19, 318-326.

Plihal, W., Born, J., 1999. Effects of early and late nocturnal sleep on priming and spatial memory. Psychophysiology. 36, 571-582.

Quirarte, G. L., Roozendaal, B., McGaugh, J. L., 1997. Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. Proc. Natl. Acad. Sci. U. S. A. 94, 14048-14053.

Radomski, M. W., Hart, L. E., Goodman, J. M., Plyley, M. J., 1992. Aerobic fitness and hormonal responses to prolonged sleep deprivation and sustained mental work. Aviat. Space Environ. Med. 63, 101-106.

Rajaratnam, S. M., Arendt, J., 2001. Health in a 24-h society. Lancet. 358, 999-1005.

Rechtschaffen, A., Bergmann, B. M., Gilliland, M. A., Bauer, K., 1999. Effects of method, duration, and sleep stage on rebounds from sleep deprivation in the rat. Sleep. 22, 11-31.

Rechtschaffen, A., Gilliland, M. A., Bergmann, B. M., Winter, J. B., 1983. Physiological correlates of prolonged sleep deprivation in rats. Science. 221, 182-184.

Riemann, D., Berger, M., Voderholzer, U., 2001. Sleep and depression--results from psychobiological studies: an overview. Biol. Psychol. 57, 67-103.

Robbins, T. W., 2002. The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology (Berl). 163, 362-380.

Rogers, A. E., 2008. The Effects of Fatigue and Sleepiness on Nurse Performance and Patient Safety.

Roman, V., Hagewoud, R., Luiten, P. G., Meerlo, P., 2006. Differential effects of chronic partial sleep deprivation and stress on serotonin-1A and muscarinic acetylcholine receptor sensitivity. J. Sleep Res. 15, 386-394.

Roman, V., Walstra, I., Luiten, P. G., Meerlo, P., 2005. Too little sleep gradually desensitizes the serotonin 1A receptor system. Sleep. 28, 1505-1510.

Romero, L. M., Dickens, M. J., Cyr, N. E., 2009. The Reactive Scope Model - a new model integrating homeostasis, allostasis, and stress. Horm. Behav. 55, 375-389.

Royo, F., Bjork, N., Carlsson, H. E., Mayo, S., Hau, J., 2004. Impact of chronic catheterization and automated blood sampling (Accusampler) on serum corticosterone and fecal immunoreactive corticosterone metabolites and immunoglobulin A in male rats. J. Endocrinol. 180, 145-153.

Samson, W. K., Resch, Z. T., 2000. The hypocretin/orexin story. Trends Endocrinol. Metab. 11, 257-262.

Sapolsky, R. M., 2000. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch. Gen. Psychiatry. 57, 925-935.

Sapolsky, R. M., Pulsinelli, W. A., 1985. Glucocorticoids potentiate ischemic injury to neurons: therapeutic implications. Science. 229, 1397-1400.

Sapolsky, R. M., Romero, L. M., Munck, A. U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55-89.

Schussler, P., Yassouridis, A., Uhr, M., Kluge, M., Weikel, J., Holsboer, F., Steiger, A., 2006. Growth hormone-releasing hormone and corticotropin-releasing hormone enhance non-rapid-eye-movement sleep after sleep deprivation. Am. J. Physiol Endocrinol. Metab. 291, E549-E556.

Schuurman, T., 1980. Hormonal correlates of agonistic behavior in adult male rats. Prog. Brain Res. 53, 415-420.

Schwartz, S., McDowell, A. W., Cole, S. R., Cornoni-Huntley, J., Hays, J. C., Blazer, D., 1999. Insomnia and heart disease: a review of epidemiologic studies. J. Psychosom. Res. 47, 313-333.

Selye H, 1950. The Physiology and Pathology of Exposures to Stress. Montreal: Acta Medica Publ.

Selye H, 1976. Stress in Health and Disease. Boston: Butterworths.

Sforza, E., Chapotot, F., Lavoie, S., Roche, F., Pigeau, R., Buguet, A., 2004. Heart rate activation during spontaneous arousals from sleep: effect of sleep deprivation. Clin. Neurophysiol. 115, 2442-2451.

Sgoifo, A., Buwalda, B., Roos, M., Costoli, T., Merati, G., Meerlo, P., 2006. Effects of sleep deprivation on cardiac autonomic and pituitary-adrenocortical stress reactivity in rats. Psychoneuroendocrinology. 31, 197-208.

Shiraishi, I., Honma, K., Honma, S., Hiroshige, T., 1984. Ethosecretogram: relation of behavior to plasma corticosterone in freely moving rats. Am. J. Physiol. 247, R40-R45.

Siegel, J. M., 1995. Phylogeny and the function of REM sleep. Behav. Brain Res. 69, 29-34.

Sobczak, S., Honig, A., van Duinen, M. A., Riedel, W. J., 2002. Serotonergic dysregulation in bipolar disorders: a literature review of serotonergic challenge studies. Bipolar. Disord. 4, 347-356.

