Involvement of 5-HT$_{1A}$ Receptors in Homeostatic and Stress-Induced Adaptive Regulations of Paradoxical Sleep: Studies in 5-HT$_{1A}$ Knock-Out Mice

Benjamin Boutrel,[1] **Christelle Monaca,**[1] **René Hen,**[2] **Michel Hamon,**[1] **and Joëlle Adrien**[1]

Institut National de la Santé et de la Recherche Médicale U288, NeuroPsychoPharmacologie Moléculaire, Cellulaire et Fonctionnelle, Faculté de Médicine Pitié-Salpêtrière, 75634 Paris Cedex 13, France, and ²Center for Neurobiology and Behavior, Columbia University, New York, New York 10032

For the last two decades, the involvement of 5-HT$_{1A}$ receptors in the regulation of vigilance states has been studied extensively thanks to pharmacological tools, but clear-cut conclusion has not been reached yet. By studying mutant mice that do not express this receptor type (5-HT$_{1A}$−/−) and their wild-type 129/Sv counterparts, we herein demonstrate that 5-HT$_{1A}$ receptors play key roles in the control of spontaneous sleep–wakefulness cycles, as well as in homeostatic regulation and stress-induced adaptive changes of paradoxical sleep. Both strains of mice exhibited a diurnal sleep–wakefulness rhythm, but 5-HT$_{1A}$−/− animals expressed higher amounts of paradoxical sleep than wild-type mice during both the light and the dark phases. In wild-type mice, pharmacological blockade of 5-HT$_{1A}$ receptors by WAY 100635 (0.5 mg/kg, i.p.) promoted paradoxical sleep, whereas the 5-HT$_{1A}$ agonist 8-OH-DPAT (0.25–1 mg/kg, s.c.) had an opposite effect. In contrast, none of the 5-HT$_{1A}$ receptor ligands affected sleep significantly in 5-HT$_{1A}$−/− mice. However, 5-HT$_{1B}$ receptor stimulation by CP 94253 (1–3 mg/kg, i.p.) induced a reduction in paradoxical sleep in both strains, this effect being more pronounced in 5-HT$_{1A}$−/− mutants. Finally, in contrast to wild-type mice, 5-HT$_{1A}$−/− mutants did not exhibit any rebound of paradoxical sleep after either a 9 hr instrumental paradoxical sleep deprivation or a 90 min immobilization stress. Altogether, these data indicate that, in the mouse, 5-HT$_{1A}$ receptors participate in the spontaneous and homeostatic regulation, as well as in stress-induced adaptive changes of paradoxical sleep.

Key words: sleep–wakefulness; 5-HT$_{1A}$ receptors; sleep deprivation; immobilization stress; knock-out mice; paradoxical sleep homeostasis

The implication of serotonin (5-hydroxytryptamine, 5-HT) receptors of the 5-HT$_{1A}$ type in the regulation of vigilance states has been the matter of numerous investigations using appropriate pharmacological tools (Portas et al., 2000; Ursin, 2002). In particular, the inhibitory effect of systemic treatment with various 5-HT$_{1A}$ receptor agonists, notably the prototypical one 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), on paradoxical sleep (PS) is considered as a key observation in support of the idea that 5-HT$_{1A}$ receptors play an important role in the regulation of this vigilance state in mammals (Dzoljic et al., 1992; Monti and Jantos, 1992; Quattrochi et al., 1993; Tissier et al., 1993; Driver et al., 1995). These receptors are located on both somas and dendrites of serotonergic neurons in raphe nuclei (somatodendritic autoreceptors) (Vergé et al., 1986; Sotelo et al., 1990) and target neurons receiving serotonergic projections (postsynaptic receptors) (Kia et al., 1996; Riad et al., 2000). The PS inhibition induced by 5-HT$_{1A}$ receptor agonists would result from activation of postsynaptic receptors (Tissier et al., 1993),

notably those at pontine level (Sanford et al., 1994; Horner et al., 1997; Thakkar et al., 1998). In contrast, activation of somatodendritic 5-HT$_{1A}$ autoreceptors in anterior raphe nuclei would induce a PS enhancement (Portas et al., 1996; Bjorvatn et al., 1997).

However, the specific involvement of 5-HT$_{1A}$ receptors in sleep–wakefulness regulations remains questionable because none of the ligands used so far is really selective of 5-HT$_{1A}$ receptors. Indeed, even 8-OH-DPAT, which is classically considered as a selective 5-HT$_{1A}$ agonist (Hoyer et al., 1994), acts at 5-HT$_7$ receptors at relatively low doses (Wood et al., 2000). An alternative strategy to assess the potential role of 5-HT$_{1A}$ receptors in sleep–wakefulness regulation under baseline conditions, as well as after various behavioral challenges (Sallanon et al., 1983; Houdouin et al., 1991a; Cespuglio et al., 1995; Gonzalez et al., 1996; Gonzalez and Valatx, 1998), is now possible thanks to the availability of knock-out mice that do not express this receptor type (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Phenotypical characterization of these mutants showed that they exhibit marked behavioral alterations, in particular increased responses in anxiety-relevant tests, in sharp contrast with the apparent decrease in anxiety-like behaviors in mutant mice that do not express 5-HT$_{1B}$ receptors (Zhuang et al., 1999). Interestingly, the latter 5-HT$_{1B}$−/− mutants show increased amounts of PS under baseline conditions but no rebound of this sleep stage after its selective deprivation (Boutrel et al., 1999). Whether 5-HT$_{1A}$−/− mutants also exhibit alterations in PS regulation, possibly opposite to those observed in 5-HT$_{1B}$−/− mutants, was an interesting question to be addressed with regard to the well established relationships between stress–anxiety-driven

behaviors and sleep–wakefulness (Cespuglio et al., 1995; Marinesco et al., 1999; Vazquez-Palacios and Velazquez-Moctezuma, 2000).

All of these considerations led us to investigate the characteristics of sleep–wakefulness regulations in 5-HT$_{1A}$−/− mice compared with wild-type counterparts, first, under baseline conditions and in response to the administration of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor ligands, and second, after selective PS deprivation or immobilization stress.

MATERIALS AND METHODS

All of 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 0315 to J.A.).

All mice used for these studies were of the 129/Sv strain. Those used for spontaneous sleep–wakefulness analysis, PS deprivation, and immobilization stress were produced by heterozygous breeding, and their genotype was determined according to the method of Ramboz et al. (1998). Other groups of mice, produced from homozygous breeding of knock-out and wild-type strains, were used for pharmacological experiments.

