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Research report

Blockage of dopaminergic D_2 receptors produces decrease of REM but not of slow wave sleep in rats after REM sleep deprivation

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Abstract

Dopamine (DA) has, as of late, become singled out from the profusion of other neurotransmitters as what could be called a key substance, in the regulation of the sleep–wake states. We have hypothesized that dopaminergic D_2 receptor blockage induced by haloperidol could generate a reduction or even an ablation of rapid eye movement (REM) sleep. Otherwise, the use of the selective D_2 agonist, piribedil, could potentiate REM sleep. Electrophysiological findings demonstrate that D_2 blockage produced a dramatic reduction of REM sleep during the rebound (REB) period after 96 h of REM sleep deprivation (RSD). This reduction of REM sleep was accompanied by an increment in SWS, which is possibly accounted for the observed increase in the sleep efficiency. Conversely, our findings also demonstrate that the administration of piribedil did not generate additional increase of REM sleep. Additionally, D_2 receptors were found down-regulated, in the haloperidol group, after RSD, and subsequently up-regulated after REB group, contrasting to the D_1 down-regulation at the same period. In this sense, the current data indicate a participation of the D_2 receptor for REM sleep regulation and consequently in the REM sleep/SWS balance. Herein, we propose that the mechanism underlying the striatal D_2 up-regulation is due to an effect as consequence of RSD which originally produces selective D_2 supersensitivity, and after its period probably generates a surge in D_2 expression. In conclusion we report a particular action of the dopaminergic neurotransmission in REM sleep relying on D_2 activation.

Keywords: Dopamine; REM sleep; Haloperidol; Piribedil; Rat; D_2 receptor

1. Introduction

From the plethora of neurotransmitters that orchestrate neural function dopamine has, as of late, become singled out as what can be referred to as a key substance in the regulation of the sleep–wake states [10,11,14,27]. Recently, dopaminergic neurons present in the ventral tegmental area (VTA) and in the substantia nigra pars compacta (SNpc) have been reported to be strongly associated to the regulation and probably with the generation of electrophysiological states of sleep, especially rapid eye movement sleep [4,11].

Clinically, it is observed that patients with Parkinson's disease (PD) who have extensive loss of dopaminergic cells within the SNpc, and less so within the VTA, often have increased sleepiness, which is aggravated by the presence of dopaminergic D_2 receptor agonists [2,3,9]. Otherwise, treatment with the D_2 antagonist and antipsychotic agent haloperidol attenuates hippocampal theta and gamma oscillations which is characteristic of REM sleep [5]. For instance, REM sleep could be recovered in the dopaminergic transporter knockout (DAT-KO) mice by selective activation of the D_2, but not the D_1, suggesting a particular role of this receptor in the regulation of REM sleep [5]. Such involvement of DA has been previously reported subsequent to sleep deprivation protocols, as being directly involved in the generation of burly dopaminergic D_2 supersensitivity [17,25,26].

To evaluate the participation of dopaminergic D_2 receptors in the regulation of REM sleep of rats we resorted to record

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the electrocorticographic activity of the Brattleboro rat, which is a lineage consistent with elevated density of D_2 receptors in both the dorsal and ventral striatum [23]. We hypothesized that dopaminergic D_2 blockage induced pharmacologically by haloperidol would most likely generate a reduction or even an ablation of REM sleep, considering that physiological function is dependent on dopaminergic D_2 autoreceptor activation. Otherwise, the use of the selective D_2 agonist, piribedil, could potentiate REM sleep. In view of the influence of the dopaminergic system over sleep patterns it becomes fundamental to comprehend the physiology underlying the systems that make use of this very important neurotransmitter so that improvements can be made in treatment protocols of sleep disturbances in PD patients.

2. Experimental procedures

2.1. Subjects

All experiments were conducted in accordance with National Institutes of Health (USA) guidelines for the care and use of animals and abided by an approved animal protocol from our university's ethical committee for animal experimentation (#0737/06). Male Brattleboro rats weighing 300–350 g at the beginning of the experiments were used. These were housed individually in transparent acrylic cages and maintained in standard laboratory conditions ($22 \pm 2\,^{\circ}C$, 12 h light/dark cycle, lights on 7:00 a.m.) with food and water provided *ad libitum*. Maximal efforts were employed to reduce the number of animals used in the experiments yet enough to ensure unambiguous and reliable statistical analysis and data interpretation.

2.2. Experimental design

In order to assess the influence of dopaminergic D_2 receptors in regulating REM sleep we adopted haloperidol as a D_2 antagonist and piribedil as a selective D_2 agonist. Two distinct groups of animals, named REM sleep deprived (RSD) and non-sleep deprived (NSD), were surgically implanted electrodes for electrophysiological recording of sleep–wake states. After 1 week of recovery the animals underwent the basal recording. Subsequently, the animals were allocated for a 96 h of REM sleep deprivation protocol, and the animals from the NSD group were conducted to their home cages, being allowed to sleep freely.

