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Homeostatic Regulation of Sleep in a Genetic Model of Depression in the Mouse: Effects of Muscarinic and 5-HT_{1A} Receptor Activation

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In depressed patients, sleep undergoes marked alterations, especially sleep onset insomnia, sleep fragmentation, and disturbances of the Rapid Eye Movement (REM) sleep. Abnormalities of rest-activity rhythms and of hypothalamic–pituitary–adrenocortical function have also been described in these patients. In the present study, we examined the presence of such abnormalities in a recently developed line of mice (Helpless mice-H) that exhibit depression-like behaviors in validated tests, compared to the nonhelpless (NH) line derived from the same colony. Experiments were essentially carried out in females for which previous studies showed marked differences between H and NH lines. Compared to NH mice, the H line exhibited (i) lower basal locomotor activity, (ii) sleep fragmentation, shift towards lighter sleep stages, and facilitation of REM sleep reflected by increased amounts and decreased latency, (iii) larger response to the REM sleep promoting effect of muscarinic receptor stimulation (by arecoline). In contrast, H and NH mice were equally responsive to the REM sleep inhibitory effect of 5-HT_{1A} receptor stimulation (by 8-OH-DPAT). In addition, a deficiency in delta power enhancement after sleep deprivation was observed in the H group, and acute immobilization stress in this group failed to elicit a REM sleep rebound and was associated with a long-lasting raise in serum corticosterone levels. These results further validate H mice as a depression model and suggest they might be of particular interest for investigating the neurobiological mechanisms and possibly genetic substrates underlying sleep alterations associated with depression.
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INTRODUCTION

Depression is one of the most common psychiatric disorders in developed countries. It is associated with characteristic sleep impairments, notably difficulties in falling asleep, reduction of the rapid eye movement (REM) sleep latency, sleep fragmentation, and early morning awakenings (Adrien, 2002; Benca, 2000). In addition, abnormalities of circadian rhythms, affecting notably nycthemeral variations in rest-activity, and body temperature, have been described in depressed patients (Avery *et al*, 1999; Bunney and Bunney, 2000; Souetre *et al*, 1989).

In order to study the physiopathology of depression, several groups have attempted to develop animal models that exhibit depression-like behaviors and do respond to antidepressant therapy (Nestler *et al*, 2002; Overstreet, 1993; Willner, 1990). Studies of sleep patterns in these various models might help to elucidate the mechanisms underlying the relationship between depression and sleep abnormalities. To date, these aspects have only been studied in a few of these models, and mostly in rats (Adrien *et al*, 1991; Dugovic *et al*, 2000; Overstreet, 1993; Willner, 1990). We recently developed a line of mice selected from its long duration immobility in the tail suspension test ('Helpless' – H), compared to 'non-Helpless' (NH) mice which exhibited short duration immobility in the test (El Yacoubi *et al*, 2003). H mice exhibited several neurochemical and behavioral impairments, that mimic those found in depression, with notably an increased pressure of REM sleep and a lighter slow wave sleep (SWS) (El Yacoubi *et al*, 2003). Here,

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to further assess the relevance of H mice as a model of depression, we detail these sleep impairments and we examine the cycle of locomotor activity and body temperature over the dark–light cycle. Moreover, we monitored sleep responses to pharmacological and behavioral challenges.

Depression is schematically associated with a general increase in cholinergic and decrease in serotonergic neurotransmission (Gillin *et al*, 1991; Hasler *et al*, 2004; Kapitany *et al*, 1999; Seifritz *et al*, 1998). To test for the presence of such imbalance between cholinergic and serotonergic function in H mice, we investigated the sleep modifications induced by treatments with agonists of muscarinic and serotonergic 5-HT_{1A} receptors in both H and NH mice.

It has been well documented that sleep regulation depends on a homeostatic drive (Borbely *et al*, 1981). During recovery from sleep deprivation, this homeostatic drive notably causes an increase of slow wave activity (SWA) assessed by quantitative EEG measures, in humans (Borbely *et al*, 1981), rats (Borbely *et al*, 1981; Tobler and Borbely, 1990), as well as mice (Franken *et al*, 2001; Lena *et al*, 2004; Tobler *et al*, 1997). The magnitude of SWA depends on the duration of prior waking (Tobler and Borbely, 1990), which suggests that SWA is a marker for sleep intensity (Borbely *et al*, 1981). On the other hand, it has been shown that stress-elicited activation of the hypothalamo–pituitary–adrenocortical (HPA) axis triggers a REM sleep rebound (Boutrel *et al*, 2002; Lena *et al*, 2004; Rampin *et al*, 1991). In depressed patients, both sleep homeostasis (Armitage *et al*, 2000; Borbely, 1987) and HPA axis activity (Barden *et al*, 1995) are frequently altered. Accordingly, the effects of sleep deprivation and of immobilization stress on subsequent sleep parameters were also investigated in H *vs* NH mice.

MATERIALS AND METHODS

All the procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (Council directive # 87–848, October 19, 1987, Ministère de l'agriculture et de la forêt, Service vétérinaire de la santé et de la protection animale, permissions # 75–116 to M.H. and # 75–125 to JA).

