



Inverse benzodiazepine agonist β -CCM does not reverse learning deficit induced by sleep deprivation

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ABSTRACT

Increasing evidence indicates that sleep deprivation (SD) alters responses to pharmacological agents by affecting specific transmitter systems. The present work addressed deficits in passive avoidance (PA) performance that are seen after SD, and investigated whether treatment with the inverse benzodiazepine agonist β -CCM could prevent such deficits. Male Wistar rats were deprived of sleep for 96 h using the platform method (SD group), or were sleep deprived and then allowed to recover sleep for 24 h (SR group). Animals were treated with saline or 0.5 mg/kg β -CCM before PA training, and were tested 30 min or 24 h later. A separate set of animals was sacrificed for [³H]Ro 15-4513 binding analysis. β -CCM increased PA performance in control animals in both short and long term retention tests, whereas SD and SR animals were unaffected by the drug treatment. Interestingly, [³H]Ro 15-4513 binding was reduced in the entorhinal cortex in both SD and SR groups. These findings suggest that the lack of promnesic effects of β -CCM after SD and SR may be associated with benzodiazepine receptor downregulation in specific brain regions related to memory formation.

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Lack of sleep due to sleep loss is a common problem of modern society [8], and several studies have shown that sleep deprivation (SD) leads to impairment in learning tasks in humans and animals. However, the mechanisms underlying these effects are not well understood [30,34]. Drugs acting on the cholinergic system [14,29] have been tested as potential agents to prevent deficits in passive avoidance (PA) learning in sleep-deprived rats. In this context, previous data from this laboratory have shown that treatment with the cholinergic agonist pilocarpine during SD prevents the impairment of PA retention [5].

The cholinergic and GABA systems are closely associated within brain structures involved in memory processes and sleep [26]. GABAergic manipulations with benzodiazepine (BDZ) ligands alter acetylcholine levels [25], and influence learning of emotional memory tasks [15]. Inverse agonists of the BDZ site, acting in a way to inhibit GABA binding to GABA_A receptors, enhance performance in learning tasks [1,15,32], augment acetylcholine efflux in cortex [25] and choline uptake in hippocampus [35], and block amnesic effects induced by pharmacological agents, such as the muscarinic antagonist scopolamine [1,11].

Previous SD studies have reported alterations in the GABAergic system, including increases in GABA synthesis in localized brain regions [19,21] and augmented GABA levels after sleep deprivation [36] and recovery [3]. Nevertheless, assessments of BDZ receptor binding after 4 days of sleep deprivation have produced negative results. Mueser et al. [23] reported no changes in BDZ in cortex and brainstem homogenates, and Dubiela et al. [13] did not find any alterations in brain autoradiograms. There are however several different types of benzodiazepine receptors [2], and it is conceivable that some types might be altered after SD, such as the ones that bind to inverse BDZ agonists but not classical benzodiazepines [38].

The present work attempted to evaluate if the PA learning deficits induced by SD could be prevented by β -CCM, an inverse BDZ agonist. In addition, a [³H]Ro 15-4513 autoradiographic analysis was performed to verify potential changes in binding to this site after sleep deprivation and recovery.

One hundred and forty eight male Wistar rats, aged 3 months, were obtained from the Department of Psychobiology breeding colony. The animals were kept in groups of 5–6 in wire-mesh cages in a room under controlled temperature (23 ± 2 °C) and 12 light/dark cycle (lights on at 07:00 h), with food and water *ad libitum*. All procedures were carried out in accordance with the National Institute of Health (NIH) guidelines on animal care and were approved by the Ethical Committee of UNIFESP (CEP # 0205/03).

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Rats were sleep deprived by the modified multiple platform method [31]. Sleep deprivation was conducted by placing 10–12 rats in a large water tank (145 cm × 44 cm × 45 cm) that contained narrow platforms (6 cm in diameter). This procedure completely abolishes paradoxical sleep of the animals, based on the muscle atonia that accomplishes this sleep stage, and also decreases some fraction of slow-wave sleep [17]. The presence of multiple platforms avoids the movement restriction and isolation associated with earlier techniques of sleep deprivation. Animals in the sleep deprivation (SD) and sleep recovery (SR) group were subjected to sleep deprivation for 96 h. SR animals were returned to their home cages and allowed to sleep for 24 h after the deprivation period (started 24 h earlier than the SD group). Animals in the control (C) group remained in their home cages in the same room where sleep deprivation procedure took place.

