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ORIGINAL INVESTIGATION

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Study of a $GABA_c$ receptor antagonist on sleep-waking behavior in rats

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Abstract *Rationale:* γ-Aminobutyric acid (GABA) is the main inhibitory transmitter of the brain. The involvement of $GABA_A$ and $GABA_B$ receptors in sleep-waking processes is well established. *Objectives*: This research studied the influence of $GABA_C$ receptors. *Methods:* The rats were randomly infused in the fourth ventricle with vehicle and 25, 50, and 100 µg (1,2,5,6,-tetrahydropyridine)-methylphosphinic acid (TPMPA), a specific antagonist of $GABA_C$ receptors. *Results*: Principally at 50 µg, the molecule induced an increase of waking from 44.7% to 61.7% ($P<0.003$), which was the consequence of enhancement of both active and quiet wakefulness. Total slow wave sleep was decreased, particularly the slow-wave stage from 39% to 27.7% ($P<0.02$). Paradoxical sleep was also decreased from 14.5% to 9.1% ($P<0.01$). *Conclusions*: $GABA_C$ receptors are also involved in sleep-waking regulation. Since the sensitivity of $GABA_C$ receptors to GABA is much higher than that of $GABA_A$ and $GABA_B$ receptors, $GABA_C$ receptor modulators could be potential medications acting at low doses with fewer side effects.

Keywords $GABA_C$ · TPMPA · Sleep · Waking · Rat

Introduction

Gamma aminobutyric acid (GABA) is the main inhibitory transmitter in the brain. It is estimated that at least 20% of brain neurons are GABAergic (Parades and Agmo 1992). The first receptor sub-type to be identified was the $GABA_A$ ionotropic receptor. It consists of a Cl^- ionophore principally coupled to GABA, barbiturate, benzodiazepine, steroid, and picrotoxin binding sites (McDonald and Olsen 1994). The second receptor to be identified was the $GABA_B$ receptor (Hill and Bowery 1981) which is coupled to Ca^{2+} and K^+ ion channels and functions through a metabotropic pathway with a second messenger. The third receptor identified was the $GABA_C$. Indeed, as early as 1975 (Johnston et al. 1975) and again later (Parades and Agmo 1992), it appeared that this receptor was not sensitive to many compounds acting on $GABA_A$ and $GABA_B$ receptors. The $GABA_C$ receptor (Drew et al. 1984) was first observed in retinal tissue (Feigenspan et al. 1993; Quian and Dowling 1994), then in the central nervous system (Bormann and Feigenspan 1995; Boue-Grabot et al. 2000). The receptor comprises a Cl^- ionophore and several subunits (ρ1, ρ2, ρ3) (Ogurusu et al. 1997). It is more sensitive to GABA than $GABA_A$ and $GABA_B$ receptors and desensitization is also different (Chu et al. 1990; Quian and Dowling 1994; Chebib and Johnston 1999; Bormann 2000). The first selective antagonist of $GABA_C$ receptors, (1,2,5,6,-tetrahydropyridine)-methylphosphinic acid (TPMPA), was reported in 1996 (Murata et al. 1996; Ragozzino et al. 1996). We undertook the study of its influence on sleep-waking behavior.

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Materials and methods

Animals and surgical procedures

Seven adult male Wistar rats (280–320 g) were implanted with chronic electrodes under intraperitoneal (i.p.) thiopental (Abbot, France) anesthesia (65 mg/kg). Four silver ball (1-mm diameter) electrodes were placed bilaterally over the frontal cortex and four on the occipital cortex. A bipolar depth electrode made up of two teflon-coated (except at the tip) stainless-steel wires (1/10 mm) twisted together was stereotaxically implanted in the hippocampal CA_1 area (A, 4.5 mm; L, 2.7 mm; D, 7.3 mm) following Paxinos and Watson (1986) coordinates. Two twisted stainless-steel wires (2.5/10 mm) were bilaterally inserted in the dorsal neck muscles. A ground electrode was screwed in front of the olfactory bulb in the middle plane. All electrodes were soldered to two 7-pin connectors (Connectral, France) which were cemented (dental cement Texton, UK) to the skull of the animal. For intracerebroventricular (i.c.v.) injections, a cannula was placed in the fourth ventricle (P, 2.6 mm; L, 0 mm; D, 2.2 mm). Each rat received, at the end of surgery, a subcutaneous (s.c.) injection of 0.02 ml Baytril 5% (enrofloxacine Bayer, France) for prophylactic antibiotic