All mice used for these studies were of the H and NH lines (CD1 strain), selected across 12–14 generations for high or low spontaneous 'helplessness' in the tail suspension test, as described previously (El Yacoubi *et al*, 2003). For the study of spontaneous sleep-wakefulness patterns, both males and females were used. For all other experiments, data were collected from females only. Indeed, previous studies showed that differences between H and NH mice (regarding notably adaptive behavioural and neurobiological responses to chronic treatment with antidepressants) were larger in females than in males (El Yacoubi *et al*, 2003).

Recordings of Locomotor Activity and Body Temperature

At 2–3 months of age, female mice of both H and NH lines were anaesthetized with sodium pentobarbital (75 mg/kg intraperitoneally(i.p.)) and implanted into the abdomen with a mini-emitter (Mini-Mitter® Co, Bend, OR, USA) for monitoring locomotor activity and body temperature. After completion of surgery, animals were housed in individual cages under standard laboratory conditions (12–12 h light–dark cycle (light on at 0700), food and water *ad libitum*, $24 \pm 1^{\circ}$C ambient temperature), and were allowed 10–14 days to recover. Thereafter, each animal was recorded during 8 days, that is across two successive ovarian cycles. Data were collected on a computer with Vitalview software (Mini-Mitter® Co). Body temperature was measured at a constant acquisition rate of once per second, and averaged over 5 min-periods. For the measurement of locomotor activity, position and orientation of the abdominal emitter relative to the receiver were determined every second, and changes in these parameters were summed over 5 min-periods to yield activity counts.

Implantation of Electrodes for Polygraphic Sleep-Wakefulness Monitoring

At 2–3 months of age, male and female mice of the H and NH lines were implanted under sodium pentobarbital anaesthesia (75 mg/kg i.p.) with the classical set of electrodes (made of enameled nichrome wire, 150 μm in diameter) for polygraphic sleep monitoring. Briefly, EEG electrodes were inserted through the skull onto the dura over the right cerebral cortex (2 mm lateral and 4 mm posterior to the bregma) and over the cerebellum (at midline, 2 mm posterior to lambda), EOG electrodes were positioned under the skin on each side of the orbit, and EMG electrodes were inserted into the neck muscles. All electrodes were anchored to the skull with super-bond and acrylic cement, and soldered to a mini-connector also embedded in cement (Boutrel *et al*, 1999). After completion of surgery, animals were housed in individual cages ($20 \times 20 \times 30$ cm) and maintained under standard laboratory conditions (see above). They were allowed 10–14 days to recover, during which they were habituated to the recording conditions, that is one animal per cage, the home-cage being the recording cage.

Recording of Sleep-Wakefulness Cycles

After the animals had been habituated to the recording cable for 2 days, polygraphic recording of the spontaneous sleep wakefulness states was performed during 48 h for males and 96 h for females, beginning at 1900, that is at the onset of the dark period.

Power Spectra Analysis

The EEG signal was processed for power spectra analysis (Franken *et al*, 1998; Lena *et al*, 2004). For each animal under well-defined experimental conditions, a spectrogram was obtained and the values for power spectra were divided into four frequency bands: delta (0.5–4.99 Hz), theta (5–9.99 Hz), spindles (10–19.99 Hz), and high frequencies (20–60 Hz). The delta and theta bands were determined according to the spectral profiles in SWS and REM sleep, respectively. EEG spectrograms and power spectra for each of the four frequency bands, expressed as a percentage of

the mean EEG power over all frequency bands, were compared between H and NH mice under baseline conditions, after sleep deprivation and after immobilization stress.

Monitoring of Estrous Cycle

The estrous cycle was monitored in order to examine a possible relation with sleep modifications. For this purpose, vaginal smear samples from H and NH females were taken daily between 1700 and 1800, starting 4–5 days before recording spontaneous sleep cycles. Quantitative determination of leukocytes and epithelial cells in vaginal smears allowed identification of the phase of the 4 day-estrous cycle. Proestrous was characterized by the presence of numerous round epithelial cells and a small number of leukocytes; estrous by that of large, flat cornified cells and no leukocytes; metestrous by some cornified cells and numerous leukocytes; and diestrous by a minority of epithelial cells and a majority of leukocytes (Schwierin *et al*, 1998).

Pharmacological Treatments

Drugs were dissolved in 0.1 ml of saline, and all injections were performed at 1000, after which polygraphic recordings were obtained until 1800. The muscarinic agonist arecoline (0.025, 0.05, 0.1 mg/kg; Sigma, St Quentin Fallavier, France) was injected i.p. whereas the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (0.2, 0.4 and 0.8 mg/kg; Research Biochemicals Int., Natick, MA) was injected subcutaneously (s.c.). For baseline data, mice were injected with saline, i.p. or s.c., as appropriate. A washout period of at least 2 days for arecoline and 7 days for 8-OH-DPAT was allowed between two consecutive treatments. The effects of each dose of arecoline and 8-OH-DPAT upon each state of vigilance were analyzed for every hour following injection. For a given treatment, each animal was referred to its own baseline represented by the data obtained after injection of saline.

