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Research report

Deficits in avoidance responding after paradoxical sleep deprivation are not associated with altered [^{3}H]pirenzepine binding to M1 muscarinic receptors in rat brain

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Abstract

Previous work had indicated that animals that were sleep-deprived and then trained on a passive avoidance task show poor retention of the task 24 h later after being allowed to sleep freely again. Cholinergic involvement is suggested by the fact that this effect is prevented by treatment with the muscarinic agonist pilocarpine during sleep deprivation. The observation that similar deficits are observed in non-deprived rats after treatment with M1-selective antagonist compounds such as dicyclomine or pirenzepine cause similar impairments, and gave rise to the hypothesis that sleep deprivation might induce significant reductions in M1 binding in brain areas involved in learning and memory processes. Rats were deprived of sleep for 96 h and then either immediately killed, or allowed to recover sleep for 24 h before being killed. [^{3}H]pirenzepine binding to M1 sites was examined by quantitative autoradiography in 39 different brain areas in cage controls, sleep-deprived and sleep-recovered animals ($N=8$ per group). No significant differences among groups were found in any brain region. A separate group of animals was subjected to the sleep deprivation procedure and then trained in a simple avoidance task. Animals were then allowed to sleep and retested 24 h later. This group showed a significant impairment in the avoidance task compared to cage controls, in agreement with previous observations. These data suggest that proactive learning/memory deficits induced by sleep deprivation cannot be attributed to altered M1 binding either immediately after deprivation (when avoidance training occurs) or after sleep has recovered (when acquisition/retention are tested). The possibility remains that alterations in M1 function occur at post-membrane second messenger systems.
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1. Introduction

Evidence from different sources has suggested a link between sleep and memory. Sleep, particularly paradoxical sleep (PS), seems to have facilitatory effects on the consolidation of recently acquired information [34], whereas PS deprivation, either before or after training, may produce deleterious effects on such processes [33], although the interpretation of such effects is often controversial (for reviews see [13,31,32,39]). Studies in our laboratory have demonstrated that 96 h of PS deprivation before training impair the acquisition/retention of aversive tasks [6,12], and that this effect can be blocked by treatment with the cholinergic agonist pilocarpine during the sleep deprivation period [7]. This suggests that cholinergic alterations may underlie the memory deficits induced by sleep deprivation. Several studies have demonstrated that cholinergic manipulations interfere with a wide variety of memory and learning tasks [1,3,14]. In par-

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ticular, cholinergic blockade by muscarinic antagonists impairs acquisition of such tasks [14,23,24].

A variety of data indicate that PS deprivation induces alterations in brain cholinergic systems, including localized decreases in acetylcholine levels [5,37], acetylcholinesterase activity [9], and cholinergic receptor sensitivity [30,28,38]. Four subtypes of muscarinic receptors are currently known, identified as M1, M2, M3 and M4, and five types of muscarinic receptors (m1–m5) have been cloned [18]. While little is known about specific roles of each of the muscarinic receptors, the M1 subtype has been the most consistently implicated in learning and memory processes. This receptor is coupled to a $G_{\alpha q}$ system and occurs predominantly as a postsynaptic receptor in the central nervous system [17]. Autoradiographic studies have shown high densities of M1 binding sites in hippocampus, amygdala and cortex, all of which are brain areas where cholinergic transmission is thought to have an essential role in memory processes [4,40]. Furthermore, treatment with M1-selective antagonist compounds such as dicyclomine [14] or pirenzepine [11,23,24] cause memory impairments, an effect which is similar to the impairment observed after PS deprivation [6].

Assessments of changes in muscarinic receptor binding using non-selective ligands revealed that relatively short periods of total sleep deprivation (24 h) did not alter muscarinic binding in rat brain [41], whereas longer period (10 days) led to decreased binding in hippocampus [36]. PS-selective deprivation for the same duration caused alterations in the septal area [36]. One study using the M2-selective ligand 3H-AFDX 384 revealed widespread decreases in M2 binding in rat brain [26]. Decreased m2 mRNA expression was found in the pontine nucleus [20]. In synaptosomal preparations M2 binding was found to be decreased after 96 h of PS deprivation [29].

