REM SLEEP DEPRIVATION IN AN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a common neurological condition all over the world. Most of the cases are considered to be the result of a degenerative process affecting mainly nigrostriatal dopamine neurons, leading to a marked dopamine deficiency in the striatum. Other neurotransmitter abnormalities occur in several different regions of the brain, but, by far, the dopamine depletion is the more striking feature of the disease. Twenty years after the introduction of L-Dopa as a treatment for PD and after other new pharmacological strategies, mainly using direct dopamine agonists (bromocriptine, lisuride, pergolide) this condition still offers a great treatment challenge. Ferguson & Dement [6], Alves et al.[1] and Carlini & Lindsey [3] showed that REM sleep deprived (REM SD) rats displayed aggressive behavior after receiving amphetamine. Non-deprived control animals which received a similar dose of the drug did not display aggressiveness. Carlini & Lindsey [3] had observed that REM SD rats receiving apomorphine (a potent direct dopamine receptor agonist) also showed the same aggressive behavior, whereas non-deprived animals did not. This effect could not be blocked by pretreatment with alpha-MPT, a tyrosine-hydroxilase inhibitor [15] or by administration of L-Dopa, and was enhanced by using bromocriptine and piribedil (direct receptor agonists)[15]. These findings suggest that REM SD may be acting by producing supersensitivity of the post-synaptic dopamine receptors, both in the striatum and cortical-limbic areas. Pflug & Tölle [12] made the first attempt at using total SD in depressed patients, reporting improvement. Since then many others have been using the same procedure [2,4,9,16]. Endogenous depression has been considered as the consequence of monoamine decreased function in CNS. Although noradrenaline and serotonin may be the major neurotransmitters involved, dopamine undoubtly plays a role in the pathophysiology, since improvement of this condition is

brought by the use of drugs with action on the dopaminergic system, such as nomifensine [8], mianserine, butriptiline, maprotiline and iprindole [7,13].

All these data support the possibility that post-synaptic dopaminergic receptors become supersensitive after REM SD. This led us to the application of this procedure in an experimental model of PD obtained in the rat.

MATERIAL AND METHODS

The experiment was made using male albino Wistar rats from our own colony, weighting from 300 to 400 g, and with age ranging from 4 to 5 months. Before the experiment the animals were kept in groups of 3 in a metal cage (30x18x16 cm) and in individual large wooden cages, after the surgical procedure. During all the experiment the animals were kept at a temperature of 23º C $\pm$ 1º C, in controlled conditions of light and darkness and with water and food ad lib.

The experimental model consisted of bilateral electrolytic lesions of the nigrostriatal pathway in the lateral hypothalamic area. These lesions were obtained by a stereotaxic apparatus. The stereotaxic parameters were as follows: the electrode tip was put 3 mm behind the bregma, 2 mm laterally to the medial line on both sides and 8.5 mm bellow the dura, the bregma and lambda being put at the same level. At this point on either side of the brain the experimental animals received a cathodic electrical current of 2 mA for 10 seconds. The control animals received sham operations in which all procedures were the same, but no electric current was applied to the electrode. All animals received barbiturate anesthesia for the surgical procedure (sodium pentobarbital — 50 mg/kg). REM SD was achieved by using the inverted flower pot technique (3). According to this method the animal was placed in a small square wood platform having 6 cm of side surrounded by water. The square platforms, instead of the usual round ones, were employed in order to facilitate the maintenance of the operated animals on the top. This water tank had a device for feeding. The lenght of REM SD was 72 hours. All results were analysed using one-way analysis of variance (ANOVA) and posteriorly Student's t test for dependent or independent samples. The minimal admitted significance level was 5% (p $<$ 0.05).