Sleep Deprivation

Mice were subjected to a 6 h sleep deprivation starting at light onset (0700). For this purpose, animals were continuously observed and objects (pencil, pieces of paper, plastic tubes) were introduced into the cage to disturb the mouse as soon as it engaged in a sleeping posture (Lena *et al*, 2004; Tobler *et al*, 1997). At the end of the deprivation period, animals were left to sleep freely and were recorded for 18 h (from 1300 to 0700 the next morning).

Immobilization Stress

At least 15 days after completion of the sleep deprivation procedure, mice were immobilized for 90 min, from 1830 to 2000, by wrapping them inside a plastic grid as described previously (Boutrel *et al*, 2002; Lena *et al*, 2004). At the end of this period, they were returned to their home cage for sleep-wakefulness monitoring during 24 h. As a control, each mouse underwent a sham procedure (the animal remained free in its home cage from 1830 to 2000 and was connected a few minutes before being recorded during 24 h), 2 days prior to the immobilization stress procedure.

Serum Corticosterone Assay

Serum corticosterone levels were measured immediately and 6 h after immobilization stress. For this purpose, animals were decapitated and their blood was collected, allowed to clot and centrifuged at 1700 g for 25 min at 4°C. Serum was then collected and stored at −20°C until further analysis. Corticosterone was quantified by radioimmunoassay after extraction in ethanol (Grino *et al*, 1987). Anticorticosterone antiserum was generously given by F Héry (INSERM U297, Marseille, France). Corticosterone (Sigma) was used as standard and [^{3}H]corticosterone (87 Ci/mmol, Amersham-Pharmacia Biotech., Les Ulis, France) as radiotracer (Grino *et al*, 1987).

Data Analyses and Statistics

The changes in locomotor activity and core temperature over time were analyzed using two-way ANOVA with repeated measures over time. The circadian oscillations of these variables were assessed using the Cosinor® software (S.E.P.T.M.R., Bordeaux, France).

Polygraphic recordings were scored visually every 15 s epoch as wakefulness (W), SWS or REM sleep following classical criteria (Lena *et al*, 2004; Tobler *et al*, 1997), using the Somnologica® software (Flaga, Reykjavik, Island). SWS was subdivided into light (SWS_1) and deep (SWS_2) according to the criteria defined for rats (Neckelmann and Ursin, 1993) and adapted to mice: when delta waves were present during less than 50% of the epoch, it was scored as SWS_1, and when this percentage was higher than 50%, as SWS_2.

For analysis of the spontaneous sleep-wakefulness patterns, the amounts as well as the mean duration and number of episodes of each vigilance state for each animal were calculated for every hour throughout 48 and 96 h, in males and females, respectively, and averaged over 2 or 12 h-cycle. Sleep fragmentation was assessed by calculating the mean duration of SWS_2 episodes as well as the number of wake bouts (Dugovic *et al*, 2000). The mean values were expressed as minutes ± SEM for each line of mice, and significance was tested by performing a two-way ANOVA with repeated measures over time. In case of significance ($p < 0.05$), the F test was followed by the Student's *t*-test for means' comparisons. For the relation between sleep and the estrous cycle, sleep amounts were evaluated every 3 h-bin over four consecutive 24 h-periods.

For pharmacological experiments, the effects of each dose of arecoline and 8-OH-DPAT upon REM sleep latency (defined as the delay between sleep onset after the injection, and the first REM sleep episode) and the amounts of each state of vigilance for every hour following injection were analyzed. For a given treatment, each animal was referred to its own baseline represented by the data obtained after injection of saline. Statistical analyses were performed using two-way ANOVA for factors treatment and group, and in case of significance ($p < 0.05$), the F test was followed by the Dunnett's *t* test for means' comparisons.

For each animal, the sleep amounts and EEG power spectra values after total sleep deprivation or immobilization stress sessions were compared with their respective control values. Differences between baseline and recovery from sleep deprivation or immobilization stress were assessed by means of the paired two-tailed Student's *t*-test.

RESULTS

Locomotor Activity and Body Temperature

Both H and NH mice exhibited a diurnal rhythm of locomotor activity and body temperature characterized by higher levels during the dark than during the light period (Figure 1). However, the amplitude of the activity rhythm was significantly smaller in H compared with NH mice (in counts per 5 min (see Figure 1): 7.9 ± 0.3 and 14.1 ± 2.2, respectively, $n = 6$ in each group, $p < 0.05$), due to a lesser locomotor activity in helpless animals, with notably an absence of peaks at the beginning and the end of the light period (ANOVA: $F_{1,1430} = 11.3$, $p < 0.01$), and during the first half of the dark period ($F_{1,1430} = 7.9$, $p < 0.05$) (Figure 1a). In contrast, core temperature was globally the same in both lines during the dark period, but was lower in H compared to NH mice during the light period ($F_{1,1430} = 7.2$, $p = < 0.05$). In particular, no peaks of temperature at the beginning and at the end of the light period were observed in H mice, in contrast to those occurring in NH mice (Figure 1b).

