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Rapid eye movement sleep deprivation-induced down-regulation of beta-adrenergic receptors in the rat brainstem and hippocampus

Mario Pedrazzoli*, Marco Antonio Campana Benedito

Departamento de Psicobiologia, Faculdade de Medicina da Universidade Federal de São Paulo, Rua Botucatu, 862, CEP—04023-062, Brazil

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Abstract

Rapid eye movement (REM) sleep deprivation induces a cortical down-regulation of beta-adrenergic receptors. Down-regulation of cortical beta-adrenergic receptors is consistently observed after a number of different chronic antidepressant treatments (drugs and electroconvulsive shock). REM sleep deprivation has an antidepressant effect in humans, and in rats, it decreases immobility in the behavioral despair test, an effect also produced by antidepressant treatments. To verify whether REM sleep deprivation also affects hippocampal beta-adrenergic receptors, we carried out the binding of [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) to hippocampal membranes from rats deprived of REM sleep for 96 h. We also determined the binding of [^{3}H]-DHA to brainstem membranes, a brain region where noradrenergic nuclei are located. Rats were deprived of REM sleep using a water tank with multiple small platforms. [^{3}H-DHA] saturation conditions (concentrations ranging from 0.15 to 6 nM) were obtained in a crude hippocampus and brainstem membrane preparation. Nonspecific binding was determined using DL-propranolol in hippocampus homogenates. In the brainstem homogenates, nonspecific binding was determined in the presence of DL-propranolol or L-isoproterenol. The results obtained showed statistically significant down-regulation of beta-adrenergic receptors in both the hippocampus and the brainstem after REM sleep deprivation. In the hippocampus, there was also a significant decrease in the dissociation constant (K_D). In the brainstem, a significant decrease in K_D was observed when DL-propranolol was used to determine nonspecific binding. The down-regulation of beta-adrenergic receptors in the hippocampus and brainstem suggests the involvement of these brain areas in the antidepressant effect of REM sleep deprivation.
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Keywords: REM sleep deprivation; Hippocampus; Brainstem; Beta-adrenoceptors; [^{3}H]-Dihydroalprenolol; Binding

1. Introduction

Noradrenergic nuclei are located in the brainstem (Moore, 1982, Stanford, 1995). Locus coeruleus (LC), the main noradrenergic nucleus located in the pons, projects to several brain areas (Aston-Jones et al., 1996; Loughlin et al., 1986a,b; Swanson, 1976). The LC nucleus participates in emotional activation (Aston-Jones et al. (1996) and noradrenaline (NA), its neurotransmitter, is involved in emotional disturbances, such as depression (Aston-Jones et al., 1996, Mongeau et al., 1997, Sugrue, 1983; Vetulani, 1991).

In depressed patients, rapid eye movement (REM) sleep is altered; REM sleep latency is diminished; there are more REM sleep episodes in the first part of the night, and there are more eye movements during REM sleep (Benca et al., 1997). REM sleep deprivation has antidepressant effects in depressed patients (Vogel et al., 1980); in rats, it decreases immobility in the behavioral despair test, as do antidepressant treatments (Porsolt et al., 1978), and most forms of antidepressant drug therapy inhibit REM sleep (Benca et al., 1997; Vogel et al., 1990), although some newer wide-spectrum selective serotonin reuptake inhibitors (SSRIs) do not have this effect (Rush et al., 1998). Chronic antidepressant treatments ameliorate the symptoms of depression, and in animals, these treatments down-regulate cortical beta-adrenergic receptors (Sugrue, 1983; Vetulani, 1991), an effect also observed after REM sleep deprivation (Mogilnicka et al., 1980). This similarity between the effects of chronic antidepressant treatments and REM sleep deprivation in relation to cortical beta-adrenergic receptors suggests an involvement of brain noradrenergic neurotransmission in the antidepressant effect of REM sleep deprivation.

* Departemento de Psicobiologia, Universidade Federal de São Paulo, Rua Napoleao de Barros 925, 04024002, São Paulo, Brazil. Tel.: +55-11-539-0155; fax: +55-11-557-25092.

E-mail address: pedrazzo@psicobio.epm.br (M. Pedrazzoli).