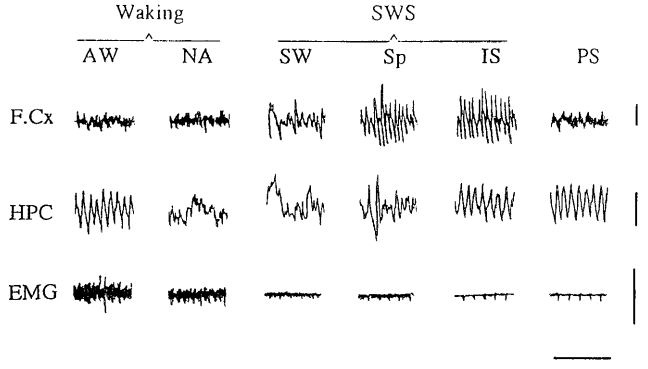

Fig. 1 Sleep-waking stages scored in the rats. Active waking (AW) with hippocampal theta rhythm is distinguished from non-active (NA) quiet waking, without theta activity. Slow wave sleep (SWS) is divided into the slow-wave (SW) stage, the first stage of sleep, then frontal cortex spindles (Sp) which increase in number, amplitude, and duration as sleep deepens. Finally, just prior to and sometimes just after paradoxical sleep, the association of high-amplitude Sp and low-frequency theta rhythm defines the intermediate stage (IS). *PS* paradoxical sleep, *F Cx.* frontal cortex, *HPC* dorsal hippocampus, *EMG* dorsal neck electromyogram. *Calibration* 1 s, 100 μV

therapy. Postoperative analgesia was performed by giving a s.c. injection (5 mg/kg) of 6-chloro-α-methylcarbozole-2-acetic acid (Carpofen, ICN, Costa Mesa, Calif.). The animals were allowed at least 15 days to recover from surgery but were cabled for habituation under artificial 12 h/12 h lighting (lights on for 12 h per day between 0900 hours and 2100 hours, increasing progressively for 33 min from 0900 hours and decreasing in the same manner before 2100 hours). The animals were located in a silent room, individually housed in transparent plexiglass cages with free access to commercial rat chow and water. The ambient temperature in the room was maintained constant at 23°C. Animals were cabled via a 19-channel rotating electrical connector. In this experiment, the principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were followed.

Experimental procedure

The i.c.v. injections were performed at 0900 hours (beginning of the light period) and the recording lasted 6 h. The rats received an injection of either vehicle or TPMPA (Tocris Cookson Ltd. U.K.) at the dose of 25, 50, or 100 μg per rat in a volume of 5 μl. The injection lasted about 2 min, and the needle remained in the cannula for at least 2 min to avoid a flow back before spreading of solution. The doses were chosen after a pre-study on the behavioral effects of different doses carried out on nine rats. The animals were injected in randomized order of administration and recorded with a wash-out period of at least 15 days between two experimental sessions.

Recording

Three channels were simultaneously recorded:

1. Frontal cortex bihemispheric activity
2. Hippocampal theta activity with the depth bipolar electrode or (sometimes) with the occipital cortex electrodes
3. Electromyogram.

Six behavioral stages were distinguished (Fig. 1). Waking was subdivided into two stages: (1) psychomotor active and/or attentive waking, characterized by frontal cortex low-voltage activity, dorsal hippocampal theta activity and high muscular tone and (2) non-active waking without theta rhythm, which corresponds to non-motivated motor activity and principally to quiet waking. During slow-wave sleep (SWS), three stages were differentiated: (1) slow waves (SW) occurring at the cortical level from sleep onset; (2) spindles (Sp) of increasing number, amplitude, and duration as SWS deepens; and (3) intermediate stage (IS) occurring prior to and sometimes just after paradoxical sleep (PS) and characterized by a short-lasting association between high-amplitude anterior spindles and low-frequency theta rhythm.

Scoring of recordings

With our scoring system (SWAS) (Pazzaglia et al. 1994), the analysis, in batch mode for this study, was performed second by second because of the polyphasic sleep-waking cycle which consisted of several short-lasting stages alternating rapidly. Electroencephalogram (EEG) variations were characterized by signal frequency and energy in specific frequency bands. For example, SWs and spindles were differentiated by their respective frequencies (1–6.5 cycles/s vs 7–16 cycles/s) and energy. The reliability of the scoring system is 93.1% of correspondence between researchers and computer.