While sleep deprivation appears to decreased M2 binding in brain, there have been no direct assessments of M1 receptor alterations after PS sleep deprivation. Kushida et al. [20] assessed expression of m1 mRNA after 72 h of PS deprivation and found no changes in various regions. It is worth noting, however, that 72-h PSD has no affect on several learning and memory tasks, while significant impairment is observed after 96 h PSD [6]. It is conceivable therefore that parallel alterations in M1 receptor may also only become apparent after periods of sleep deprivation longer than 72 h. Accordingly, the objective of this study was to assess M1 binding after 96 h of sleep deprivation. Our hypothesis is that M1 binding would be significantly decreased in brain regions involved in learning and memory processes.

2. Materials and methods

Three-month-old male Wistar rats from the Psychobiology Vivarium at the Federal University of São Paulo, were housed in groups of six, with free access to food and water at all times. Temperature was kept at 23 °C±2 and lights were on from 07:00 to 19:00 h.

2.1. Sleep deprivation procedure

Animals were randomly assigned to sleep deprivation, sleep rebound or cage control groups (N=8 per group). Sleep deprivation was induced by the modified multiple platform method [25], a procedure that in our hands completely abolishes PS, but also decreases slow-wave sleep by approximately 35% [22]. The use of multiple platforms avoids the lack of movement and isolation associated with earlier flower pot techniques of sleep deprivation. Care was also taken to test animals that were reared together, as it has been shown that the presence of unfamiliar animals in the deprivation tank contributes to the stress of the procedure [35]. Rats were killed immediately after 96 h of sleep deprivation were completed. Animals in the sleep rebound group were also subjected to sleep deprivation for 96 h (started 24 h before the previous group), but were returned to the home cage and allowed to sleep for 24 h before being killed. Animals in the cage control group remained in their home cages in the same room where deprivation procedures took place. All animals were killed on the same day between 10:00 and 11:00 h.

2.2. Autoradiographic analyses

Rats were killed by decapitation and brains were rapidly removed, frozen over dry ice and then stored at −80 °C. Twenty-micron coronal sections were cut in a Leica cryostat at −18 °C and thaw-mounted onto gelatinized slides. Slides were stored at −80 °C until processing. M1 binding assays followed the procedures of Yamamura et al. [42] with minor modifications. Slide-mounted sections were brought to room temperature and pre-incubated in Krebs buffer (pH 7.4, 120 mM NaCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 5.6 mM glucose, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and 4.7 mM KCl) for 30 min at 25 °C. Sections were then incubated in 20 nM [^{3}H]pirenzepine (75 Ci/mmol, PerkinElmer Inc., SP, Brazil) in Krebs buffer (pH 7.4), in the presence or absence of 1 μM atropine sulfate (Sigma Chemical Corp., St Louis, MO) at 25 °C for 60 min. Sections were then washed in cold (0–4 °C) buffer for 5 min, and then cold distilled water for 1 min. Sections were dried under a stream of cold air and exposed to [^{3}H] Hyperfilm (Amersham, SP, Brazil) for 4 weeks at 4 °C along with calibrated radioactive standards. Films were developed according to the manufacturer's instructions and analyzed with an MCID system (Imaging Research, St. Catharines, Ont, Canada). Anatomical regions were defined according to the atlas of Paxinos and Watson [27] and sampled without awareness of group membership.

2.3. Behavioral testing: one-trial passive avoidance task

In order to confirm the ability of the SD procedure to

induce learning deficits, a separate group of animals was subjected to the SD procedure for 96 h as described above while controls remained in their home cage. Immediately following the deprivation period, all rats were trained, starting at 10:00 h, and then placed back in their home-cages for 24 h, during which they were allowed to sleep freely. Twenty-four hours later, animals were tested for retention of the task.