Sousa, N., Paula-Barbosa, M. M., Almeida, O. F., 1999. Ligand and subfield specificity of corticoid-induced neuronal loss in the rat hippocampal formation. Neuroscience. 89, 1079-1087.

Spath-Schwalbe, E., Gofferje, M., Kern, W., Born, J., Fehm, H. L., 1991. Sleep disruption alters nocturnal ACTH and cortisol secretory patterns. Biol. Psychiatry. 29, 575-584.

Spiegel, K., Leproult, R., Van, C. E., 1999. Impact of sleep debt on metabolic and endocrine function. Lancet. 354, 1435-1439.

Srikumar, B. N., Raju, T. R., Shankaranarayana Rao, B. S., 2006. The involvement of cholinergic and noradrenergic systems in behavioral recovery following oxotremorine treatment to chronically stressed rats. Neuroscience. 143, 679-688.

Steffens, A. B., 1969. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. Physiol Behav. 4, 833-836.

Stein, L., 1966. Habituation and stimulus novelty: a model based on classical conditioning. Psychol. Rev. 73, 352-356.

ston-Jones, G., Rajkowski, J., Kubiak, P., Alexinsky, T., 1994. Locus coeruleus neurons in monkey are selectively activated by attended cues in a vigilance task. J. Neurosci. 14, 4467-4480.

Suchecki, D., Lobo, L. L., Hipolide, D. C., Tufik, S., 1998. Increased ACTH and corticosterone secretion induced by different methods of paradoxical sleep deprivation. J. Sleep Res. 7, 276-281.

Suchecki, D., Tiba, P. A., Tufik, S., 2002. Paradoxical sleep deprivation facilitates subsequent corticosterone response to a mild stressor in rats. Neurosci. Lett. 320, 45-48.

Sutcliffe, J. G., de, L. L., 2000. The hypocretins: excitatory neuromodulatory peptides for multiple homeostatic systems, including sleep and feeding. J. Neurosci. Res. 62, 161-168.

Sutcliffe, J. G., de, L. L., 2002. The hypocretins: setting the arousal threshold. Nat. Rev. Neurosci. 3, 339-349.

Symons, J. D., VanHelder, T., Myles, W. S., 1988. Physical performance and physiological responses following 60 hours of sleep deprivation. Med. Sci. Sports Exerc. 20, 374-380.

Tang, X., Liu, X., Yang, L., Sanford, L. D., 2005. Rat strain differences in sleep after acute mild stressors and short-term sleep loss. Behav. Brain Res. 160, 60-71.

Tang, X., Yang, L., Sanford, L. D., 2007. Sleep and EEG spectra in rats recorded via telemetry during surgical recovery. Sleep. 30, 1057-1061.

Tartar, J. L., Ward, C. P., Cordeira, J. W., Legare, S. L., Blanchette, A. J., McCarley, R. W., Strecker, R. E., 2008. Experimental sleep fragmentation and sleep deprivation in rats increases exploration in an open field test of anxiety while increasing plasma corticosterone levels. Behav. Brain Res.

Thompson, R. F., 2009. Habituation: a history. Neurobiol. Learn. Mem. 92, 127-134.

Thrivikraman, K. V., Huot, R. L., Plotsky, P. M., 2002. Jugular vein catheterization for repeated blood sampling in the unrestrained conscious rat. Brain Res. Brain Res. Protoc. 10, 84-94.

Tiemeier, H., Pelzer, E., Jonck, L., Moller, H. J., Rao, M. L., 2002. Plasma catecholamines and selective slow wave sleep deprivation. Neuropsychobiology. 45, 81-86.

Tobler, I., Murison, R., Ursin, R., Ursin, H., Borbely, A. A., 1983. The effect of sleep deprivation and recovery sleep on plasma corticosterone in the rat. Neurosci. Lett. 35, 297-300.

Tochikubo, O., Ikeda, A., Miyajima, E., Ishii, M., 1996. Effects of insufficient sleep on blood pressure monitored by a new multibiomedical recorder. Hypertension. 27, 1318-1324.

Tononi, G., Cirelli, C., 2006. Sleep function and synaptic homeostasis. Sleep Med. Rev. 10, 49-62.

Ulrich-Lai, Y. M., Herman, J. P., 2009. Neural regulation of endocrine and autonomic stress responses. Nat. Rev. Neurosci. 10, 397-409.

Ursin, R., 2002. Serotonin and sleep. Sleep Med. Rev. 6, 55-69.

Van den Buuse, M., 1994. Circadian rhythms of blood pressure, heart rate, and locomotor activity in spontaneously hypertensive rats as measured with radio-telemetry. Physiol Behav. 55, 783-787.

Van den Buuse, M., Van Acker, S. A., Fluttert, M., De Kloet, E. R., 2001. Blood pressure, heart rate, and behavioral responses to psychological "novelty" stress in freely moving rats. Psychophysiology. 38, 490-499.

