RAPID EYE MOVEMENT (REM) SLEEP DEPRIVATION INDUCES A DECREASE IN NEURONAL NUCLEAR VOLUME IN THE LOCUS COERULEUS, HIPPOCAMPUS AND CINGULATE CORTEX OF THE RAT

Mario Pedrazzoli and Marco Antonio Campana Benedito

Departamento de Psicobiologia - Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brasil

Running title: Neuronal nuclear volume after REM sleep deprivation

Corresponding author
Mario Pedrazzoli
Departamento de Psicobiologia
Universidade Federal de São Paulo
Rua Botucatu, 862 - 1° andar
040023-062 - São Paulo, Brazil
Phone: 55-11-5539-0155
Fax: 55-11-572-5092
e-mail:pedrazzo@psicobio.epm.br

Acknowledgments

Mario Pedrazzoli was a recipient of a fellowship from CAPES. This work was partially supported by Associação Fundo de Incentivo à Psicofarmacologia (AFIP). We thank Dr. Manuel de Jesus Simões for his help in the histological procedures. We thank Ms. Inês Mônaco for typing of the manuscript.

Abstract

Literature findings have suggested that brain morphological changes may underlie behavioral disturbances such as depression. In depressed patients, alteration in the volume of some brain regions were described and in laboratory animals antidepressant treatments change neuronal nuclei volume and axon densities in some brain regions. Rapid eye movement (REM) sleep deprivation has antidepressant effect in humans and in animals models of depression; moreover it induces changes in brain neurotransmission similar to those obtained after chronic antidepressant drug treatment (i.e., down-regulation of beta-adrenergic receptors and decreased synthesis of noradrenaline-stimulated cAMP). The aim of this work was to determine if REM sleep deprivation would induce morphological changes in the brains of rats. The effects of REM sleep deprivation on the nuclear volume of neurons from the locus coeruleus, the main noradrenergic nucleus in the brain, hippocampus (area CA_1) and cingulate cortex, two brain areas innervated by locus coeruleus, were studied. The results obtained showed that REM sleep deprivation significantly decreased the nuclear volume of neurons in the locus coeruleus and in cingulate cortex and hippocampus, whereas stress significantly decreased the mean nuclear volume of neurons only from the hippocampus. A change in cell nuclear volume suggests a change in its metabolic activity, therefore, our data provide an anatomical basis for further studies of neuron's morphology in brain structures after REM sleep deprivation.

Key words: REM sleep deprivation, locus coeruleus, hippocampus, cingulate cortex, neuron nuclear volume, neuronal morphology

INTRODUCTION

Literature data report morphological changes in brain tissue in depression (Sheline Yi et al., 1998; Rajkowska et al., 1999) and after antidepressant treatments (Nakamura et al., 1991; Kitayama et al., 1997; Magarinos et al., 1999). These data indicate that morphological alteration in brain tissue can underlie biochemical changes that would lead to mood disorders or antidepressive effects.

Rapid eye movement (REM) sleep deprivation is a procedure that claimed to posses antidepressant effects on endogenous depression (Vogel, 1980) as well as being effective in animal models of depression (Porsolt et al., 1978). Increased noradrenergic neurotransmission is induced by antidepressant treatments; down-regulation of beta-adrenergic receptors and decreased synthesis of cyclic adenosine monophosphate (cAMP) are classical effects resulting from chronic antidepressive treatments (Sugrue, 1983). REM sleep deprivation also induces increased central nervous system (CNS) noradrenergic neurotransmission shown by an increase in brain noradrenaline (NA) turnover (Schildkraut, 1972), which leads to cortical beta-adrenergic receptor down-regulation (Mogilnicka et al., 1980) and a consequent decrease in the synthesis of cortical NA-induced cAMP (Troncone et al., 1986). These similar effects

between antidepressive treatments and REM sleep deprivation suggest the existence of other similarities between them with respect to noradrenergic neurotransmission.

Locus coeruleus (LC) is the main noradrenergic nucleus in the CNS and has been implicated in the mechanism of action of antidepressant drugs (Nestler et al., 1990; Kostowski et al., 1986). Among other effects, imipramine, a classical tricyclic antidepressant, induces a decrease in the nuclear size of culture glial and nerve cells in the rat cingulate cortex (CC) (Bal-Klara & Smialowska, 1987) and chronic treatment decreases the nuclear size of LC and CC cortex neurons *in situ* (Smialowska et al., 1988).