Surgery

Male wild-type (5-HT$_{1A}$+/+) and mutant (5-HT$_{1A}$−/−) mice were used at 2–3 months of age (body weight, 22–26 gm). Animals were implanted under sodium pentobarbital anesthesia (70–75 mg/kg, i.p.) with the classical set of electrodes (made of enameled nichrome wire, 150 μm in diameter) for polygraphic sleep monitoring as described previously (Boutrel et al., 1999). In brief, EEG electrodes were inserted through the skull onto the dura over the right cortex (2 mm lateral and 4 mm posterior to the bregma) and over the cerebellum (at midline, 2 mm posterior to lambda), electrooculography electrodes were positioned subcutaneously on each side of the orbit, and EMG electrodes were inserted into the neck muscles. All electrodes were anchored to the skull with Superbond (Limoge-Lendais et al., 1994) and acrylic cement and soldered to a miniconnector also embedded in cement. After completion of surgery, animals were housed in individual cages (20 × 20 × 30 cm) and maintained under standard laboratory conditions: 12 hr light/dark cycle (light on at 7:00 A.M.), 24 ± 1°C ambient temperature, and food and water available *ad libitum*. The animals were allowed 7–10 d to recover and habituate to the recording conditions.

Pharmacological treatments

Drugs were dissolved in 0.1 ml of saline, except for the 5-HT$_{1B}$ agonist 3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-b]pyridine (CP 94253), which was dissolved in 0.1 ml of warm distilled water. All injections were performed between 9:30 and 10:00 A.M.; CP 94253 and the 5-HT$_{1A}$ antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide (WAY 100635) were injected intraperitoneally, whereas 8-OH-DPAT was injected subcutaneously. For baseline data, mice were injected intraperitoneally or subcutaneously with the vehicle only, as appropriate. A washout period of at least 2 d for CP 94253 and WAY 100635 and 7 d for 8-OH-DPAT was allowed between two consecutive treatments.

PS deprivation

Mice were placed for 9 hr, starting at 10:00 A.M., on platforms (control conditions, 7.5 cm in diameter, 3 cm high; deprivation conditions, 3.5 cm in diameter, 4 cm high) surrounded by water (2 cm deep) (Boutrel et al., 1999) at an ambient temperature of 24°C, with access to food and water *ad libitum*. At the end of this period, they were returned to their home cage and allowed to recover for 12 hr (from 7:00 P.M. to 7:00 A.M. the next morning). Each mouse underwent the paired control and deprivation procedures (separated by at least 1 week).

Immobilization stress

At least 10 d after completion of the deprivation procedure, mice were immobilized for 90 min, from 6:30 to 8:00 P.M., by wrapping them inside a plastic grid. At the end of this period, they were returned to their home cage for sleep–wakefulness monitoring. Each mouse underwent, first, the

control procedure (the animal remained free in its home cage and was connected a few minutes before being recorded) and, second (2–3 d later), the immobilization procedure.

Polygraphic recordings

For the study of spontaneous sleep–wakefulness cycles, each animal was recorded for 48 hr, beginning at 7:00 P.M., i.e., at the onset of the dark period. For pharmacological studies, sleep–wakefulness parameters were recorded for 8 hr after injections, i.e., from 10:00 A.M. to 6:00 P.M.. For PS deprivation experiments, recordings were performed from the beginning of the deprivation period (at 10:00 A.M.) and continued until 12 hr after the end of deprivation, and, for the stress procedure, recordings were performed for 12 hr after the end of immobilization challenge.

Data analysis and statistics

Polygraphic recordings were scored manually every 15 sec epoch using Somnologica software (Flaga, Reykjavik, Iceland), and the amounts of each state of vigilance [wakefulness (W), slow-wave sleep (SWS), and PS] were calculated per hour.

Spontaneous sleep–waking cycles. For each animal, the amounts of vigilance states for every hour throughout 48 hr were averaged for the light and the dark phases. The mean values (expressed as minutes ± SEM) for each strain of mice were used for calculating the ANOVA for the genotype. In case of significance ($p < 0.05$), the F test was followed by the Student's t test for mean comparisons.

Pharmacological experiments. The effects of each dose of a given compound on each state of vigilance were analyzed for every 2 hr period after injection and are expressed as minutes ± SEM. For a given treatment, each animal was referred to its own baseline, represented by the data obtained after injection of vehicle. Statistical analyses were performed using ANOVA for factors treatment and strain, and in case of significance ($p < 0.05$), the F test was followed by the *post hoc* Fisher's test for assessing the effect of each dose of compounds.

PS deprivation and immobilization stress. For each animal, the PS amounts during the small platform condition and the following 12 hr recovery period and those during the 12 hr post-stress period were compared with their respective control values (i.e., PS amounts during the large platform condition and the corresponding control recovery period and those during the same period with no stress, respectively). Paired t tests were performed to assess statistical significance of the data.

Chemicals

The following drugs were used: WAY 100635 (0.5 mg/kg, i.p.; Wyeth Research, Princeton, NJ), 8-OH-DPAT (0.25–1.0 mg/kg, s.c. ; Research Biochemicals, Natick, MA), and CP 94253 (1–3 mg/kg i.p.; Pfizer, Groton, CT).

RESULTS

Spontaneous sleep–wakefulness cycles

All mice exhibited a clear-cut circadian sleep–wakefulness rhythm, with larger amounts of sleep during the light period than during the dark one (Fig. 1). However, 5-HT$_{1A}$−/− mutants differed significantly ($p < 0.05$) from wild-type mice by a greater amount of PS, during both the light (approximately +39%) and the dark (approximately +45%) phase (Table 1). This enhancement was accounted for by an increase in the mean duration of PS episodes during the light phase (1.25 ± 0.04 vs 1.08 ± 0.02 min; mean ± SEM; $n = 8$ in each group; $p < 0.05$) and by an increase in the number of PS episodes during the dark phase (35.6 ± 2.5 vs 26.8 ± 2.5; $p < 0.05$).

In contrast, the amounts of W and SWS were identical in both groups throughout the entire circadian period (Fig. 1, Table 1).