Van den, B. M., 1994. Circadian rhythms of blood pressure, heart rate, and locomotor activity in spontaneously hypertensive rats as measured with radio-telemetry. Physiol Behav. 55, 783-787.

Van der Borght, K., Ferrari, F., Klauke, K., Roman, V., Havekes, R., Sgoifo, A., Van der Zee, E. A., Meerlo, P., 2006. Hippocampal cell proliferation across the day: increase by running wheel activity, but no effect of sleep and wakefulness. Behav. Brain Res. 167, 36-41.

Van Dongen, H. P., Maislin, G., Mullington, J. M., Dinges, D. F., 2003. The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep. 26, 117-126.

van Erp, A. M., Kruk, M. R., Meelis, W., Willekens-Bramer, D. C., 1994. Effect of environmental stressors on time course, variability and form of self-grooming in the rat: handling, social contact, defeat, novelty, restraint and fur moistening. Behav. Brain Res. 65, 47-55.

van Gaalen, M. M., Stenzel-Poore, M., Holsboer, F., Steckler, T., 2003. Reduced attention in mice overproducing corticotropin-releasing hormone. Behav. Brain Res. 142, 69-79.

Vassalli, A., Dijk, D. J., 2009. Sleep function: current questions and new approaches. Eur. J. Neurosci. 29, 1830-1841.

Vgontzas, A. N., Mastorakos, G., Bixler, E. O., Kales, A., Gold, P. W., Chrousos, G. P., 1999. Sleep deprivation effects on the activity of the hypothalamic-pituitary-adrenal and growth axes: potential clinical implications. Clin. Endocrinol. (Oxf). 51, 205-215.

Vgontzas, A. N., Zoumakis, E., Bixler, E. O., Lin, H. M., Follett, H., Kales, A., Chrousos, G. P., 2004. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. J. Clin. Endocrinol. Metab. 89, 2119-2126.

von Treuer, K., Norman, T. R., Armstrong, S. M., 1996. Overnight human plasma melatonin, cortisol, prolactin, TSH, under conditions of normal sleep, sleep deprivation, and sleep recovery. J. Pineal Res. 20, 7-14.

Walker, J. M., Berger, R. J., 1980. Sleep as an adaptation for energy conservation functionally related to hibernation and shallow torpor. Prog. Brain Res. 53, 255-278.

Walker, M. P., Stickgold, R., 2004. Sleep-dependent learning and memory consolidation. Neuron. 44, 121-133.

Walker, M. P., Stickgold, R., 2006. Sleep, memory, and plasticity. Annu. Rev. Psychol. 57, 139-166.

Weiss, J. M., 1972. Influence of psychological variables on stress-induced pathology. Ciba Found. Symp. 8, 253-265.

Weitzman, E. D., Fukushima, D., Nogeire, C., Roffwarg, H., Gallagher, T. F., Hellman, L., 1971. Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. J. Clin. Endocrinol. Metab. 33, 14-22.

Whyte, D. G., Johnson, A. K., 2007. Lesions of the anteroventral third ventricle region exaggerate neuroendocrine and thermogenic but not behavioral responses to a novel environment. Am J Physiol Regul. Integr. Comp Physiol. 292, R137-R142.

Wimmer, F., Hoffmann, R. F., Bonato, R. A., Moffitt, A. R., 1992. The effects of sleep deprivation on divergent thinking and attention processes. J. Sleep Res. 1, 223-230.

Zhong, X., Hilton, H. J., Gates, G. J., Jelic, S., Stern, Y., Bartels, M. N., Demeersman, R. E., Basner, R. C., 2005. Increased sympathetic and decreased parasympathetic cardiovascular modulation in normal humans with acute sleep deprivation. J. Appl. Physiol. 98, 2024-2032.

Zohar, D., Tzischinsky, O., Epstein, R., Lavie, P., 2005. The effects of sleep loss on medical residents' emotional reactions to work events: a cognitive-energy model. Sleep. 28, 47-54.

Nederlandse samenvatting

ACHTERGROND

De exacte functie van slaap is onbekend, maar dat slaap belangrijk is, blijkt uit het feit dat mensen ongeveer een derde van hun leven aan slapen besteden. Slaap is belangrijk voor verschillende processen zoals energiebalans, temperatuurregulatie, leren, geheugen, cognitie, stemming en motoriek. Tevens beïnvloedt slaap onder andere hormonale en immunologische processen. In de huidige samenleving hebben steeds meer mensen te maken met een tekort aan slaap, waarbij niet alleen de hoeveelheid slaap per dag afgenomen is, maar ook een verstoring van slaap een veel voorkomend probleem is. Beiden hebben gezondheidsproblemen tot gevolg. Niet alleen de exacte functie van slaap is onbekend, ook de mechanismen verantwoordelijk voor de negatieve effecten van een slaaptekort zijn onbekend. Meerdere hypothesen zijn geformuleerd en één daarvan suggereert betrokkenheid van de stresssystemen.

