INTRODUCTION

Frequently disrupted and restricted sleep is a widespread problem in our modern around-the-clock society\textsuperscript{1,2}. In the long run, insufficient sleep may have serious repercussions for health and well-being. Indeed, sleep complaints and restricted sleep have been identified as risk factors for various diseases including, for example, mood disorders such as depression\textsuperscript{3}. One pathway along which restricted sleep might affect the sensitivity to disease is by altering central and neuroendocrine stress systems. The stress systems in our brain and body play a critical role in adapting to a continuously changing and challenging environment\textsuperscript{4,5}. Stress hormones not only support metabolic processes and physical activity under acute stress but also affect brain function, cognition and mood. Therefore, effects of sleep loss on these stress systems may have direct functional consequences for the way we perform and deal with everyday challenges.

The present study in rats examined the effect of chronic partial sleep deprivation on the sensitivity of the serotonin-1A (5HT-1A) receptor system and the corticotropin-releasing hormone (CRH) receptor system. Both of these systems play an important role in coordinating stress responses, which includes regulation of the hypothalamic-pituitary-adrenal (HPA) axis, one of the main neuroendocrine stress systems\textsuperscript{4,5}. Also, alterations in both the serotonergic and CRH system have been implicated in the pathophysiology of depression\textsuperscript{8,9}.

METHODS

Sleep restriction

Adult male Wistar rats were subjected to a protocol of chronic sleep restriction allowing them only 4h of undisturbed rest per day at the beginning of the light phase, their normal resting phase\textsuperscript{10,11}. The remainder of the time, animals were kept awake by placing them in slowly rotating wheels driven by an engine at constant speed (0.4 m/min). Since the sleep deprivation procedure includes mild forced locomotion, we used forced activity control rats to test whether effects of sleep restriction might be due to forced activity rather then sleep loss per se. Animals of the forced activity group were placed in wheels similar to the ones that were used for sleep restriction. However, these wheels rotated at double speed (0.8 m/min) for half the time (10h). With this protocol, rats walked the same distance as sleep restricted ones, but had sufficient time for sleep (14h).
Pharmacological challenge tests
To investigate whether sleep loss alters the sensitivity of the serotonin-1A and/or CRH receptor system and thereby changes HPA axis reactivity, sleep restricted and control rats received an intravenous injection of ovine CRH (0.5 μg/kg; American Peptide Company, Sunnyvale, CA, USA) or an injection with the serotonin-1A receptor agonist 8-OH-DPAT (0.1 mg/kg; Sigma, St. Louis, MO, USA). The pharmacological challenges were performed after 7 and 8 days of restricted sleep and blood samples were taken to measure ACTH and corticosterone responses. The magnitude of these hormone responses served as read out for the sensitivity to CRH and 8-OH-DPAT.

Blood sampling and hormone measurements
The rats were equipped with permanent heart catheters that allowed for iv injections of the pharmaeca and at the same time permitted repeated blood sampling without disturbing the animals. To measure the ACTH and corticosterone responses, blood samples were taken shortly before as well as 5, 15, and 60 min after the injections with CRH or 8-OH-DPAT. Blood was collected in pre-chilled tubes containing EDTA as anti-coagulant. Samples were centrifuged and the plasma was stored at -80°C until radioimmunoassay analysis of hormones (ICN Biomedicals, Costa Mesa, CA, USA).

RESULTS
Injection of CRH induced clear ACTH and corticosterone responses in all rats (Figure 1A and B). However, the ACTH response was lower in sleep restricted rats compared to the response in forced activity control animals and home cage control animals. Yet, despite the lower ACTH response, the corticosterone response in sleep restricted rats was not different from the response in the control rats.

Also injection of the serotonin-1A receptor agonist 8-OH-DPAT resulted in a clear HPA-axis response in all animals (Figure 1C and D). On average, the ACTH response of the sleep restricted rats was lower than that of the home cage control animals, which in turn was lower than that of the forced activity control rats. In contrast, the CORT response to 8-OH-DPAT was not significantly different between sleep restricted and control rats.

DISCUSSION
The data show that rats subjected to chronic partial sleep deprivation have reduced pituitary ACTH responses upon stimulation of CRH and serotonin-1A receptors. This finding is in line with an earlier study showing a similar blunted ACTH response to a real stressor. Together these findings imply that losing too much sleep alters neuroendocrine stress reactivity and suggest that these changes may be mediated by decreased sensitivity of CRH and serotonin-1A receptors in the pituitary and/or in other central structures. The data further show that, despite an attenuated ACTH release, sleep restricted rats had an adrenal corticosterone response that was not different from that of controls animals. An unchanged corticosterone response in the face of blunted ACTH levels can be explained by increased ACTH sensitivity in the adrenal cortex, which indicates that sleep restriction alters regulation of the HPA-axis at multiple levels.

Importantly, reduced serotonin-1A receptor sensitivity and blunted ACTH responses to CRH have been reported in depressed patients. In other words, our studies show that
experimental restriction of sleep changes receptor systems involved in stress and regulation of the HPA axis in a direction that is similar to what is seen in affective disorders. Therefore, this study supports the hypothesis that disrupted and restricted sleep may result in a brain condition that is more vulnerable to malfunction and disease.

Figure 1. Neuroendocrine responses to an intravenous infusion of corticotropin releasing hormone (CRH) or the serotonin-1A agonist 8-OH-DPAT in sleep restricted rats, forced activity controls, and home cage controls (n=8 to 10 each). Chronic sleep restriction resulted in an attenuated ACTH responses to both CRH and 8-OH-DPAT (A,C) whereas the corticosterone response was not altered (B,D). Significant differences: a = sleep restricted vs home cage controls; b = sleep restricted vs forced activity controls (p<0.05, posthoc Tukey test following ANOVA).

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