Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 478 (2003) 121–130
www.elsevier.com/locate/ejphar

Differential effects of the 5-HT_{1A} receptor agonist flesinoxan given locally or systemically on REM sleep in the rat

Jaime M. Monti*, Héctor Jantos

Department of Pharmacology and Therapeutics, Clinics Hospital, 2833/602 Zudañez Street, Montevideo 11300, Uruguay

Received 6 March 2003; received in revised form 14 August 2003; accepted 22 August 2003

Abstract

The effects of flesinoxan, a selective 5-HT_{1A} receptor agonist on spontaneous sleep, were studied in adult rats implanted for chronic sleep recordings. Flesinoxan was administered systemically or infused directly into the dorsal raphe nucleus, the left laterodorsal tegmental nucleus or the medial pontine reticular formation. Systemic administration of flesinoxan (0.03 and/or 0.06 μmol/kg) significantly increased wakefulness and sleep latencies, and reduced rapid eye movement (REM) sleep and the number of REM periods, during the first and/or second 2-h period after treatment. Direct infusion of the 5-HT_{1A} receptor agonist (0.06 and/or 0.12 nmol) into the dorsal raphe nucleus induced a significant increment of REM sleep and augmented the number of REM periods during the second and/or third 2-h period of recording. Microinjection of flesinoxan (0.03, 0.06 and/or 0.12 nmol) into the laterodorsal tegmental nucleus reduced REM sleep and the number of REM periods, and augmented REM sleep latency during the first, second and/or third 2-h recording period. Finally, direct infusion of flesinoxan (0.48 nmol) into the medial pontine reticular formation decreased REM sleep and the number of REM periods, and increased REM sleep latency during the first and second 2 h of recording. Our results indicate that the 5-HT_{1A} receptor is involved in the inhibitory effect of serotonin on brainstem structures that act to promote and to induce REM sleep.

Keywords: Sleep; REM sleep; Dorsal raphe nucleus; Laterodorsal tegmental nucleus; Medial pontine reticular formation; 5-HT_{1A} receptor

1. Introduction

A number of hypotheses have proposed the involvement of different nuclei, pathways and neurotransmitters in order to explain the regulation of rapid eye movement (REM) sleep. The reciprocal interaction hypothesis of REM sleep generation identifies cholinergic neurons in the laterodorsal and the pedunculopontine tegmental nuclei as promoting REM sleep, and inhibition of these neurons by serotonergic afferents from the dorsal raphe nucleus and by noradrenergic afferents from the locus coeruleus (McCarley and Hobson, 1975; Hobson et al., 1998). The REM sleep induction region of the medial pontine reticular formation includes predominantly glutamatergic neurons, which are in turn activated by efferent connections of the pedunculopontine tegmental/laterodorsal tegmental nuclei. In addition to cholinergic projections from the pedunculopontine tegmental and the laterodorsal tegmental nuclei, the REM sleep induction zone receives various aminergic inputs. According to Semba (1993), serotonergic afferents represent a mean of 44% of all aminergic/cholinergic projections to the REM sleep induction zone, with the heaviest projections arising from the dorsal raphe nucleus and the median raphe nucleus.

The 5-HT_{1A} receptor is located on the soma and the dendrites (somatodendritic autoreceptor) of serotonergic neurons, and at postsynaptic sites. Stimulation of the somatodendritic 5-HT_{1A} receptor inhibits the firing rate of serotonergic neurons, whereas activation of the postsynaptic receptor induces inhibitory responses on target structures. Brain areas rich in 5-HT_{1A} receptors include the pedunculopontine tegmental/laterodorsal tegmental nuclei, the medial pontine reticular formation and some raphe nuclei, particularly the dorsal raphe nucleus (Kia et al., 1996a; Tohyama and Takatsuji, 1998).

Currently, a number of ligands are available that show affinity for the 5-HT_{1A} receptor. They include 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) and flesinoxan, which are agonists at pre- and postsynaptic sites. 8-OH-DPAT was until recently considered a selective 5-HT_{1A}

* Corresponding author. Tel.: +598-2-710-58-07; fax: +598-2-487-27-07.
E-mail address: jmonti@mednet.org.uy (J.M. Monti).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.08.039

receptor agonist. However, it is now known that the compound is also a partial agonist for the 5-HT_7 receptor (Vanhoenacker et al., 2000).

Direct infusion or microdialysis perfusion of 8-OH-DPAT into the dorsal raphe nucleus significantly increased REM sleep in rats and cats (Portas et al., 1996; Bjorvatn et al., 1997; Monti et al., 2002). Direct administration of flesinoxan into the dorsal raphe nucleus induced also a significant increment of REM sleep in the rat. Pretreatment with the selective 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide (WAY 100635) prevented the increase of REM sleep induced by flesinoxan (Monti et al., 2002). On the other hand, microinjection of serotonin (5-HT) or 8-OH-DPAT into the laterodorsal tegmental nuclei selectively inhibited REM sleep in the cat and rat (Sanford et al., 1994; Horner et al., 1997).

In the present study to further elucidate the mechanisms of REM sleep, we examined the effects on REM sleep occurrence of direct microinjection of the selective 5-HT_{1A} receptor agonist flesinoxan into the laterodorsal tegmental nucleus or the medial pontine reticular formation of the rat. The effects of intra-dorsal raphe nucleus injection of flesinoxan on REM sleep were previously published by our group (Monti et al., 2002). For comparative purposes, the data included in this study were newly collected using a different group of implanted animals.

2. Methods

2.1. Animals

Four different groups of male Wistar rats weighing 350–400 g were included in the study. All rats were cared for and used in strict accordance to the European Community guidelines for the use of experimental animals. All procedures were approved by the Institutional Animal Care and Use Committee of the Medical School, Montevideo, Uruguay.

2.2. Surgical procedures

The animals were anesthetized with sodium pentobarbital (40.0 mg/kg, i.p.) and implanted with Nichrome® electrodes (200-μm diameter) for chronic sleep recordings of electro-