Studies laten veelal zien dat onthouding van slaap (slaapdeprivatie) geassocieerd is met stresssysteem activatie. Om die reden wordt slaapdeprivatie vaak gezien als een stressor. Activatie van de stresssystemen zorgt voor een scala aan processen die het mogelijk maken voor het organisme om met de situatie om te gaan. Chronische stresssysteem activatie heeft echter ongunstige effecten en kan leiden tot gezondheidsschade. In de studies beschreven in dit proefschrift ligt de focus op het nader begrijpen van de effecten van acute slaapdeprivatie bij ratten en de betrokkenheid van de stresssystemen. De effecten van acute slaapdeprivatie op de stressrespons, stresssysteem reactiviteit en aandachtsconcentratie zijn bestudeerd. Onze hypothese was dat slaapdeprivatie een effect heeft op de stresssystemen en daardoor de stressrespons tijdens situaties die stresssysteem activatie veroorzaken beïnvloedt, als ook de aandachtsconcentratie. De meer – en nieuwwaarde van de uitgevoerde studies ten opzichte van de huidige verzameling aan slaapdeprivatie studies is dat we stress verstorende factoren hebben gereduceerd, terwijl hormonale, fysiologische, gedragsmatige en elektro-

encefalografische (EEG) parameters simultaan, frequent en voor langere duur in vrij bewegende dieren werden gemeten. Dit gaf tevens de mogelijkheid om correlatie analyses te doen die belangrijk zijn voor het correct interpreteren van de resultaten.

DEFINITIE VAN STRESS

Om de resultaten van onze studies juist te kunnen interpreteren, hebben we ons eerst bezig gehouden met de definitie van stress. Over het algemeen wordt een situatie als stressvol beoordeeld wanneer het activatie van de fysiologische stresssystemen tot gevolg heeft. Echter, activatie van deze fysiologische systemen gebeurt niet alleen tijdens stressvolle situaties maar tijdens elke situatie die gepaard gaat met gedragsmatige activiteit. De fysiologische stressrespons bestaat uit activatie van het sympathico-adrenomedullair (SAM) systeem en de hypothalamus-hypofyse-bijnier-as (HPA-as). Activatie van het SAM-systeem zorgt voor het vrijkomen van adrenaline en noradrenaline in het bloed die onder andere verantwoordelijk zijn voor het mobiliseren van energie efficiënt transport van glucose en zuurstof naar de spieren door toename in bloeddruk en hartslag. HPA-as activatie zorgt voor het vrijkomen van glucocorticoïden (cortisol in mensen en corticosteron in dieren), die ook een rol spelen bij het leveren van energie en het reguleren van de energie balans. Onze hypothese is daarom dat een deel van de fysiologische activatie die optreedt tijdens een bepaalde situatie, een reflectie is van de energie die nodig is voor het gedrag wat gaande is, en dus van de vereiste metabole activiteit, en niet per se van de mate waarin de situatie aversief of negatief is. De proeven zijn gebaseerd op het idee dat een daadwerkelijke stressvolle situatie dient te voldoen aan twee criteria; de situatie dient zowel onvoorspelbaar als oncontroleerbaar te zijn voor het organisme. In hoofdstuk 2 en 5 hebben we de hypothese verder onderzocht door dieren bloot te stellen aan twee verschillende situaties en de stressrespons te analyseren samen met de mate van activiteit van de dieren.

In hoofdstuk 2 hebben we ratten bloot gesteld aan ‘novelty exposure’. Hierbij werden ratten gedurende vijftien minuten in een lege kooi geplaatst, identiek aan hun thuiskooi, maar dan zonder water, voedsel en bodembedekking. In de literatuur wordt deze situatie veelal beschreven als een stressor omdat het activatie van de

stresssystemen tot gevolg heeft. Naar onze mening bevat deze situatie echter weinig oncontroleerbare en onvoorspelbare elementen. Onze resultaten laten zien dat er inderdaad een hormonale en fysiologische stressrespons optreedt tijdens 'novelty exposure', maar dat de activatie van de stresssystemen nauw correleert met de mate van gedragsmatige activiteit van de dieren. Deze correlatie was zowel tijdens als na 'novelty exposure' zichtbaar. De stresssysteem activatie was mild met een snel en compleet herstel na beëindiging van de 'novelty exposure'. Tevens vertoonden de dieren geen stress gerelateerd gedrag. Deze resultaten tezamen tonen aan dat de gemeten hormonale en fysiologische respons als gevolg van 'novelty exposure' een reflectie is van de vereiste metabole activiteit voor het normale gedrag dat werd geïnduceerd door de situatie en niet van de mate waarin de situatie aversief of negatief is. Dus, ondanks dat 'novelty exposure' leidt tot stresssysteem activatie, voldoet deze situatie, op basis van onze metingen en onze visie, niet aan de definitie van een stressor. Deze eerste studie gaf ons tevens de bevestiging dat we in staat waren om op hoge frequentie simultaan corticosteron, hartslag, bloeddruk, lichaamstemperatuur, activiteit, gedrag en EEG te meten terwijl stress verstorende factoren succesvol gereduceerd werden.