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In rats, REM sleep deprivation increases brainstem tyrosine hydroxylase activity (Sinha et al., 1973), the rate-limiting step in NA synthesis (Kumer and Vrana, 1996), and increase levels of tyrosine hydroxylase mRNA expression in the LC (Basheer et al., 1998; Porka-heiskanen et al., 1995). REM sleep deprivation also decreases brainstem monoamineoxidase (MAO)-A activity (Perez and Benedito, 1997; Thakkar and Mallick, 1993), an enzyme involved in the intraneuronal NA inactivation (Finberg, 1995). MAO-A inhibitors are antidepressant drugs that induce cortical (Sugrue, 1983; Vetulani, 1991) and brainstem (Cohen et al., 1982) down-regulation of beta-adrenergic receptors.

Higher brainstem tyrosine hydroxylase activity, LC tyrosine hydroxylase mRNA and lower brainstem MAO-A activity suggest that REM sleep deprivation boosts noradrenergic activity, thus providing evidence for a noradrenergic mechanism underlying its antidepressant effect.

The LC is the sole source of noradrenergic input to the hippocampus (Foote et al., 1983), a brain region belonging to the limbic system and probably involved in the effect of antidepressant treatments (Mongeau et al., 1997). Antidepressant treatments were shown to raise extracellular NA levels in the hippocampus (L'Hereux et al., 1986; Seo et al., 1999a,b) and homogenate saturation binding studies have found down-regulation of beta-adrenergic receptor in this brain region after chronic antidepressant treatments (Bergstrom and Kellar, 1979; Seo et al., 1999a,b; Stanford and Nutt, 1982; Stanford et al., 1983; Tiong and Richardson, 1990). Single-concentration autoradiographic binding studies also showed a decrease in beta-adrenergic receptors in the hippocampus (Biegon and Israeli, 1986; Duncan et al., 1989; Ordway et al., 1988).

A study reporting a single concentration autoradiographic binding found decreased beta-adrenergic receptor binding in hippocampal areas after 96 h of REM sleep deprivation in rats (Hipolide et al., 1998). However, this study did not determine maximum binding (B_{max}) or the dissociation constant (K_D). Therefore, to address this issue, we carried out homogenate saturation binding studies in hippocampal membranes of REM sleep-deprived rats. We also assayed beta-adrenergic receptors in the brainstem because it has been shown that REM sleep deprivation induces biochemical changes in noradrenergic neurotransmission in this brain area (Perez and Benedito, 1997; Sinha et al., 1973; Thakkar and Mallick, 1993) and chronic antidepressant treatment also down-regulates brainstem beta-adrenergic receptors (Cohen et al., 1982).

2. Material and methods

2.1. Subjects

Naive adult Wistar male rats, 3 months old, from our own colony were used in the experiment. After weaning, the rats were kept in plastic cages (60 × 50 × 22 cm; 6 rats per cage) under a controlled light–dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) and temperature (22 ± 2 °C). The rats had free access to food (Purina labchow) and tap water until they were sacrificed.

3. REM sleep deprivation

The rats were deprived of REM sleep for 96 h by the 'flowerpot' method (Hipolide et al., 1998; Van Hulzen and Cohen, 1981) in water tanks measuring 150 × 44 × 44 cm (10 rats per tank). The platforms (5.5 cm in diameter) were immersed in water up to 2 cm from their upper surfaces. REM sleep deprivation started at 9:00 a.m. Control rats remained in their home cages in the same room. All rats were sacrificed 96 h after the beginning of REM sleep deprivation.

3.1. Homogenate preparation

After decapitation, the brains were excised and washed with cold saline 0.9% to remove blood. The brain regions (hippocampus and brainstem) were dissected on a paper-filter-covered Petri dish, kept cold with crushed ice. The brainstem was obtained by cutting between the cerebral peduncles and the caudal end of medulla oblongata (between sections A11 and A4, described in Craigie's Neuroanatomy of the Rat). The hippocampus structure became visible taking out the cortex; the all-hippocampus formation was separated from other brain structures and dissected as a whole. Tissue was homogenized at a 10% (w/v) in cold 50 mM Tris–HCl buffer pH 7.0 using an Ultraturrax. The homogenates were centrifuged at 49,000 × *g* for 20 min. After discarding the supernatant, the pellets were resuspended and centrifuged once again. Finally, after discarding the supernatants, the pellets were kept at −20 °C until assayed. For the hippocampus, tissue from three rats was pooled to yield the necessary volume for binding assays.