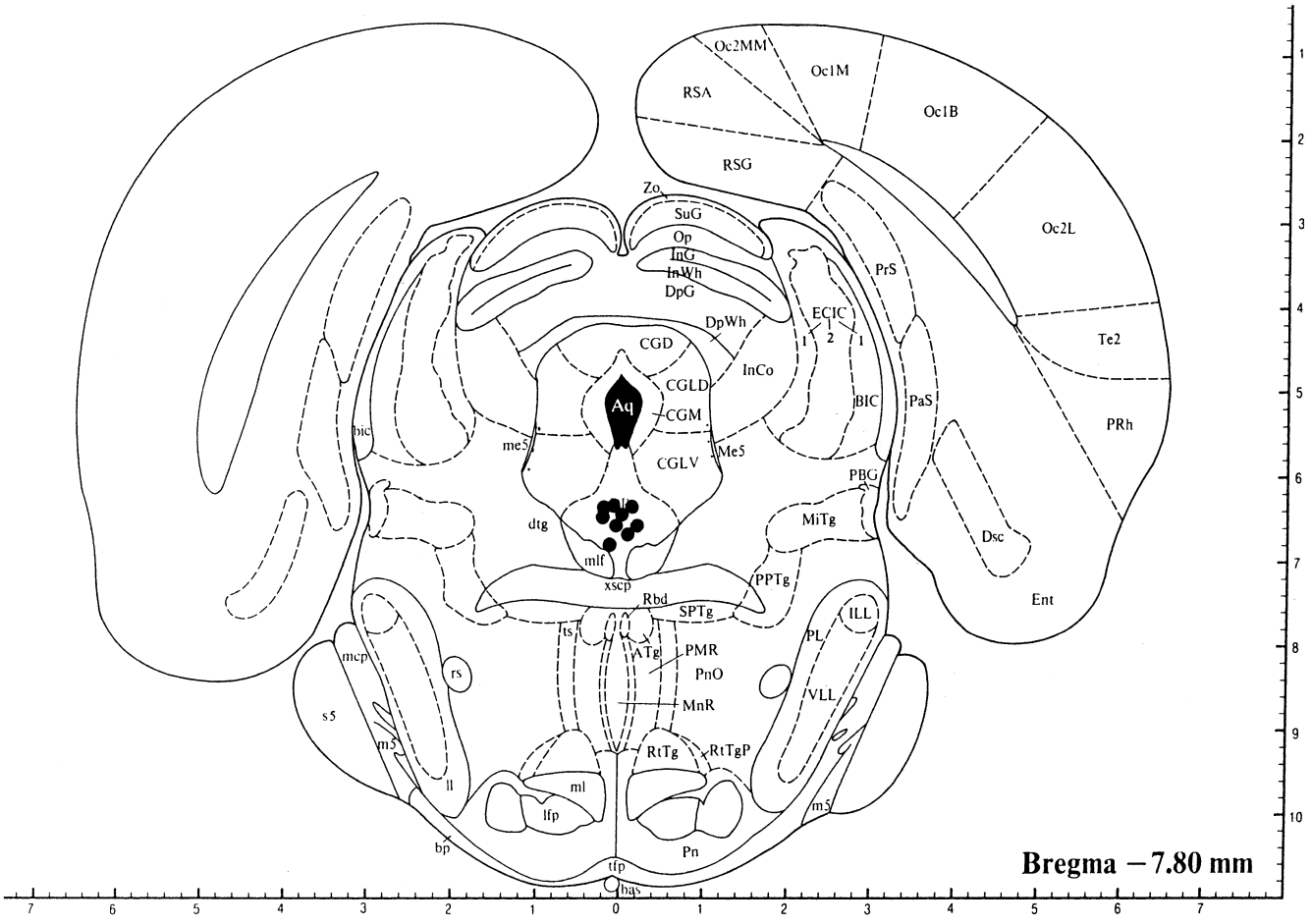

Fig. 1. Schematic drawing of flesinoxan (●) injection sites in the dorsal raphe nucleus of the rat. Microinjection sites correspond to experiment 2 (group 2). Section according to Paxinos and Watson (1986).

encephalogram (EEG) and electromyogram (EMG) activities, through placement on the frontal and the occipital cortex for the former, and on the neck musculature for the latter. In addition, a guide cannula was inserted and maintained in the dorsal raphe nucleus (group 2), the left laterodorsal tegmental nucleus (group 3) or the medial pontine reticular formation (group 4); the final coordinates were: dorsal raphe nucleus: AP 7.8, L 0.0, V-6.4; laterodorsal tegmental nucleus: AP 8.8, L 0.6, V-6.6; and medial pontine reticular formation: AP 8.6, L 1.0, V − 8.1 (Paxinos and Watson, 1986). The tip of the tubular guide (gauge 26) for drug injection was left 2 mm above the corresponding neuroanatomical structure to minimize cellular damage at the injection site. The animals were treated postoperatively for 4 days with the antibiotic cefradine 50.0 mg/kg. A topical antibiotic cream (neomycin) was also applied to the implant incision. Drug or vehicle was injected into the dorsal raphe nucleus, the laterodorsal tegmental nucleus or the medial pontine reticular formation with an injection cannula (28 gauge), which extended 2.0 mm beyond the guide, in a 0.25-μl volume over a 2-min period. On completion of the study, the rats were sacrificed under pentobarbital anesthesia and cannulae placements were defined histologically. Correct cannula/injection sites were assessed using the atlas of Paxinos and Watson (1986) following a 0.25-μl injection of Fast-green dye into the dorsal raphe nucleus, the laterodorsal tegmental nucleus or the medial pontine reticular formation. All data in this report are derived from animals whose injection site was within the limits of the corresponding neuroanatomical structure.

2.3. Recording and sleep scoring

The animals were housed individually in a temperature-controlled room (23 ± 1 °C) under a 12-h light/12-h dark cycle (the lights went on at 0500 h) and with food and water ad libitum. Ten days after surgery, the animals were habituated to a soundproof chamber fitted with slip-rings and cable-connectors, and to the injection procedure. Drugs were always administered during the light phase of the 12-h light/12-h dark cycle, at approximately 0730 h. A 4 × 4 Latin Square was always used to merge effects of both the drug and the time elapsed during the protocol. Initially, three rats were randomly assigned to each groups