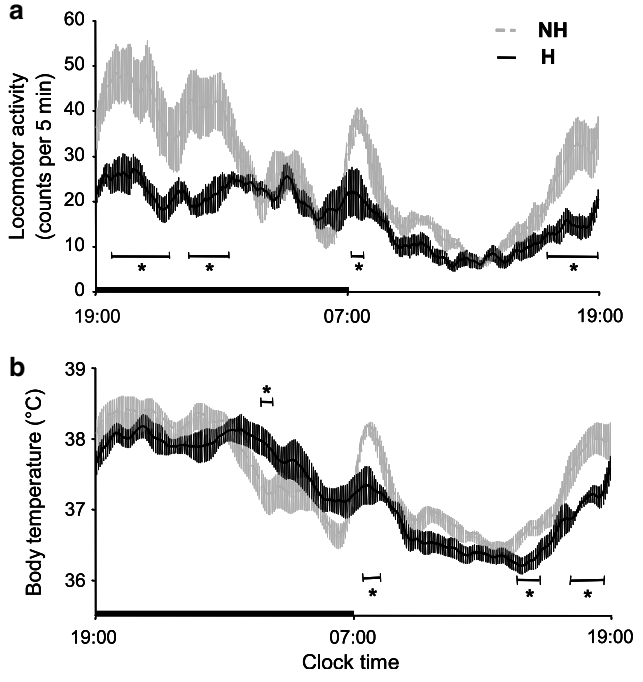

Figure 1 Locomotor activity (a) and body temperature (b) in NH (*dashed line*) and H (*solid line*) mice. Data (mean ± SEM of 6 animals in each group) are expressed every 5 min as counts for activity and as °C for temperature. *$p < 0.05$, significantly different between H and NH mice; unpaired Student's *t* test.

Sleep and Wakefulness

Under baseline conditions. Both lines of mice exhibited similar EEG spectra (Table 1) and diurnal rhythm of sleep and wakefulness, with larger amounts of sleep during the light period than during the dark one (Figure 2). However, marked differences in the amounts of sleep and wakefulness were observed between the two lines. For both males and females, H mice exhibited significantly lower levels of wakefulness (ANOVA: $F_{1,220} = 23.0$, $p < 0.001$) and greater amounts of REM sleep ($F_{1,220} = 21.2$, $p < 0.001$) and SWS_1 ($F_{1,220} = 78.0$, $p < 0.001$) across the 24 h, compared to NH mice (Figure 2, Table 2). SWS_2 was increased at the beginning of the dark period (Figure 2) but represented in the whole a smaller proportion of total sleep in H than in NH mice (Table 2). These modifications were accompanied by a decrease in the mean duration of SWS_2 episodes (in min: females, H: 1.31 ± 0.12 *vs* NH: 2.21 ± 0.23, $p < 0.05$; males, H: 1.00 ± 0.05 *vs* NH: 1.58 ± 0.25, $p < 0.05$), and an increase in the number of wakefulness episodes notably in females (H: 151.17 ± 6.15 *vs* NH: 107.89 ± 15.51/24 h, $p < 0.05$), both parameters providing an index of sleep fragmentation (Dugovic *et al*, 2000).

In females, amounts of sleep and wakefulness varied across the estrous cycle only in NH mice, with less time spent in REM sleep between 2100 and 2300 at the proestrous period (in minutes: 2.6 ± 0.5 *vs* 4.9 ± 1.1 in estrous, $p < 0.05$). There were no other changes in the vigilance states in NH mice, and EEG spectra did not significantly vary across the estrous cycle in either NH or H mice (not shown).

With respect to both spontaneous sleep-wakefulness characteristics and responses in depression-related behavioural tests (El Yacoubi *et al*, 2003), females of the H line were generally more affected than males. Accordingly, the following studies were performed only with females.

Pharmacological activation of muscarinic and 5-HT_{1A} receptors. Under vehicle injection, REM sleep latency was significantly shorter in H than in NH mice (in minutes: H: 16.5 ± 3.6 *vs* NH: 30.2 ± 4.2, $n = 8$-9, $p < 0.01$).

Acute administration of the muscarinic agonist arecoline (0.025–0.1 mg/kg, i.p.) did not modify REM sleep amounts (during 2 h after injection: NH: $F_{3,29} = 1.4$, $p < 0.3$; H: $F_{3,21} = 1.6$, $p = 0.2$; not shown) but produced a significant decrease in REM sleep latency in both mouse lines (NH: $F_{3,32} = 10.2$, $p < 0.0001$; H: $F_{3,23} = 8.4$, $p < 0.001$) (Figure 3a).