On day 0 the animals were operated upon. Twenty nine animals completed the experiment and formed 4 groups: 6 non-lesioned and non-deprived (NL-ND) animals; 9 non-lesioned and deprived (NL-D); 6 lesioned and non-deprived (L-ND); and 8 lesioned and deprived (L-D). On days 3 and 7 the animals were individually observed in the open-field. From day 7 to 10 the animals from groups NL-D and L-D were REM SD whereas the control animals were kept in their own individual cages. On day 10 all animals were observed in the open-field, the same happening on day 14 and 21. A second REM SD was performed from days 21 to 24, when the last observation in the open-field was made. The observation consisted on rating the ambulation (number of squares invaded by the animal), rearing (number of rearing movements), grooming (time spent by the animal in grooming behavior) and latency (time spent by to animal to start the ambulation). The observation period was 3 minutes.

Having completed the experiment, the brains were removed, fixed in 10% formalin for 30 days, cut in the frontal plane in 0.05 mm slices which were examined through an entomoscopic equipment, for confirmation of the lesion location.

RESULTS

Figure 1 represents the results concerning the item ambulation. As can be seen, both groups of lesioned animals (L-ND and L-D), before REM SD, were significantly different of the two groups of non-lesioned animals at the 3rd day post-surgery. This difference became less striking at the 7th day after the surgery. At the 10th day,
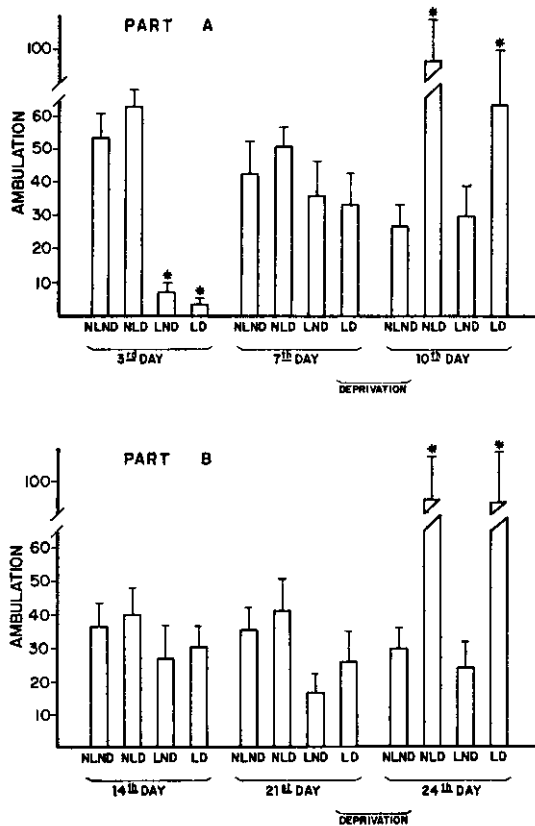

Fig 1 — Effect of REM SD on ambulation. Asterisk indicates a significant difference at a level of 5% in comparison with non-lesioned non-deprived (NL-ND), non-lesioned deprived (NL-D) and lesioned non-deprived (L-ND) controls.