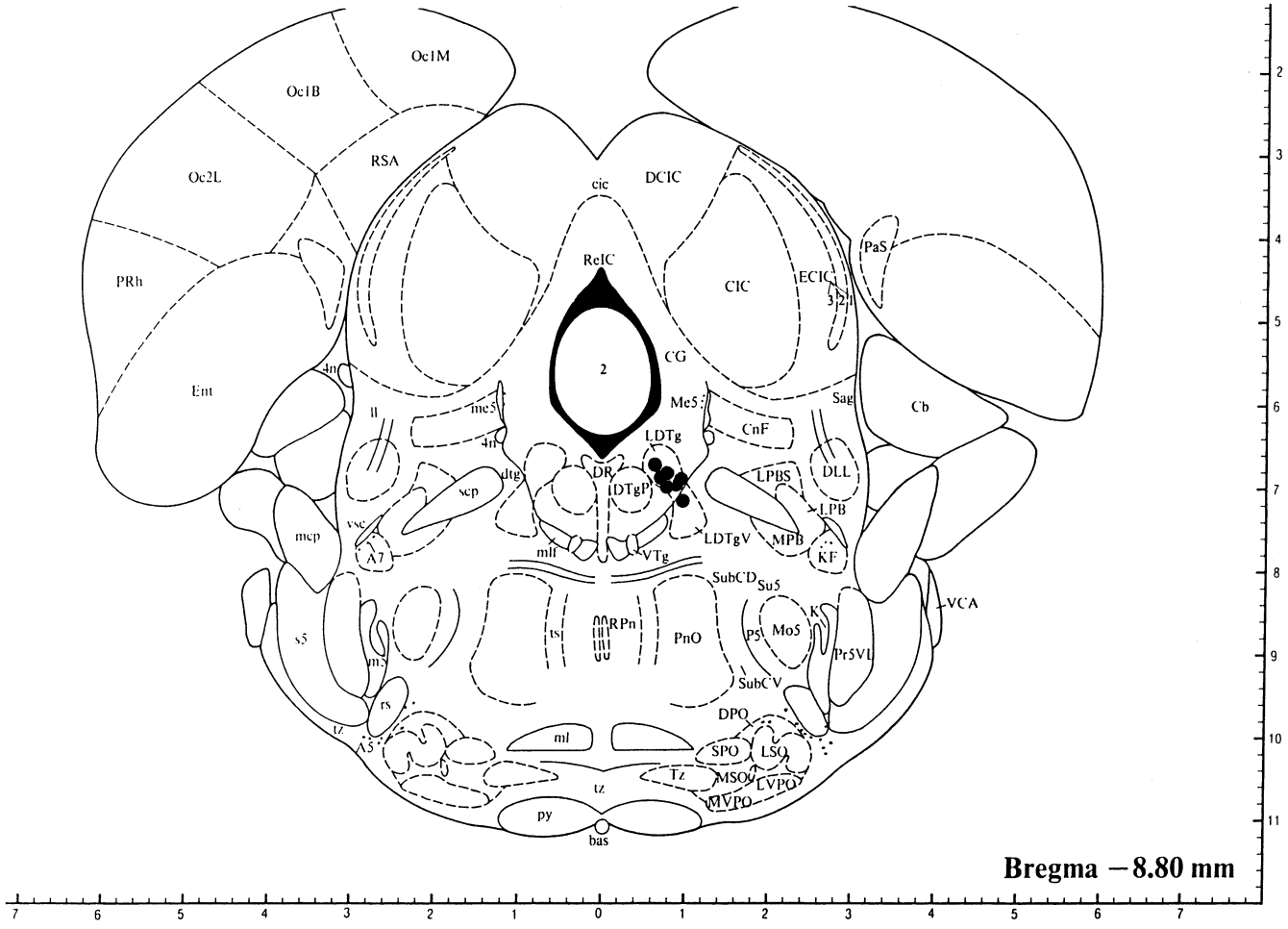

Fig. 2. Schematic drawing of flesinoxan (●) injection sites in the laterodorsal tegmental nucleus of the rat. Microinjection sites correspond to experiment 3 (group 3). Section according to Paxinos and Watson (1986).

A, B, C, and D. Electrographic activity of 25-s epochs was scored by a trained rater (H.J.) blind to the treatment received by the animals. The predominant activity of each epoch was assigned to one of the following categories: wakefulness, light sleep, slow wave sleep, and REM sleep. Slow wave sleep and REM sleep latencies, and the number of REM periods were also determined (Monti et al., 1988).

2.4. Experimental design

The effects of the 5-HT_{1A} receptor agonist flesinoxan were studied in four different groups of animals according to the following experimental paradigms.

2.4.1. Experiment 1 (group 1)

Flesinoxan (Solvay-Duphar, The Netherlands) 0.015, 0.03 or 0.06 μmol/kg or saline was administered s.c. ($n=9$).

2.4.2. Experiment 2 (group 2)

Flesinoxan 0.03, 0.06 or 0.12 nmol or vehicle (saline) was infused into the dorsal raphe nucleus ($n=9$).

2.4.3. Experiment 3 (group 3)

Flesinoxan 0.03, 0.06 or 0.12 nmol or vehicle (saline) was infused into the laterodorsal tegmental nucleus ($n=7$).

2.4.4. Experiment 4 (group 4)

Flesinoxan 0.12, 0.24 or 0.48 nmol or vehicle (saline) was infused into the medial pontine reticular formation ($n=7$).

Flesinoxan was dissolved in an isotonic NaCl solution. Subcutaneous injections were given in a final volume of 1.0 ml/kg. Flesinoxan has an elimination half-life of 7–9 h in the rat (Grof et al., 1993). Thus, in all experiments at least 3 days were allowed to elapse between injections to avoid long-lasting and rebound effects on sleep.

2.5. Statistics

A one-way analysis of variance (ANOVA) using dose as the between subject-factor was performed, with multiple post-hoc comparisons carried out with the Dunnett Multiple Comparisons Test when the ANOVA indicated significance ($P<0.05$).