A passive avoidance apparatus consisting of two acrylic boxes, each measuring 30×21×30 cm, and connected by a sliding door, was used. The clear box (white acrylic) was the safe compartment, whereas the box where the animals received shock (dark compartment) was made of black acrylic containing some white squares on its walls. The ceiling was made of transparent acrylic. The floor of the apparatus was made of parallel metallic rods, each 0.4 cm in diameter, 1.2 cm apart from each other, and connected to a Ugo Basile/Passive Avoidance Controller CAT 7551 Model electric shock generator. Testing was performed on 2 consecutive days (training and testing days). Immediately after the end of 96 h of PS deprivation each animal was placed in the clear compartment of the apparatus, with the sliding door closed. Ten seconds later, the door was opened. As soon as the animal crossed to the dark compartment the door was closed, latency to enter was recorded, and the animal received five footshocks of 1 mA/s, with a 30-s interval between shocks. The animal was then removed from the apparatus and placed in its original home-cage. Twenty-four hours later, each animal was placed again in the clear compartment of the apparatus. The sliding door was opened 10 s later and, again, latency to cross to the dark compartment was recorded. Each animal was allowed 540 s to cross to the dark compartment. If it did not do so, it was removed from the apparatus and a latency of 540 s was recorded.

3. Results

3.1. Effects of SD on avoidance responding

Results are shown in Fig. 1. A two-way repeated measures ANOVA indicated a significant main effect of PS deprivation ($F_{1,13}$=11. 63, $P<0.005$) and a significant interaction between PS deprivation and day ($F_{1,13}$=11.28, $P<0.006$). Post hoc analysis using Duncan Multiple Range Tests revealed that on day 1 (training) all animals behaved similarly ($P>0.75$); however, on day 2 (test), cage control animals displayed significantly longer latencies to enter the dark compartment compared to PS deprived animals ($P<0.005$).

3.2. [^{3}H]pirenzepine autoradiography

Binding to M1 receptors did not differ statistically among PS-deprived, rebound and controls in any of the 39 brain regions analyzed (Table 1 and Fig. 2). Nevertheless, a general trend was apparent towards decreased binding in the sleep deprived group (22 out of 39 regions) and towards increased binding in the rebound group (30 of the 39 regions) compared to the cage control group. The latter trend was significantly different from chance levels, using a sign test ($P<0.0004$).

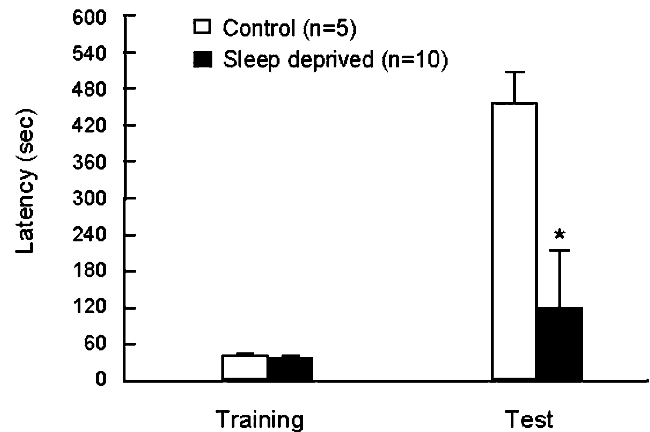

Fig. 1. Effects of pre-training PS deprivation on passive avoidance performance. Training was conducted immediately after 96 h of sleep deprivation and retention was conducted 24 h later, after PS-deprived rats had been allowed to sleep. Data are means±sem. Groups Ns are shown in parentheses; *$p<0.005$ compared to the Cage Control group.

4. Discussion

To our knowledge this is the first investigation of the effects of sleep deprivation on M1 binding in brain. No significant group differences were found in any of 39 brain regions analyzed, including regions involved in learning and memory processes, paradoxical sleep generation and sensory information processing. These results are in line with the observations of Kushida et al. [20] of no significant alterations in regional brain expression of m1 mRNA, assessed by in situ hybridization, after 72 h of PS deprivation.

While the present results and those of Kushida et al. [20] found no significant differences in M1 receptor expression or density after PS deprivation, evidence from pharmacological manipulations consistently point to altered sensitivity of M1 agents after sleep deprivation. For example, administration of the selective M1 antagonist biperidine both in humans and laboratory animals induced significant decreases in sleep rebound after sleep deprivation, in doses that do not induce significant alterations in basal levels of PS [30,28]. While this suggests that sleep deprivation induces changes in M1 receptor sensitivity, the present findings indicate that such changes, to the extent that they do occur, cannot be accounted for by binding changes, either immediately after sleep deprivation or after sleep recovery.