Table 1 Power Spectra of the EEG Frequency Bands During SWS_2 and REM Sleep in Female NH and H mice

	SWS_2		REM	
% Total power	**NH**	**H**	**NH**	**H**
Delta (0.5–4.99 Hz)	34.4 ± 2.8	40.4 ± 3.9	24.7 ± 2.9	20.1 ± 2.2
Theta (5–9.99 Hz)	28.2 ± 0.7	27.8 ± 1.4	33.1 ± 1.4	38.7 ± 2.4
Spindles (10–19.99 Hz)	23.8 ± 1.1	20.6 ± 1.6	25.4 ± 1.6	23.1 ± 0.9
High frequency (20–60 Hz)	13.6 ± 1.4	11.1 ± 1.6	16.9 ± 1.4	18.3 ± 2.4

Results (mean ± SEM of 10 and 12 animals, respectively) are expressed for every animal as percentage of the total power during 24 h.

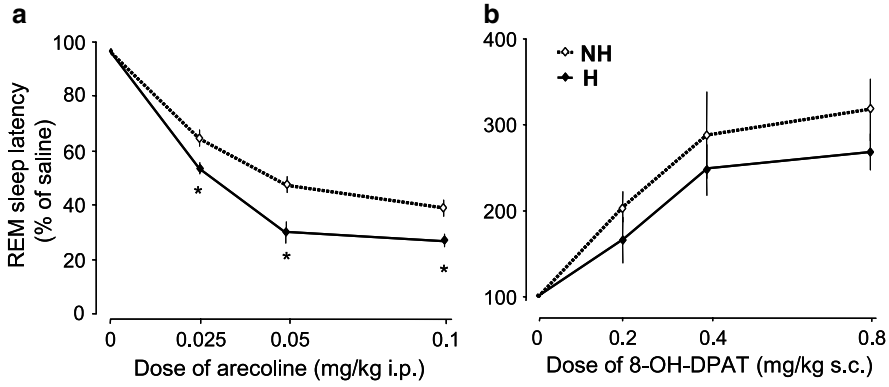

Figure 3 Effects of the muscarinic receptor agonist arecoline (a) and of the 5-HT_{1A} receptor agonist 8-OH-DPAT (b) at various doses on REM sleep latency in NH (*dotted line*) and H (*solid line*) mice. Data (mean ± SEM of 9 and 8 animals, respectively, for arecoline, and of 7 and 5 animals, respectively, for 8-OH-DPAT) are expressed as percent of REM sleep latency in saline-treated mice (0 on abscissa). *$p < 0.05$, significantly different from NH mice; *post hoc* Dunnett's test.

Table 3 Effects of the 5-HT_{1A} Receptor Agonist 8-OH-DPAT at Various Doses on W, SWS_2 and REM Sleep in NH and H Mice

		8-OH-DPAT mg/kg		
		0.2	**0.4**	**0.8**
W	NH	109.5 ± 29.5	125.8 ± 16.7	151.6 ± 21.9*
	H	138.4 ± 18.0	154.5 ± 7.0*	162.6 ± 10.3*
SWS_2	NH	88.9 ± 20.7	88.9 ± 16.6	72.3 ± 17.6*
	H	84.1 ± 11.4	69.1 ± 8.5*	55.4+19.1*
REM sleep	NH	68.8 ± 8.1*	28.3 ± 6.0*	20.6 ± 9.4*
	H	54.9 ± 8.2*	28.4 ± 9.6*	13.7 ± 4.3*

Data (mean ± SEM of 7 and 5 animals, respectively) are expressed as percent of saline treatment.
*$p < 0.05$, significantly different from saline; paired Student's *t* test.

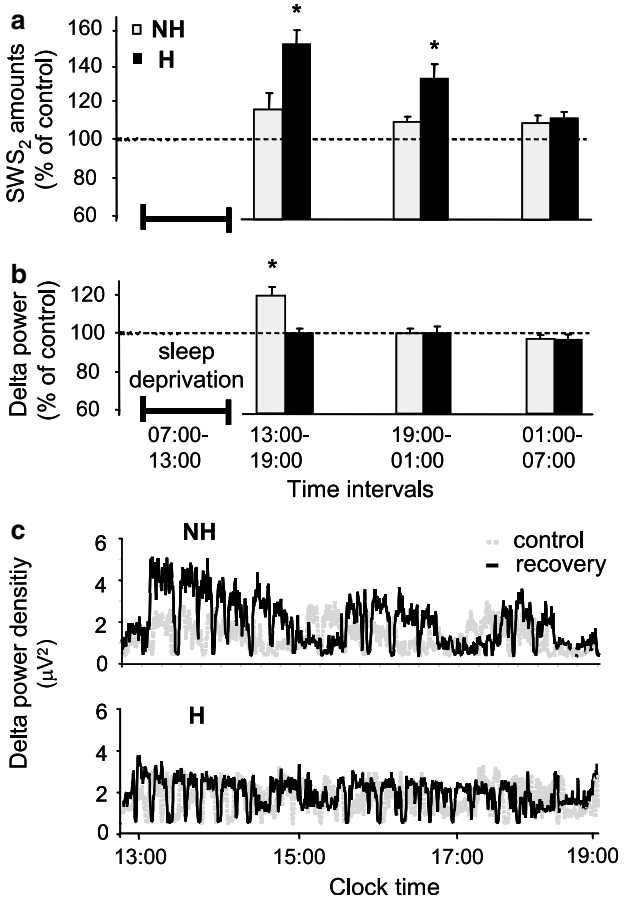

Figure 4 Effects of a 6 h-sleep deprivation starting at light onset on (a) SWS_2 amounts and (b) delta power, during three consecutive 6 h intervals of the recovery period after sleep deprivation. Data (mean ± SEM of 8 NH mice (grey bars) and 8 H mice (black bars)) are expressed as percent of the paired values obtained under control conditions. *$p < 0.05$, significantly different from respective control value; paired Student's *t* test. (c) Example of EEG delta power in NH (top) and H (bottom) mice during control (dotted line) and recovery (solid line) periods.