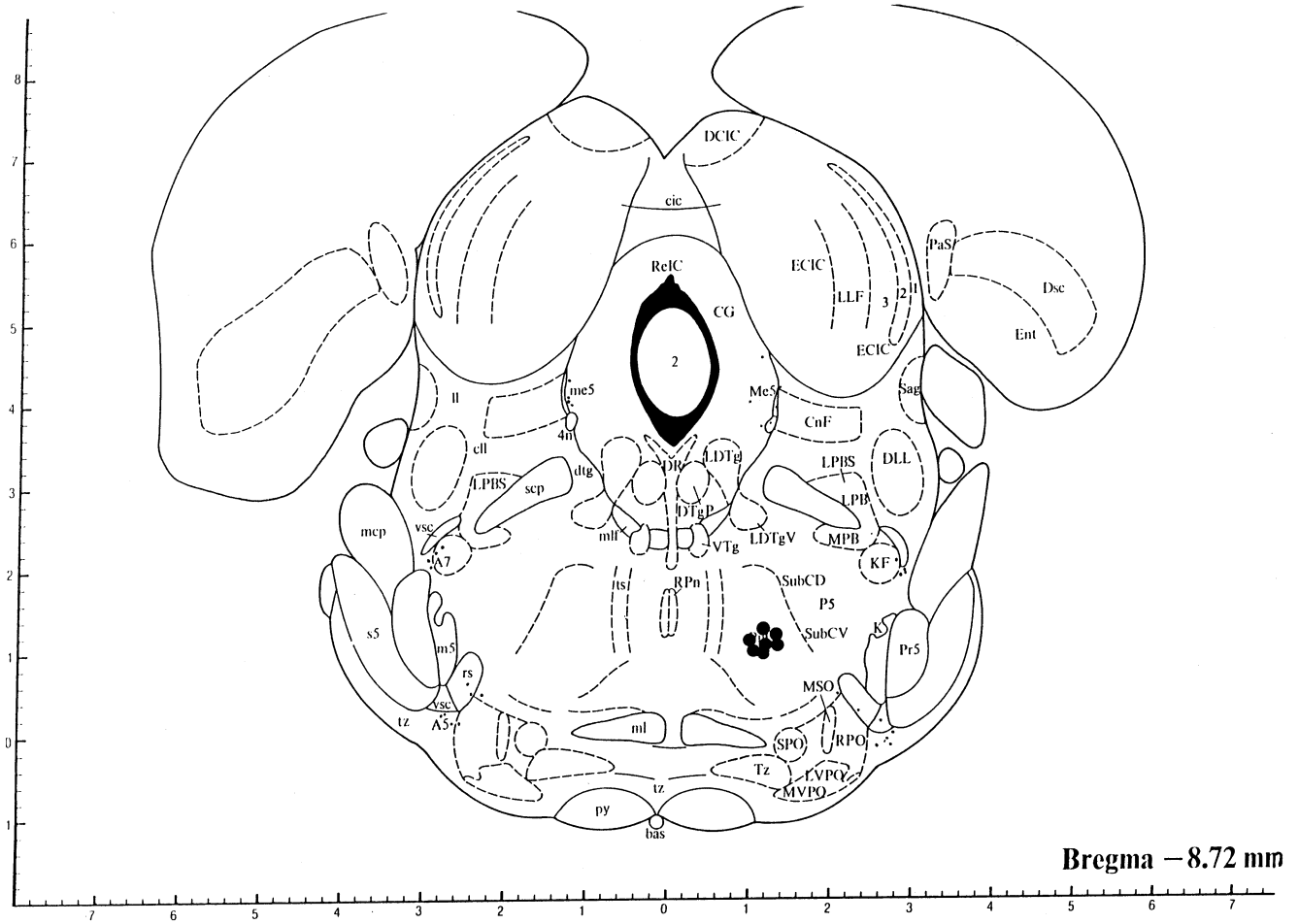

Fig. 3. Schematic drawing of flesinoxan (●) injection sites in the medial pontine reticular formation of the rat. Microinjection sites correspond to experiment 4 (group 4). Section according to Paxinos and Watson (1986).

3. Results

Data from three animals that received flesinoxan by the s.c. route were lost due to technical difficulties. The histological analysis of the injection sites showed that 23 of the 36 animals originally included in the study received microinjections of flesinoxan that were confined within the limits of the corresponding neural structure (dorsal raphe nucleus = 9; laterodorsal tegmental nucleus = 7; medial pontine reticular formation = 7). The data from the 23 rats are summarized in the anatomical schematic of Figs 1, 2 and 3. In those animals where the microinjections of flesinoxan were not confined within the limits of the corresponding neural structure, REM sleep values remained almost unchanged or showed erratic changes.

3.1. Effects of s.c. injection of flesinoxan

Following the administration of 0.06 μmol/kg flesinoxan by s.c. route, wakefulness was increased during the first 2-h recording period ($F_{(3,24)} = 3.31$, $P < 0.03$), whereas REM sleep was reduced during the first and the second 2-h periods after treatment ($F_{(3,24)} = 12.59$, $P < 0.001$, and $F_{(3,24)} = 5.19$, $P < 0.006$, respectively). The 0.03-μmol/kg dose induced a similar effect on REM sleep during the first 2 h of recording ($F_{(3,24)} = 12.59$, $P < 0.001$) (Fig. 4). Slow wave sleep latency was significantly increased after 0.06 μmol/kg flesinoxan ($F_{(3,24)} = 3.77$, $P < 0.02$). Moreover, the 5-HT_{1A} receptor agonist (0.03 and 0.06 μmol/kg) increased REM sleep latency ($F_{(3,24)} = 12.19$, $P < 0.0001$) and reduced the number of REM periods ($F_{(3,24)} = 25.93$, $P < 0.0001$) during the first 2-h period (Table 1).

3.2. Dorsal raphe nucleus: effects of microinjection of flesinoxan

After flesinoxan was microinjected into the dorsal raphe nucleus, REM sleep was increased by the 0.12-nmol dose during the second and the third 2 h of recording ($F_{(3,24)} = 5.52$, $P < 0.005$, and $F_{(3,24)} = 3.84$, $P < 0.05$, respectively). The 0.06-nmol dose of the 5-HT_{1A} receptor agonist augmented REM sleep only during the second 2 h of recording ($F_{(3,24)} = 5.52$, $P < 0.005$). Values of wakefulness, light sleep, and slow wave sleep showed slight but inconsistent changes that did not attain significance (Fig. 5). Injected at this site, flesinoxan did not significantly modify sleep latencies. However, the number of REM periods was increased after the 0.12-nmol dose during the second 2-h period ($F_{(3,24)} = 8.26$, $P < 0.0006$) (Table 2).

3.3. Laterodorsal tegmental nucleus: effects of microinjection of flesinoxan

Following the microinjection of 0.06 nmol flesinoxan into the laterodorsal tegmental nucleus, REM sleep was suppressed during the first 2-h period after treatment ($F_{(3,18)} = 2.93$, $P < 0.05$). Moreover, REM sleep was significantly reduced by the entire range of doses during the second 2 h of recording ($F_{(3,18)} = 16.94$, $P < 0.0001$), and

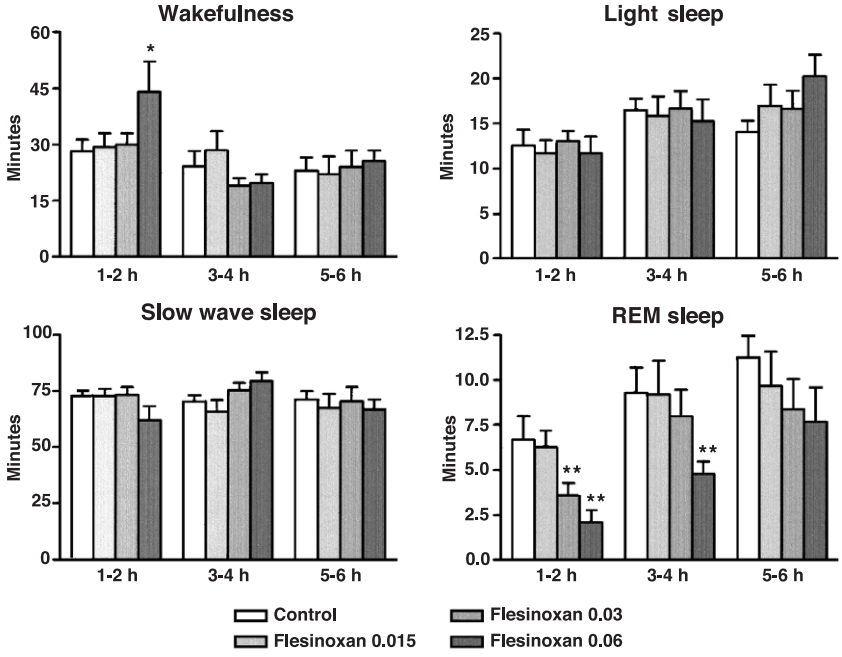

Fig. 4. Effects of flesinoxan administered by the s.c. route on sleep and wakefulness. Nine animals were in each experimental group. Ordinate: time in min of behavioral stage according to EEG criteria. Abscissae: wakefulness and sleep per 2-h recording expressed as mean values ± S.E.M. The doses are in μmol/kg. Compared with control values: *$P < 0.05$; **$P < 0.01$ (Dunnett Multiple Comparisons Test).