The scored data were expressed as a percentage by 2-h periods (first, second and third) and 6 h, i.e., the total recording duration. A significant F test (>2.9, $P<0.04$) was followed by a paired-sample student's t-test.

Results

Waking

Total waking was increased for the 6-h recording at 25 μg because of an increase during the two first 2-h periods (Fig. 2). It was the result of a continuous increase of quiet wakefulness. At 50 μg, the increase of total waking was present for the three 2-h periods. The same variations were observed for non-active waking, while for active-waking only the first and second 2-h period showed an increase, as did the total recording duration (Fig. 2). No variation was obtained at 100 μg, a dose which often induced some behavioral disturbances.

Slow-wave sleep

With 25 μg TPMPA, the global SWS was decreased during the first 2 h of recording (Fig. 3). This was the consequence of a decrease in the SW and IS. With 50 μg, TPMPA decreased the global SWS only during the first 2-h, second 2-h, and 6-h periods. This was the consequence of the SW stage decrease during the same periods. The only one effect obtained at 100 μg involved the spindles, which decreased during the first and third 2-h periods and over the total duration.

Paradoxical sleep

After 50 μg TPMPA, there was a decrease of PS for the second 2-h period and total recording time, while, after 100 μg, there was a decrease during the first 2-h period (Fig. 4).

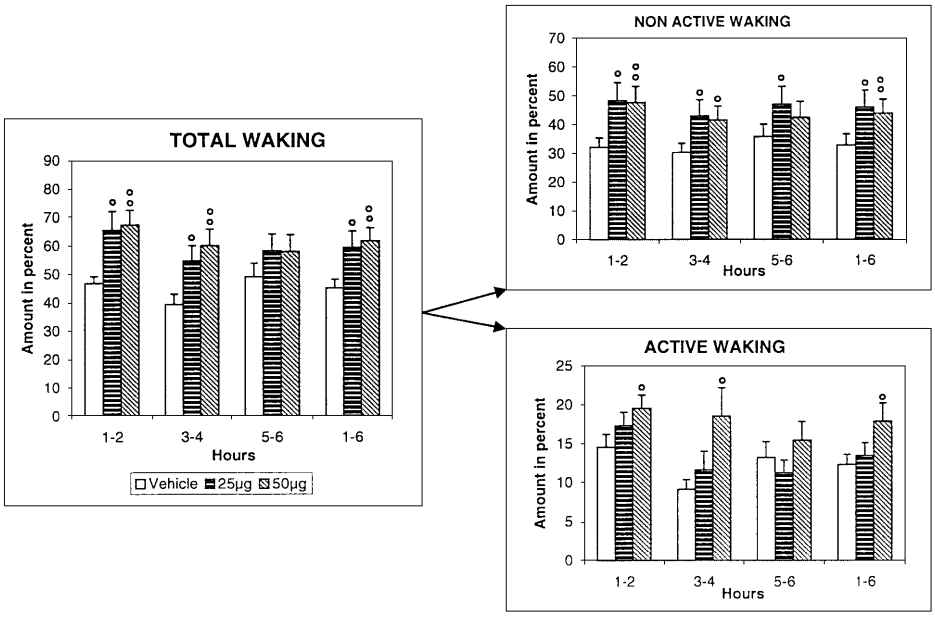

Fig. 2 Influence of the γ-amino-butyric acid $(GABA)_C$ receptor antagonist TPMPA on waking. Total waking is increased at both doses. At the low dose, it is consecutive to the enhancement of non-active waking only, At the higher dose, active waking is also increased. ○ $P<0.05$; ○○ $P<0.01$