after the REM SD (groups NL-D and L-D), a large increase (p $<$ 0.05) in the ambulatory behavior was evident in both groups of animals submitted to SD procedure comparing with the other two groups. The same finding appeared on day 24th, after the second period of SD. Figure 2 displays the results for the rearing behavior. They are similar to the ambulatory behavior, expressing also a large increase of rearing in the SD groups (p $<$ 0.05). Latency for ambulation and grooming behavior showed different results on day 3 after surgery, when comparing lesioned and non-lesioned animals, but failed to show any statistically significant difference in REM SD rats throughout the whole experiment.
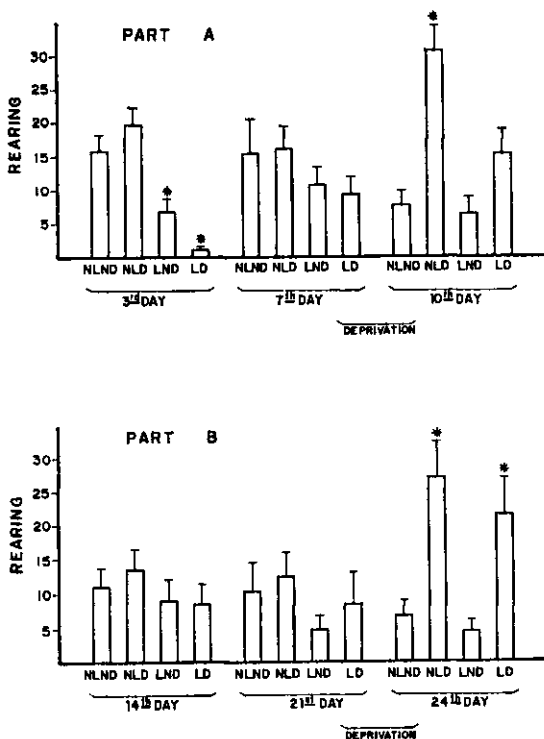
Fig. 2 — Effect of REM SD on rearing behavior. Asterisk indicates a significant difference at the level of 5% in comparison with non-lesioned non-deprived (NL-ND), non-lesioned deprived (NL-D) and lesioned non-deprived (L-ND) controls.

COMMENTS

Motor impairment is one of the characteristic signs of Parkinson's disease. In the operated rats a striking feature was the reduction in ambulation and rearing observed in the first post-operative days. However, from days 3 to 7, the animals were able to partially recover from this impairment without any manipulation. Nonetheless, REM SD clearly improved ambulation and rearing behavior when these two aspects of motor behavior were measured immediately after the deprivation periods (see figures 1 and 2). In a similar animal model, Marshall et al.[11], studied some activation stimuli (ice water swimming, forced swimming and the exposure of the rats to a colony of cats) in the motor behavior and showed a significant increase in certain motor parameters after the animals were exposed to these stimuli. This work was done to correlate the results with the "paradoxical kinesia" which was observed in some PD patients. The REM SD might share a similar mechanism to explain its action in the animal model.

As shown by the histological examination (Fig. 3), the extensive lesions of the nigrostriatal pathways would prevent any pre-synaptic action by the
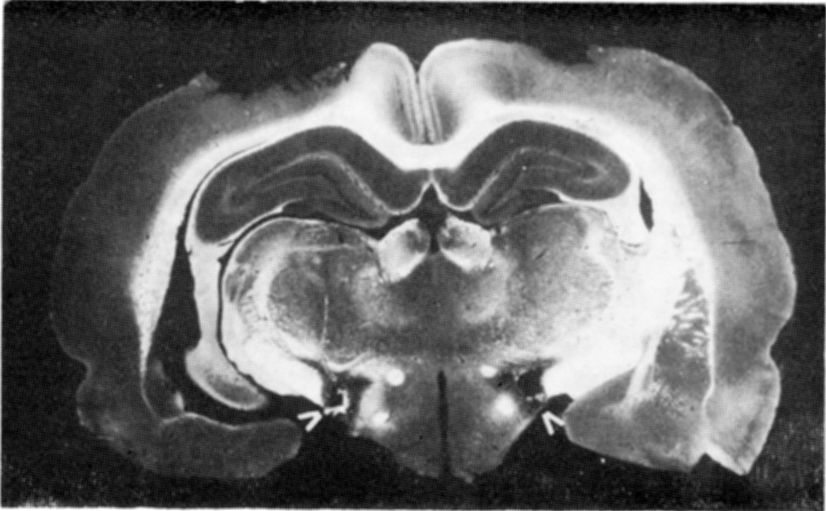
Fig. 3 — Histological demonstration of lesions on the nigrostriatal pathways (arrows). The lesions were induced by a 2mA electrical current with 10 seconds of duration stereotaxically directed.