Table 1
Effects of flesinoxan administered by the s.c. route on sleep latencies and number of REM periods

Group	SWS latency (min)	REMS latency (min)	Number of REM periods		
			1–2 h	3–4 h	5–6 h
Control	9.4 ± 2.1	52.9 ± 6.5	3.3 ± 0.3	5.3 ± 0.7	5.6 ± 0.9
Flesinoxan					
0.015	10.7 ± 1.6	42.2 ± 5.7	3.4 ± 0.5	4.6 ± 0.6	3.9 ± 0.5
0.03	8.6 ± 1.8	78.2 ± 8.1[a]	1.9 ± 0.4[b]	5.7 ± 0.5	4.0 ± 0.6
0.06	19.8 ± 5.3[a]	90.8 ± 12.5[b]	1.0 ± 0.3[b]	5.2 ± 0.9	4.0 ± 0.8

All values are means ± S.E.M. Nine animals were in each experimental group. The doses are in μmol/kg. Compared with control values: [a]$P<0.05$; [b]$P<0.01$ (Dunnett Multiple Comparisons Test). SWS = slow wave sleep; REMS = REM sleep.

Table 2
Effects of flesinoxan administered directly into the dorsal raphe nucleus on sleep latencies and number of REM periods

Group	SWS latency (min)	REMS latency (min)	Number of REM periods		
			1–2 h	3–4 h	5–6 h
Control	14.4 ± 2.3	47.8 ± 7.3	4.2 ± 0.6	5.6 ± 1.0	6.1 ± 1.1
Flesinoxan					
0.03	12.9 ± 2.4	47.8 ± 7.1	3.6 ± 0.8	5.2 ± 0.8	6.2 ± 0.8
0.06	10.4 ± 1.8	36.0 ± 6.9	4.4 ± 0.5	7.7 ± 0.8	5.9 ± 0.7
0.12	10.9 ± 1.3	45.1 ± 4.6	4.3 ± 0.8	9.0 ± 1.0[b]	8.4 ± 0.8

All values are means ± S.E.M. Nine animals were in each experimental group. The doses are in nmol. Compared with control values: [b]$P<0.01$ (Dunnett Multiple Comparisons Test).

after the 0.12-nmol dose during the third 2-h period ($F_{(3,18)}=4.16$, $P<0.02$). Wakefulness, light sleep, and slow wave sleep were slightly but not significantly modified (Fig. 6). REM sleep latency was increased after the 0.12-nmol dose ($F_{(3,18)}=2.86$, $P<0.05$), whereas the number of REM periods was reduced during the second and the third 2-h periods ($F_{(3,18)}=7.87$, $P<0.001$, and $F_{(3,18)}=3.85$, $P<0.02$, respectively). Values corresponding to this variable were also reduced after the 0.06-nmol dose during the second 2-h recording period ($F_{(3,18)}=7.87$, $P<0.001$) (Table 3).

3.4. Medial pontine reticular formation: effects of microinjection of flesinoxan

In the rats recorded after receiving flesinoxan, REM sleep was reduced significantly after the 0.48-nmol dose during the first and second 2 h of recording ($F_{(3,18)}=7.36$, $P<0.002$, and $F_{(3,18)}=4.89$, $P<0.01$, respectively) (Fig. 7). Wakefulness, light sleep, and slow wave sleep were slightly but not significantly modified. Flesinoxan (0.48 nmol) significantly increased REM sleep latency ($F_{(3,18)}=8.9$, $P<0.0008$). In addition, the number of REM periods was significantly reduced after the 0.24-nmol dose during the first and second 2-h periods ($F_{(3,18)}=12.93$, $P<0.0001$, and $F_{(3,18)}=7.47$, $P<0.001$, respectively); values corresponding to this variable were also reduced after the 0.48-nmol dose during the whole recording period ($F_{(3,18)}=4.09$, $P<0.02$) (Table 4).

4. Discussion

The main finding of this study is that the effects of flesinoxan on sleep variables differed depending on the site and method of administration. The systemic administration of flesinoxan increased wakefulness and reduced REM

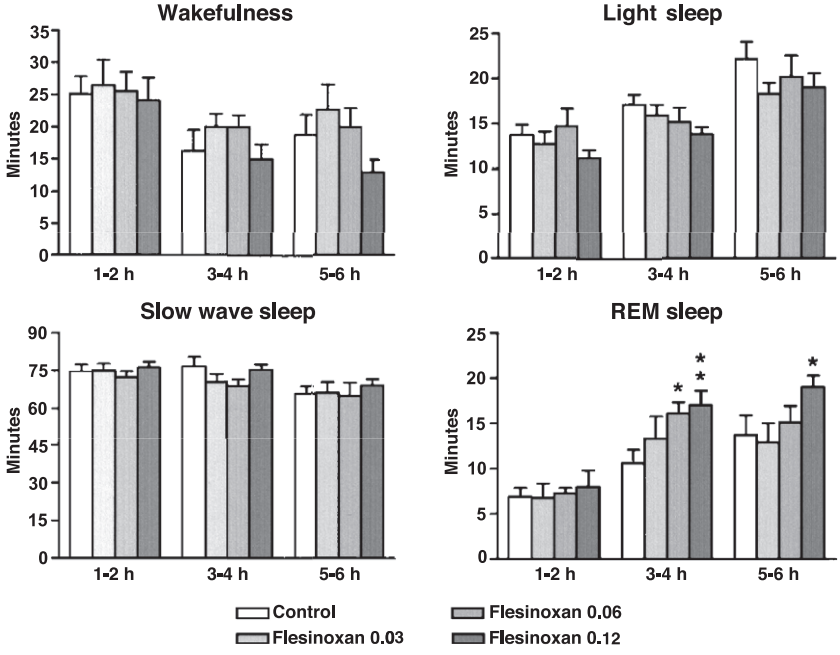

Fig. 5. Effects of flesinoxan microinjected into the dorsal raphe nucleus on sleep and wakefulness. Nine animals were in each experimental group. Ordinate and abscissae as in Fig. 1. The doses are in nmols. Compared with control values: *$P<0.05$; **$P<0.01$ (Dunnett Multiple Comparisons Test).

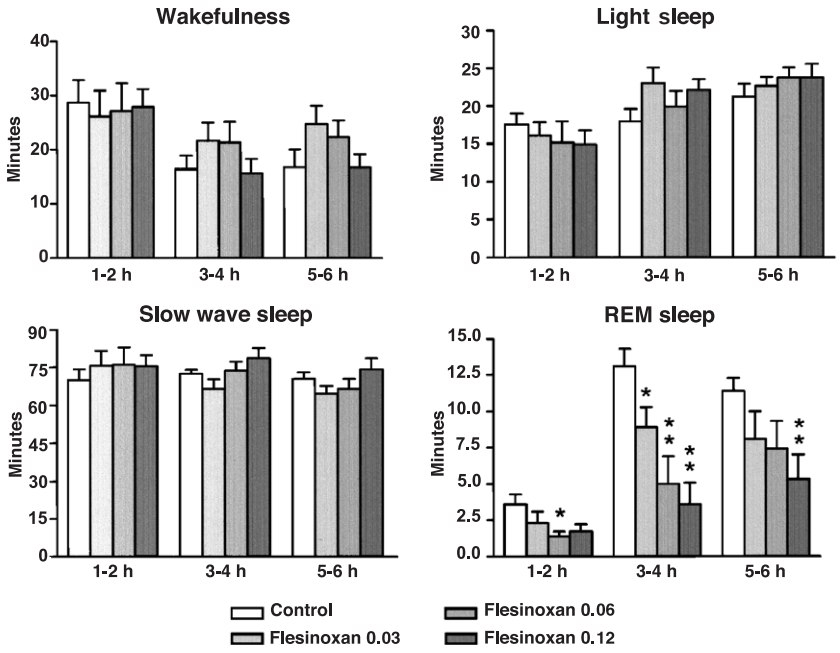

Fig. 6. Effects of flesinoxan microinjected into the left laterodorsal tegmental nucleus on sleep and wakefulness. Seven animals were in each experimental group. Ordinate and abscissae as in Fig. 1. The doses are in nmols. Compared with control values: *$P<0.05$; **$P<0.01$ (Dunnett Multiple Comparisons Test).