In hoofdstuk 5 werden de dieren getraind om op een pedaaltje te drukken voor een voedselbeloning. Dit is een zogenaamde 'operant-conditioning taak'. Wanneer dit goed is aangeleerd, kan het gebruikt worden om zogenaamde 'frustrative non-reward stress' te induceren; het drukken op het pedaaltje wordt dan ineens niet meer beloond (extinctie). Dit geeft een onvoorspelbare en oncontroleerbare situatie. Onze resultaten laten zien dat wanneer het dier gedurende dertig minuten de taak uitvoert en wel een voedselbeloning krijgt, er stresssysteem activatie optreedt die geassocieerd is met de mate van gedragsmatige activiteit van de dieren. De gemeten fysiologische en hormonale respons lijkt in deze situatie dus een reflectie te zijn van de vereiste metabole activiteit. Wanneer de dieren gedurende dertig minuten niet beloond werden, was er ook stresssysteem activatie zichtbaar, evenals een toename in gedragsmatige activiteit. De mate van gedragsmatige activiteit nam echter af gedurende de dertig minuten van de taak. De dieren werden niet beloond en verloren motivatie. Deze afname in activiteit ging gepaard met een afname in SAM-systeem activiteit terwijl de corticosteron spiegels hoog bleven. Deze dissociatie tussen gedragsmatige activiteit en HPA-as activatie was nog duidelijker zichtbaar na het

beëindigen van de taak. Dit wijst op een vertraagd herstel van de corticosteron respons die niet geassocieerd is met de mate van gedragsmatige activiteit van de dieren. Deze bevindingen versterken onze hypothese dat een daadwerkelijk stressvolle situatie wordt gekenmerkt door een dissociatie tussen fysiologische stresssysteem activatie en gedragsmatige activiteit, en door oncontroleerbaarheid en onvoorspelbaarheid.

Beide studies tonen aan dat het belangrijk is om de fysiologische en hormonale respons samen met de gedragsmatige activiteit te analyseren om zo stresssysteem activatie correct te kunnen interpreteren in termen van stress of in termen van metabole ondersteuning van gedrag.

SLAAPDEPRIVATIE EN DE STRESSSYSTEMEN

Studies naar de effecten van een slaaptekort op de stresssystemen laten geen eenduidige resultaten zien. Veel studies tonen een activatie van de stresssystemen maar er zijn ook studies die geen effect of zelfs een afname laten zien als gevolg van slaapdeprivatie. Deze verschillen zouden veroorzaakt kunnen zijn door verschillen in slaapdeprivatie methode. In hoofdstuk 2 en 5 lieten we zien dat stresssysteem activatie voor een groot deel afhankelijk is van de mate van fysieke en gedragsmatige activiteit van de dieren. Ook de mate van mentale activiteit en stress is van invloed op de stresssysteem activatie. Zo wordt een hogere stresssysteem activatie gezien wanneer organismen tijdens de slaapdeprivatie periode fysiek of mentaal actief zijn of wanneer de slaapdeprivatie methode aversief is. Verschillende effecten kunnen ook ontstaan door verschillen in slaapdeprivatie duur. In hoofdstuk 3, 4 en 5 hebben we ratten blootgesteld aan 4 of 6 uur slaapdeprivatie. Aangezien ratten normaal ongeveer 12 uur per dag slapen werd de hoeveelheid slaap met een derde tot de helft gereduceerd. Dit werd gedaan door de zogenaamde ‘gentle handling’ slaapdeprivatie methode. De dieren werden wakker gehouden door ze te aaien of, als dit niet meer voldoende was, door ze te verplaatsen in de kooi. De dieren werden van te voren hieraan gewend. Door deze methode te gebruiken hebben we geprobeerd zo weinig mogelijk stress te induceren. Ook induceert deze methode weinig mentale en fysieke activiteit.

Onze studies tonen aan dat deze 'gentle handling' slaapdeprivatie methode effectief is in het induceren van een slaaptekort. Tijdens de periode van slaapdeprivatie waren er enkel zogenaamde 'Non Rapid Eye Movement (NREM) microsleeps' te zien maar verder waren de dieren wakker. Deze NREM 'microsleeps' zijn praktisch onvermijdelijk. Tevens laten de resultaten een NREM slaap rebound zien na het beëindigen van de slaapdeprivatie. Dit was tot 1,5 uur na het beëindigen van de slaapdeprivatie nog zichtbaar en geeft aan dat de dieren extra zijn gaan slapen om het tekort in te halen. De resultaten beschreven in hoofdstuk 4 laten ook nog een toename in NREM slaap delta power zien, wat aangeeft dat de slaapdiepte verhoogd was.