Pharmacological data

Activation or blockade of 5-HT$_{1A}$ receptors

In wild-type mice, 8-OH-DPAT (0.25–1 mg/kg, s.c.) induced, during the first 2 hr period after injection, a dose-related inhibition of PS (ANOVA; $F_{(3,15)} = 20.4$; $p < 0.0001$) (Fig. 2) and SWS (ANOVA; $F_{(3,15)} = 15.9$; $p < 0.0001$; data not shown), as well as a concomitant increase in W (ANOVA; $F_{(3,15)} = 21.0$; $p < 0.0001$;
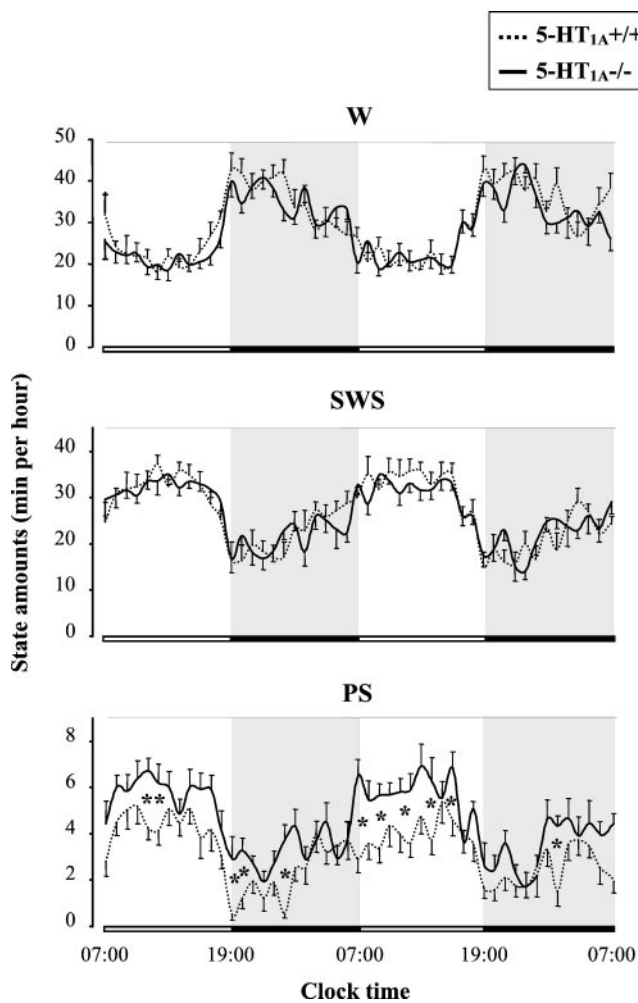
Figure 1. Circadian variations of W, SWS, and PS in 5-HT$_{1A}$+/+ (*dotted line*) and 5-HT$_{1A}$−/− (*solid line*) mice. Data (mean ± SEM of 9 and 8 animals, respectively) are expressed as minutes per hour during two consecutive light/dark cycles (lights on from 7:00 A.M. to 7:00 P.M.). *$p <$ 0.05, significant difference between groups; Student's *t* test.

data not shown). These initial modifications of sleep–wakefulness states were significant for all doses of 8-OH-DPAT tested (*p <* 0.001; *post hoc* Fisher's test). They were followed by a rebound of PS observed between 6 and 8 hr after the injection, which was significant (*p <* 0.05; Fisher's test) for the doses of 0.25 and 0.5 mg/kg 8-OH-DPAT (Fig. 2). In contrast, 8-OH-DPAT had no effect on sleep or wakefulness amounts in 5-HT$_{1A}$−/− mice, except for a nonsignificant PS enhancement (125.3 ± 18.6% of baseline; *n* = 6; *p* = 0.17) during the first 2 hr after injection at the dose of 1 mg/kg (Fig. 2). On the whole, the difference between strains during this period was highly significant for the three states of vigilance (ANOVA; $F_{(1,36)}$ = 43.1, 21.5, and 32.0; *p <* 0.001 for PS, SWS, and W, respectively).

Blockade of 5-HT$_{1A}$ receptors by WAY 100635 (0.5 mg/kg, i.p.) induced in 5-HT$_{1A}$+/+ mice, but not in 5-HT$_{1A}$−/− mutants, a significant increase in PS amounts (*p* = 0.001; paired Student's *t* test) during the first 4 hr after the injection (Fig. 3) and no modifications in W and SWS in any strain (data not shown).

Activation of 5-HT$_{1B}$ receptors
5-HT$_{1B}$ receptor activation by CP 94253 (1–3 mg/kg, i.p.) in-duced, during the first 4 hr after injection, a significant reduction

in PS amounts in wild-type mice (ANOVA; $F_{(3,23)}$ =3.6; *p* = 0.028), as well as in mutants (ANOVA; $F_{(3,23)}$ = 9.6; *p <* 0.001). This reduction reached significance for the doses of 2 and 3 mg/kg in wild-type (*p* = 0.023 and 0.007, respectively; *post hoc* Fisher's test) and mutant (*p <* 0.001 for both doses) mice and was more pronounced in 5-HT$_{1A}$−/− animals (ANOVA; $F_{(1,46)}$ = 9.63; *p* = 0.003) (Fig. 4). In contrast, neither W nor SWS were significantly affected by CP 94253 in both groups of mice (data not shown).

Paradoxical sleep deprivation
During PS deprivation (small platform), mice of both groups exhibited nearly the same amounts of SWS (data not shown) but only ~20% of PS compared with those observed under control conditions (large platform) (Fig. 5). During the recovery period, PS amounts were significantly enhanced in 5-HT$_{1A}$+/+ mice compared with those under control conditions (Fig. 5, Table 2), notably during the first 6 hr (+76.4 ± 22.3%; *n* = 6; *p <* 0.05; paired Student's *t* test). This increase was accounted for by an increase in the number of PS episodes (Table 2) with no modifi-cation of their mean duration (1.21 ± 0.11 vs 1.11 ± 0.10 min; NS; paired Student's *t* test). In contrast, in 5-HT$_{1A}$−/− mice, only a slight but not significant increase in PS amounts was observed after PS deprivation compared with those under control condi-tions (Fig. 5, Table 2) (+20.6 ± 16.9% during the first 6 hr of the recovery period; *n* = 6; NS; paired Student's *t* test), and neither the number (Table 2) nor the mean duration (1.22 ± 0.10 vs 1.16 ± 0.05 sec; NS; paired Student's *t* test) of PS episodes were altered during the recovery period in PS-deprived compared with nondeprived mutants. In addition, no modifications in W and SWS amounts were observed after PS deprivation in wild-type and 5-HT$_{1A}$−/− mice (data not shown).

Immobilization stress
After 90 min of immobilization, wild-type mice, but not 5-HT$_{1A}$−/− mutants, exhibited a significant increase in PS amounts during the 12 hr recovery period compared with the control conditions [respectively, +27.5 ± 10.6% (*n* = 9; *p <* 0.05) and −0.2 ± 7.0% (*n* = 7; NS); paired Student's *t* test]. This PS rebound in wild-type mice was mainly observed during the sec-ond half of the night (Table 2), especially for the last 3 hr (Fig. 6), and was accounted for by an increase in the number of PS episodes (Table 2), with no change of their mean duration (1.20 ± 0.05 vs 1.06 ± 0.07 min; NS; paired Student's *t* test). In contrast, no significant modifications in the amounts of W and SWS after the immobilization procedure were observed in wild-type and 5-HT$_{1A}$−/− mice (data not shown).