sleep. On the other hand, the microinjection of the 5-HT_{1A} receptor agonist into the dorsal raphe nucleus induced an increment of REM sleep, whereas its direct application into the laterodorsal tegmental nucleus or the medial pontine reticular formation had the opposite effect.

It took 3–4 h before a significant effect on REM sleep became apparent after flesinoxan microinjection into the dorsal raphe nucleus. Interestingly, Bonvento et al. (1992) found that the reduction of 5-HT output in the striatum and the frontal cortex was delayed after intra-dorsal raphe nucleus microinjection of 8-OH-DPAT in the rat. Becquet et al. (1990) described a decreased 5-HT release in the striatum of cats during the perfusion of *p*-chlorophenylethylamine, a 5-HT-releasing drug, in the dorsal raphe nucleus. The reduced 5-HT release was still evident at least 1 h after the withdrawal of *p*-chlorophenylethylamine, a time when 5-HT release in the dorsal raphe nucleus had almost regained normal levels. Thus, as proposed by Ferré et al. (1994) the decreased 5-HT release in the striatum and the frontal cortex may not be an immediate consequence of the suppression of serotonergic activity by 8-OH-DPAT or *p*-chlorophenylethylamine, but could depend on the critical reduction of a terminal 5-HT releasable pool. A similar mechanism could account for the long delay before a significant increase of REM sleep was observed after flesinoxan microinjection into the dorsal raphe nucleus.

Table 3
Effects of flesinoxan administered directly into the left laterodorsal tegmental nucleus on sleep latencies and number of REM periods

Group	SWS latency (min)	REMS latency (min)	Number of REM periods		
			1–2 h	3–4 h	5–6 h
Control	7.6 ± 1.7	64.7 ± 12.0	2.4 ± 0.5	7.4 ± 0.7	6.0 ± 0.5
Flesinoxan					
0.03	5.9 ± 2.1	80.9 ± 14.4	1.6 ± 0.6	5.6 ± 1.0	4.1 ± 0.8
0.06	9.3 ± 3.0	86.4 ± 12.9	0.9 ± 0.3	3.3 ± 1.0[b]	4.0 ± 1.0
0.12	8.1 ± 3.4	107.0 ± 21.2[a]	1.4 ± 0.4	3.0 ± 1.2[b]	3.0 ± 1.0[a]

All values are the means ± S.E.M. Seven animals were in each experimental group. The doses are in nmol. Compared with control values: [a]$P<0.05$; [b]$P<0.01$ (Dunnett Multiple Comparisons Test).

Moderate to dense projections of the dorsal raphe nucleus have been observed to reach the laterodorsal tegmental nucleus and the medial pontine reticular formation (Honda and Semba, 1994; Vertes and Kocsis, 1994). In addition, serotonergic afferents innervate non-cholinergic, presumptively glutamatergic, neurons of the REM sleep induction zone of the medial pontine reticular formation, with the heaviest projections arising from the dorsal raphe nucleus and the median raphe nucleus (Semba, 1993). Serotonergic receptors have been characterized in the laterodorsal tegmental/pedunculopontine tegmental nuclei and the medial pontine reticular formation. In this respect, the binding density levels of 5-HT_{1A} receptors in these structures vary from low to medium (Sanford et al., 1995; Kia et al., 1996a,b; Tohyama and Takatsuji, 1998). Recently, Strecker et al. (1999) quantified spontaneous levels of extracellular 5-HT in the pedunculopontine tegmental nucleus during sleep and wakefulness in the cat. Extracellular serotonin levels were highest during wakefulness, and progressively lower during slow wave sleep and REM sleep. Furthermore, Thakkar et al. (1998) showed that local microdialysis perfusion of the partial 5-HT_{1A} receptor agonist 8-OH-DPAT into areas where cholinergic laterodorsal tegmental and pedunculopontine tegmental neurons are located almost

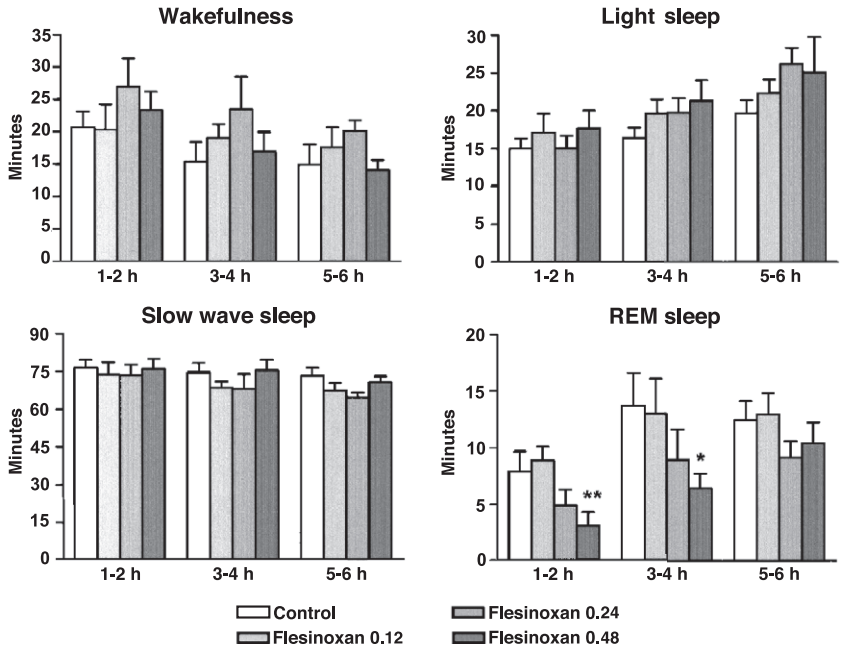

Fig. 7. Effects of flesinoxan microinjected into the medial pontine reticular formation on sleep and wakefulness. Seven animals were in each experimental group. Ordinate and abscissae as in Fig. 1. The doses are in nmols. Compared with control values: *$P<0.05$; **$P<0.01$ (Dunnett Multiple Comparisons Test).

completely suppressed the discharge activity of REM-on neurons. On the other hand, 8-OH-DPAT had minimal or no effect on the Wake-REM-on cells.

