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Sleep disorders in Parkinson's disease: The contribution of the MPTP non-human primate model

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ABSTRACT

To replicate the sleep–wake disorders of Parkinson's disease (PD) and to understand the temporal relationship between these sleep disturbances and the occurrence of parkinsonism, we performed long-term continuous electroencephalographic monitoring of vigilance states in unrestrained rhesus monkeys using an implanted miniaturized telemetry device and tested the effect of MPTP intoxication on their sleep–wake organization. MPTP injection yielded a dramatic disruption of sleep–wake architecture with reduced sleep efficacy that persisted years after MPTP administration. Primary deregulation of REM sleep and increased daytime sleepiness occurring before the emergence of motor symptoms were a striking feature of the MPTP effect. This was concomitant with a breakdown of dopaminergic homeostasis, as evidenced by decreased dopamine turnover measured after a single MPTP injection. In the long term, partial re-emergence of REM sleep paralleled the partial adaptation to parkinsonism, the latter being known to result from compensatory mechanisms within the dopaminergic system. Altogether, these findings highlight the suitability of the MPTP model of PD as a tool to model the sleep/wake disturbances of the human disease. Ultimately, this may help in deciphering the specific role of dopamine depletion in the occurrence of these disorders.

Introduction

Sleep disturbances, excessive daytime sleepiness (EDS) and rapid eye movement (REM) sleep deregulation are among the most frequent and disabling non-motor manifestations of Parkinson's disease (PD) (Arnulf et al., 2002; Ghorayeb et al., 2007; Schenck et al., 1996). They may precede the cardinal motor features of the disease by years and may serve as early biomarkers of the premotor phase of PD (Abbott et al., 2005; Happe et al., 2007; Postuma and Montplaisir, 2006; Schenck et al., 1996). The pathophysiology of these disorders is far from being understood. In particular, it is still debated as to whether the underlying mechanisms of sleep disturbances in PD are related to dopamine (DA) deficiency. By contrast, the pathophysiology of the motor symptoms of PD has rapidly advanced due to the development of animal models including the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkey (Bezard et al., 1997; Langston et al., 1984). However, the relevance of the MPTP model remains almost unexplored with regard to sleep–wake disorders that occur in PD (Almirall et al., 1999), we investigated the suitability of the non-human primate model of PD to replicate the sleep–wake disturbances of the human disease and examined the temporal relationship between sleep parameter modifications and the occurrence of parkinsonian motor signs. To this end, we used a totally implantable telemetric device that allows long-term, continuous and simultaneous electroencephalographic (EEG), electrooculographic (EOG), electromyographic (EMG) and locomotor general activity monitoring in unrestrained freely moving monkeys. Beyond the scope of PD, this model would allow the investigation of sleep disturbances in the context of dopamine depletion.

Abbreviations: 5-HT, 5-hydroxytryptamine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EEG, electroencephalogram; EOG, electrooculogram; EMG, electromyogram; EDS, excessive daytime sleepiness; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; IR, immunoreactivity; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NE, norepinephrine; PBS, phosphate buffered saline; PD, Parkinson's disease; REM sleep, rapid eye movement sleep; RBD, REM sleep behavior disorder; SE, Sleep efficacy; SWS, slow wave sleep; TH, tyrosine hydroxylase; TST, total sleep time; WASO, wake after sleep onset.

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Materials and methods

Animals

Sixteen adult female rhesus monkeys (*Macaca mulatta*, Station de primatologie CNRS, Rousset, France) weighing 5 to 7 kg were used for this study. Seven of them were used for the sleep study part of the experiment while the nine others were used for *ex vivo* dosage of neurotransmitters. This diurnal species was chosen since it has been previously shown to best reproduce the human organization of the sleep and wake cycles (Balzamo et al., 1977; Kripke et al., 1968). All procedures were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) recommendations for laboratory animal care and use, and in accordance with the guidelines of the French Agriculture and Forestry Ministry. All efforts were made to minimize the number of animals used. Animals were housed in individual primate cages under controlled conditions of humidity (50%±20% relative humidity), temperature (24 °C±2 °C) and light (12-hour light/12-hour dark cycles, time lights on 8:00 a.m.). Food and water were available *ad libitum*. During the whole experiment, the animals roomed together with other animals in their home facility. By allowing almost normal social interactions through visual and auditory contacts, this reassuring environment provided normal restorative sleep in these animals as evidenced by our baseline sleep data.