DISCUSSION
Regulation of sleep–wakefulness cycles under baseline conditions
In the present work, we found that knock-out mice, which do not express the 5-HT$_{1A}$ receptor, and their wild-type counterparts exhibit similar circadian sleep–wakefulness cycles with predom-inance of wakefulness during the dark period and sleep during the light one. These data are comparable with those generally obtained in mice (Tobler et al., 1997) and notably in the 129/Sv strain (Boutrel et al., 1999), which shares the same genetic back-ground as the present ones. However, 5-HT$_{1A}$−/− mutants dif-fered from 5-HT$_{1A}$+/+ wild-type mice by higher amounts of PS during the entire circadian period, whereas wakefulness and SWS amounts were similar in both groups. Because we used in this part

Table 1. Amounts of W, SWS, and PS in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice under normal conditions

Genotype	Period	W	SWS	PS
5-HT$_{1A}$+/+ ($n = 9$)	24 hr	720.5 ± 25.0	641.9 ± 21.5	77.6 ± 5.7
	12 hr light	282.6 ± 13.3	387.8 ± 10.1	49.6 ± 4.4
	12 hr dark	437.9 ± 13.3	254.2 ± 12.5	27.9 ± 1.8
5-HT$_{1A}$−/− ($n = 8$)	24 hr	696.5 ± 25.1	634.2 ± 25.9	109.3 ± 3.6*
	12 hr light	271.0 ± 11.4	380.0 ± 11.6	68.9 ± 3.2*
	12 hr dark	425.5 ± 19.2	254.2 ± 18.1	40.4 ± 2.2*

Results are expressed as minutes (mean ± SEM of n animals) during the 12 hr light (7:00 A.M. to 7:00 P.M.) and dark (7:00 P.M. to 7:00 A.M.) periods and during the complete nycthemeral cycle (24 hr). *$p < 0.05$, significantly different from 5-HT$_{1A}$+/+ group; Student's t test.
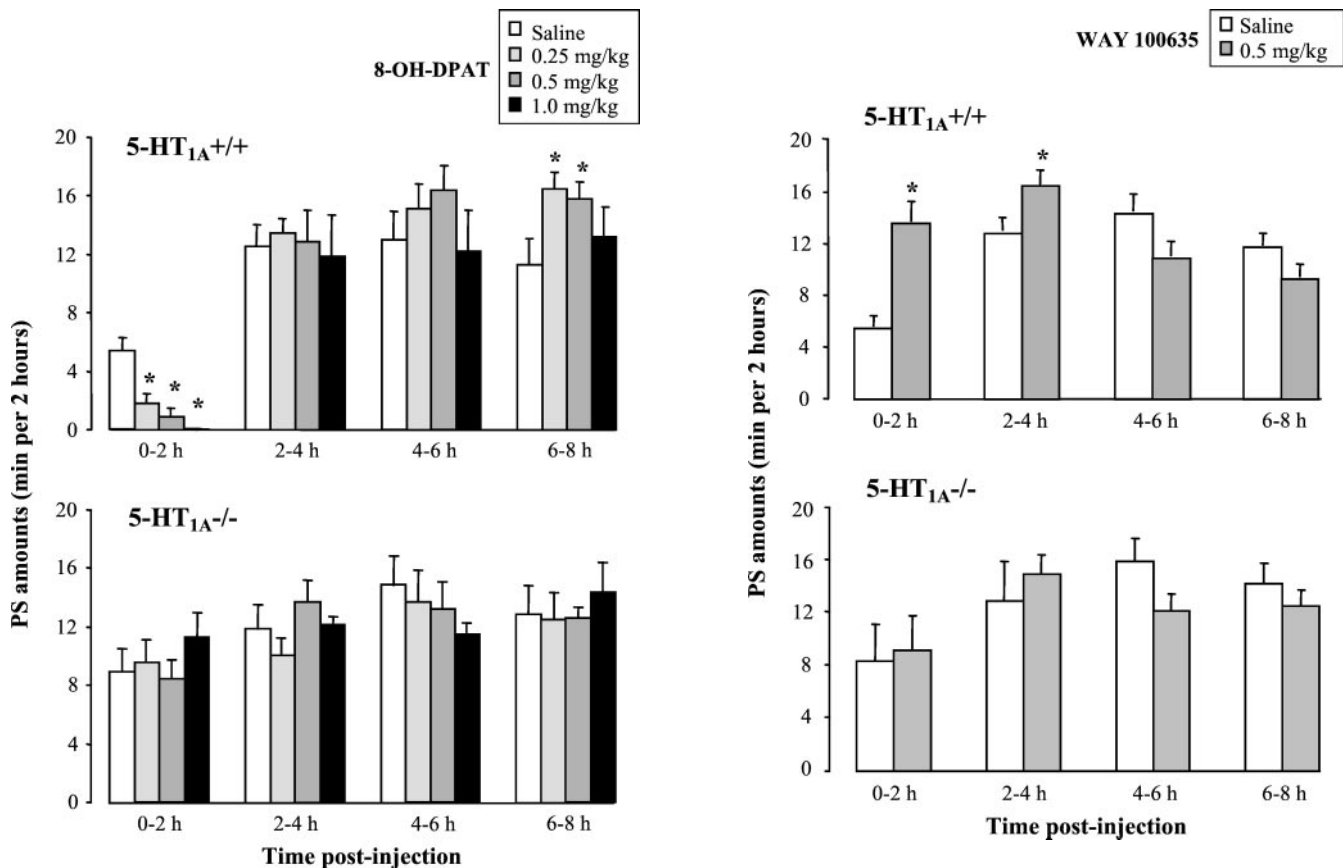

Figure 2. Effects of the 5-HT$_{1A}$ agonist 8-OH-DPAT on paradoxical sleep in 5-HT$_{1A}$+/+ (*top*) and 5-HT$_{1A}$−/− (*bottom*) mice during four successive 2 hr periods after injection. Data (mean ± SEM of 5 and 6 animals, respectively) are expressed as minutes per 2 hr after subcutaneous injection of saline (*open bars*) or 8-OH-DPAT at various doses (*filled bars*). *$p < 0.05$, significantly different from saline; *post hoc* Fisher's test.