Iwakiri et al. (1993) measured extracellular levels of endogenous serotonin in the medial pontine reticular formation of intact cats and found that they were at their highest during wakefulness. As the animals entered slow wave sleep, 5-HT levels decreased to about 90%; during REM sleep the levels of this neurotransmitter were at their lowest (60–50%). Stevens et al. (1992) examined the action of serotonin on medial pontine reticular formation neurons using intracellular recordings of rat brainstem slices in vitro. Serotonin induced a hyperpolarization associated with a decrease in input resistance in 34% of the neurons. This response was mimicked by 8-OH-DPAT and was blocked by the nonselective 5-HT_{1A} receptor antagonist spiperone.

Thus, our findings tend to indicate that the flesinoxan-dependent activation of postsynaptic 5-HT_{1A} receptors, after its microinjection into the laterodorsal tegmental nucleus or the medial pontine reticular formation, is responsible for the REM sleep suppression. As mentioned before, microinjection of 5-HT or 8-OH-DPAT into the laterodorsal tegmental nucleus selectively suppressed REM sleep in the cat and rat (Sanford et al., 1994; Horner et al., 1997). Since all serotonin receptors bind the natural ligand 5-HT, the response could not be associated with the activation of a given receptor. Moreover, it is now known that 8-OH-DPAT binds not only to the 5-HT_{1A} receptor but also to 5-HT_7 receptor (Vanhoenacker et al., 2000). This implies that the increase or reduction of REM sleep after local 8-OH-DPAT administration, previously related to the activation of the 5-HT_{1A} receptor might also be, at least partly, a 5-HT_7 mediated response.

Table 4
Effects of flesinoxan administered directly into the medial pontine reticular formation on sleep latencies and number of REM periods

Group	SWS latency (min)	REMS latency (min)	Number of REM periods		
			1–2 h	3–4 h	5–6 h
Control	7.9 ± 2.4	35.7 ± 6.1	4.7 ± 0.7	5.9 ± 1.0	5.4 ± 0.5
Flesinoxan					
0.12	8.1 ± 2.8	35.3 ± 7.4	4.3 ± 0.4	4.9 ± 0.5	5.3 ± 0.8
0.24	10.0 ± 3.7	45.4 ± 10.0	2.4 ± 0.5[b]	3.4 ± 1.0[b]	3.9 ± 0.7
0.48	7.0 ± 2.8	87.1 ± 16.1[b]	1.7 ± 0.6[b]	2.9 ± 0.5[b]	3.4 ± 0.6[a]

All values are the means ± S.E.M. Seven animals were in each experimental group. The doses are in nmol. Compared with control values: [a]$P<0.05$; [b]$P<0.01$ (Dunnett Multiple Comparisons Test).

How can the effect of systemic administration of flesinoxan on wakefulness be understood? Jones (1994) proposed that wakefulness is maintained by neurons within the brainstem reticular formation, which in turn excite cells in the nonspecific thalamo-cortical projection system, the posterior hypothalamus, and the basal forebrain. All these neural structures activate in turn the cerebral cortex and the limbic system (septal nuclei, hippocampal formation, amygdala). The neurotransmitters involved in the arousal process include glutamate, acetylcholine, norepinephrine, serotonin, histamine, and orexin (Jones, 1994; Monti, 1982, 1995; Monti and Monti, 2000; Baghdoyan and Lydic, 2002; Pace-Schott and Hobson, 2002). Pharmacological studies, particularly those employing selective agonists of 5-HT_{1A}, 5-HT_{1B}, 5-$HT_{2A/C}$ or 5-HT_3 receptors, support the proposal that serotonin facilitates the cortical processes of wakefulness (Monti and Jantos, 1992; Monti et al., 1990, 1995; Ponzoni et al., 1993). In this respect, it has been

consistently shown that systemic injection of 8-OH-DPAT into different species increases wakefulness and reduces EEG power density in the low frequency range (Dugovic and Wauquier, 1987; Dzoljic et al., 1989; Monti et al., 1990; Van Proosdij and Ruigt, 1994). Although the process by which flesinoxan facilitates the state of wakefulness is still unknown, it should be mentioned that many of the non-cholinergic neurons in the basal forebrain, including gamma-amino butyric acid (GABA)-ergic cells, are hyperpolarized by serotonin released from the dorsal raphe nucleus (Parnavelas, 1990; Detari et al., 1999). Thus, inhibition of GABAergic neurons by flesinoxan could account, at least in part, for its facilitatory effects on wakefulness. In other words, the 5-HT_{1A} receptor agonist may attenuate GABAergic input and thereby indirectly increase the release of acetylcholine (and glutamate) at cortical sites.

In conclusion, our results indicate that the 5-HT_{1A} receptor is involved in the inhibitory effect of serotonin on brainstem structures that act to promote and induce REM sleep.

References

Baghdoyan, E.A., Lydic, R., 2002. Neurotransmitters and neuromodulators regulating sleep. In: Bazil, C.W., Malow, B.A., Sammaritano, M.R. (Eds.), Sleep and Epilepsy: The Clinical Spectrum. Elsevier, Amsterdam, pp. 17–44.

Becquet, D., Faudon, M., Hery, F., 1990. The role of serotonin release and autoreceptors in the dorsalis raphe nucleus in the control of serotonin release in the cat caudate nucleus. Neuroscience 39, 639–647.

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.

Bonvento, G., Scatton, B., Claustre, Y., Rouquier, L., 1992. Effect of local injection of 8-OH-DPAT into the dorsal or medial raphe nuclei on extracellular levels of serotonin in serotonergic projection areas in the rat brain. Neurosci. Lett. 137, 101–104.

Detari, L., Rasmusson, D.D., Semba, K., 1999. The role of basal forebrain neurons in tonic and phasic activation of the cerebral cortex. Prog. Neurobiol. 58, 249–277.

Dugovic, C., Wauquier, A., 1987. 5-HT2 receptors could be primarily involved in the regulation of slow wave sleep in the rat. Eur. J. Pharmacol. 137, 145–146.

Dzoljic, M.R., Saxena, P.R., Ukponmwan, O.E., 1989. 5-HT1 receptor agonists enhance alertness. Sleep Res. 16, 88.

Ferré, S., Cortes, R., Artigas, F., 1994. Dopaminergic regulation of the serotonergic raphe-striatal pathway: microdialysis studies in freely moving rats. J. Neurosci. 14, 4839–4846.

Grof, P., Joffe, R., Kennedy, S., Persad, E., Syrotiuk, J., Bradford, D., 1993. An open study of oral flesinoxan, a 5-HT_{1A} receptor agonist, in treatment-resistant depression. Int. Clin. Psychopharmacol. 8, 167–172.

Hobson, A.J., Stickgold, P., Pace-Schott, E.F., 1998. The neurophysiology of REM sleep dreaming. NeuroReport 9, R1–R14.