Other studies have suggested that sleep deprivation induces a decrease in muscarinic receptor sensitivity. Thus,

Table 1
[^{3}H]pirenzepine binding to M1 receptors after sleep deprivation[a]

	Cage controls (n=8)	Sleep deprived (n=8)	Rebound (n=8)
Accumbens n., core	192.2±5.1	195.5±5.5	204.9±4.7
Accumbens n., shell	159.2±6.0	157.3±6.6	169.5±3.0
Caudate-putamen	132.1±4.1	136.1±8.2	139.6±5.1
Amygdala			
Central n.	60.1±4.2	57.4±3.7	56.1±3.2
Basolateral n.	140.5±10.1	137.3±10.9	138.0±6.2
Lateral n.	136.5±7.4	147.6±6.4	142.3±10.8
Medial n.	41.4±1.6	43.9±3.3	47.8±3.4
Basolateral n.	66.7±17.8	48.1±9.1	66.3±14.5
Cerebral cortex			
Orbital	106.0±3.1	99.1±3.6	111.2±4.5
Frontal, associative	119.9±4.4	110.2±5.7	124.9±5.1
Cingulate, areas 1 and 2	117.3±6.1	116.0±6.1	114.9±7.1
Entorhinal, internal	113.0±7.8	107.6±18.4	115.2±4.5
Entorhinal, external	148.1±7.9	140.0±16.0	149.9±5.7
Hypothalamus			
Preoptic a., lateral	10.6±0.8	10.0±1.0	8.7±1.3
Preoptic a., medial	12.6±1.5	11.8±0.5	10.8±1.8
Anterior a.	12.7±1.8	14.4±1.1	16.8±1.4
Lateral a.	9.2±0.9	11.0±1.4	9.5±1.0
Mammilary body	18.6±0.8	22.8±1.3	20.6±1.3
Hippocampal formation			
CA1	213.8±9.4	202.9±5.8	215.7±7.9
CA3	112.1±7.7	105.1±5.3	119.8±4.7
Stratumoriens	111.5±7.0	109.8±16.0	122.7±4.9
Pyramid cell I.	96.4±5.3	90.7±11.4	103.4±4.6
Stratum lucidum	120.1±7.3	113.2±16.7	129.3±5.8
Stratum radiatum	105.3±7.3	94.2±11.8	102.4±6.0
CA4	133.3±7.7	127.5±6.3	150.6±13.4
Dentate gyrus	219.4±10.1	214.2±6.2	233.0±7.6
Thalamus			
Anterodorsal n.	23.4±1.4	26.4±1.8	28.1±1.7
Central medial n.	24.2±3.7	26.7±1.0	28.0±1.1
Reticular n.	12.8±0.8	15.1±0.9	14.9±1.6
Mediodorsal n., medial part	31.9±3.0	35.8±1.4	37.5±0.9
Mediodorsal n., lateral part	25.9±1.9	29.7±1.0	29.1±0.9
Posterolateral n.	17.0±1.8	19.2±1.1	18.7±1.2
Geniculate n., lateroventral	8.3±0.7	10.8±1.7	9.8±0.9
Geniculate n., laterodorsal	20.5±1.0	18.0±2.0	22.2±1.6
Periacqueductal gray, lateral	10.5±1.3	12.3±1.0	12.9±1.4
Periacqueductal gray, dorsomedial	9.2±1.4	10.2±1.1	9.8±1.3
Pedunculopontine n.	5.6±1.5	4.0±1.1	4.8±1.2
Laterodorsal tegmental n.	18.2±1.2	13.7±0.8	16.7±2.0
Locus coeruleus	8.8±2.7	9.5±2.4	11.5±1.9

[a] Values are means±sem in pmol/g tissue. No significant group differences were found for any of the regions.