procedure. There are some evidences that "denervation supersensitivity" occurs in striatal neurons of parkinsonian patients. Lee et al.[10] studied the binding of 3H-apomorphine and 3H-haloperidol to DA-receptors in the putamen and caudate nucleus of 6 parkinsonian patients and in neurologically normal patients. The 3H-apomorphine binding in the putamen of parkinsonian patients was significantly decreased as compared to controls. Lee et al.[10] explained the decreased binding of 3H-apomorphine as being the consequence of nigral cells loss, as, according to them, this ligand would be binding predominantly to the pre-synaptic receptors. On the other hand, the 3H-haloperidol binding, mainly in the putamen, was significantly increased. This fact could be the result of an increase in the number and/or in the affinity of the post-synaptic dopaminergic receptors, what brings support to the supersensitivity denervation theory in PD. An alternative explanation could be given by the agonist-antagonist concept of post-synaptic dopaminergic receptors [5,14]. According to the authors the dopaminergic receptors could exist under two inter-convertible forms: the agonist form, in which there is a greater affinity for stimulation agents (dopamine and apomorphine); and the antagonist form with greater affinity for the blocking agents (haloperidol, for example). It is conceivable that under certain circumstances (neurological disease, drugs, stress, etc.) one form could predominate over the other and REM SD could act by favoring the presence of the agonist form.

In parkinsonian brains the post-synaptic dopamine receptors are more sensitive to haloperidol, as compared to apomorphine [10]. If the SD soul revert this trend, bringing the dopamine receptors more sensitive to agonist agents, this would be a major breakthrough in the treatment of PD. In addition, it is

well known that chronic L-Dopa therapy produces a decrease of the post-synaptic dopamine receptors, this fact being one possible cause of the progressive deterioration of its effectiveness in the treatment in the long range.   The SD, if really acting as we are proposing, could induce an increase in the affinity of these receptors to the drug.

Therefore, although we do not have as yet a satisfactory explanation for the improvement brought about by REM SD to the lesioned rats, we feel that a clinical trial of sleep deprivation in parkinsonian patients should be tried.

SUMMARY

Previous investigations have shown that REM sleep deprived (REM SD) rats display an enhanced response to dopamine agonists. This action seems to be mediated through a supersensitivity of dopamine post-synaptic receptors. Accordingly, REM SD was performed on rats with an experimental model of Parkinson's disease.   The animals were bilaterally lesioned in the nigrostriatal pathway through a stereotaxically directed electrical current.   Seven days after the surgery the animals were REM SD for 72 hours and immediatelly after the end of this period were observed in an open field for ambulation, rearing, grooming, and latency.   In comparison with non-deprived rats there was a significant increase in ambulation and rearing, a response that appeared again after a second REM SD period on day 21th after the surgery.   These data of improvement of two parameters of an experimental model of Parkinson's disease suggest that SD may be useful in this condition.

RESUMO

Privação de sono REM em um modelo experimental da doença de Parkinson.
Investigação prévia mostrou que ratos privados de sono (REM SD) mostram acentuação de resposta a agonistas dopaminérgicos. As evidências indicam que essa ação parece ser mediada por supersensibilização de receptores dopaminérgicos pós-sinápticos.   Com base nisso, foi feita REM SD em ratos com modelo experimental da doença de Parkinson, nos quais foi feita lesão eletrolítica bilateral de ambas as vias nigro-estriatais.   Sete dias após a cirurgia os animais eram submetidos a REM SD por 72 horas.   Imediatamente após o final deste período era feita observação em campo aberto para a ambulação, "rearing", "grooming" e latência.   Em comparação com ratos não-privados foi observado aumento significativo na ambulação e "rearing", resposta que reapareceu após um segundo período de REM SD, realizado 21 dias após a cirurgia. Estes dados, de melhora de dois parâmetros de modelo experimental da doença de Parkinson, sugerem que a privação de sono pode ser útil nesta doença.

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