We laten verder zien dat acute 'gentle handling' slaapdeprivatie een milde activatie van het SAM-systeem en de HPA-as geeft en dat deze volledig hersteld zijn 45 minuten na beëindiging van de slaapdeprivatie. De milde stresssysteem activatie kan het gevolg zijn van het slaaptekort dat werd geïnduceerd. Echter, correlatie analyses toonden een correlatie tussen de mate van fysieke activiteit van de dieren en de gemeten fysiologische en hormonale respons. Deze correlatie was zowel tijdens als na slaapdeprivatie zichtbaar. Dit wijst erop dat een deel van de stresssysteem activatie tijdens slaapdeprivatie nodig was om de fysieke activiteit, geïnduceerd om de dieren wakker te houden, te ondersteunen. De geïnduceerde fysieke activiteit was niet hoger dan de activiteit die onder normale omstandigheden wordt gezien als de dieren wakker zijn. De gedragsmatige activiteit van dieren tijdens slaapdeprivatie kan de stresssystemen dus sterk beïnvloeden en daarom is het belangrijk dat hier rekening mee gehouden wordt wanneer de resultaten van slaapdeprivatie studies worden geïnterpreteerd.

Op basis van onze resultaten blijkt 'gentle handling' slaapdeprivatie weinig stressvol te zijn. Er was een milde stresssysteem activatie te zien die nauw gerelateerd was aan de fysieke activiteit die werd geïnduceerd. Toch bemerkten we dat sommige dieren geagiteerd reageerden tegen het einde van de slaapdeprivatie periode. Dit was niet terug te zien in de gemeten fysiologische en hormonale respons. Omdat dit vooral tegen het einde van de slaapdeprivatie periode te zien was, denken we dat dit het gevolg is van een toename in slaaptekort en niet zozeer van stress of de slaapdeprivatie procedure, maar dit dient verder onderzocht te worden.

Verschillende brein mechanismen zijn betrokken bij het effect van slaapdeprivatie op de stresssystemen. In dit proefschrift zijn de mogelijke onderliggende mechanismen in het brein niet onderzocht, maar is de betrokkenheid van verschillende systemen wel bediscussieerd.

SLAAPDEPRIVATIE EN STRESSSYSTEEM REACTIVITEIT

Slaapdeprivatie kan niet alleen rechtreeks de fysiologische en hormonale stresssystemen activeren (basale stressrespons), maar kan ook een effect hebben op de reactiviteit van de stresssystemen op bepaalde nieuwe situaties (geactiveerde stressrespons). In hoofdstuk 4 hebben we dit onderzocht door de dieren na het beëindigen van de slaapdeprivatie bloot te stellen aan 'novelty exposure' en in hoofdstuk 5 aan 'frustrative non-reward stress'. Beide situaties geven stresssysteem activatie maar, zoals besproken, is deze activatie voor 'novelty exposure' een reflectie van de fysieke activiteit van de dieren, waar deze voor de 'frustrative non-reward' situatie een reflectie is van de aversiviteit. De dieren kregen de kans om 1,5 uur te herstellen na beëindiging van de slaapdeprivatie voordat ze werden blootgesteld aan de situatie. Deze herstelperiode werd geïnduceerd om te voorkomen dat de dieren in een geagiteerde staat zouden worden blootgesteld aan de situaties. Onze studies laten zien dat er na deze 1,5 uur nog steeds een slaaptekort was.

De resultaten laten zien dat acute slaapdeprivatie geen effect heeft op de gemeten hormonale en fysiologische respons tijdens 'novelty exposure' en 'frustrative non-reward stress'. Ook had voorafgaande slaapdeprivatie geen effect op de fysieke activiteit en het gedrag van de dieren tijdens beide situaties. Tevens vonden we geen effect van slaapdeprivatie op de fysiologisch en hormonale respons en het gedrag tijdens de situatie waarbij de dieren wel beloond werden in de 'operant-conditioning taak'. Ook het herstel van de stressrespons na het beëindigen van de verschillende situaties was niet beïnvloed door voorafgaande slaapdeprivatie. Dus, ondanks dat er een slaaptekort was geïnduceerd, had dit geen effect op de hormonale en fysiologische respons, en op het gedrag, tijdens de situaties waaraan de dieren werden blootgesteld.

Er zijn weinig studies waarbij dieren worden blootgesteld aan een potentieel stressvolle situatie nadat ze zijn gedepriveerd van slaap. De studies die zijn gedaan, verschillen in slaapdeprivatie duur, slaapdeprivatie methode en type situatie waardoor het lastig is de studies met elkaar te vergelijken.