Subsequent to the RSD procedure groups received a single intraperitoneal (i.p.) injection of saline 0.9% or haloperidol (3.0 mg/kg) or piribedil (8.0 mg/kg). Immediately afterwards the distinct groups initiated the sleep–wake evaluation (initiated between 7:30 and 8:30 a.m.) for a period up to 48 h, called rebound period. The first 24 h of recording concentrated all the alterations reported. The following 24 h did not present statistical differences among the groups tested, indicating a clearance of the effects of the RSD and of the drugs to which subjects were exposure.

In parallel, a different set of animals underwent the same experimental design to determine the protein expressions of D_1, D_2 and tyrosine hydroxylase (TH). This set of animals was decapitated (between 7:30 and 9:00 a.m.) immediately after: drug administrations, RSD and REB. (For more details of the experimental design see Fig. 1.)

2.3. Stereotaxic surgery for sleep–wake cycle recording

Rats were distributed at random into the three groups named saline, haloperidol and piribedil. Animals were anesthetized with diazepam (10 mg/kg, i.p.) and ketamine (90 mg/kg, i.p.) and they were mounted in a classical stereotaxic frame (Insight Instruments). Body temperature was maintained at 37 °C with a regulated electric heating pad (Harvard Apparatus). Two bipolar electrodes with four stainless-steel screws (Ø 0.9 mm) were placed into the skull through small holes bored into the right lateral fronto-parietal (one pair) and in the left medial fronto-parietal (another pair) in order to monitor bipolar electroencephalogram (EEG). The free ends of the electrodes were soldered to a socket that was attached to the skull with acrylic dental cement. Two nickel–chromium flexible wires were inserted into the neck muscles in order to record the electromyogram (EMG). All the rats received penicillin (20,000 U in 0.1 ml, i.m.) and sodium diclofenac (25 mg/ml, i.p.) after surgery. One week after surgery, the sockets were connected via flexible recording cables and a commutator to a polygraph and computer. After 3 days with the cables the rats progressively habituated

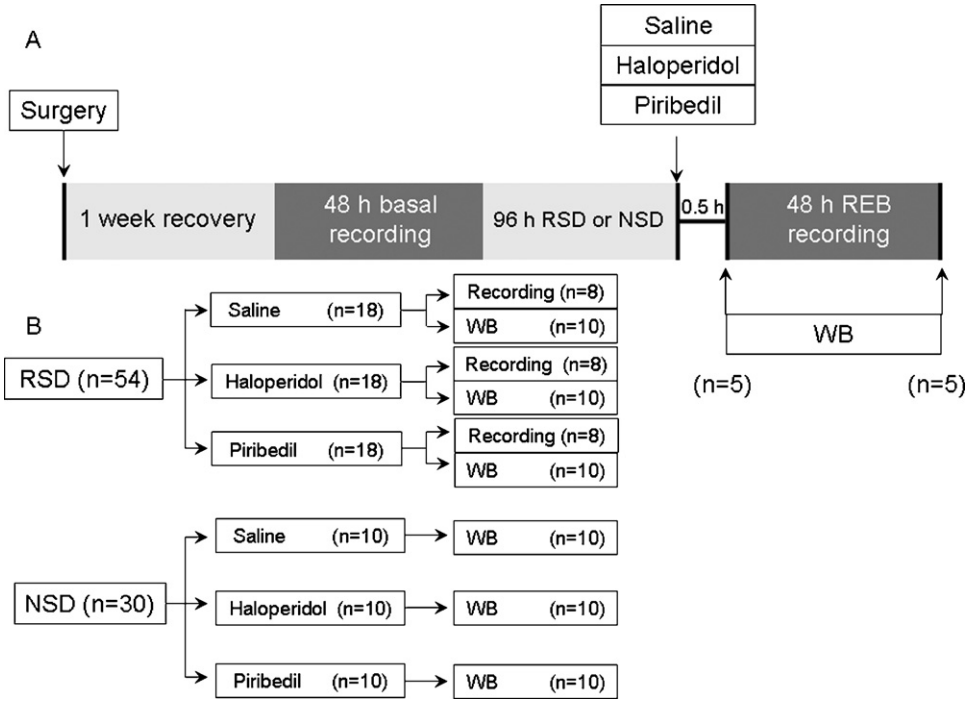

Fig. 1. Schematic representation of the experimental design. Panel A depicts a time-line illustration (not represented in scale) of the sequence of events throughout the experiments. Panel B illustrates the subdivisions of groups, with the respective amostral numbers for each examination. REM sleep deprivation, RSD; non-sleep deprived, NSD; rebound, REB; Western blotting, WB.