Figure 3. Effects of blockade of 5-HT$_{1A}$ receptors on paradoxical sleep in 5-HT$_{1A}$+/+ (*top*) and 5-HT$_{1A}$−/− (*bottom*) mice during four successive 2 hr periods after treatment. Data (mean ± SEM of 6 and 4 animals, respectively) are expressed as minutes per 2 hr after injection of saline (*white bars*) or the 5-HT$_{1A}$ antagonist WAY 100635 at the dose of 0.5 mg/kg intraperitoneally (*gray bars*). *$p < 0.05$, significantly different from saline; paired Student's t test.

of the study only mice derived from heterozygous breeding, it is most probable that the difference in PS amounts is accounted for by the 5-HT$_{1A}$ gene disruption rather than by some heterogeneity in the genetic background between both groups of mice (Gerlai, 1996). This is further supported by the fact that pharmacological blockade of 5-HT$_{1A}$ receptors with WAY 100635 induced in wild-type mice an increase in PS amounts, thereby mimicking the change that occurred spontaneously in mutants lacking 5-HT$_{1A}$ receptors. Accordingly, both the larger amounts of spontaneous PS in 5-HT$_{1A}$−/− mutants and the PS enhancement after 5-HT$_{1A}$ receptor blockade in wild-type mice indicate that these receptors mediate a tonic inhibitory influence on PS in this species.

The lack of significant effect of 8-OH-DPAT on sleep–wake-fulness cycle in 5-HT$_{1A}$−/− mutants would suggest that 5-HT$_7$ receptors at which this ligand acts as a partial agonist (Wood et al., 2000) are not involved in the regulation of vigilance states, at least in the mouse. This conclusion is in line with previous results showing that the effects of 8-OH-DPAT on sleep and wakefulness in the mouse were completely prevented by the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 (Boutrel et al., 1999). In any case, additional investigations are needed to assess the possible implication of 5-HT$_7$ receptors in sleep–wakefulness regulation because Hagan et al. (2000) reported recently a decrease in PS after administration of a 5-HT$_7$ receptor antagonist in rats. Indeed, we did find a tendency to an increase in PS amounts in 5-HT$_{1A}$−/− mice treated with 8-OH-DPAT (Fig. 2), which would
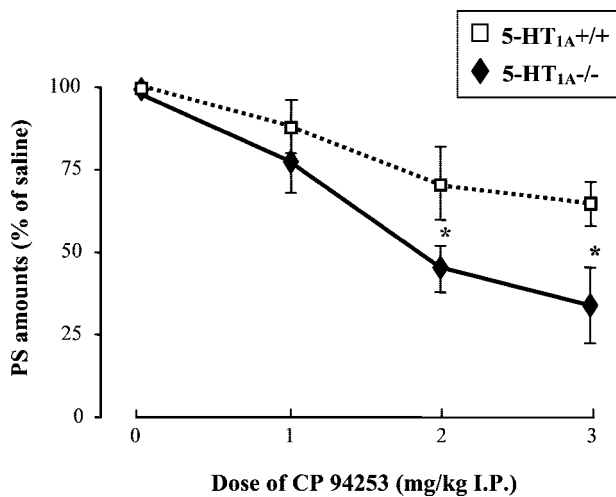
Figure 4. Effects of the 5-HT$_{1B}$ agonist CP 94253 at various doses on paradoxical sleep in 5-HT$_{1A}$+/+ (*dotted line*) and 5-HT$_{1A}$−/− (*solid line*) mice during the first 4 hr period after injection. Data (mean ± SEM of 7 animals in each group) are expressed as percentages of PS amounts in saline-treated mice (*0* on *abscissa*). *$p < 0.05$, significant difference between groups; Fisher's test.
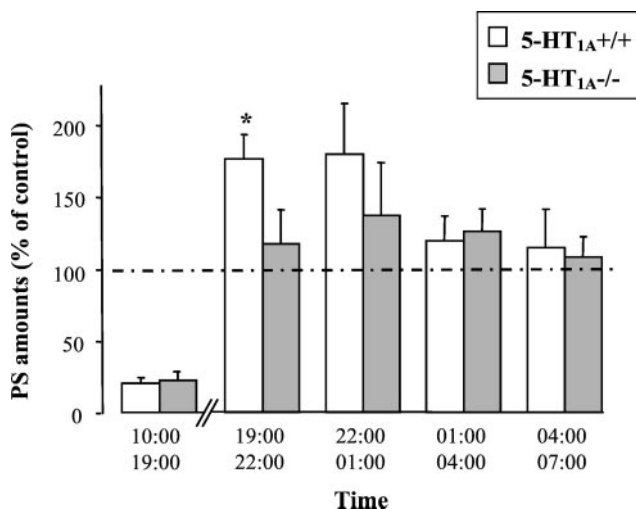

Figure 5. Paradoxical sleep amounts observed during 9 hr of PS deprivation and 12 hr thereafter, in 5-HT$_{1A}$+/+ (*white bars*) and 5-HT$_{1A}$−/− (*gray bars*) mice. Data (mean ± SEM of 6 animals in each group) are expressed as percentage of paired values obtained under control conditions (large platform). *$p < 0.05$, significant difference between groups; Student's *t* test.

fit with the idea of 5-HT$_7$ receptor stimulation exerting a facilitatory influence on PS expression in rodents.

According to the reciprocal interaction model for PS regulation (McCarley and Massaquoi, 1992), 5-HT exerts an inhibitory influence on mesopontine cholinergic "PS-on" neurons (Honda and Semba, 1994), notably through postsynaptic 5-HT$_{1A}$ receptors (Sanford et al., 1994; Horner et al., 1997). On the contrary, 5-HT might have a facilitatory influence on PS (Portas et al., 1996; Bjorvatn et al., 1997) or SWS (Sakai and Crochet, 2001) through the activation of somatodendritic 5-HT$_{1A}$ autoreceptors in anterior raphe nuclei. In the present work, the increase of PS amounts observed after both pharmacological and genetic inactivation of 5-HT$_{1A}$ receptors, together with the unchanged levels of SWS,

support the view that mainly postsynaptic 5-HT$_{1A}$ receptors are involved in the physiological regulation of PS (Tissier et al., 1993).

Compensation at 5-HT$_{1B}$ receptors in 5-HT$_{1A}$−/− mutants

Interestingly, we found that 5-HT$_{1B}$ receptor activation by CP 94253 caused a more pronounced reduction of PS amounts in 5-HT$_{1A}$−/− mutants than in wild-type mice, thereby suggesting that 5-HT$_{1B}$ receptors are supersensitive in these mutants. A similar adaptation of 5-HT$_{1A}$ receptors was apparent in 5-HT$_{1B}$ knock-out mice in which 8-OH-DPAT was found to be more potent than in paired wild-type mice to inhibit PS expression (Boutrel et al., 1999). Because both 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors appear to be involved in a 5-HT-mediated inhibitory control of PS in mice (Boutrel et al., 1999; present study), these data would support the idea that a compensatory increase in the functioning of one receptor occurs after inactivation of the other. Such compensatory changes in 5-HT$_{1A}$−/− and 5-HT$_{1B}$−/− mice have been reported previously but with variations from one brain area to another (Bouwknecht et al., 2001; Knobelman et al., 2001), illustrating the complexity of adaptive processes affecting these receptors. With regard to sleep–wakefulness mechanisms, whether 5-HT$_{1B}$ receptors in pontine nuclei possibly controlling PS expression (Boutrel et al., 1999) are supersensitive or upregulated in 5-HT$_{1A}$−/− mutants is an important question to be addressed in future investigations.