Apparatus

For the sleep monitoring part of the experiment, seven animals were chronically implanted with a radio-telemeter transmitter for continuous and long-term recording. Of these, five were healthy animals (monkeys #1 to #5) and served to test the time course of sleep disturbances and motor impairment appearance after MPTP administration. The remaining two animals (monkeys #6 and #7) were previously exposed to MPTP and exhibited stable and chronic parkinsonism for several years (3 years) and served to characterize the sleep–wake architecture deterioration in the chronic parkinsonism condition. The transmitter implant consisted of a sterile disk-shaped three-channel biopotential device (D70-EEE, Data Sciences International, St Paul, MN, USA) for measuring EEG, EOG and EMG signals with a sampling rate of 500 Hz. The device also allowed the continuous evaluation of the general level of locomotor activity in the implanted animals. An incorporated magnetically activated switch enabled the implanted transmitter to be switched on and off externally. Extending from the transmitter body were flexible leads consisting of one ground lead and 3 channels, each made of two 60 cm helical stainless-steel electrode wires enclosed in flexible silicone tubing. Biopotential signs were transmitted to two receivers mounted on the primate cage and then forwarded to a data exchange matrix serving as a multiplexer and connected through a ribbon cable to a computer for data recording and storage.

After premedication with 1 mg/kg diazepam (Valium, Roche), 0.05 mg/kg atropine sulfate and 10 mg/kg ketamine chlorhydrate given intramuscularly, animals were intubated and anesthetized with isoflurane volatile anesthetic. The transmitter was implanted within the abdominal muscle layers. The electrode biopotential leads were tunneled subcutaneously to the skull and the electrode tips affixed unilaterally and secured with dental acrylic cement at the level of the orbital arch bone (EOG channel) and the parieto-occipital part of the skull (EEG channel). The two remaining EMG leads were sutured to the lateral trapezius muscle.

Prophylactic antibiotic cover was started 1 day preoperatively and provided for 2 additional weeks postoperatively by subcutaneous administration of amoxicillin (Duphamox* LA, Fort Dodge A.H.) every other day. Analgesia was provided on the day of the surgery by a single intramuscular injection of 2 mg/kg ketoprofene (Ketofen*, Merial, France) that was re-administered once a day for 10 days.

Experimental design

Animals were allowed to recover from surgery for 1 month. The transmitter was then switched on and continuous data collection started for a 2- to 4-week period to establish the baseline sleep and daytime vigilance natural organization and alternation in healthy animals (monkeys #1 to 5) prior to MPTP administration and to characterize the sleep architecture in monkeys with chronic parkinsonism (monkeys #6 and 7). Subsequently, and following the same intoxication paradigm that was applied to monkeys #6 and #7, monkeys #1, #2 and #3 received 0.5 mg/kg of MPTP intravenously. A total of three injections for monkey #1 and four injections for monkeys #2 and #3 were sufficient to induce progressive and severe parkinsonism (Ghorayeb et al., 2000). Monkeys #4 and #5 received saline and served as controls. A continuous 2-week post-MPTP recording period was available for analysis in all animals, and in monkeys #2 and #3 data collection was pursued until 3 months post-MPTP when chronic parkinsonian symptoms were stable.

Clinical monitoring

Motor status was monitored each afternoon during a 15-min observation period using a semi-quantitative scale of primate parkinsonism assessing the following clinical symptoms: tremor, general level of spontaneous activity, body posture, vocalization, freezing, rigidity and movements of each arm (Imbert et al., 2000). Disability score ranged from 0 (normal) to 25 (maximal parkinsonian severity). The general level of daytime and nighttime locomotor activity was continuously and simultaneously recorded by the implanted radio telemetry transmitter throughout the experimental design (Barcia et al., 2004). In addition, continuous infrared video monitoring was performed to detect the occurrence of any sleep-related movement disorders before and after MPTP injection.

Recording and analysis of vigilance states

Sleep data were analyzed from 8 pm (lights off) to 8 am (lights on). To investigate the monkeys' natural propensity to sleep during the daytime in their home facility, staging of vigilance states during the day was also performed from 8 am to 8 pm.

Sleep staging was performed offline on SleepSign™ for Animal software (Kissei Comtec Co., Ltd, Matsumoto, Nagano, Japan). Scoring of sleep staging was manually performed according to the Rechtschaffen and Kales criteria for the scoring of sleep in humans (Rechtschaffen and Kales, 1968). Vigilance stage scoring was performed, as in humans, in 30-second epochs so that the whole epoch was assigned to one stage. The vigilance stages identified were wake, light sleep (stages 1–2), slow wave sleep (stages 3–4) (SWS) and REM sleep. Wake was considered when low amplitude mixed-frequency EEG with high amplitude EOG and excessive muscle tone were observed (Fig. 1A). Stage 1 was scored when slow eye movements with a low voltage, mixed-frequency EEG and regular and lowered muscle tone appeared, whereas stage 2 was scored with the first appearance of specific features common to human stage 2, namely K complexes and spindles, and absence of eye movements (Fig. 1B). As in humans, SWS was considered present when slow frequency but high amplitude EEG waveforms occurred. Sleep was staged 3 when these waveforms accounted for more than 25% but less than 50% of the epoch, and stage 4 was staged when more than 50% of the epoch was occupied by these waves (Fig. 1C). REM sleep was considered when the background EEG returned to a low-voltage mixed-frequency activity with characteristic sawtooth waves, prolonged phasic eye movements and absent muscle tone (Fig. 1D).