Immediately following the deprivation period, animals received one i.p. injection of either methyl- β -carboline-3-carboxylate (β -CCM, Sigma, 0.5 mg/kg/ml) or vehicle solution. Thus, rats were allocated to 6 groups, according to the sleep intervention (C, SD, SR) and treatment (vehicle, β -CCM). β -CCM was dissolved in 0.1N HCl and diluted in distilled water. Fifteen minutes later, the animals were submitted to a step-through passive avoidance (PA) task. The PA apparatus consisted of two acrylic boxes, each measuring 21 cm × 26 cm × 27.5 cm, connected by a sliding door. One of the boxes was the safe compartment, with white acrylic walls, whereas the other box was the aversive compartment, made of black acrylic with some white squares on the walls. 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 an electric shock generator (AVS Projetos Especiais, São Paulo, Brazil). The PA performance was evaluated using two interval sets between training and test sessions: in the short term PA retention, as described elsewhere [13], each animal was placed in the safe compartment of the apparatus, with the sliding door closed. Ten seconds later, the door was opened. As soon as the animal crossed to the aversive compartment with its four paws, the door was closed, latency to enter was recorded, and the animal received one footshock (0.8 mA/1 s). Fifteen seconds later, the animal was removed from the apparatus and returned to its original place, i.e. control and sleep recovered animals were returned to their home cages, while sleep-deprived animals were returned to the water tank. Thirty minutes later, the retention session took place, where each animal was placed again in the safe compartment of the apparatus, and the latency to cross to the aversive compartment was recorded without any shock presentation. Each animal was allowed 400 s to cross to the aversive compartment. If it did not do so, the animal was removed from the apparatus and a latency of 400 s was recorded. In the long term PA retention, the procedure was similar to the one just described, except that the retention session was performed 24 h later after training, and all groups, including sleep-deprived rats, were allowed to sleep in their home cages between sessions. The maximal latency to cross to the aversive compartment was set at 300 s.

A separate set of animals was subjected to the sleep deprivation procedure as described above (each group $n=8-9$), and sacrificed by decapitation immediately after 96 h of SD or after 96 h plus 24 h of sleep recovery. Brains were rapidly removed, frozen over dry ice, and stored at -80°C . Later, 20 μm coronal sections were cut on a Leica cryostat at -20°C , thaw-mounted onto gelatinized microscope slides and stored at -80°C before the binding assays. Slide-mounted sections were allowed to equilibrate to room temperature and then preincubated in 50 mM Tris–NaCl buffer (120 mM NaCl, 5 mM KCl, 2 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2 mM MgCl_2 , pH 7.5) for 30 min at 23°C to remove endogenous ligands. Sections were incubated with 5 nM [^3H]Ro 15-4513 (Perkin–Elmer, 40.0 Ci/mmol) in buffer solution pH 7.0 for 60 min at 37°C . Sections

were then rinsed in cold buffer, and then cold distilled water, air-dried and exposed to Kodak Biomax X-rays films (Kodak, SP, Brazil) for four weeks along with calibrated radioactive standards. Films were developed on Kodak D-19 according to the manufacturer's instructions. Computer-assisted densitometry was performed with the MCID system (InterFocus Imaging, Linton, UK). Brain regions were defined according to the atlas of Paxinos and Watson [24] and sampled without knowledge of group membership of the animals.

Latency data in passive avoidance were analyzed by two-way repeated measure ANOVAs, with group as the between subject factor and session as the within subject factor. Binding data were analyzed by one-way ANOVA for each brain region, and post hoc analyses were carried out using Fisher's LSD test, with the level of significance set at $p < 0.05$.

Results of the short term PA retention are shown in Fig. 1A. A two-way repeated measures ANOVA indicated significant main effects of group ($F_{5,47} = 2.614$, $p < 0.05$) and session ($F_{1,47} = 25.7826$, $p < 0.0001$), and a significant interaction between them ($F_{5,47} = 2.499$, $p < 0.05$). Fisher's LSD post hoc analyses revealed that all animals behaved similarly in the training session ($p > 0.05$); however, on the test session, control animals treated with β -CCM displayed significant higher latencies than control animals treated with vehicle ($p < 0.05$) and SD animals treated with β -CCM ($p < 0.001$). The SD group treated with vehicle displayed lower latencies compared to C animals treated with vehicle ($p < 0.05$).