DISCUSSION

The main objective of this study was to investigate the locomotor activity, body temperature and sleep-wakefulness regulation in a novel model of depression derived from selective breeding in mice (El Yacoubi *et al*, 2003). Several differences were found between H and NH mice in this study. Indeed, the H line exhibited flattened activity rhythms, an enhanced cholinergic sensitivity of REM sleep, and abnomal responses to sleep deprivation and acute stress. Therefore, a large number of the deficiencies observed in depressed patients were also found in the H line.

Locomotor Activity and Sleep-Wakefulness Cycles Under Baseline Conditions

The two mouse lines exhibited circadian rhythms for sleep and wakefulness under light–dark conditions. However, H mice expressed a lower level of locomotor activity than NH mice during both the light and the dark phases that grossly paralleled their reduced amounts of wakefulness. In addition, the amplitude of locomotor activity rhythm was smaller in H mice. These results are similar to those

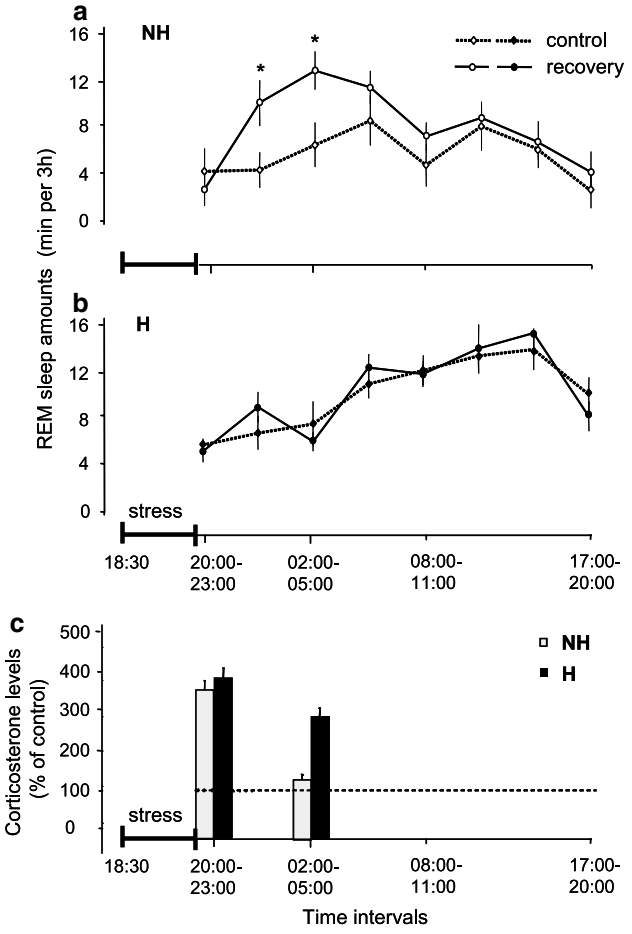

Figure 5 (a, b) REM sleep amounts during 23 h after 90 min of immobilization stress in NH (top) and H (bottom) mice. Data (mean ± SEM of 6 animals in each group) are expressed as minutes per 3 h after sham conditions (dotted line) and after immobilization stress (solid line). *$p < 0.05$, significantly different from respective sham control value, paired Student's *t* test. (c) Corticosterone levels (bars; at two times: 2000 and 0200) are expressed as percent of controls at the same time (mean ± SEM of 6 animals in each group). *$p < 0.05$, significantly different from respective control values, unpaired Student's *t* test.

reported for other animal models of depression (Overstreet, 1993; Solberg *et al*, 2001) and are reminiscent of the behavioural inhibition and the global flattening of circadian rhythms observed in depressed patients (Bunney and Bunney, 2000; Souetre *et al*, 1989).

In NH mice, the changes in core temperature across the 24 h period were directly correlated with those of locomotor activity (Bunney and Bunney, 2000). In contrast, in H mice, the core temperature was lower than in NH mice during the light period, but it rose to NH levels during the dark period. Interestingly, depressed patients also exhibit disruptions of the body temperature rhythm (Avery *et al*, 1999; Bunney and Bunney, 2000).