PS rebound after selective PS deprivation or immobilization stress

In agreement with previous reports, wild-type mice were presently found to exhibit a PS rebound after either selective PS deprivation (Sallanon et al., 1983; Adrien and Dugovic, 1984; Gonzalez et al., 1996; Boutrel et al., 1999) or immobilization stress (Cespuglio et al., 1995; Gonzalez et al., 1995; Gonzalez and Valatx, 1998; Meerlo et al., 2001). Interestingly, the rebound after deprivation started immediately at the beginning of the recovery period, whereas that after immobilization stress was delayed by 3–6 hr. Such differences in the time courses of rebound in the two experimental procedures, which have also been observed in rats (Adrien and Dugovic, 1984; Houdouin et al., 1991a), probably reflect two different mechanisms. Indeed, PS deprivation (small platform), in contrast to immobilization stress, does not enhance plasma corticosterone levels (compared with paired control conditions, large platform) (Porkka-Heiskanen et al., 1995). Accordingly, immediate PS rebound would not be attributable to the stress inherent to the platform technique but would rather reflect sleep homeostatic properties (Barbato and Wehr, 1998). On the other hand, the delay in PS rebound after immobilization stress may be accounted for by increased levels of corticosterone just after the stress, because this hormone exerts inhibitory influence on PS (Bradbury et al., 1998; Marinesco et al., 1999). Indeed, under our conditions, preliminary data showed that plasma corticosterone levels increased markedly just after immobilization and returned to baseline only 4–6 hr later (our unpublished observations), i.e., at the time of PS rebound onset.

In contrast to wild-type animals, 5-HT$_{1A}$−/− mutants did not exhibit any PS rebound after PS deprivation or immobilization stress. This has been found previously with 5-HT$_{1B}$−/− mice after selective PS deprivation (Boutrel et al., 1999) and immobilization stress (our unpublished data). Accordingly, it can be inferred that both 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors play key roles in the homeostatic regulation of PS and in the PS adaptive response

Table 2. Characteristics of paradoxical sleep in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice during 12 hr after a 9 hr PS deprivation or a 90 min immobilization stress

		Control conditions		Experimental conditions	
		Amounts (min)	Number	Amounts (min)	Number
PS deprivation					
5-HT$_{1A}$+/+ ($n = 6$)	0–6 hr	12.5 ± 1.5	11.3 ± 1.0	20.8 ± 1.6*	17.2 ± 1.6*
	6–12 hr	18.3 ± 1.1	16.2 ± 1.5	20.1 ± 1.7	18.0 ± 2.6
5-HT$_{1A}$−/− ($n = 6$)	0–6 hr	20.2 ± 2.9**	16.5 ± 1.8**	23.1 ± 2.7	19.7 ± 2.2
	6–12 hr	25.8 ± 1.9**	21.2 ± 2.2	28.3 ± 2.2**	24.7 ± 1.8
Immobilization stress					
5-HT$_{1A}$+/+ ($n = 9$)	0–6 hr	12.2 ± 1.1	10.3 ± 1.1	15.9 ± 2.2	15.0 ± 2.0*
	6–12 hr	19.1 ± 1.1	16.1 ± 1.2	23.4 ± 1.5*	22.1 ± 1.5*
5-HT$_{1A}$−/− ($n = 7$)	0–6 hr	19.2 ± 2.1**	16.6 ± 1.8**	17.0 ± 1.8	15.1 ± 1.1
	6–12 hr	24.7 ± 3.0	21.4 ± 2.2**	25.1 ± 1.5	23.1 ± 1.6

The data (mean ± SEM of n animals) represent the amounts of PS expressed as minutes and the number of PS episodes for the 0–6 and 6–12 hr periods of recovery after control (large platform) and PS deprivation (small platform) procedure (top) and control and immobilization stress (bottom). *$p < 0.05$, significantly different from control conditions; paired Student's t test. **$p < 0.05$, significantly different from 5-HT$_{1A}$+/+ group; unpaired Student's t test.
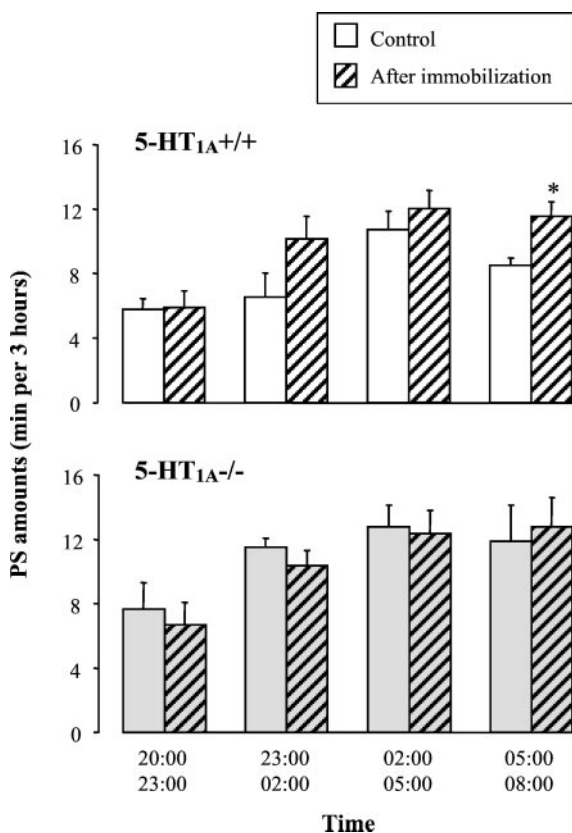

Figure 6. Paradoxical sleep amounts observed during 12 hr after 90 min of immobilization in 5-HT$_{1A}$+/+ (*top*) and 5-HT$_{1A}$−/− (*bottom*) mice. Data (mean ± SEM of 9 and 7 animals, respectively) are expressed as minutes per 3 hr under control conditions (*white* and *gray solid bars*) and after immobilization stress (*hatched bars*). *$p < 0.05$, significantly different from respective control value; paired Student's t test.

to acute stress. Interestingly, previous studies also showed an absence of sleep rebound when 5-HT neurotransmission had been impaired (Sallanon et al., 1983; Houdouin et al., 1991a,b). Altogether, these data support the idea that the serotonergic system, in addition to the corticotropin-releasing hormone–noradrenergic one (Gonzalez et al., 1995, 1996; Gonzalez and Valatx, 1998), underlie the PS rebound in response to both

conditions. Such a lack of PS rebound after acute stress in 5-HT$_{1A}$−/− mice might be linked to a prolactin deficit (Meerlo et al., 2001) and/or increased corticosterone levels. Indeed, in 5-HT$_{1A}$ (and 5-HT$_{1B}$) knock-out mice, but not in their wild-type counterparts, plasma corticosterone levels had not returned to baseline, even 6 hr after the end of immobilization (our unpublished observations), thereby accounting for a sustained prevention of PS rebound in these mutants.