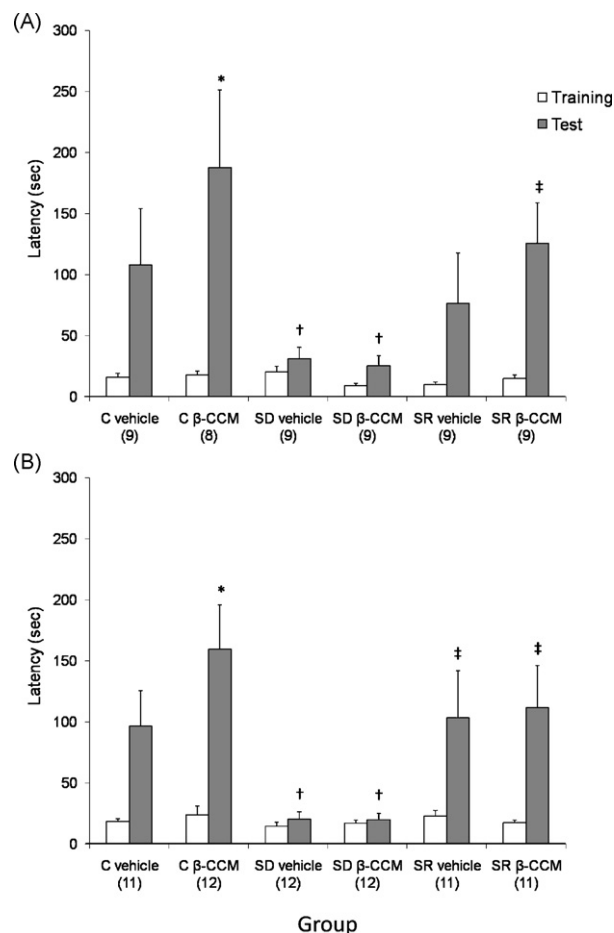


Fig. 1. Effects of β -CCM (0.5 mg/kg) treatment on short (A) and long term (B) retention of passive avoidance of sleep deprived and recovered animals. C: control group, SD: sleep deprivation group, SR: sleep recovery group. Groups Ns are shown in parentheses. Data are means \pm SEM in seconds; * $p < 0.05$ vs. respective vehicle-treated group; † $p < 0.05$ vs. respective control group; ‡ $p < 0.05$ vs. respective sleep deprivation group (Fisher's LSD test).

Table 1
[³H] Ro 15-4513 binding after sleep deprivation and recovery.

Region	Group		
	C	SD	SR
	(8)	(9)	(9)
Cortex			
Frontal association	0.346 ± 0.025	0.323 ± 0.011	0.359 ± 0.015
Cingulate 1	0.336 ± 0.013	0.310 ± 0.008	0.333 ± 0.014
Cingulate 2	0.359 ± 0.011	0.338 ± 0.012	0.348 ± 0.011
Entorhinal	0.297 ± 0.012	0.255 ± 0.011**	0.262 ± 0.009*
Hippocampal formation			
CA1	0.297 ± 0.020	0.261 ± 0.018	0.245 ± 0.013
CA3	0.267 ± 0.016	0.245 ± 0.012	0.247 ± 0.011
Dentate gyrus dorsal limb	0.302 ± 0.016	0.274 ± 0.015	0.278 ± 0.013
Dentate gyrus ventral limb	0.200 ± 0.008	0.185 ± 0.015	0.165 ± 0.010
Medial septal nucleus	0.208 ± 0.014	0.193 ± 0.019	0.186 ± 0.010
Basal nucleus	0.273 ± 0.009	0.253 ± 0.010	0.247 ± 0.014

Values are means ± SEM in uCi/g tissue; Groups Ns are shown in parentheses.

* $p < 0.05$ vs. control group.

** $p < 0.01$ vs. control group.

The SD group treated with β -CCM also displayed lower latencies compared to SR animals treated with β -CCM ($p < 0.05$).