Both H and NH mice exhibited similar circadian variations of sleep and wakefulness, with a predominance of wakefulness during the dark period, and of sleep during the light period. These rhythms are classically observed in mice of various genetic backgrounds (Boutrel *et al*, 1999; Boutrel *et al*, 2002; Franken *et al*, 1998; Lena *et al*, 2004; Tobler *et al*, 1997). The comparison between H and NH mice confirms and extends our previous data (El Yacoubi *et al*, 2003). Globally, H mice had fragmented sleep, with notably a shorter duration of SWS bouts and a greater number of wake episodes, higher amounts of SWS_1 and REM sleep, and a shorter latency of this stage. These sleep-wakefulness alterations in H mice are consistent with the data obtained in various depression models in the rat (Adrien *et al*, 1991; Dugovic *et al*, 2000; Nestler *et al*, 2002; Shiromani *et al*, 1988). Furthermore, they are reminiscent of sleep impairments observed in depressed patients, notably the sleep fragmentation, the shift towards lighter sleep stages, and the enhanced REM sleep pressure (Benca, 2000; Gillin *et al*, 1991).

With regard to the estrous cycle, in NH mice, no influence was observed on W or SWS, but REM sleep amounts were significantly decreased at the end of the dark period during the proestrous compared to the estrous phase. These data are in agreement with previous reports in rats, where low levels of REM sleep, but also of SWS, were found in the proestrous phase (Fang and Fishbein, 1996; Schwierin *et al*, 1998; Zhang *et al*, 1995). However, in contrast to rats (Schwierin *et al*, 1998), NH mice exhibited no variations in the EEG power spectra across the estrous cycle. The reduction of REM sleep during proestrous, when plasma levels of progesterone and estradiol are high (Schwierin *et al*, 1998), could be explained by the inhibitory influence of ovarian hormones on REM sleep (Fang and Fishbein, 1996; Schwierin *et al*, 1998). It has also been observed in women, who express reduced amounts of REM sleep in the luteal compared to the follicular phase (Baker *et al*, 2001), and only slight modifications of SWS all along the ovarian cycle (Driver *et al*, 1996).

In contrast to NH mice, no changes in W, SWS or REM sleep across the estrous cycle were observed in H mice. This might reflect a dysregulation of the hypothalamo–pituitary–gonadal axis in these animals, as described in depressed women (Meller *et al*, 2001; Young *et al*, 2000). However, no problems in breeding have been observed in H mice (El Yacoubi *et al*, 2003).

Muscarinic- and 5-HT_{1A}-Receptor-Mediated Regulations of REM Sleep

Sleep patterns are under the regulatory influence of several neurotransmitter systems among which the cholinergic and serotoninergic ones are prominent, notably regarding REM sleep expression (Pace-Schott and Hobson, 2002; Shiromani *et al*, 1988). In particular, REM sleep is enhanced following treatments with cholinergic agonists in rats (Baghdoyan, 1997), mice (Coleman *et al*, 2004), and humans (Seifritz *et al*, 1998; Sitaram and Gillin, 1980). In contrast, REM sleep is inhibited by 5-HT_{1A} agonists (Boutrel *et al*, 1999; Driver *et al*, 1995; Tissier *et al*, 1993).

In the present work, we found that activation of muscarinic receptors by arecoline decreased REM sleep latency. Interestingly, this arecoline-induced facilitation of REM sleep was more marked in H than in NH mice, as observed in depressed patients compared with control subjects (Sitaram and Gillin, 1980). This enhanced facilitation could reflect a cholinergic hypersensitivity at the level of pontine REM sleep control system (Gillin *et al*, 1991; Seifritz *et al*, 1998). Along this line, the larger amounts of

REM sleep in H mice under baseline conditions might be explained, at least in part, by a high muscarinic tone (Coleman *et al*, 2004), just as in the case of FSL (Flinders Sensitive Line) rats, another validated model of depression (Overstreet, 1993).

In contrast to muscarinic receptors, activation of 5-HT_{1A} receptors by 8-OH-DPAT induced a dose-dependent reduction of REM sleep amounts and an increase in REM sleep latency in both mouse lines, as observed in other mouse strains (Boutrel *et al*, 1999), in rats (Tissier *et al*, 1993), and also in humans (Driver *et al*, 1995). This effect is most probably accounted for by an agonist action of 8-OH-DPAT at 5-HT_{1A} but not 5-HT_7 receptors (Wood *et al*, 2000). Indeed, selective blockade of 5-HT_{1A} receptors (by WAY 100,635) either enhanced REM sleep (Monaca *et al*, 2003), or completely suppressed the REM sleep inhibitory effect of 8-OH-DPAT in mice (Boutrel *et al*, 2002). In contrast, blockade of 5-HT_7 receptors has been found to reduce REM sleep (Thomas *et al*, 2003).

Interestingly, this REM sleep inhibitory response to 5-HT_{1A} receptor activation was similar in H and NH mice. This is in agreement with results obtained in depressed patients *vs* controls (Gillin *et al*, 1996; Seifritz *et al*, 1998), but it contrasts with the augmented hypothermia response to the same agonist found in H mice (El Yacoubi *et al*, 2003). This apparent discrepancy is due to the fact that the sleep response is mediated by post-synaptic 5-HT_{1A} receptors (Tissier *et al*, 1993), possibly at pontine level (Horner *et al*, 1997), whereas the hypothermic response is mediated by pre-synaptic (auto-) receptors (Goodwin *et al*, 1985). Indeed, the latter receptors are functionally hypersensitive in H mice (El Yacoubi *et al*, 2003), whereas the post-synaptic ones are unchanged, as observed under various experimental conditions (Fabre *et al*, 2000, Hensler, 2003).