Because PS amounts for the recovery period after deprivation or immobilization stress in wild-type mice were similar to those previously observed at baseline in 5-HT$_{1A}$−/− mutants, it can also be proposed that the latter have reached a maximum level of PS production as a result of the absence of 5-HT-mediated inhibitory control. In this scheme, the 5-HT system, notably through 5-HT$_{1A}$ and 5-HT$_{1B}$ (Boutrel et al., 1999) receptors, would play a predominant role in all adaptive mechanisms involving PS. In any case, the absence of PS rebound after PS deprivation, as well as after immobilization stress, raises the question of the existence of common mechanisms underlying the homeostatic regulations of PS and the PS-mediated adaptations to stress, notably those involving 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors.

It is interesting to note that both 5-HT$_{1A}$−/− and 5-HT$_{1B}$−/− (Boutrel et al., 1999) mutants exhibit, on the one hand, a spontaneous increase in PS amounts and a lack of PS adaptation to PS deprivation or a stressful condition, and, on the other hand, abnormal behaviors (anxiety-like for the 5-HT$_{1A}$−/− and aggressiveness for the 5-HT$_{1B}$−/− mice). The present results indicate that these abnormal behaviors are associated with sleep alterations, a situation that is also often described in humans suffering from mood disorders (Gillin, 1998; Van Praag, 1998) and that an altered 5-HT neurotransmission could be, at least, a common factor in these disorders. In this respect, it has to be emphasized that depression is associated with both an increased pressure of paradoxical sleep (Kupfer, 1976; Gillin, 1998) and a general decrease in postsynaptic 5-HT$_{1A}$ receptor density in various brain regions (Drevets et al., 2000; Sargent et al., 2000) in humans. The increased expression of PS observed herein in 5-HT$_{1A}$−/− mice would suggest that such a sleep anomaly in depressed patients is underlain, at least in part, by the reported downregulation of 5-HT$_{1A}$ receptors in these patients.

REFERENCES

Adrien J, Dugovic C (1984) Presence of a paradoxical sleep (PS) inducing factor in the cerebrospinal fluid of PS-deprived rats. Eur J Pharmacol 100:223–226.

Barbato G, Wehr TA (1998) Homeostatic regulation of REM sleep in humans during extended sleep. Sleep 21:267–276.

Bjorvatn B, Fagerland S, Eid T, Ursin R (1997) Sleep/waking effects of a selective 5-HT$_{1A}$ receptor agonist given systemically as well as perfused in the dorsal raphe nucleus in rats. Brain Res 770:81–88.

Boutrel B, Franc B, Hen R, Hamon M, Adrien J (1999) Key role of 5-HT$_{1B}$ receptors in the regulation of paradoxical sleep as evidenced in 5-HT$_{1B}$ knock-out mice. J Neurosci 19:3204–3212.

Bouwknecht JA, Van der Gugten J, Hijzen TH, Maes RAA, Hen R, Olivier B (2001) Corticosterone responses in 5-HT$_{1B}$ receptor knock-out mice to stress or 5-HT$_{1A}$ receptor activation are normal. Psychopharmacology 153:484–490.

Bradbury MJ, Dement WC, Edgar DM (1998) Effects of adrenalectomy and subsequent corticosterone replacement on rat sleep state and EEG power spectra. Am J Physiol 275:R555–R565.

Cespuglio R, Marinesco S, Baubet V, Bonnet C, El Kafi B (1995) Evidence for a sleep-promoting influence of stress. Adv Neuroimmunol 5:145–154.

Drevets WC, Frank E, Price JC, Kupfer DJ, Greer PJ, Mathis C (2000) Serotonin type-$_{1A}$ receptor imaging in depression. Nucl Med Biol 27:499–507.

Driver HS, Flanigan MJ, Bentley AJ, Luus HG, Shapiro CM, Mitchell D (1995) The influence of ipsapirone, a 5-HT$_{1A}$ agonist, on sleep patterns of healthy subjects. Psychopharmacology 7:186–192.

Dzoljic MR, Ukponmwan O, Saxena PR (1992) 5-HT$_1$-like receptor agonists enhance wakefulness. Neuropharmacology 31:623–633.

Gerlai R (1996) Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? Trends Neurosci 19:177–181.

Gillin JC (1998) Are sleep disturbances risk factors for anxiety, depressive and addictive disorders? Acta Psychiatr Scand [Suppl] 393:39–43.

Gonzalez MM, Valatx JL (1998) Involvement of stress in the sleep rebound mechanism induced by sleep deprivation in the rat: use of alpha-helical CRH (9–41). Behav Pharmacol 9:655–662.

Gonzalez MM, Debilly G, Valatx JL, Jouvet M (1995) Sleep increase after immobilization stress: role of the noradrenergic locus coeruleus system in the rat. Neurosci Lett 202:5–8.

Gonzalez MM, Valatx JL, Debilly G (1996) Role of the locus coeruleus in the sleep rebound following two different sleep deprivation methods in the rat. Brain Res 740:215–226.

Hagan JJ, Price GW, Jeffrey P, Deeks NJ, Stean T, Piper D, Smith MI, Upton N, Medhurst AD, Middlemiss DN, Riley GJ, Lovell PJ, Bromidge SM, Thomas DR (2000) Characterization of SB-269970-A, a selective 5-HT$_7$ receptor antagonist. Br J Pharmacol 130:539–548.

Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P, Parsons LH, Tecott LH (1998) Elevated anxiety and antidepressant-like responses in serotonin 5-HT$_{1A}$ receptor mutant mice. Proc Natl Acad Sci USA 95:15049–15054.

Honda T, Semba K (1994) Serotonergic synaptic input to cholinergic neurons in the rat mesopontine tegmentum. Brain Res 647:299–306.

Horner RL, Sanford LD, Annis RA, Pack AI, Morrison AR (1997) Serotonin at the laterodorsal tegmental nucleus suppresses rapid-eye-movement sleep in freely behaving rats. J Neurosci 17:7541–7552.

Houdouin F, Cespuglio R, Gharib A, Sarda N, Jouvet M (1991a) Detection of the release of 5-hydroxyindole compounds in the hypothalamus and the n. raphe dorsalis throughout the sleep-waking cycle and during stressful situations in the rat: a polygraphic and voltammetric approach. Exp Brain Res 85:153–162.