pilocarpine-induced yawning is significantly reduced after PS deprivation, an effect which was hypothesized to be due to altered M2 receptor function [38]. A comprehensive autoradiographic study did subsequently reveal a widespread reduction in binding to M2 receptors labeled with [^{3}H]AFDX 384 after 96 h of sleep deprivation [26]. Similarly, using the non-selective muscarinic ligand [^{3}H]QNB, Tsai et al. [36] found a reduction in muscarinic binding in hippocampal homogenates after 10 days of total sleep deprivation. In contrast to the observed reductions in M2 receptors after PS deprivation, no changes were seen in M1 binding in the present study. It is therefore possible that altered sensitivity to muscarinic compounds after PS deprivation may be largely due to changes in M2, rather than M1 receptors. Although [^{3}H]pirenzepine appears at present to be the ligand of choice for the characterization of M1 receptor in rat brain, it should be noted that this ligand also shows activity at m4 cloned receptors. For this reason, M4 binding could mask M1 changes in areas that also express M4, but the proportion of M4 compared to

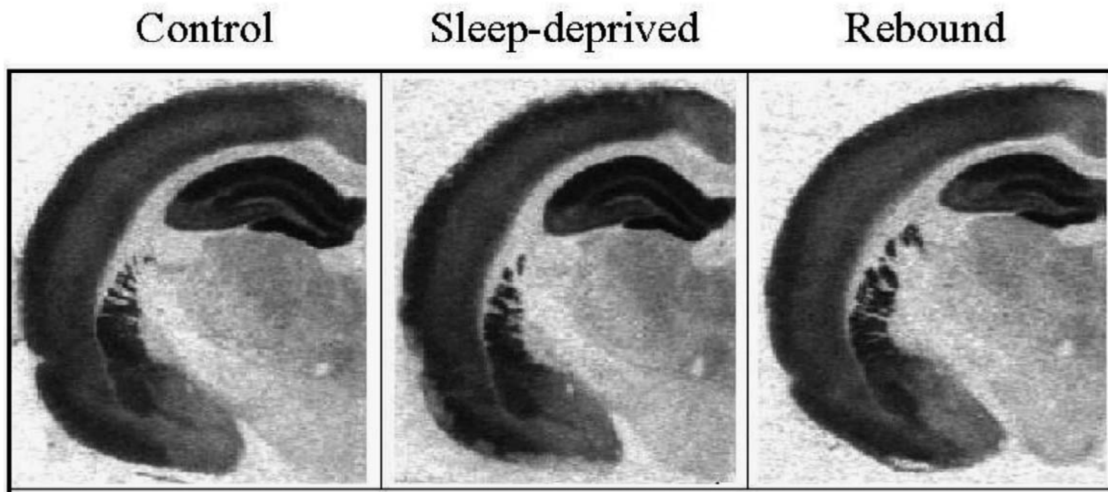

Fig. 2. Illustration of absence of changes in [^{3}H]pirenzepine binding in brains from control, sleep-deprived and sleep rebound animals.

M1 vary in different brain areas. In situ hybridization and immunoreactivity work suggests that most of the muscarinic receptors in areas such as caudate-putamen and olfactory tubercle belong mainly to the M4 class whereas M1 predominate in areas more directly involved in memory process such as hippocampus, dentate gyrus, amygdala, and cerebral cortex (see [10]). Nonetheless, the preceding considerations suggest that at least in some brain areas such as caudate-putamen and olfactory tubercle the binding signal analyzed here may not be exclusively attributable to M1 sites.