Honda, T., Semba, K., 1994. Serotonergic synaptic input to cholinergic neurons in the rat pontine tegmentum. Brain Res. 647, 299–308.

Horner, R.L., Sanford, L.D., Annis, D., Pack, A.I., Morrison, A.R., 1997. Serotonin at the laterodorsal tegmental nucleus suppresses rapid-eye-movement sleep in freely behaving rats. J. Neurosci. 17, 7541–7552.

Iwakiri, H., Matsuyama, K., Mori, S., 1993. Extracellular levels of serotonin in the medial pontine reticular formation in relation to sleep–wake cycle in cats: a microdialysis study. Neurosci. Res. 18, 157–170.

Jones, B., 1994. Basic mechanisms of sleep–wake states. In: Kryger, M.H., Roth, T., Dement, W.C. (Eds.), Principles and Practice of Sleep Medicine. Saunders, Philadelphia, pp. 145–162.

Kia, H.K., Brisorgueil, M.J., Hamon, M., Calas, A., Vergé, D., 1996a. Ultrastructural localization of 5-hydroxytryptamine1A receptors in the rat brain. J. Neurosci. Res. 46, 697–708.

Kia, H.K., Miquel, M.-C., Brisorgueil, M.J., Daval, G., Riad, M., El Mestikawy, S., Hamon, M., Verge, D., 1996b. Immunocytochemical localization of serotonin$_{1A}$ receptors in the rat central nervous system. J. Comp. Neurol. 365, 289–305.

McCarley, R.W., Hobson, J.A., 1975. Neuronal excitability modulation over the sleep cycle: a structural and mathematical model. Science 189, 58–60.

Monti, J.M., 1982. Catecholamines and the sleep–wake cycle: I. EEG and behavioral arousal. Life Sci. 30, 1145–1157.

Monti, J.M., 1995. Pharmacology of the histaminergic system. In: Kales, A. (Ed.), The Pharmacology of Sleep. Springer, Berlin, pp. 117–142.

Monti, J.M., Jantos, H., 1992. Dose-dependent effects of the 5-HT_{1A} receptor agonist 8-OHDPAT on sleep and wakefulness in the rat. J. Sleep Res. 1, 169–175.

Monti, J.M., Monti, D., 2000. Role of dorsal raphe nucleus serotonin 5-HT_{1A} receptor in the regulation of REM sleep. Life Sci. 66, 1999–2012.

Monti, J.M., Hawkins, M., Jantos, H., Labraga, P., Fernández, J., 1988. Biphasic effects of dopamine D-2 receptor agonists on sleep and wakefulness in the rat. Psychopharmacology 95, 395–400.

Monti, J.M., Orellana, C., Boussard, M., Jantos, H., Labraga, P., Olivera, S., Alvariño, F., 1990. 5-HT receptor agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and 8-OH-DPAT increase wakefulness in the rat. Biog. Amines 7, 145–151.

Monti, J.M., Monti, D., Jantos, H., Ponzoni, A., 1995. Effects of selective activation of the 5-HT_{1B} receptor with CP-94,253 on sleep and wakefulness in the rat. Neuropharmacology 34, 1647–1651.

Monti, J.M., Jantos, H., Monti, D., 2002. Increased REM sleep after intradorsal raphe nucleus injection of flesinoxan or 8-OHDPAT: prevention with WAY 100635. Eur. Neuropsychopharmacol. 12, 47–55.

Pace-Schott, E.F., Hobson, J.A., 2002. Basic mechanisms of sleep: new evidence of the neuroanatomy and neuromodulation of the NREM–REM cycle. In: Davis, K.L., Charney, D., Coyle, J.T., Nemeroff, C. (Eds.), Neuropsychopharmacology—The Fifth Generation of Progress. Lippincott Williams & Wilkins, Philadelphia, pp. 1859–1877.

Parnavelas, J.G., 1990. Neurotransmitters in the cerebral cortex. In: Uylings, H.B.M., Van Eden, C.G., De Bruin, J.P.C., Corner, M.A., Feenstra, M.G.P. (Eds.), Progress in Brain Research, vol. 85. Elsevier, Amsterdam, pp. 13–29.

Paxinos, G.,Watson, C., 1986. The Rat Brain. Academic Press, Sidney.

Ponzoni, A., Monti, J.M., Jantos, H., 1993. The effects of selective activation of the 5-HT_3 receptor with m-chlorophenylbiguanide on sleep and wakefulness in the rat. Eur. J. Pharmacol. 249, 259–264.

Portas, C.M., Thakkar, M., Rainnie, D., McCarley, R.W., 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.

Sanford, L.D., Ross, R.J., Seggos, A.E., Morrison, A.R., Ball, B.A., Mann, G.L., 1994. Central administration of two 5-HT receptor agonists: effect on REM sleep initiation and PGO waves. Pharmacol. Biochem. Behav. 49, 93–100.

Sanford, L.D., Tejani-Butt, S.M., Ross, R.J., Morrison, A.R., 1995. Amygdaloid control of alerting and behavioral arousal in rats: involvement of serotonergic mechanisms. Arch. Ital. Biol. 134, 81–99.

Semba, K., 1993. Aminergic and cholinergic afferents to REM sleep induction regions of the pontine reticular formation in the rat. J. Comp. Neurol. 330, 543–556.

Stevens, D.R., McCarley, R.W., Greene, R.W., 1992. Excitatory amino-acid-mediated responses and synaptic potentials in medial pontine

reticular formation neurons in the rat in vitro. J. Neurosci. 12, 4188–4194.

Strecker, R.E., Thakkar, M.M., Porkka-Heiskanen, T., Dauphin, L.J., Bjørkum, A.A., McCarley, R.W., 1999. Behavioral state-related changes of extracellular serotonin concentration in the pedunculopontine tegmental nucleus: a microdialysis study in freely moving animals. Sleep Res. Online 2, 21–27.

Thakkar, M.M., Strecker, R.E., McCarley, R.W., 1998. Behavioral state control through differential serotonergic inhibition of the mesopontine cholinergic nuclei: a simultaneous unit recording and microdialysis study. J. Neurosci. 18, 5490–5497.

Tohyama, M., Takatsuji, K., 1998. Atlas of Neuroactive Substances and their Receptors in the Rat. Oxford Univ. Press, Oxford.

Vanhoenacker, P., Haegeman, G., Leysen, J.E., 2000. 5-HT_7 receptors: current knowledge and future prospects. TIPS 21, 70–77.

Van Proosdij, J., Ruigt, G., 1994. Effects of selective serotonergic agonists and antagonists on rat sleep–waking behavior and sleep-stage dependent power spectra. In: Coenen, A.M.L. (Ed.), Sleep–Wake Research in The Netherlands. Drukkerij Elinkwijk, Utrecht, pp. 121–127.

Vertes, R.P., Kocsis, B., 1994. Projections of the dorsal raphe nucleus to the brainstem: PHA-L analysis in the rat. J. Comp. Neurol. 340, 11–26.