REM sleep deprivation, a procedure where no drugs are introduced in the organism and which has antidepressant effect in humans (Vogel, 1980), could serve as a model to detect morphological changes in brain that could be related to its antidepressant effect. Therefore, we studied the effects of REM sleep deprivation on the nuclear volume of LC neurons and two other structures innervated by this nucleus, the hippocampus (HI) and the CC (Foote et al., 1983; Loughlin et al., 1986 a and b).

MATERIAL AND METHODS

Subjects

The experiments were carried out on 12 adult male Wistar rats from the animal facility of the Departamento de Psicobiologia, weighing 250-300g. Animals were kept in a room under controlled 12:12h light/dark cycle (lights on from 07:00h to 19:00h) and temperature (22^{o}C). Food and water were provided *ad libitum* until the animals were sacrificed.

REM sleep deprivation

Rats rats were assigned to three different groups: 1) REM sleep-deprived group (REMd); 2) Large platform group, as partial REM sleep deprived group (PREMd) and 3) Control group (CTR). Rats in the REMd group were REM sleep deprived by the flower-pot procedure (Cohen & Dement, 1965). The animals were placed in an individual chamber on a platform 6cm in diameter surrounded by water until 1cm below the platform top, for 96h. In the PREMd group the rats remained in a similar environment as the REMd group but on a larger platform (14cm in diameter); in this group the animals are maintained in the same stress conditions (humidity and isolation) as the REMd group, but are only partially REM sleep deprived (Machado et al., 2004). Rats in the CTR group remained in the same room, individually housed. REM sleep deprivation started at 08:00h.

Histological procedure

After 96h of REM sleep deprivation all rats were perfused with a 9% NaCl solution and after that with a 10% formalin solution. After removal, the brains were post-fixed with formalin for 7 days, dehydrated, cleared and embedded in paraffin according to routine histological procedures. Brains were sliced in the frontal plane in 7μm consecutive sections, which were grouped in sets of 3 slices per slide. To avoid measuring the same nuclei, of the three sections mounted on each slide the next consecutive three were discarded and only one per slide was used for measurements. The slices were stained with hematoxylin-eosin.

Volume measurement

The evaluation of each brain region was performed under light microscopy using a KPL 8x Carl Zeiss lens. Neuronal nuclei volumes of the selected structures were calculated according to the formula of ellipsoid revolution ($V = d_1.d_2^2 / 1.91$, where d_1 and d_2 are the perpendicular diameters of the cell nucleus and where $d_1 > d_2$) (Bal & Smialowska, 1987). The neurons were recognized and differentiated from the glia cells by their larger size, by the fact that the nucleus was centralized in the cell and was perfectly visible, and by the presence of a single nucleolus and little or no granulation in the cytoplasm of the LC and CC. In the HI, neurons were perfectly visible in the CA_1 field in the line of cells that delimit the Amon's horn and no glia cells were observed between these neurons. For each rat and anatomical structure, about 100 neurons' nuclei were measured in similar fields and for all regions studied. The neurons to be measured were chosen randomly in the entire region field. Three anatomical regions were evaluated: 1) Locus coeruleus; 2) Hippocampus (CA1 field) and 3) Anterior part of the cingulate cortex.

Statistics

Data were statistically analyzed by means of the One-way Analysis of Variance (ANOVA) and post-hoc comparison among groups was done by the Newman-Keuls Multiple range test ($p<0.05$).

RESULTS

Locus Coeruleus - One-way ANOVA detected a statistically significant change in the LC neuron nuclei volume ($F_{(2,975)} = 7.2$; $p<0.008$) (Figure 1) and the post-hoc statistical analysis showed a significant decrease in REMd group compared to both CTR ($p<0.05$) and PREMd ($p<0.001$).