Several studies have suggested that sleep deprivation impairs memory processes, and this has been confirmed in the present study. The previous observation that this effect can be prevented by pilocarpine treatment during sleep deprivation period [7] suggests that cholinergic alterations that occur during sleep deprivation, including a decrease in acetylcholine released in the forebrain [5,37] and changes in acetylcholinesterase activity [9] may play a role in these deficits. We hypothesized that parallel alterations in M1 receptor might become apparent after periods of sleep deprivation that produce memory deficits [6,7,12]. Although a number of studies have demonstrated a close relationship between M1 receptor function and learning and memory [11,14,23,24], the absence of binding changes in this receptor suggests that other types of alterations in the cholinergic system may be responsible for the cognitive deficits. Pilocarpine is a non-selective agonist, and it is possible that its effects on memory relate primarily to effects on M2 and not M1. However, there is controversy about the involvement of M2 receptors in memory processes. There are indications that activation of this receptor may have negative effects on memory, since intraperitoneal administration of the M2 antagonist AF-DX 116 improves performance in passive avoidance tests [2]. However, intrahippocampal injections of this compound induce memory defects [23]. A possible involvement of other cholinergic systems might also be considered, since nicotinic systems have been implicated in memory [21]. However, Tsai et al. [36] found no changes in nicotinic binding after sleep deprivation. Non-cholinergic systems that may be involved in memory processes and are susceptible to sleep deprivation effects may include various neuropetides [15,19]. Nevertheless, the fact that memory impairment after sleep deprivation was prevented by pilocarpine does argue strongly for a role of muscarinic receptors in the process.

The possibility may also be considered that the avoidance deficits induced by sleep deprivation, as observed here and in previous studies, do not reflect learning and memory deficits but changes in sensory systems, possibly in brain areas that are not primarily involved in learning and memory. For example, sleep deprivation might affect sensory processes, involving brainstem, thalamic and cortical areas. Of note, cholinergic systems in the brainstem have been implicated in sensory processing [8]. Recent observations in this laboratory revealed that sleep deprivation interferes with reactivity to footshock; sleep-deprived rats do not have different thresholds for perceiving footshock, but do differ from non-deprived rats in threshold for vocalization in response to increased shock intensity [12]. This may suggest that sleep deprivation does indeed affect sensory processing. If this is the case, our results suggest that altered M1 binding in cortex, thalamus or brainstem is not an important part of this process.

Given the fact that avoidance deficits are seen after animals that were allowed to sleep for 24 h after the sleep deprivation period, it is significant that no alterations in M1 binding were found at that particular point in time. Tsai et al. [36] hypothesized that PS rebound might produce a decrease in muscarinic binding in response to increased ACh release that occurs during paradoxical sleep. In the specific case of M1 receptors, the present

results and the previous findings of Tsai et al. [36] do not support this hypothesis. It remains nevertheless possible that M1 receptor effects do not manifest themselves at the level of receptor expression or binding, but involve alterations in second messenger systems coupled to this receptor [16].

Although we did not observe significant group differences, it is interesting to note that most of the cortical and hippocampal structures analyzed showed a trend towards decreased binding immediately after sleep deprivation and a trend toward increased binding after 24 h of sleep rebound. A similar trend was observed in the pedunculopontine nucleus and laterodorsal tegmental nucleus. These trends were not observed in other brain regions. Their possible implications are not clear, but suggest the need to further explore the effects of PS deprivation on the cholinergic system. Microdialysis studies in cortical structures both during PS deprivation and after sleep rebound may be useful to elucidate the effects of sleep deprivation on cholinergic neurotransmission.