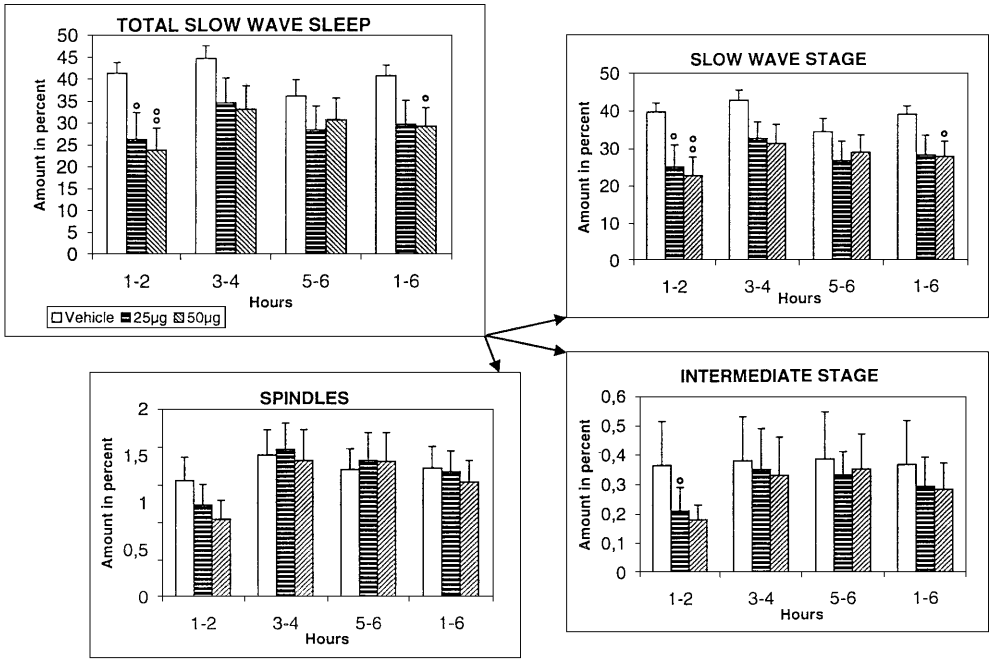

Fig. 3 Total slow-wave sleep is decreased by the low dose during the first 2 h as a result of the decreased duration of the slow-wave stage. With 50 µg TPMPA, for the same reason, total slow-wave sleep is decreased throughout the recording period (6 h). ○ $P<0.05$; ○○ $P<0.01$

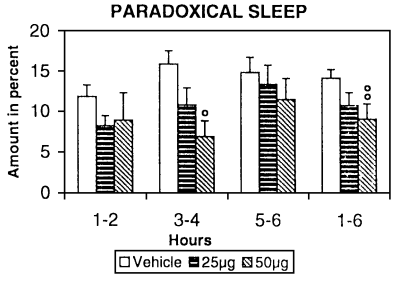

Fig. 4 The higher dose induces a decrease of paradoxical sleep during the 6-h recording period. ○ $P<0.05$; ○○ $P<0.01$

Discussion

The results show that TPMPA, the first specific antagonist of the $GABA_C$ receptor, increases waking and decreases SWS and PS. The increase of waking was seen with the low dose (25 µg), but the middle dose (50 µg) gave the more effective results, while the high dose gave rise to some behavioral abnormalities: it seemed to be slightly too high. The increase of waking first concerns quiet wakefulness, which was be seen with the 25-µg dose. With 50 µg, active waking was also increased. It should be mentioned that although the experiment took place

during daytime, the amount of waking in the control condition seems slightly high. We have no explanation, since the animals were habituated to the cables and were in a quiet environment. The only unusual fact is that the progressive light appearance and extinction we adopted was more functional. The increase of wakefulness induced by the compound is associated with a decrease of the SW stage, which can be observed as early as during the first 2 h with the low dose. With 50 µg, this decrease was combined with a decrease of PS. All these influences are rather long-lasting since they cover the 6-h recording period.

It has been previously shown that the $GABA_A$ receptor complex modulates sleep-waking behavior. Indeed, barbiturates have long been known to induce sleep. More precisely, these modulators of GABA receptor function promote SWS and inhibit PS (Oswald 1968). In rats (Gottesmann 1964, 1996) and cats (Gottesmann et al. 1984; Gottesmann 1996), they massively increase the IS at the expense of PS. This is probably the consequence of the observed inhibition of pontine reticular activation responsible for PS characteristics (Gottesmann 1967, 1969, 1996 for review). Benzodiazepines, second-generation hypnotics, also act by favoring sleep, particularly stage II in humans, and inhibiting PS (Gaillard et al. 1973). In rats, most of them also increase the IS at the expense of PS (Gandolfo et al. 1994). Zolpidem and zopiclone, third-generation hypnotics, induce sleep (Stutzmann et al. 1993) but decrease the IS and PS during the first hours after administration (Gottesmann et al. 1994, 1998; Gauthier et al. 1997a for review), Finally, even neurosteroids (or neurosteroid analogs), which also bind to the $GABA_A$ receptor complex, favor sleep (Edgar et al.1997) and extend the IS (Lancel et al. 1996, 1997).