3.2. [^{3}H]-Dihydroalprenolol binding assay

Frozen pellets were resuspended in cold Tris–HCl (50 mM, pH 7.0). [^{3}H]-Dihydroalprenolol ([^{3}H]-DHA) binding assay was carried out according to Bylund and Snyder (1976) with modifications. A 100-μl aliquot of homogenate was incubated in triplicate for 20 min in the presence of [^{3}H]-DHA (25 μl, diluted in 50 mM Tris–HCl pH 7.0). The final incubation volume was 250 μl, made up of 50 mM Tris–HCl buffer pH 7.0. Nonspecific binding in duplicate was carried out in the presence of DL-propranolol (10 μM final concentration in the assay). For the brainstem, nonspecific binding was also carried out in the presence of L-isoproterenol (10 μM final concentration; Stone and U'Prichard, 1981). Saturation curves were obtained using 6 to 8 [^{3}H]-DHA concentrations in a 0.15–6 nM final concentration in the assay. Incubation ended by adding 5 ml of cold

50 mM Tris–HCl buffer pH 7.7 to the tubes. The solution was filtered under vacuum and tissue trapped in Whatman GF/B filters. Filters were washed four times with 5 ml of cold 50 mM Tris–HCl buffer pH 7.7. Then, the filters were placed in scintillation vials and air dried overnight. After adding 10 ml of scintillation liquid (Instagel), the vials were shaken for 20 min and radioactivity was counted. Specific binding was determined by subtracting nonspecific from total binding. The results are expressed as fmol of bound [^{3}H]-DHA/mg protein. B_{max} and K_D were calculated using a nonlinear regression analysis (Graph Pad Prizim Software, San Diego, CA). Proteins were assayed using albumin as the standard (Lowry et al., 1951).

3.3. Reagents

All reagents were analytical grade (Sigma, St. Louis, MO). The water used to make solution was bidistilled. L-[propyl-2,3-^{3}H] Dihydroalprenolol (s.a. 50 Ci/mmol) was obtained from Amersham Life Science (Little Chalfont, Buckinghamshire, England).

3.4. Statistics

Unpaired Student's t test was used to compare the results. The level of significance was set at $P \leq .05$.

4. Results

As Table 1 shows, REM sleep deprivation induced a significant decrease of [^{3}H]-DHA B_{max} binding to beta-adrenergic receptors in the hippocampus ($t=2.9$, $df=11$, $P<05$). A significance decrease in the affinity constant (K_D; Table 1) was also observed after REM sleep deprivation ($t=3.2$, $df=11$, $P<.05$), Fig. 1 shows an example of the binding saturation analysis in the hippocampus. REM sleep deprivation also induced a decrease in the B_{max} of [^{3}H]-DHA in the brainstem (Table 1, Fig. 2) using either DL-propranolol ($t=2.7$, $df=8$, $P<.05$) or L-isoproterenol ($t=2.3$, $df=10$, $P<.05$) to determine nonspecific binding. As Table 1 shows, REM sleep deprivation induced a decrease in K_D ($t=2.9$, $df=8$, $P<.05$) only when DL-propranolol was used in the incubation medium ($t=0.05$, $df=10$, $P>.05$ for L-isoproterenol groups), Fig. 3 shows an example of the binding saturation analysis in the brainstem using isoproterenol.