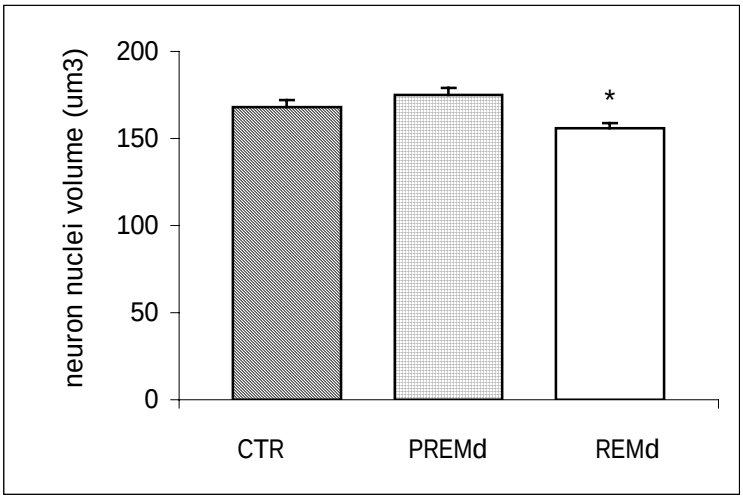

Figure 1. Locus Coeruleus neurons nuclei volume in rats deprived of REM sleep. CTR (control group, N=300), PREMd (partial REM sleep deprivation, N=300), REMd (REM sleep deprived group, N=378). The values are mean ± S.E.M. * different from control and PREMd groups, $p<0.05$

Hippocampus **-** A statistically significant change was observed in the HI neuron nuclei volume ($F_{(2,938)} = 90.6$; $p<0.0001$) and subsequent post-hoc statistical analysis showed a significant difference between PREMd and REMd groups compared to CTR group ($p<0.001$) (Figure 2).

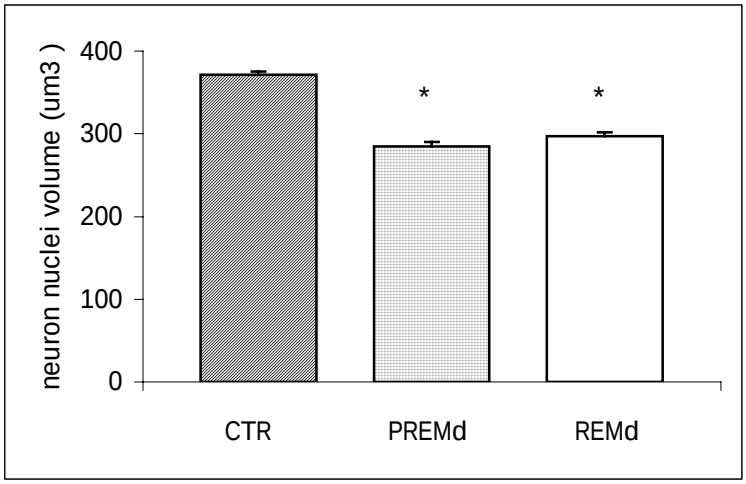

Figure 2. Hippocampus neuronal nuclei volume in rats deprived of REM sleep. CTR (control group), PREMd (partial REM sleep deprivation), REMd (REM sleep deprived group. The values are mean ± SEM. * different from the control group $p<0.05$.

Cingulate cortex - Cingulate cortex neuron nuclei volume change significantly after REM sleep deprivation ($F_{(2,1010)} = 14.3$, $p<0.0001$), post-hoc statistical comparison among groups showed a significant decrease in REMd group and both CTR ($p<0.001$) and PREMd ($p<0.001$) groups (Figure 3).