In summary, the sleep/wakefulness alterations described in H mice mimic the alterations observed in depressed patients (Lustberg and Reynolds, 2000). As suggested for these patients, they could be accounted for by an imbalance between cholinergic and serotonergic tones. Such imbalance would be essentially due to an increase in cholinergic tone which would result notably in enhanced REM sleep (Gillin *et al*, 1991) and more frequent awakenings (Lustberg and Reynolds, 2000).

Effect of Sleep Deprivation

After 6 h of sleep deprivation, NH mice exhibited a significant increase in SWA during the first few hours of the recovery period, with no change in the total duration of SWS. These data confirm the results obtained in mice of other lines (Franken *et al*, 2001; Lena *et al*, 2004; Tobler *et al*, 1997), and are consistent with those in humans after one night of sleep deprivation (Borbely *et al*, 1981; Dijk and Beersma, 1989).

In contrast to NH mice, the H line exhibited no change in SWA, indicating an impairment of the homeostatic regulation of sleep in these mice, just like in depressed patients (Armitage *et al*, 2000; Borbely, 1987). Recombinant offsprings of H and NH mice might represent an interesting paradigm to investigate the genetic control of such a deficit in sleep homeostasis (Franken *et al*, 2001).

Effect of Acute Stress

A REM sleep rebound (50% over baseline) was observed in NH mice from the 4th to the 9th h after the immobilization stress, as reported in mice of other lines (Boutrel *et al*, 2002; Lena *et al*, 2004) and in rats (Bouyer *et al*, 1998; Rampin *et al*, 1991). In contrast, H mice expressed no increase of REM sleep amounts after stress.

The impact of acute stress on sleep involves notably the HPA axis (Bouyer *et al*, 1998; Steiger, 2002). Indeed, we observed that 6 h after cessation of the immobilization stress, corticosterone levels had decreased to basal values in NH mice, but were still elevated in H mice, thereby preventing the occurrence of the REM sleep rebound (Boutrel *et al*, 2002). Interestingly, the return of corticosterone to baseline level is under cholinergic control in the hippocampus (Han *et al*, 2002). Thus, a functional deficit of hippocampal muscarinic receptors in H mice could be responsible, at least in part, for the delay in corticosterone decline after stress, and in turn for the absence of REM sleep rebound. Finally, like in other models of depression (Ladd *et al*, 2004; Prathiba *et al*, 1998) and in depressed patients (Arborelius *et al*, 1999), dysregulation of HPA axis is observed in H mice (El Yacoubi *et al*, 2003), an impairment which would alter their stress-induced adaptive response of REM sleep.

Physiopathological Implications

In terms of the relation between sleep disorders and depression, the present model can lead to various comments and questions. Firstly, like most models, it does not entirely mimic the very complex pattern of sleep impairments associated with depression. In particular, one would expect to observe in a depression model some manifestations of insomnia, and notably an increase in wakefulness (Lustberg and Reynolds, 2000). This was not the case for H mice that exhibited decreased wakefulness. In the same manner, we found no abnormality of delta power during deep SWS in H mice, whereas this is a constant feature of depression, especially in women (Armitage *et al*, 2000). However, depressed patients suffer also decreased sleep continuity, difficulties falling asleep and maintaining sleep, and early morning awakenings. Globally, the present model mimics these characteristics, illustrated by sleep fragmentation and the frequent awakenings. In addition, deficits in locomotor activity, REM sleep alterations, and sleep homeostasis deficiency also evoke the impairments found in depressed patients (Armitage *et al*, 2000; Lustberg and Reynolds, 2000).

Along this line, one can ask whether sleep impairments in H mice represent trait- or state-dependent characteristics. In depressed patients, sleep disorders may persist in a milder form during periods of remission, suggesting they may be a trait marker of depression (Lustberg and Reynolds, 2000). In the same manner, the sleep impairments observed in H mice might be trait-related. As in patients, sleep disorders, and notably decreased REM sleep latency, should aggravate under conditions of a 'depressive episode' (Lustberg and Reynolds, 2000). Analysis of sleep patterns in H *vs* NH mice after chronic stress should allow to clarify this point.

In summary, H mice exhibit sleep alterations that include fragmented sleep, lighter SWS, increased REM sleep pressure, and lack of influence of the ovarian cycle on sleep. Moreover, their response to behavioural and pharmacological challenges indicates changes in the muscarinic component of the REM sleep regulatory mechanisms, and deficits in adaptive responses to sleep deprivation and acute stress. Since these alterations evoke those found in depressed patients, this model might be of particular interest for investigating mechanisms and molecular targets for alleviating depression-related sleep disorders.

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