SLAAPDEPRIVATIE EN AANDACHTSCONCENTRATIE

We waren niet alleen geïnteresseerd in de effecten van acute slaapdeprivatie op de geactiveerde stressrespons, we waren ook geïnteresseerd in het effect op de aandachtsconcentratie. Meerdere studies tonen aan dat slaapdeprivatie de aandachtsconcentratie negatief beïnvloedt, maar er zijn maar weinig studies die verschillende aandachtsconcentratie parameters bestudeerd hebben. Tevens verschillen de huidige studies in slaapdeprivatie methode, -duur en type aandachtsconcentratie test wat het lastig maakt om de resultaten te vergelijken.

De resultaten van hoofdstuk 5 laten al zien dat acute slaapdeprivatie de prestatie, impulsiviteit en motivatie tijdens de ‘operant-conditioning taak’ niet beïnvloedt en dat ook de stressrespons niet is beïnvloed. In hoofdstuk 6 hebben we dit verder onderzocht door de dieren te trainen in de zogeheten '5-choice serial reaction time task’ (5-CSRTT). Voor het uitvoeren van deze taak wordt het ratten aangeleerd om te reageren op een licht stimulus die willekeurig en voor korte tijd wordt gepresenteerd in 1 van de 5 gaten die aanwezig zijn in een wand van de set-up. Wanneer het dier het lichtsignaal ziet en binnen de gestelde tijd reageert door de neus in het gaatje te drukken waar het lichtsignaal voor korte tijd te zien was, verdient het een voedselbeloning. Deze test maakt het mogelijk om de selectieve en de volgehouden aandacht opgesplitst te onderzoeken. Selectieve aandacht is de mogelijkheid om de aandacht te richten op een gebeurtenis. Volgehouden aandacht is de mogelijkheid om de aandacht voor langere duur vast te houden. Onze resultaten laten zien dat voorafgaande slaapdeprivatie niet van invloed is op de selectieve en volgehouden aandacht, als gemeten in de 5-CSRTT. Ook de motivatie en impulsiviteit van de dieren waren niet beïnvloed.

Twee andere studies die slaapdeprivatie hebben gecombineerd met de 5-CSRTT tonen wel een effect van voorafgaande slaapdeprivatie op de selectieve en

volgehouden aandacht. Verschillen in het aanleren van de taak en de prestatie in de 5-CSRTT kunnen een oorzaak zijn voor het vinden van verschillende effecten. Ook een verschil in slaapdeprivatie methode en -duur speelt zeer waarschijnlijk een rol. Tevens hebben wij de dieren de kans gegeven om voor korte tijd te herstellen van de slaapdeprivatie om te voorkomen dat de dieren geagiteerd de taak zouden uitvoeren. Dit is niet gedaan in andere studies. Het is belangrijk dat er een gestandaardiseerd design komt met onder andere duidelijke training protocollen en een standaard slaapdeprivatie methode zodat de resultaten tussen studies beter vergeleken kunnen worden en elkaar kunnen aanvullen.

Het bestuderen van de onderliggende mechanismen in het brein die betrokken zouden kunnen zijn bij de effecten van acute slaapdeprivatie op de aandachtconcentratie, viel buiten de onderzoeksvraag van dit proefschrift. Wel bediscussiëren we in dit proefschrift verschillende betrokken systemen. Studies tonen aan dat slaapdeprivatie en stresssysteem activatie mechanismen beïnvloeden die belangrijk zijn voor de aandachtconcentratie. Er is een interessante neurobiologische overlap tussen de processen betrokken bij aandachtconcentratie, stresssysteem activatie en slaap.

CONCLUSIES

De studies in dit proefschrift bevestigen dat het experimenteel diermodel, dat wij hebben ontwikkeld, het mogelijk maakt om meerdere hormonale, fysiologische, EEG, gedragsmatige en aandachtsconcentratie parameters te meten terwijl stress verstorende factoren gereduceerd worden. Tevens bevestigen onze studies dat het belangrijk is, voor een correcte interpretatie van de resultaten, om stresssysteem activatie samen met de gedragsmatige activiteit te bestuderen.

Het doel van onze studies was om te bekijken of slaapdeprivatie, door middel van de stresssystemen, de aandachtsconcentratie en stresssysteem reactiviteit zou beïnvloeden. We hebben aangetoond dat acute slaapdeprivatie, indien toegepast volgens ons model, de stresssystemen mild beïnvloedt en dat er geen effect is op de hormonale en fysiologische respons, de aandachtsconcentratie en op gedragmatige parameters wanneer het dier daarna wordt blootgesteld aan ‘novelty exposure’,

'frustrative non-reward stress', een 'operant-conditioning taak' of de 5-CSRTT. Dit kan worden verklaard door het complete herstel van de stresssysteem activatie na beëindiging van de slaapdeprivatie en de nauwe correlatie van de fysiologische en hormonale respons met de mate van fysieke activiteit van de dieren. Ondanks dat we geen negatieve effecten zien, ondersteunen onze resultaten wel onze hypothese dat de stresssystemen in belangrijke mate betrokken zijn bij de consequenties van slaapdeprivatie.