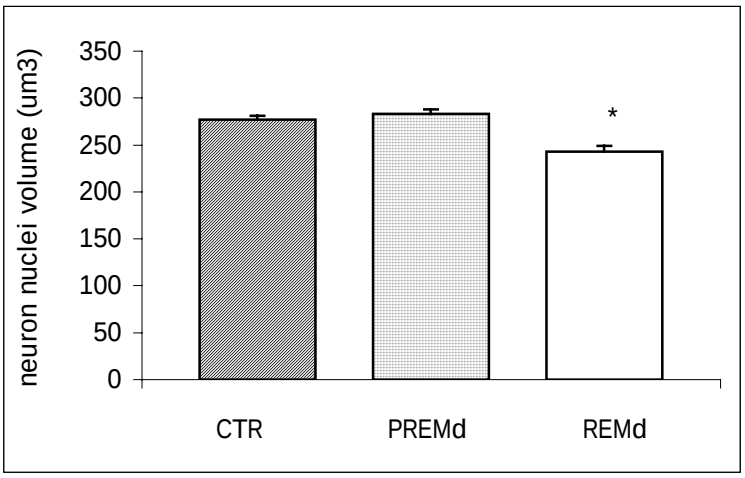

Figure 3. Cingulate Cortex neuronal nuclei volume in rats deprived of REM sleep. CTR (control group), PREMd (partial REM sleep deprivation), REMd (REM sleep deprived group. The values are mean ± SEM. * different from the control group, $p<0.05$.

DISCUSSION

The results obtained in this study showed a decrease in the nuclear volume of LC, HI and CC induced by REM sleep deprivation (Figures 1-3). The decrease observed in the neuronal nuclear volume of the HI after REM sleep deprivation was very consistent and a large difference (20 - 25%) in the neuronal nuclear volume was observed in REMd groups compared to the control group (Figure 3).

Locus coeruleus innervates both the HI and CC (Foote et al., 1983; Loughlin et al., 1986 a and b); however, as REM sleep deprivation has also been shown to alter neurotransmitters other than NA (Hery et Al 1970; Wojcik & Radulovacki, 1981; Camarini & Benedito, 1997) it is not possible at this moment to discard the participation of other neurotransmitters in the changes observed in these structures.

LC is not a homogeneous structure from a cellular point of view. Data in the literature have shown that LC can be anatomically divided in different regions and it is composed by

different type cells, project to different brain regions, with some specificity, depending on the cell type and its location; for instance, efferent projections to HI originate solely from the dorsal segment of LC whereas efferent projections to the cerebral cortex appear to be distributed throughout the central part of the LC (Loughlin et al., 1986 a and b). We measured neuronal nuclear volume randomly throughout the LC; moreover, our method did not differentiate the cell types. Therefore, other experiments should be designed to verify the possibility that specific LC cell types and sub-regions are responsible for the changes obtained in this study.

Data in the literature have demonstrated the involvement of the LC in the effects of antidepressant treatments (Nestler et al., 1990; Kostowski et al., 1986). Chronic imipramine decreases LC neuronal nuclear volume (Smialowska et al., 1988) and our data showed a similar pattern of change induced by REM sleep deprivation. The difference between the effect of imipramine, which induces a larger change in the nuclear volume of neurons, and REM sleep deprivation which induced a smaller decrease in neuron nuclei volume can not be considered unexpected as the drug is supposed to distribute throughout the brain, thus probably affecting more neurons, while REM sleep deprivation, a behavioral disturbance, is probably affecting limited neuronal circuits in the region.

In the CC, imipramine was shown to decrease the neuronal nuclear volume and we also observed a decrease in the nuclear volume induced by REM sleep deprivation in this brain region. Chronic imipramine does not induce any change in HI neuronal nuclear volumes, whereas our results showed that partial and total REM sleep deprivation did.

Rapid eye movement sleep deprivation has been shown to induce an increase in LC tyrosine hydroxylase mRNA levels and noradrenergic mRNA transporter (Porkka-Heiskanen et al., 1995; Basheer et al., 1998). In the brainstem, where LC is located, REM sleep deprivation was shown to induce an increase in the activity of tyrosine hydroxylase (Sinha et al., 1973) and a decrease in monoamine oxidase A activity (Perez & Benedito, 1997), enzymes responsible, respectively, for the synthesis and degradation of NA. REM deprivation also decreases the number of beta-adrenergic receptors in the brainstem (Pedrazzoli & Benedito, 1997). These biochemical changes induced by REM sleep deprivation indicates that the decrease in the nuclear volume of LC neurons after REM sleep deprivation may reflect complex effects of REM sleep deprivation in LC neurons types.