In the same way, $GABA_B$ receptor modulators modify sleep-waking behavior. In rats, the receptor antagonist CGP 35348 increases SWS and PS in one study (Puigcerver et al. 1996) and increases wakefulness and PS in another (Gauthier et al. 1997b). The findings of the last study are more in accordance with expected physiological results, since these two behavioral stages are characterized by activation of the midbrain (Moruzzi and Magoun 1949; Steriade 1996) and pons (Gottesmann 1967, 1969; McCarley and Hobson 1971; Vertes 1977).

Consequently, all three kinds of GABA receptors are involved in sleep-waking regulation. The increase of sleep with agonists and of waking with antagonists by peripheral or i.c.v. administration suggests that the receptors could be situated in the brain stem (Xi et al. 1999; Maloney et al. 2000) and/or in the target structures of the ventrolateral preoptic nucleus (Gallopin et al. 2000). However, since $GABA_C$ receptors have the highest sensitivity to GABA, this recently discovered receptor could have major functional influences. Specific agonists, if identified, could be interesting compounds for clinical use as hypnotics and perhaps antiepileptics, acting at low doses, thus with fewer side effects than current medications. The idea that antagonists could be effective on narcoleptic attacks remains hypothetical at present, although findings suggest GABAergic participation in recent medication improving this disease (Lin et al. 2000).

References

Borman J (2000) The 'ABC' of GABA receptors. Trends Pharmacol Sci 21:16–19

Bormann J, Feigenspan A (1995) $GABA_C$ receptors. Trends Neurosci 18:515–518

Boue-Grabot E, Taupignon A, Tramu G, Garret M (2000) Molecular and electrophysiological evidence for a GABAC receptor in therotropin-secreting cells. Endocrinology 141:1627–1632

Chebib M, Johnston GAR (1999) The 'ABC' of GABA receptors: a brief review. Clin Exp Pharmacol Physiol 26:937–940

Chu DCM, Albin RL, Young AB, Penney JB (1990) Distribution and kinetics of GABAB binding sites in rat central nervous system: a quantitative autoradiographic study. Neuroscience 34:341–357

Drew CA, Johnston GAR, Weatherby RP (1984) Bicuculine insensitive GABA receptors: studies on the binding of (–)-baclofen to rat cerebellar membranes. Neurosci Lett 52:317–321

Edgar DM, Seidel WF, Gee KW, Lan NC, Field G, Xia H, Hawkinson JE, Wieland S, Carter RB, Wood PL (1997) CCD-3693: an orally bioavailable analog of the endogenous neuroactive steroid, pregnanolone, demonstrates potent sedative hypnotic actions in the rat. J Pharmacol Exp Ther 282: 420–429

Feigenspan A, Wässle H, Bormann J (1993) Pharmacology of GABA receptor Cl^- channels in rat retinol bipolar cells. Nature 361:159–162

Gaillard JM, Schultz P, Tissot R (1973) Effects of three benzodiazepines (nitrazepam, flunitrazepam and bromazepam) on sleep of normal subjects, studied with an automatic scoring system. Pharmacopsychiatry 6:207–217

Gallopin T, Fort P, Eggermann E, Caull B, Luppi PH, Rossier J, Audinat E, Mühlethaler M, Serafin M (2000) Identification of sleep-promoting neurons in vitro. Nature 404:992–995

Gandolfo G, Scherschlicht R, Gottesmann C (1994) Benzodiazepines promote the intermediate stage at the expense of paradoxical sleep in the rat. Pharmacol Biochem Behav 49: 921–927

Gauthier P, Arnaud C, Stutzmann JM, Gottesmann C (1997a) Influence of zopiclone, a new generation hypnotic, on the intermediate stage and paradoxical sleep in the rat. Psychopharmacology 130:139–143

Gauthier P, Arnaud C, Gottesmann C (1997b) Influence of a $GABA_B$ receptor antagonist on sleep-waking cycle in the rat. Brain Res 773:8–14

Gottesmann C (1964) Données sur l'activité corticale au cours du sommeil profond chez le Rat. C R Seances Soc Biol Fil 158:1829–1834

Gottesmann C (1967) Recherche sur la psychophysiologie du sommeil chez le Rat. Presses du Palais-Royal, Paris

Gottesmann C (1969) Etude sur les activités électrophysiologiques phasiques chez le Rat. Physiol Behav 4:495–504

Gottesmann C (1996) The transition from slow wave sleep to paradoxical sleep: evolving facts and concepts of the neurophysiological processes underlying the intermediate stage of sleep. Neurosci Biobehav Rev 20:367–387