Results of the long term PA retention are shown in Fig. 1B. A two-way repeated measures ANOVA indicated significant main effects of group ($F_{5,63} = 4.092$, $p < 0.005$) and session ($F_{1,63} = 34.703$, $p < 0.0001$), and a significant interaction ($F_{5,63} = 3.717$, $p < 0.01$). Fisher's LSD post hoc analyses revealed that all animals behaved similarly in the training session ($p > 0.05$); on the test session, control animals treated with β -CCM displayed significantly higher latencies than control animals treated with vehicle ($p < 0.05$) and SD animals treated with β -CCM ($p < 0.0001$). The SD group treated with vehicle displayed lower latencies compared to control and SR animals treated with vehicle ($p < 0.05$ and $p < 0.01$, respectively). The SD group treated with β -CCM also displayed lower latencies compared to SR animals treated with β -CCM ($p < 0.005$).

Results of [³H]Ro 15-4513 binding are shown in Table 1. One-way ANOVA indicated differences in the entorhinal cortex ($F_{2,23} = 4.5154$, $p < 0.05$). Post hoc analyses revealed significant binding reductions in SD and SR groups compared to control group in the entorhinal cortex ($p < 0.01$ and $p < 0.05$, respectively).

Previous work from this laboratory had found passive avoidance deficits when sleep-deprived rats were allowed short [13] or long term [4,5,10,22] retention intervals between training and test sessions. In the present experiment, the performance of sleep recovered animals in the short term retention test was not different from that of controls or SD animals (Fig. 1A), showing an intermediate performance, as shown previously [13]. Interestingly, after a 24 h interval between training and test sessions, sleep recovered animals performed as good as controls and better than the SD group (Fig. 1B). A similar result was obtained by McDermott et al. [20] after sleep depriving rats for 72 h and allowing 24 h of recovery; these authors evaluated rats in another emotional memory task – contextual fear conditioning – with a 24 h interval between training and test sessions. It seems plausible that short and long term memory processes are differentially modulated after sleep recovery, which could be due to a prolonged sleep recovery within long periods between acquisition and retrieval sessions in comparison to short periods.

Based on previous studies showing a promnesic effect of β -CCM on the passive avoidance task [1,32], we sought to verify if treatment with this drug could prevent learning deficits induced by sleep deprivation. Contrary to our hypothesis, β -CCM treatment had no effect on sleep deprived and sleep recovered animals, while augmenting the passive avoidance response of control animals in both short and long term retention paradigms (Fig. 1). While there

is evidence that cholinergic transmission participates in the prevention of memory deficits after SD [5,14,29], it is surprising that a BDZ inverse agonist had no effect, as several reports show that BDZ receptor ligands influence cholinergic activity in brain areas associated with memory processes [25,35]. A tentative explanation arising from our binding results is suggested below.

Our findings with [³H]Ro 15-4513 binding revealed decreases in the entorhinal cortex of sleep deprived and sleep recovered animals in comparison to controls (Fig. 2). A previous study from our group has shown [³H]flunitrazepam binding reduction only in the frontal cortex of sleep recovered rats [13]. This could be due to different affinities of the two ligands for specific sites of the receptor complex, as well as differences in their respective distributions [2]. For instance, BDZ receptors containing α_4 subunits, which are found mainly in the hippocampus and cortex [38], do not bind classical benzodiazepines but bind Ro 15-4513 and beta carbolines with high affinity [39]. In addition, there are reports of changes in [³H]Ro 15-4513 binding in other behavioral paradigms besides sleep deprivation (alcohol consumption, electroconvulsive shock) which were not followed by [³H]flunitrazepam binding changes [7,18].

The inverse agonists β -CCM and Ro 15-4513 share a similar BDZ binding profile [2], and the present evidence of reduced [³H]Ro 15-4513 binding after sleep deprivation and recovery in areas associated to emotional memory formation – specially in the entorhinal cortex [27] – raises interesting possibilities to explain the absence of β -CCM effects in these conditions. Bicuculline and flumazenil, which are respectively antagonists of GABA_A and BDZ receptors, increase acetylcholine efflux in entorhinal slices [12], while local administration of bicuculline in this region immediately after training improves PA performance [16]. Moreover, intra-entorhinal infusion of muscimol prior to PA training blocks amnesic effects induced by the same drug applied after training [37]. As these studies provide evidence for a participation of entorhinal GABA_A receptors in memory formation, it seems plausible that endogenous modifications in the BDZ site might interfere with pharmacological responses of BDZ agents. Studying two mouse strains selected for their resistance and sensitivity to β -CCM induced seizures, Chapouthier et al. [6] found strong decreases in [³H]flumazenil B_{max} after β -CCM administration in resistant mice. Interestingly, mice from the resistant strain performed worse in memory tasks than mice from the sensitive strain [33]. Therefore, it is conceivable that the observed [³H]Ro 15-4513 binding reduction in the entorhinal cortex is associated with the lack of β -CCM promnesic action in sleep deprived and recovered animals.