There are indications of the involvement of the anterior CC and HI in depression and also on the effects of antidepressant drugs (Mayberg et al., 1997; Ebert & Ebneier, 1996; Bench et al., 1995; Mongeauet al., 1997). In a study with depressed patients using positron

emission tomography it was shown that baseline metabolic rates for the CC were significantly higher in the depressed patients who responded to sleep deprivation than in normal control subjects (Wu et al., 1992). The cingulate cortex has the densest noradrenergic innervation of the rat's cerebral cortex (Fuxe et al., 1968) and electrophysiological data show that repetitive stimulation of the LC evokes strong inhibition of the firing rate of the rat's CC neurons (Dillier et al., 1978). REM sleep deprivation induced a decrease in the anterior CC neurons' nuclei. These data may indicate a possible involvement of the noradrenergic innervation to the CC in the antidepressant effect of REM sleep deprivation. There is a lack of data regarding the biochemical effect of REM sleep deprivation in this brain region. Therefore further studies are necessary to explore this possibility.

Corticosteroid hormones are implicated in mood disorders. REM sleep deprivation has antidepressant effects in humans and in rats and it activates the hypothalamic-pituitary-adrenal axis as shown by higher plasma levels of ACTH and corticosterone (Suchecki et al., 1998). Corticosterone is uptaken by brain tissue and its cytoplasmic receptors are located in several brain areas but the highest level is found in the HI (Sapolsky et al., 1983). Excess of glucocorticoids alters dendritic morphology of hippocampal neurons (Sapolsky, 1996) and down-regulates corticosterone receptors (Spencer et al., 1991). Corticosterone operates at the level of gene regulation and modulates its own mRNA receptors (Chao et al., 1998), neurotransmitter receptor levels in HI (Biegon et al., 1985) and neurotransmitter-stimulated cAMP synthesis (Harrelson et al., 1987). Prolonged exposure to corticosterone accelerates the process of cell loss in the HI of rats and analysis of size distribution of hippocampal cell bodies shows a loss of certain types of neurons (Sapolsky et al., 1985). Data regarding corticosterone effect on HI and the higher levels of this hormone after REM sleep deprivation (Suchecki et al., 1998) suggests a possible involvement of the corticosteroid on the change of neuronal nuclei volume induced by REM sleep deprivation as seen in our study.

Some authors have used nuclear volume as an index of metabolic and functional activity (Bal & Smialowska, 1987; Smialowska et al., 1988; Bubenick & Monier, 1972). Therefore it is possible that the morphological changes obtained in our study may correspond to metabolic/functional changes produced by the treatment in some cell type nuclei.

In conclusion, our data showed that REM sleep deprivation induced a decrease in neuronal nuclear volume in the LC, HI and CC. Whether these changes are involved in the antidepressant effect of REM sleep deprivation needs to be clarified.

REFERENCES

Bal, A. and Smialowska, M. The influence of some antidepressant drugs on the nuclear volume of rat cingular cortex cells in culture. Neuroscience, 22: 671-674, 1987.

Basheer, R., Magnes, M., McCarley, R.W. and Shiromani, P. REM sleep deprivation increases the levels of tyrosine hydroxylase and norepinephrine transporter mrna in the Locus Coeruleus. Mol. Res., 57: 235-240, 1998.

Bench, C.J., Frackowiak, R.S.J. and Dolan, R.J. Changes in regional cerebral blood flow on recovery from depression. Psychol. Med., 25, 247-51, 1995.

Biegon, A., Rainbow, T.C. and McEwen, B.S. Corticosterone modulation of neurotransmitter receptors in rat hippocampus: a quantitative autoradiographic study. Brain Res., 332: 309-314, 1985.

Brown, E.S., Rush, A.J. and McEwen, B.S. Hippocampal remodeling and damage by corticosteroids: implications for mood disorders. Neuropsychopharmacology, 21: 474-484, 1999.

Bubenick, G. and Monier, M. Nuclear size variations in cells of locus coeruleus during sleep, arousal and stress. Exper. Neurol., 35: 1-12, 1972.

Camarini, R. and Benedito, M.A.C. Rapid eye movement (REM) sleep deprivation reduces rat frontal cortex acetylcholinesterase activity. Braz. J. Med. Biol. Res., 30: 641-647, 1997.

Chao, H.M., MA, L.Y., McEwen, B.S. and Sakai, R.R. Regulation of glucocorticoid receptor and mineralocorticoid receptor messenger ribonucleic acids by selective agonists in the rat hippocampus. Endocrinology, 139: 1810-1814, 1998.