Houdouin F, Cespuglio R, Jouvet M (1991b) Effects induced by the electrical stimulation of the nucleus raphe dorsalis upon hypothalamic release of 5-hydroxyindole compounds and sleep parameters in the rat. Brain Res 565:48–56.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PPA (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 46:157–203.

Kia HK, Miquel MC, Brisorgueil MJ, Daval G, Riad M, El Mestikawy S, Hamon M, Vergé D (1996) Immunocytochemical localization of serotonin 5-HT$_{1A}$ receptors in the rat central nervous system. J Comp Neurol 365:289–305.

Knobelman DA, Hen R, Blendy JA, Lucki I (2001) Regional patterns of compensation following genetic deletion of either 5-hydroxytryptamine$_{1A}$ or 5-hydroxytryptamine$_{1B}$ receptor in the mouse. J Pharmacol Exp Ther 298:1092–1100.

Kupfer DJ (1976) REM latency: a psychobiologic marker for primary depressive disease. Biol Psychiatry 11:159–174.

Limoge-Lendais I, Robert C, Degrange M, Goldberg M, Stinus L, Limoge A (1994) Study on Superbond adhesion to the skull for chronic electrode implantation in the rat. Neurosci Protocol 70:1–11.

Marinesco S, Bonnet C, Cespuglio R (1999) Influence of stress duration on the sleep rebound induced by immobilization in the rat: a possible role for corticosterone. Neuroscience 92:921–933.

McCarley RW, Massaquoi SG (1992) Neurobiological structure of the revised limit cycle reciprocal interaction model of REM cycle control. J Sleep Res 1:132–137.

Meerlo P, Easton A, Bergmann BM, Turek FW (2001) Restraint increases prolactin and REM sleep in C57BL/6J but not in BALB/cJ mice. Am J Physiol 281:R846–R854.

Monti JM, Jantos H (1992) Dose-dependent effects of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT on sleep and wakefulness in the rat. J Sleep Res 1:169–175.

Parks CL, Robinson PS, Sibille E, Shenk T, Toth M (1998) Increased anxiety of mice lacking the serotonin$_{1A}$ receptor. Proc Natl Acad Sci USA 95:10734–10739.

Porkka-Heiskanen T, Smith SE, Taira T, Urban JH, Levine JE, Turek FW, Stenberg D (1995) Noradrenergic activity in rat brain during rapid eye movement sleep deprivation and rebound sleep. Am J Physiol 268:R1456–R1463.

Portas CM, Thakkar M, Rainnie D, McCarley RW (1996) Microdialysis perfusion of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in the dorsal raphe nucleus decreases serotonin release and increases rapid eye movement sleep in the freely moving cat. J Neurosci 16:2820–2828.

Portas CM, Bjorvatn B, Ursin R (2000) Serotonin and the sleep/wake cycle: special emphasis on microdialysis studies. Prog Neurobiol 60:13–35.

Quattrochi JJ, Mamelak AN, Binder D, Williams J, Hobson JA (1993) Dose-related suppression of REM sleep and PGO waves by the serotonin-1 agonist eltoprazine. Neuropsychopharmacology 8:7–13.

Ramboz S, Oosting R, Aït Amara D, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R (1998) Serotonin receptor$_{1A}$ knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci USA 95:14476–14481.

Riad M, Garcia S, Watkins KC, Jodoin N, Doucet E, Langlois X, El Mestikawy S, Hamon M, Descarries L (2000) Somatodendritic localization of 5-HT$_{1A}$ and preterminal axonal localization of 5-HT$_{1B}$ receptors in adult rat brain. J Comp Neurol 417:181–194.

Sakai K, Crochet S (2001) Role of dorsal raphe in paradoxical sleep generation in the cat: no evidence for serotonergic mechanism. Eur J Neurosci 13:103–112.

Sallanon M, Janin M, Buda C, Jouvet M (1983) Serotoninergic mechanisms and sleep rebound. Brain Res 268:95–104.

Sanford LD, Ross RJ, Seggos AE, Morrison AR, Ball WA, Mann GL (1994) Central administration of two 5-HT receptor agonists: effect on REM sleep initiation and PGO waves. Pharmacol Biochem Behav 49:93–100.

Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C, Meyer J, Gunn RN, Grasby PM, Cowen PJ (2000) Brain serotonin$_{1A}$ receptor binding measured by positron emission tomography with [^{11}C]WAY-100635: effects of depression and antidepressant treatment. Arch Gen Psychiatry 57:174–180.

Sotelo C, Cholley B, El Mestikawy S, Gozlan H, Hamon M (1990) Direct immunohistochemical evidence for the existence of 5-HT$_{1A}$ autoreceptors on serotoninergic neurons in the midbrain raphe nuclei. Eur J Neurosci 2:1144–1154.

Thakkar MM, Strecker RE, McCarley RW (1998) Behavioral state control through differential serotonergic inhibition in the mesopontine cholinergic nuclei: a simultaneous unit recording and microdialysis study. J Neurosci 18:5490–5497.

Tissier M, Lainey E, Fattaccini C, Hamon M, Adrien J (1993) Effects of ipsapirone, a 5-HT$_{1A}$ agonist, on sleep/wakefulness cycles: probable post-synaptic action. J Sleep Res 2:103–109.

Tobler I, Deboer T, Fisher M (1997) Sleep and sleep regulation in normal and prion protein-deficient mice. J Neurosci 17:1869–1879.

Ursin R (2002) Serotonin and sleep. Sleep Med Rev 6:55–67.

Van Praag HM (1998) Anxiety and increased aggression as pacemakers of depression. Acta Psychiatr Scand [Suppl] 393:81–88.

Vazquez-Palacios G, Velazquez-Moctezuma J (2000) Effect of electric foot shocks, immobilization, and corticosterone administration on the sleep-wake pattern in the rat. Physiol Behav 71:23–28.

Vergé D, Daval G, Marcinkiewicz M, Patey A, El Mestikawy S, Hamon M (1986) Quantitative autoradiography of multiple 5-HT$_1$ receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. J Neurosci 6:3474–3482.

Wood M, Chaubey M, Atkinson P, Thomas DR (2000) Antagonist activity of meta-chlorophenypiperazine and partial agonist activity of 8-OH-DPAT at the 5-HT$_7$ receptor. Br J Pharmacol 396:1–8.

Zhuang X, Gross C, Santarelli L, Compan V, Trillat AC, Hen R (1999) Altered emotional states in knockout mice lacking 5-HT$_{1A}$ or 5-HT$_{1B}$ receptors. Neuropsychopharmacology 21:52S–60S.