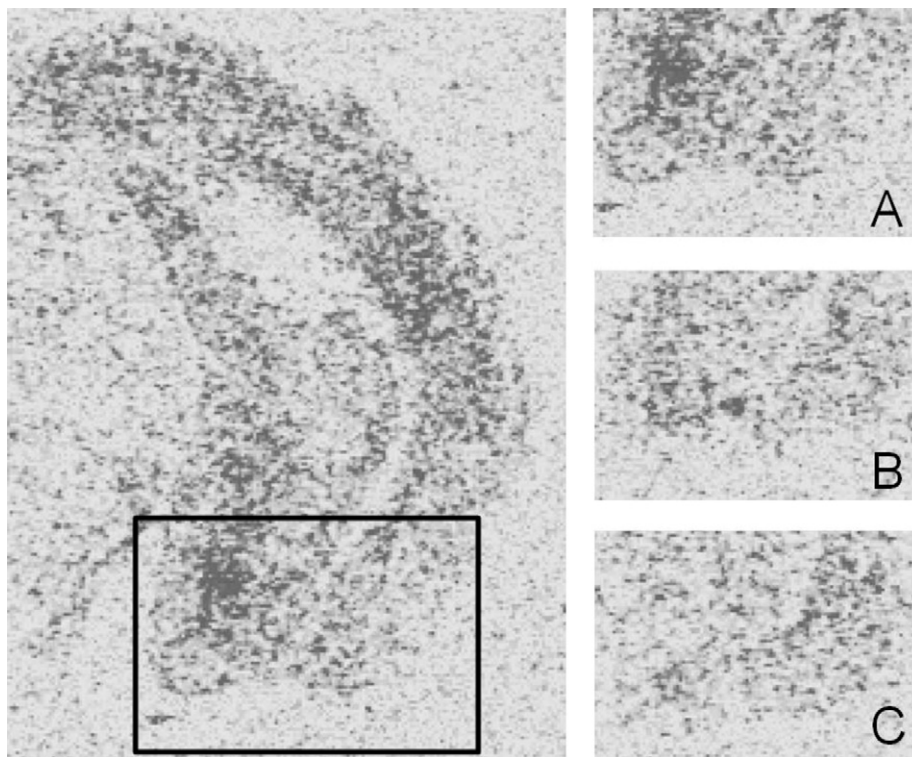


Fig. 2. Illustration of decreased [^3H]Ro 15-4513 binding in the entorhinal cortex after sleep deprivation and recovery. (A) Control; (B) sleep deprived; (C) sleep recovered.

The inefficacy of β -CCM to block the deleterious effects of SD on the PA task could be related to other modifications of the GABAergic system besides its receptors, as there is evidence of augmented GABA levels in several brain regions after sleep deprivation [19,36] and recovery [3]. Increased GABAergic transmission through pharmacological inhibition of either GABA degradation or uptake reduces proconvulsive effects of BDZ inverse agonists [9,28]. Hence, it could be argued that promnesic effects of β -CCM after SD can only be obtained if both receptor BDZ inverse agonist binding and GABA levels are normalized. This hypothesis should be subjected to further experiments.

In conclusion, the present findings show that sleep deprivation and recovery prevent the promnesic action of β -CCM in both short and long term retention of passive avoidance memory, which may be due to downregulation of BDZ inverse agonist binding sites in the entorhinal cortex after sleep deprivation.