Cohen, H.B., Dement, W.C. Sleep: changes in threshold to electroconvulsive shock in rats after deprivation of "paradoxical" phase. Science, 150, 1318-9, 1965.

Dillier, N., Laszlo, J., Muller, B., Koella, W.P. and Olpe, H.R. Activation of an inhibitory noradrenergic pathway projecting from the locus coeruleus to the cingulate cortex of the rat. Brain Res., 154, 61-68, 1978.

Ebert, D. and Ebneier, K.P. The role of the cingulate gyrus in depression: from functional anatomy to neurochemistry. Biol. Psychiat., 39: 1044-1050, 1996.

Foote, S.L., Bloom, F.E. and Aston-Jones, G. Nucleus locus coeruleus: new evidence of anatomical and physiological specificity. Physiol. Rev., 63: 844-914, 1983.

Fuxe, K., Hamberger, B. and Hokfelt, T. Distribution of noradrenaline nerve terminals in cortical areas of the rat. Brain Res., 8: 125-131, 1968.

Harrelson, A.L., Rostene, W. and McEwen, B.S. Adrenocortical steroids modify neurotransmitter-stimulated cyclic amp accumulation in the hippocampus and limbic brain of the rat. J. Neurochem., 48: 1648-1655, 1987.

Hery, F., Pujol, J.F., Lopez, M., Macon, J. and Glowinski, J. Increased synthesis and utilization of serotonin in the central nervous system of the rat during paradoxical sleep deprivation. Brain Res., 21: 391-393, 1970.

Kitayama, I., Yaga, T., Kayahara, T., Nakano, K., Murase, S., Otani, M. and Nomura, J. Long-term stress degenerates, but imipramine regenerates, noradrenergic axons in the rat cerebral cortex. Biol. Psychiat., 42: 687-696, 1997.

Kostowski, W., Plazinick, A. and Danyz, W. The role of locus coeruleus - limbic noradrenergic transmission in the action of antidepressant drugs. Psychopharmacol. Bull., 22: 512-22, 1986.

Loughlin, S.E., Foote, S.L. and Bloom, F.E. Efferent projections of nucleus locus coeruleus: topographic organization of cells of origin demonstrated by three-dimensional reconstruction. Neuroscience, 18: 291-306, 1986a.

Loughlin, S.E., Foote, S.L. and Grzanna, R. Efferent projections of nucleus locus coeruleus: morphologic subpopulations have different targets. Neuroscience, 18: 307-319, 1986b.

Magarinos, A.M., Deslandes, A. and McEwen, B.S. Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. Eur. J. Pharmacol., 371: 113-122, 1999.

Machado, R. B., Hipólide, D. C., Benedito-Silva, A. A. and Tufik, S. Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery. Brain Res., 1004: 45-51, 2004.

Mayberg, H.S., Braunan, S.K., Mahurin, R.K., Jerabek, P.A., Brickman, J.S., Tekell, J.S., Silva, J.A., McGinnis, S., Glass, T.G., Martin, C.C. and Fox, P.T. Cingulate function in depression: a potential predictor of treatment response. Neuroreport, 8: 1057-1061, 1997.

Mogilnicka, E., Arbilla, S., Depoortere, H. and Langer, S.Z. REM sleep deprivation decreases density of ^{3}H-dihydroalprenolol and ^{3}H-imipramine binding sites in the rat cerebral cortex. Eur. J. Pharmacol., 65: 288-292, 1980.

Mongeau, R., Blier, P. and De Montigny, C. The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effect of antidepressant treatments. Brain Res. Rev., 3: 145-195, 1997.

Nakamura, S. Axonal sprouting of noradrenergic locus coeruleus neurons following repeated stress and antidepressant treatment. Prog. Brain Res., 88: 587-598, 1991.

Nestler, E.J., McMahon, A., Sabban, E.L., Tallman, J.F. and Duman, R.S. Chronic antidepressant administration decreases the expression of tyrosine hydroxylase in the rat locus coeruleus. Proc. Natl. Acad. Sci. (USA), 87: 7522-7526, 1990.

