

Rapid eye movement sleep deprivation induces a decrease in neuronal nuclear volume in the locus coeruleus, hippocampus and cingulate cortex of the rat

Mario Pedrazzoli*, Marco Antonio Campana Benedito

Department of Psychobiology - Universidade Federal de São Paulo (UNIFESP) - São Paulo (SP), Brazil.

Abstract

Background: 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).

Objective and methods: 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 CA1) and cingulate cortex, two brain areas innervated by locus coeruleus, were studied.

Results: 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.

Conclusion: 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.

Keywords: REM sleep deprivation, locus coeruleus, hippocampus, cingulate cortex, neuron nuclear volume, neuronal morphology.

Introduction

Literature data report morphological changes in brain tissue in depression (1,2) and after antidepressant treatments (3-5). 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 possess antidepressant effects on endogenous depression (6) as well as being effective in animal models of depression (7). 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

*Correspondence:

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

treatments (8). REM sleep deprivation also induces increased central nervous system (CNS) noradrenergic neurotransmission shown by an increase in brain noradrenaline (NA) turnover (9), which leads to cortical beta-adrenergic receptor down-regulation (10) and a consequent decrease in the synthesis of cortical NA-induced cAMP (11). 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 (12,13). 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) (14) and chronic treatment decreases the nuclear size of LC and CC cortex neurons in situ (15).

REM sleep deprivation, a procedure where no drugs are introduced in the organism and which has antidepressant effect in humans (6), 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 (16-18).

Material and Methods

Subjects

The experiments were carried out on 12 adult male Wistar rats from the animal facility of the Departamento de Psicobiología, 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°C). Food and water were provided ad libitum until the animals were sacrificed.

REM sleep deprivation

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 (19). The animals were placed in an individual chamber on a platform 6 cm in diameter surrounded by water until 1 cm below the platform top, for 96 h. In the PREMd group the rats remained in a similar environment as the REMd group but on a larger platform (14 cm 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 (20). Rats in the CTR group remained in the same room, individually housed. REM sleep deprivation started at 08:00h.

Histological procedure

After 96 h 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 7 μ m 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 ($V = d1 \cdot d2^2 / 1.91$, where $d1$ and $d2$ are the perpendicular diameters of the cell nucleus and where $d1 > d2$) (14). 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 CA1 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 ($p < 0.001$).

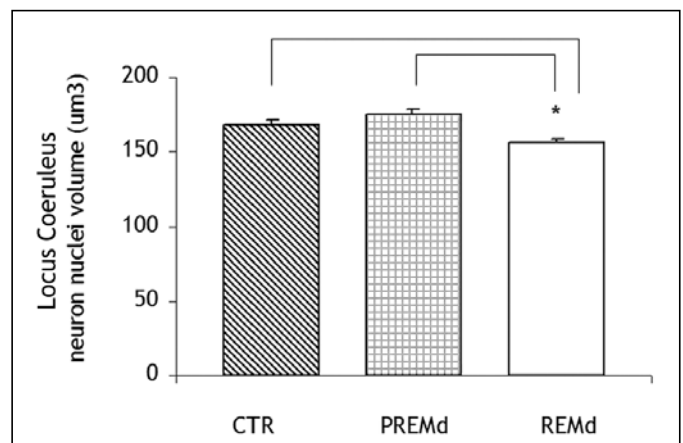


Figure 1. Locus Coeruleus neuronal nuclei volume in rats deprived of REM sleep. CTR (control group, N=300), PREMd (partial REM sleep deprivation, N=300), REMd (REM sleep deprived group, N=378). The values are mean \pm SEM * different from control and PREMd groups, $p < 0.05$

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).

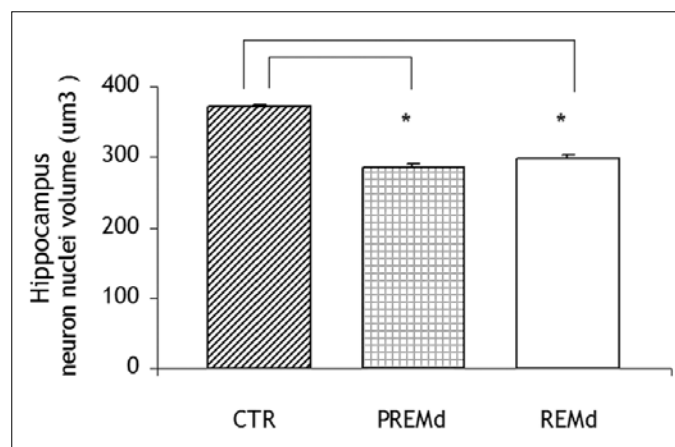


Figure 2. Hippocampus neuronal nuclei volume in rats deprived of REM sleep. CTR (control group), PREMd (partial REM sleep deprivation), REMd (REM sleep deprived group). The values are mean \pm SEM * different from the control group $p < 0.05$

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).

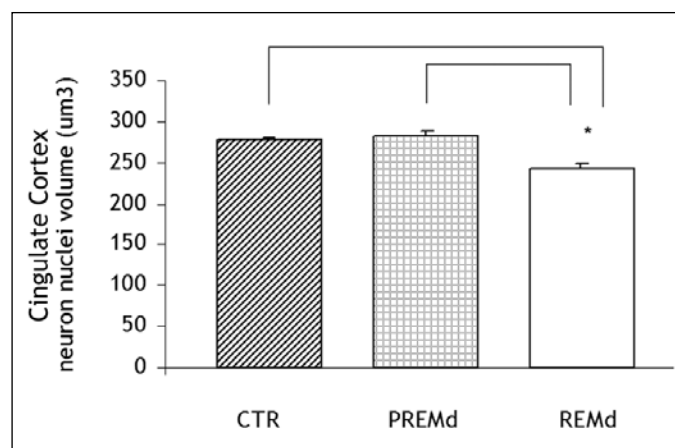


Figure 3. Cingulate Cortex neuronal nuclei volume in rats deprived of REM sleep. CTR (control group), PREMd (partial REM sleep deprivation), REMd (REM sleep deprived group). The values are mean \pm SEM * different from the control group, $p < 0.05$.

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 2).

Locus coeruleus innervates both the HI and CC (16-18); however, as REM sleep deprivation has also been shown to alter neurotransmitters other than NA (21-23) 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 (17,18). 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 (12,13). Chronic imipramine decreases LC neuronal nuclear volume (15) 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 (24,25). In the brainstem, where LC is located, REM sleep deprivation was shown to induce an increase in the activity of tyrosine hydroxylase (26) and a decrease in monoamine oxidase A activity (27), enzymes responsible, respectively, for the synthesis and degradation of NA. REM deprivation also decreases the number of beta-adrenergic receptors in the brainstem (28). 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 (29-32). 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 (33). The cingulate cortex has the densest noradrenergic innervation of the rat's cerebral cortex (34) and electrophysiological data show that repetitive stimulation of the LC evokes strong inhibition of the firing rate of the rat's CC neurons (35). 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 (36). 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 (37). Excess of glucocorticoids alters dendritic morphology of hippocampal neurons (38) and down-regulates corticosterone receptors (39). Corticosterone operates at the level of gene regulation and modulates its own mRNA receptors (40), neurotransmitter receptor levels in HI (41) and neurotransmitter-stimulated cAMP synthesis (42). 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 (43). Data regarding corticosterone effect on HI and the higher levels of this hormone after REM sleep deprivation (36) 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 (14,15,44). 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.

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