Gottesmann C, Gandolfo G, Zernicki B (1984) Intermediate stage of sleep in the cat. J Physiol Paris 79:365–374

Gottesmann C, Trefouret S, Depoortere H (1994) Influence of Zolpidem, a novel hypnotic, on the intermediate stage and paradoxical sleep in the rat. Pharmacol Biochem Behav 47: 359–362

Gottesmann C, Gandolfo G, Arnaud C, Gauthier P (1998) The intermediate stage and paradoxical sleep in the rat: influence of three generation of hypnotics. Eur J Neurosci 10:409–414

Hill DR, Bowery NG (1981) ^{3H}-baclofen and ^{3H}-GABA bind to bicuculine insensitive $GABA_B$ sites in rat brain. Nature 290: 149–152

Johnston GAR, Curtis DR, Beart PM, Game CJA, McCulloch RM, Twitclin B (1975) Cis-and trans-4-aminocrotonic acid as GABA analogues of restricted conformation. J Neurochem 24: 157–160

Lancel M, Faulhaber J, Holsboer F, Ruppert R (1996) Progesterone induces changes in sleep comparable to those of agonists $GABA_A$ receptor modulations. Am J Physiol 34:E763–E772

Lancel M, Faulhaber J, Schiffelholz T, Romeo E, Di Michele F, Holsboer F, Ruppecht R (1997) Allopregnanolone affects sleep in a benzodiazepine-like fashion. J Pharmacol Exp Ther 282:1213–1218

Lin JS, Gervasoni D, Hou Y, Vanni-Mercier G, Rambert F, Frydman A, Jouvet M (2000) Effects of amphetamine and modafinil on the sleep-wake cycle during experimental hypersomnia induced by sleep deprivation in the cat. J Sleep Res 9:89–96

Maloney KJ, Mainville L, Jones BE (2000) c-Fos expression in Gabaergic, serotonergic, and other neurons of the pontomedullary reticular formation and raphe after paradoxical sleep deprivation and recovery. J Neurosci 20:4669–4679

McCarley RW, Hobson JA (1971) Single neuron activity in cat giganto cellular tegmental field: selectivity of discharge in desynchronized sleep. Science 174:1250–1252

McDonald RL, Olsen RW (1994) $GABA_A$ receptor channels. Annu Rev Neurosci 17:569–602

Moruzzi G, Magoun HW (1949) Brain stem reticular formation and activation of the EEG. Electroencephalogr Clin Neurophysiol 1:455–473

Murata Y, Woodward RM, Miledi R, Overman LE (1996) The first selective antagonist for $GABA_C$ receptor. Bioorg Med Chem 6:2073–2076

Ogurusu T, Eguchi G, Schingai R (1997) Localization of γ-aminobutyric acid (GABA) receptor π3 subunit in rat retina. Neuroreport 8:925–927

Oswald I (1968) Drugs and sleep. Pharmacol Rev 20:273–303

Parades RG, Agmo A (1992) GABA and behavior: the role of receptor subtypes. Neurosci Biobehav Rev 16:145–170

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic Press, New York

Pazzaglia JC, Gauthier P, Gottesmann C (1994) A new automatic sleep-waking scoring system in the rat. J Sleep Res 3[Suppl 1]:195

Puigcerver A, Van Luijtelaar ELJM, Drinkenburg WHI, Coenen ALM (1996) Effects of the $GABA_B$ antagonist CGP 35348 on sleep-wake states, behavior and spike-wave discharges in old rats. Brain Res Bull 40:157–162

Quian H, Dowling JE (1994) Pharmacology of novel GABA receptors found on rod horizontal cells of the white perch retina. J Neurosci 14:4299–4307

Ragozzino D, Woodward RM, Murata Y, Eusebi F, Overman LE, Miledi R (1996) Design an in vitro pharmacology of a selective γ-aminobutyric $acid_c$ receptor antagonist. Mol Pharmacol 50:1024–1030

Steriade M (1996) Arousal: revisiting the reticular system. Science 272:225–226

Stutzmann JM, Delahaye C, Alain H (1993). Zopiclone. Données de pharmacologie expérimentale et de clinique. Thérapie 48: 33–42

Vertes RP (1977) Selective firing of rat pontine giganto cellular neurons during movement and REM sleep. Brain Res 128: 146–152

Xi MC, Morales FR, Chase MH (1999) A GABAergic pontine reticular system is involved in the control of wakefulness and sleep. Sleep Res Online 12:43–48