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References

- [1] F. Anglade, J.C. Bizot, R.H. Dodd, C. Baudoin, G. Chapouthier, Opposite effects of cholinergic agents and benzodiazepine receptor ligands in a passive avoidance task in rats, *Neuroscience Letters* 182 (1994) 247–250.
- [2] E.A. Barnard, P. Skolnick, R.W. Olsen, H. Mohler, W. Sieghart, G. Biggio, C. Braestrup, A.N. Bateson, S.Z. Langer, International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acid A receptors: classification on the basis of subunit structure and receptor function, *Pharmacological Reviews* 50 (1998) 291–313.
- [3] L. Bettendorff, M. Sallanon-Moulin, M. Touret, P. Wins, I. Margin-Eanu, E. Schofeniels, Paradoxical sleep deprivation increases the content of glutamate and glutamine in rat cerebral cortex, *Sleep* 19 (1996) 65–71.
- [4] O.F.A. Bueno, L.L. Lobo, M.G. Oliveira, E.B. Gugliano, A.C. Pomarico, S. Tufik, Dissociated paradoxical sleep deprivation effects on inhibitory avoidance and conditioned fear, *Physiology and Behavior* 56 (1994) 775–779.
- [5] O.F.A. Bueno, M.G. Oliveira, L.L. Lobo, P.R. Morais, F.H. Melo, S. Tufik, Cholinergic modulation of inhibitory avoidance impairment induced by paradoxical sleep deprivation, *Progress in Neuro-psychopharmacology & Biological Psychiatry* 24 (2000) 595–606.
- [6] G. Chapouthier, J.M. Launay, P. Venault, C. Breton, P.L. Roubertoux, W.E. Crusio, Genetic selection of mouse lines differing in sensitivity to a benzodiazepine receptor inverse agonist, *Brain Research* 787 (1998) 85–90.
- [7] M. Clark, Sensitivity of the rat hippocampal GABA(A) receptor alpha 4 subunit to electroshock seizures, *Neuroscience Letters* 250 (1998) 17–20.
- [8] H.R. Colten, B.M. Altevogt (Eds.), *Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem*, National Academies Press, Washington, DC, 2006, p. 424.
- [9] N.O. Dalby, E.B. Nielsen, Comparison of the preclinical anticonvulsant profiles of tiagabine, lamotrigine, gabapentin and vigabatrin, *Epilepsy Research* 28 (1997) 63–72.
- [10] M. Dametto, D. Suchecki, O.F.A. Bueno, K.M. Moreira, S. Tufik, M.G. Oliveira, Social stress does not interact with paradoxical sleep deprivation-induced memory impairment, *Behavioural Brain Research* 129 (2002) 171–178.
- [11] T.M. DeLorey, R.C. Lin, B. McBrady, X. He, J.M. Cook, J. Lamah, G.H. Loew, Influence of benzodiazepine binding site ligands on fear-conditioned contextual memory, *European Journal of Pharmacology* 426 (2001) 45–54.
- [12] M. Díez-Ariza, M.J. Ramírez, B. Lasheras, J. Del Río, Differential interaction between 5-HT₃ receptors and GABAergic neurons inhibiting acetylcholine release in rat entorhinal cortex slices, *Brain Research* 801 (1998) 228–232.
- [13] F.P. Dubiela, M.G. Oliveira, K.M. Moreira, J.N. Nobrega, S. Tufik, D.C. Hipolide, Learning deficits induced by sleep deprivation and recovery are not associated with altered [(3)H]muscimol and [(3)H]flunitrazepam binding, *Brain Research* 1037 (2005) 157–163.
- [14] P.F. Harris, D.H. Overstreet, J. Orbach, Disruption of passive avoidance memory by REM sleep deprivation: methodological and pharmacological considerations, *Pharmacology Biochemistry & Behavior* 17 (1982) 1119–1122.
- [15] I. Izquierdo, J.H. Medina, GABA receptor modulation of memory: the role of endogenous benzodiazepines, *Trends in Pharmacological Sciences* 12 (1991) 260–265.
- [16] T. Luft, G.S. Pereira, M. Cammarota, I. Izquierdo, Different time course for the memory facilitating effect of bicuculline in hippocampus, entorhinal cortex, and posterior parietal cortex of rats, *Neurobiology of Learning & Memory* 82 (2004) 52–56.
- [17] R.B. Machado, D.C. Hipolide, A.A. Benedito-Silva, S. Tufik, Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery, *Brain Research* 1004 (2004) 45–51.
- [18] M. Mhatre, A.K. Mehta, M.K. Ticku, Chronic ethanol administration increases the binding of the benzodiazepine inverse agonist and alcohol antagonist [3H]RO15-4513 in rat brain, *European Journal of Pharmacology* 153 (1988) 141–145.