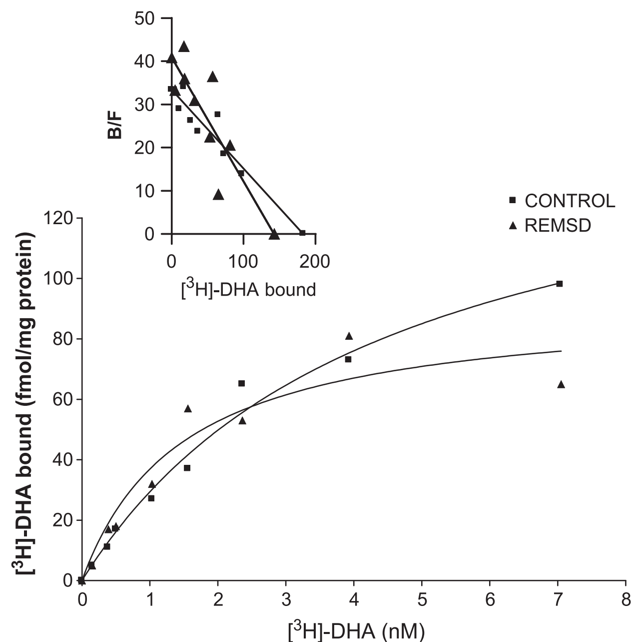

Fig. 1. A nonlinear regression analysis of a saturation biding assay of hippocampal membranes from a control and a REMSD rat using propranolol as the could drug. The parameters obtained were $B_{max}=142.4$ and $K_D=3.48$ for the control and $B_{max}=92.0$ and $K_D=1.49$ for the REMSD rat. Inset: the Scatchard plot of the data.

5. Discussion

The data obtained in this study showed a down-regulation of beta-adrenergic receptors in the rat's brainstem and hippocampus after 96 h of REM sleep deprivation. The study also found a significant decrease in the dissociation constant (K_D) in both brain regions using DL-propranolol to determine nonspecific binding; however, there was no change in brainstem K_D when L-isoproterenol was used.

The down-regulation of hippocampal beta-adrenergic receptors after REM sleep deprivation suggests a sustained

Table 1
β-adrenergic receptors binding (B_{max}) and dissociation affinity constant (K_D)

Group	Brain region	B_{max} (fmol/mg of protein)		K_D (nM)	
Control	Hippocampus	111 ± 17 (6)		2.3 ± 0.6	
REMSD	Hippocampus	86 ± 12 * (7)		1.2 ± 0.7 *	
		Propranolol	Isoproterenol	Propranolol	Isoproterenol
Control	Brainstem	81 ± 23 (5)	25 ± 8 (6)	2.4 ± 0.7	1.6 ± 0.4
REMSD	Brainstem	47 ± 13 * (5)	16 ± 5 * (6)	1.2 ± 0.7 *	1.6 ± 0.6

Maximum binding and dissociation constant affinity of [^{3}H]-DHA to hippocampal and brainstem membranes from REM–sleep-deprived rats and controls. For the brainstem, the nonspecific binding was obtained using DL-propranolol or L-isoproterenol. The number of animals per group (n) is shown in parenthesis. The values are mean ± S.D.

* Significantly different from its respective control, $P<.05$.

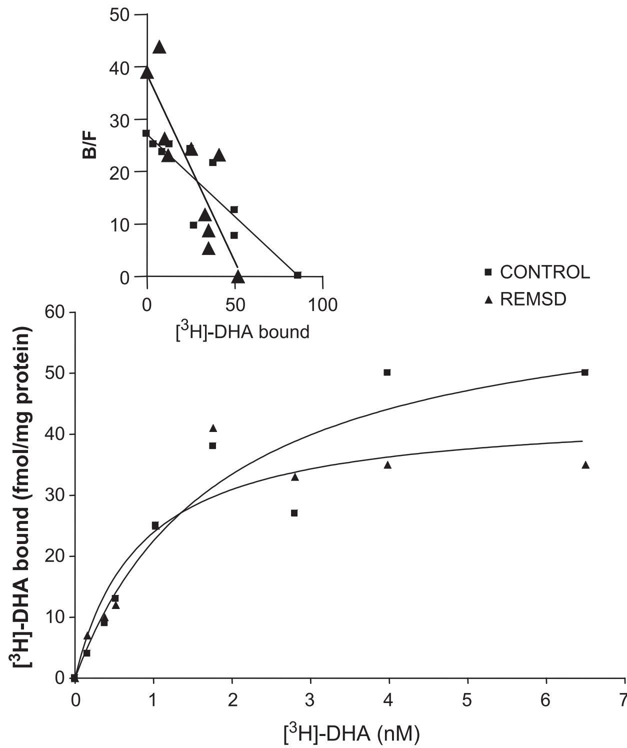

Fig. 2. A nonlinear regression analysis of a saturation biding assay of brainstem membranes from a control and a REMSD rat using propranolol as the could drug. The parameters obtained were B_{max} = 65 and K_D = 1.88 for the control and B_{max} = 44 and K_D = 0.8 for the REMSD rat. Inset: the Scatchard plot of the data.