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References

[1] S.G. Anagnostaras, S. Maren, J.R. Sage, S. Goodrich, M.S. Fancelow, Scopolamine and pavlovian fear conditioning in rats: dose–effect analysis, Neuropsychopharmacology 21 (1999) 731–744.
[2] C.M. Baratti, J.W. Opezzo, D.S.R. Kopf, DSR, Facilitation of memory storage by the acetylcholine M2 muscarinic receptor antagonist AF-DX 116, Behav. Neural Biol. 60 (1993) 69–74.
[3] R.T. Bartus, R.L. Dean, B. Beer B, A.S. Lippa, The cholinergic hypothesis of geriatric memory dysfunction, Science 217 (1982) 408–417.
[4] T.I. Bonner, N.J. Buckley, A.C. Young, M.R. Brann, Identification of a family of muscarinic acetylcholine receptor genes, Science 237 (1987) 237.
[5] M.B. Bowers Jr., E.L. Hartmann, D.X. Freedman, Sleep deprivation and brain acetylcholine, Science 153 (1966) 1416–1417.
[6] O.F.A. Bueno, L.L. Lobo, M.G.M. Oliveira, E.B. Gugliano, A.C. Pomarico, S. Tufik, Dissociated paradoxical sleep deprivation effects on inhibitory avoidance and conditioned fear, Physiol. Behav. 56 (1994) 775–779.
[7] O.F.A. Bueno, M.G.M. Oliveira, L.L. Lobo, P.R. Morais, F.H.M. Melo, S. Tufik, Cholinergic modulation of inhibitory avoidance impairment induced by paradoxical sleep deprivation, Prog. Neuropsychopharmacol. Biol. Psychiatry 24 (2000) 595–606.
[8] L.L. Butcher, Cholinergic neurons and networks, in: G. Paxinos (Ed.), The Rat Nervous System, 2nd Edition, Academic Press, San Diego, 1995, pp. 1003–1015.
[9] R. Camarini, M.A. Benedito, Rapid eye movement (REM) sleep deprivation reduces rat frontal cortex acetylcholinesterase (EC 3.1.1.7) activity, Braz. J. Med. Biol. Res. 30 (1997) 641–647.
[10] M.P. Caulfield, Muscarinic receptors—characterization, coupling and function, Pharmacol. Ther. 58 (1993) 319–379.
[11] M.P. Caulfield, G.A. Higgins, D.W. Straughan, Central administration of the muscarinic receptor subtype—selective antagonist pirenzepine selectively impairs passive avoidance learning in the mouse, J. Pharm. Pharmacol. 35 (1983) 131–132.
[12] M. Dametto, D. Suchecki, O.F.A. Bueno, K.M. Moreira, S. Tufik, M.G.M. Oliveira, Social stress does not interact with paradoxical sleep deprivation-induced memory impairment, Behav. Brain Res. 129 (2002) 171–178.
[13] K. Dujardin, A. Guerrien, P. Leconte, Sleep, brain activation and cognition, Physiol. Behav. 47 (1990) 1271–1278.
[14] R.V. Fornari, K.M. Moreira, M.G.M. Oliveira, Effects of the selective M1 muscarinic receptor antagonist dicyclomine on emotional memory, Learn Mem. 7 (2000) 287–292.
[15] I. Gozes, A. Bardea, A. Reshef, R. Zamostiano, S. Zhukovsky, S. Rubinraut et al., Neuroprotective strategy for Alzheimer disease: Intranasal administration of a fatty neuropeptide, Proc. Natl. Acad. Sci. USA 93 (1996) 427–432.
[16] L. Graves, A. Pack, T. Abel, Sleep and memory: a molecular perspective, Trends Neurosci. 24 (2001) 237–243.
[17] E.C. Hulme, Muscarinic acetylcholine receptors: typical G-coupled receptors, Symp. Soc. Exp. Biol. 44 (1990) 39–54.
[18] E.C. Hulme, N.M.J. Birdsall, N.J. Buckley, Muscarinic receptor subtypes, Annu. Rev. Pharmacol. Toxicol. 30 (1990) 633–673.
[19] A. Jimenez-Anguiano, F. Garcia-Garcia, J.L. Mendoza-Ramirez, A. Duran-Vazquez, R. Drucker-Colin, Brain distribution of vasoactive intestinal peptide receptors following REM sleep deprivation, Brain Res. 22 (1996) 37–46.
[20] C.A. Kushida, R.K. Zoltoski, J.C. Gillin, The expression of m1–m3 muscarinic receptor mRNAs in rat brain following REM sleep deprivation, Neuroreport 21 (1995) 1705–1708.
[21] E.D. Levin, Nicotinic receptor subtypes and cognitive function, J. Neurobiol. 53 (2002) 633–640.
[22] R.B. Machado, D.C. Hipólide, S. Tufik, Continuous sleep monitoring (EEG and EMG) in rats using two different methods of paradoxical sleep deprivation, Sleep 24 (Suppl.) (2001) A237–A238.
[23] W.S. Messer Jr., G.J. Thomas, W. Hoss, Selectivity of pirenzepine in the central nervous system, Brain Res. 407 (1987) 37–45.
[24] W.S. Messer Jr., M. Bohnett M, J. Stibbe, Evidence for a preferential involvement of M1 muscarinic receptors in representational memory, Neurosci. Lett. 116 (1990) 184–189.
[25] G.P. Nunes Jr., S. Tufik, Validation of the modified multiple platform method of paradoxical sleep deprivation in rats, Sleep Res. 23 (1994) 419.
[26] G.P. Nunes Jr., S. Tufik, J.N. Nobrega, Decreased muscarinic receptor binding in rat brain after paradoxical sleep deprivation: an autoradiographic study, Brain Res. 645 (1994) 247–252.
[27] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, New York, 1986.
[28] R.J. Salin-Pascual, A. Jimenez-Anguiano, D. Granados-Fuentes, R. Drucker-Colin, Effects of biperiden on sleep at baseline and after 72 h of REM sleep deprivation in the cat, Psychopharmacology 106 (1992) 540–542.
[29] R.J. Salin-Pascual, M. Diaz-Munoz, L. Rivera-Valerdi, L. Ortiz-Lopez, C. Blanco-Centurion, Decrease in muscarinic M2 receptors from synaptosomes in the pons and hippocampus after REM sleep deprivation in rats, Sleep Res. Online 1 (1998) 19–23.
[30] R.J. Salin-Pascual, D. Granados-Fuentes, L. Galicia-Polo, E. Nieves, J. Echeverry, Biperiden administration in normal sleep and after rapid eye movement sleep deprivation in healthy volunteers, Neuropsychopharmacology 5 (1991) 97–102.
[31] J.M. Siegel, The REM sleep–memory consolidation hypothesis, Science 294 (2001) 1058–1063.
[32] C. Smith, Sleep states and learning: a review of the animal literature, Neurosci. Biobehav. Rev. 9 (1985) 157–168.
[33] C. Smith, P.T. Wong, Paradoxical sleep increases predict successful learning in a complex operant task, Behav. Neurosci. 105 (1991) 282–288.