- [19] S. Majumdar, B.N. Mallick, Increased levels of tyrosine hydroxylase and glutamic acid decarboxylase in locus coeruleus neurons after rapid eye movement sleep deprivation in rats, *Neuroscience Letters* 338 (2003) 193–196.
- [20] C.M. McDermott, G.J. LaHoste, C. Chen, A. Musto, N.G. Bazan, J.C. Magee, Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons, *Journal of Neuroscience* 23 (2003) 9687–9695.
- [21] D. Micic, V. Karadzic, L.M. Rakic, Changes of gamma-aminobutyric acid, glutamic acid and aspartic acid in various brain structures of cats deprived of paradoxical sleep, *Nature* 215 (1967) 169–170.
- [22] K.M. Moreira, D.C. Hipolide, J.N. Nobrega, O.F.A. Bueno, S. Tufik, M.G. Oliveira, Deficits in avoidance responding after paradoxical sleep deprivation are not associated with altered [³H]pirenzepine binding to M1 muscarinic receptors in rat brain, *Brain Research* 977 (2003) 31–37.
- [23] T. Mueser, L. Isaac, M. Radulovacki, Neither REM sleep deprivation nor rebound influences 3H-diazepam binding in the rat brain, *Physiology & Behavior* 31 (1983) 237–239.
- [24] G. Paxinos, C. Watson, *The Rat Brain in Stereotaxic Coordinates*, fourth ed., Academic Press, New York, 1998, p. 256.
- [25] M. Sarter, J.P. Bruno, Trans-synaptic stimulation of cortical acetylcholine and enhancement of attentional functions: a rational approach for the development of cognition enhancers, *Behavioural Brain Research* 83 (1997) 7–14.
- [26] M. Sarter, J.P. Bruno, Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal fore-brain by telencephalic and brainstem afferents, *Neuroscience* 95 (2000) 933–952.
- [27] E.E. Schenberg, J.C. Soares, M.G. Oliveira, Effects of pre- or post-training entorhinal cortex AP5 injection on fear conditioning, *Physiology and Behavior* 86 (2005) 508–515.
- [28] L. Schmid, M. Bottlaender, E. Brouillet, C. Fuseau, M. Maziere, Vigabatrin modulates benzodiazepine receptor activity in vivo: a positron emission tomography study in baboon, *Journal of Pharmacology and Experimental Therapeutics* 276 (1996) 977–983.
- [29] D.M. Skinner, D.H. Overstreet, J. Orbach, Reversal of memory-disruptive effects of rem-sleep deprivation by physostigmine, *Behavioral Biology* 18 (1976) 189–198.
- [30] C. Smith, Sleep states and memory processes, *Behavioural Brain Research* 69 (1995) 137–145.
- [31] D. Suchecki, S. Tufik, Social stability attenuates the stress in the modified multiple platform method for paradoxical sleep deprivation in the rat, *Physiology & Behavior* 68 (2000) 309–316.
- [32] P. Venault, G. Chapouthier, L.P. de Carvalho, J. Simiand, M. Morre, R.H. Dodd, J. Rossler, Benzodiazepine impairs and beta-carboline enhances performance in learning and memory tasks, *Nature* 321 (1986) 864–866.
- [33] P. Venault, D. Beracochea, M. Valteau, C. Joubert, G. Chapouthier, Mouse lines selected for difference in sensitivity to beta-CCM also differ in memory processes, *Behavioural Brain Research* 173 (2006) 282–287.
- [34] M.P. Walker, R. Stickgold, Sleep, memory, and plasticity, *Annual Review of Psychology* 57 (2006) 139–166.
- [35] T.J. Walsh, R.W. Stackman, D.F. Emerich, L.A. Taylor, Intraseptal injection of GABA and benzodiazepine receptor ligands alters high-affinity choline transport in the hippocampus, *Brain Research Bulletin* 31 (1993) 267–271.
- [36] S.X. Wang, Q.S. Li, Effects of sleep deprivation on gamma-amino-butyric acid and glutamate contents in rat brain, *Di Yi Jun Yi Da Xue Xue Bao* 22 (2002) 888–890.
- [37] P. Williner, M. Bianchin, R. Walz, M. Bueno e Silva, M.S. Zanatta, I. Izquierdo, Muscimol infused into the entorhinal cortex prior to training blocks the involvement of this area in post-training memory processing, *Behavioural Pharmacology* 4 (1993) 95–99.
- [38] W. Wisden, D.J. Laurie, H. Monyer, P.H. Seeburg, The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon, *Journal of Neuroscience* 12 (1992) 1040–1062.
- [39] W. Yang, J.A. Drewe, N.C. Lan, Cloning and characterization of the human GABAA receptor alpha 4 subunit: identification of a unique diazepam-insensitive binding site, *European Journal of Pharmacology* 291 (1995) 319–325.