increase in NA release in this brain region during REM sleep deprivation. This effect does not seem to involve a decrease in NA metabolism because no change was found in either MAO-A or -B activity in the hippocampus of rats deprived of REM sleep for 96 h (Perez and Benedito, 1997).The decrease in beta-adrenergic receptor B_{max} in hippocampal membranes after REM sleep deprivation is in accordance with the trend observed in a previous single concentration autoradiographic binding study (Hipolide et al., 1998); but in addition to the qualitative decrease of the receptors reported by the latter, we showed that both maximum binding (B_{max}, a quantitative measure) and K_D (a receptor affinity state measure) were altered in hippocampal crude membrane preparation from REM sleep-deprived rats.

Although receptor maximum number decreased, there was an increased affinity to the ligand. This decrease in K_D indicates a conformational change of the binding site and was not observed in the hippocampus after chronic antidepressant treatment (Biegon and Israeli, 1986; Stanford and Nutt, 1982; Stanford et al., 1983). Therefore, this difference is indicative of a different mechanism underlying the down-regulation of beta-adrenergic receptors induced by REM sleep deprivation.

Endogenous levels of hippocampal NA decreased in the rat hippocampus deprived of REM sleep for 72 h, when compared to the home-cage control group (Porka-heiskanen et al., 1995). Endogenous levels are not the best estimate of synaptic levels; however, it could be an indication of higher turnover of NA. A higher turnover of hippocampal NA could explain the down-regulation of beta-adrenergic receptor in this brain area after REM sleep deprivation. A similar effect of REM sleep deprivation on hippocampal B_{max} beta-adrenergic receptors has already been observed in chronic antidepressant treatment (Seo et al., 1999a,b; Stanford and Nutt, 1982, Stanford et al., 1983; Tiong and Richardson, 1990), which suggests the involvement of this brain area in the antidepressant effect of REM sleep deprivation.

Our own study showed that REM sleep deprivation induced a down-regulation of beta-adrenergic receptors in the brainstem. This effect of REM sleep deprivation on the brainstem was also observed after chronic antidepressant treatment (Cohen et al., 1982). The reported increase in tyrosine hydroxylase (Sinha et al., 1973) and decrease in MAO-A (Perez and Benedito, 1997) activities in the brainstem of REM sleep-deprived rats indicate a higher NA availability in this brain region; this may explain the observed down-regulation of beta-adrenergic receptors. Besides a decrease in the B_{max}, we also observed a significant decrease in the K_D when DL-propranolol was used to

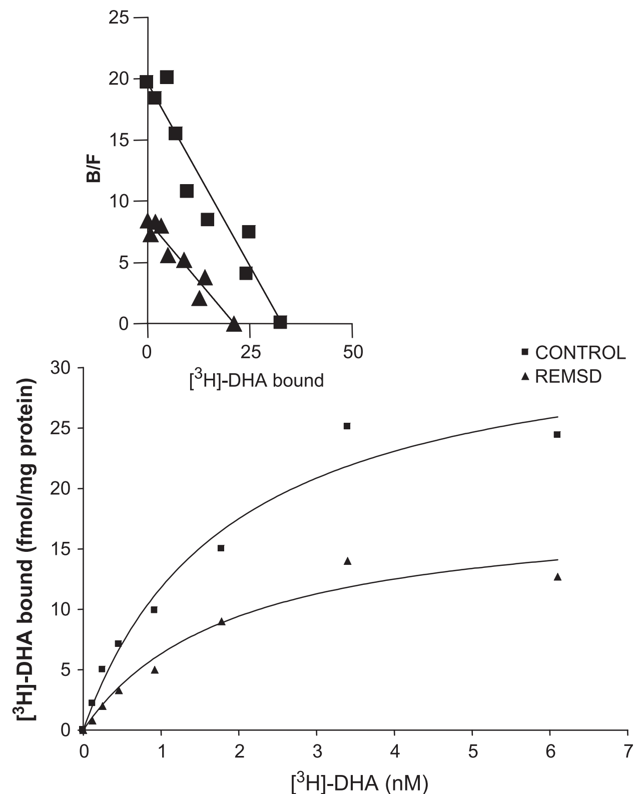

Fig. 3. A nonlinear regression analysis of a saturation biding assay of brainstem membranes from a control and an REMSD rat using isoproterenol as the could drug. The parameters obtained were B_{max} = 33.8 and K_D = 1.8 for the control and B_{max} = 18.6 and K_D = 1.9 for the REMSD rat. Inset: the Scatchard plot of the data.

determine nonspecific binding; however, this change was no longer observed when L-isoproterenol was used.