Pedrazzoli, M. and Benedito, M.A.C. Effects of paradoxical sleep deprivation on hippocampal and brainstem β-adrenoceptors in rat brain. J. Sleep Res., 7(Suppl. 2): 203, 1997.

Perez, N.M. and Benedito, M.A.C. Activities of monoamineoxidase (MAO) A and B in discrete regions of rat brain after rapid eye movement (REM) sleep deprivation. Pharmacol. Biochem. Behav., 58: 605-8, 1997.

Porkka-Heiskanen, T., Smith, S.E., Taira, T., Urban, J.H., Levine, J.E., Turek, E.W. and Stenberg, D. Noradrenergic activity in rat brain during rapid eye movement sleep deprivation and rebound sleep. Am. J. Physiol., 268: R1456-R1463, 1995.

Porsolt, R.D., Anton, G., Blavet, N. and Jalfre, N. Behavioral despair in rats: a new model sensitive to antidepressive treatments. Eur. J. Pharmacol., 49: 479-491, 1978.

Rajkowska, G., Miguel-Hidalgo, J.J., Wei, J., Dilley, G., Pittman, S.D., Meltzer, H.Y., Overholser, J.C., Roth, B.C., Stockmeier, C.A. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol. Psychiat., 45: 1085-1098, 1999.

Sapolsky, R.M., Krey, L.C. and McEwen, B.S. Prolonged glucocorticoid exposure reduces hippocampal neuron number: implication for aging. J. Neurosci., 5: 1222-1227, 1985.

Sapolsky, R.M., McEwen, B.S. and Rainbow, T.C. Quantitative autoradiography of [^{3}H]-corticorterone receptors in rat brain. Brain Res., 271: 331-334, 1983.

Sapolsky, R.M. Stress, glucocorticoids and damage to the nervous system: the current state of confusion. Stress, 1: 1-19, 1996.

Schildkraut, J.J. Turnover and metabolism of norepinephrine in rat brain after 72 hours on a D-deprivation island. Psychopharmacology, 27: 17-27, 1972.

Sheline, Y.I., Gado, M.H. and Price, J.L. Amygdala core nuclei volumes are decreased in recurrent major depression. Neuroreport, 9: 2023-2028, 1998.

Sinha, A.K., Ciaranello, R.D., Dement, W.C. and Barchas, J.D. Tyrosine hydroxylase activity in rat brain following REM deprivation. J. Neurochem., 20, 1289-90, 1973.

Smialowska, M., Bal-Klara, A. and Smialowski, A. Chronic imipramine diminishes the nuclear size of neurons in the locus coeruleus, cingulate cortex but not hippocampus of rat brain. Neuroscience, 26: 803-807, 1988.

Spencer, R.L., Miller, A.H., Stein, M. and McEwen, B.S. Corticosterenone regulation of type I and type II adrenal steroid receptors in brain, pituitary, and immune tissue. Brain Res., 549: 236-246, 1991.

Suchecki, D., Lobo, L.L., Hipolide, D. and Tufik, S. Increased ACTH and corticosterone secretion induced by different methods of paradoxical sleep deprivation. J. Sleep Res., 7: 276-281, 1998.

Sugrue, M.F. Chronic antidepressant therapy and associated changes in central noradrenergic receptor functioning. Pharmacol. Ther., 21: 1-33, 1983.

Troncone, L.R.P., Braz, S., Benedito, M.A.C. and Tufik, S. REM sleep deprivation induces a decrease in norepinephrine-stimulated ^{3}H-cyclic amp accumulation in slices from rat brain. Pharmacol. Biochem. Behav., 25: 223-225, 1986.

Vogel, G. Improvement of depression by REM sleep deprivation. Arch. Gen. Psychiat., 37: 247-253, 1980.

Wojcik, W.J. and Radulovacki, M. Selective increase in brain dopamine metabolism during REM sleep rebound in the rat. Physiol. Behav., 27: 305-12, 1981.

Wu, J.C., Gillin, J.C., Buchsbaum, M.S., Hershey, T., Johnson, J.C. and Bunney, W.E. Effect of REM sleep deprivation on brain metabolism of depressed patients. Am. J. Psychiat., 149: 538-43, 1992.