[34] C. Smith, L. Lapp, Increases in number of REMS and REM density in humans following an intensive learning period, Sleep 14 (1991) 325–330.

[35] D. Suchecki, S. Tufik, Social stability attenuates the stress in the modified multiple platform method for paradoxical sleep deprivation in the rat, Physiol. Behav. 68 (2000) 309–316.

[36] L.L. Tsai, B.M. Bergmann, B.D. Perry, A. Rechtschaffen, Effects of chronic sleep deprivation on central cholinergic receptors in rat brain, Brain Res. 642 (1994) 95–103.

[37] K. Tsuchiya, M. Toru, T. Kobayashi, Sleep deprivation: changes of monoamines and acetylcholine in rat brain, Life Sci. 98 (1969) 867–873.

[38] S. Tufik, L.R. Troncone, S. Braz, A.R. Silva-Filho, B.G. Neumann, Does REM sleep deprivation induce subsensitivity of presynaptic dopamine or postsynaptic acetylcholine receptors in the rat brain?, Eur. J. Pharmacol. 140 (1987) 215–219.

[39] R.P. Vertes, K.E. Eastman, The case against memory consolidation in REM sleep, Behav. Brain Sci. 23 (2000) 793–1121.

[40] S.J. Wall, R.P. Yasuda, F. Hory, S. Flagg, B.M. Martin, E.I. Ginns et al., Production of antisera selective for m1 muscarinic receptors using fusion proteins: distribution of m1 receptors in rat brain, Mol. Pharmacol. 39 (1991) 643–649.

[41] A. Wirz-Justice, I. Tobler, M.S. Kafka, D. Naber, P.J. Marangos, A.A. Borbely et al., Sleep deprivation: effects on circadian rhythms of rat brain neurotransmitter receptors, Psychiatry Res. 5 (1981) 67–76.

[42] H.I. Yamamura, J.K. Wamsley, P. Deshmukh, W.R. Roeske, Differential light microscopic autoradiographic localization of muscarinic cholinergic receptors in the brainstem and spinal cord of the rat using [^{3}H]pirenzepine, Eur. J. Pharmacol. 91 (1983) 147–149.