Because [^{3}H]-DHA labels both beta 1- and beta 2-adrenoceptors (Neve et al., 1986) and the ratio beta 1/beta 2 receptor is around 1 in the brainstem (Rainbow et al., 1984), the decrease in the B_{max} observed in our study may represent a change involving both types of adrenergic receptors and these data are not in full agreement with the previous single concentration autoradiographic binding studies that did not show significant changes in either beta 1- or beta 2-adrenergic receptors in brainstem areas (with few exceptions) after 96 h of REM sleep deprivation (Hipolide et al., 1998).

It has been shown that DL-propranolol is not suitable for determining nonspecific binding in the brainstem (Stone and U'Prichard, 1981). We used L-isoproterenol, as recommended, or DL-propranolol, to determine nonspecific binding. In both approaches, there was a down-regulation of receptors in the brainstem; however, the decrease in B_{max} obtained by using DL-propranolol may also involve a decrease in other binding sites not related to adrenergic receptors (Stone and U'Prichard, 1981).

Noradrenergic nuclei are all located in the brainstem (Moore, 1982; Stanford, 1995). Therefore, the down-regulation of beta-adrenergic receptors in this brain area after REM sleep deprivation cannot be attributed solely to a change in LC neuron activity; it may also involve changes in the activities of other brainstem noradrenergic nuclei. However, the LC is the main noradrenergic-synthesizing nuclei in the central nervous system (Aston-Jones et al., 1995) and alterations in these nuclei may have important physiological consequences. Central noradrenergic neurons are known to be involved in responses to stress, and because REM sleep deprivation is a stressful procedure, several attempts have been made to eliminate the stressors involved in the platform method. A large water tank, as used in our experiment, containing an excess of platforms, is intended to avoid the immobility present in the single platform procedure. Moreover, the larger water tank also eliminates isolation stress and allows rats to continue interacting.

Some stressors (restraint, footshock, isolation and novelty) were shown not to change brainstem or hippocampal beta-adrenergic receptors assayed using [^{3}H]-DHA, ^{125}I-Iodo-pindolol or ^{125}I-Iodocyanopindolol (Areso and Frazer, 1991; Brannan et al., 1995; Cohen et al., 1986; Hilakivi-Clarke et al., 1991; Nomura et al., 1981; Stone and Platt, 1982; Yamanaka et al., 1987); however, the effect of stressors on beta-adrenergic receptors is still a controversial matter, and in general, the evidence points to a varying patterns of effects of stress on beta-adrenergic receptors (Stanford, 1995).

A recent report has showed an increase in plasma corticosterone levels in rats kept on multiple large and small platforms and single large and small platforms; it also showed an increase in plasma ACTH levels in rats kept on multiple large and small platforms (Suchecki et al., 1998). These data on increased stress hormone levels after REM sleep deprivation indicate that stress is probably either an unavoidable component of the REM sleep deprivation procedure or that, at least in relation to the brain noradrenergic systems, REM sleep deprivation itself is a stressor. If this hypothesis holds true, even eliminating all other possible stressors in the REM sleep deprivation procedure, brain noradrenergic activity increase would still be observed. Because our method (multiple platforms) aims to minimize the stress of the sleep deprivation environment and we found similar results to other REM sleep deprivation studies (Hipolide et al., 1998, Mogilnicka et al., 1986), it seems likely that, if the present results are associated with stress, they would reflect a specific stress factor associated with sleep loss rather than a general stress effect.

In conclusion, we showed that REM sleep deprivation induced a down-regulation of beta-adrenergic receptors in the hippocampus and brainstem. This down-regulation of beta-adrenergic receptors suggests the involvement of brain noradrenergic neurotransmission in the antidepressant effect of REM sleep deprivation.

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