De studies in deze thesis geven meer inzicht in de complexe relatie tussen slaaptekort en stresssysteem activatie, maar er blijven nog veel vragen onbeantwoord. Zo is het belangrijk om ook de achterliggende neurobiologische mechanismen verder te onderzoeken. Met name de overlappende mechanismen die betrokken zijn bij slaap, stresssysteem activatie en aandachtsconcentratie zijn interessant. Deze kunnen meer inzicht geven in de relatie tussen slaaptekort en de stresssystemen en hun consequenties op stress reactiviteit en de aandachtsconcentratie.

Ondanks dat wij laten zien dat er geen effect is van acute slaapdeprivatie op stress reactiviteit en de aandachtsconcentratie kan herhaaldelijk korte slaapdeprivatie andere effecten hebben. Het is goed mogelijk dat de dieren na herhaaldelijk acute slaapdeprivatie niet voldoende kunnen herstellen. Een incompleet herstel wordt ook gezien bij chronische slaapdeprivatie studies. We verwachten dat chronische slaapdeprivatie of herhaaldelijke acute slaapdeprivatie de stresssystemen activeert en er geen compleet herstel is na beëindiging van de slaapdeprivatie. We verwachten daarom een dissociatie te zien tussen de stresssysteem activatie en de gedragsmatige respons. We verwachten ook dat hierdoor de stresssysteem reactiviteit en aandachtsconcentratie aangetast is wanneer het dier na beëindiging van de slaapdeprivatie wordt blootgesteld aan bepaalde situaties. Het zou daarom interessant zijn om ons diermodel verder te gebruiken en de slaapdeprivatie duur te verlengen of de acute slaapdeprivatie dagelijks te herhalen. Ons diermodel biedt een uitstekende basis voor zulke vervolg studies.

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Wieteke

Curriculum Vitae

Wieteke Beerling was born on January 15 in the year 1983 in Drachten, the Netherlands. She attended the Athenaeum at the Drachtster Lyceum in Drachten and graduated in the year 2001. After that she studied Biology, with the Behavioural & Neurosciences specialization and with the Research variant, at the University of Groningen in the Netherlands. Her first research project was performed at the Department of Behavioural Physiology under the supervision of Dr. B. Buwalda where she studied the behavioural and neurobiological indicators of neurotoxicity caused by methotrexate. The second project was performed at the Department of Neuroendocrinology and was supervised by Prof. Dr. G. van Dijk. The aim of this project was to study the involvement of the MCH and MSH system in obesity and the possible treatment with the MCH antagonist. For the third project she moved to Belgium for seven months where she worked under the supervision of Dr. W.H.I.M. Drinkenburg and Prof. Dr. J.M. Koolhaas at the Department of Neuroscience Research of Janssen Research & Development, a division of Janssen Pharmaceutica N.V., Beerse, Belgium. In this research project, she studied the stress challenges in paradigms for psychiatric drug discovery and the effects of CO_2 and 8-OH-DPAT on neuroendocrine, physiological and behavioural variables. This led to a PhD project after Wieteke graduated in 2007. The PhD project was performed at the same department of Janssen Research & Development under supervision of Dr. W.H.I.M. Drinkenburg, in collaboration with the Department of Behavioural Physiology at the University of Groningen in the Netherlands under the supervision of Prof. Dr. J.M. Koolhaas and Dr. P. Meerlo. The work she performed during this period has led to the present thesis. Since 2011 she works as Clinical Research Associate at PPD in Bennekom in the Netherlands.

List of publications

Beerling W, Koolhaas JM, Ahnaou A, Bouwknecht JA, de Boer SF, Meerlo P, Drinkenburg WHIM. Physiological and hormonal responses to novelty exposure in rats are mainly related to ongoing behavioral activity. Physiology & Behavior 2011, 103:412-20.

Beerling W, Koolhaas JM, Meerlo P, Raeymaekers LJ, Heylen AML, Ahnaou A, Drinkenburg WHIM. Sleep deprivation by gentle handling in rats does not affect the physiological, behavioral and hormonal responses to novelty exposure. Sleep-Wake Research in the Netherlands, Annual Proceedings of the NSWO 2010, 21:55-58.

Beerling W, Ahnaou A, Raeymaekers LJ, Heylen AML, Geys HM, Meerlo P, Koolhaas JM, Drinkenburg WHIM. Sleep deprivation by gentle handling in rats: is it stressful? Sleep-Wake Research in the Netherlands, Annual Proceedings of the NSWO 2009, 20:49-52.

Seigers R, Schagen SB, **Beerling W**, Boogerd W, van Tellingen O, van Dam FS, Koolhaas JM, Buwalda B. Long-lasting suppression of hippocampal cell proliferation and impaired cognitive performance by methotrexate in the rat. Behavioral Brain Research 2008, 186:168-75.