Interleukin-1β Mediates Sleep Alteration in Rats With Rotenone-Induced Parkinsonism

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Study Objective: Recently, the pathogenesis of Parkinson disease (PD) has been focused on microglial activation, especially the subsequent increase of cytokines. A body of clinical evidence suggests that sleep is altered in patients with PD; however, there is a lack of understanding of the basic cellular mechanism. This study was designed to elucidate the influence of brain interleukin (IL)-1β on sleep changes, in addition to the dopaminergic and γ-aminobutyric acid (GABA)-ergic systems, in an animal PD model.

Design: We employed a long-term subcutaneous infusion of rotenone, a mitochondrial complex-I inhibitor, to induce a parkinsonism-like model in rats. Behavioral tests and tyrosine hydroxylase immunocytochemistry were used for confirmation of PD in this animal model. Pharmacologic agonist and antagonists were administered centrally to test the involvement of dopamine, GABA, and IL-1 in rotenone-induced sleep alteration. Protein expression of cytokines, ie, IL-1β and tumor necrosis factor α (TNF-α), in 5 distinct brain regions was also determined by Western blot and enzyme-linked immunosorbent assay (ELISA).

Setting: Sleep-recording equipment in the National Taiwan University and China Medical University.

Participant and Interventions: Male Sprague-Dawley rats were implanted with electromyograph electrodes, a thermistor, and an intracerebroventricular guide cannula. Chronic infusion of rotenone was given by an Alzet minipump implanted subcutaneously on the back of each rat.

Measurement and Results: We found that locomotion activity was reduced, slow-wave sleep (SWS) was increased during the dark (active) phase and decreased during the light (rest) period, and rapid eye movement sleep (REM) was enhanced in the dark period after rotenone treatment. This rotenone PD animal model successfully causes loss of tyrosine hydroxylase-immunopositive neurons in the substantia nigra; induces the events of sleep disturbance, such as excessive daytime sleepiness and insomnia during the nighttime, that are seen in patients with PD; and suppresses locomotion. Our results that intracerebroventricular administration of dopamine and blockade of GABA in the brain have less significant effect on rotenone-induced sleep alteration suggest that the sleep disturbance is not primarily mediated by the disruption of dopaminergic and GABAergic systems in the current PD rat model. The expression of TNF-α was not altered by rotenone. However, the results of enhanced expression of IL-1β in the hypothalamus after rotenone and that of the blockade of sleep alteration, but not the locomotion activity, by intracerebroventricular administration of an IL-1 receptor antagonist implies that increased IL-1β in the hypothalamus mediates sleep alteration, but not the locomotion, in rats with rotenone-induced parkinsonism.

Conclusion: These observations suggest that rotenone-induced sleep-wake alteration is dominated by central increase of somnogenic IL-1.

Keywords: parkinsonism, sleep, IL-1, dopamine, and GABA

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INTRODUCTION

THE PREDOMINANT LESION IN PARKINSON DISEASE (PD) IS THE DEGENERATION OF PIGMENTED NEURONS IN THE PARS COMPACTA OF THE SUBSTANTIA NIGRA (SNpc) and deficient dopamine in the striatum. In addition to the motor symptoms, most patients with PD experience sleep disturbances, particularly insomnia and excessive daytime sleepiness (EDS). Both the pathology of PD and dopaminergic drugs may contribute to a much higher than expected frequency of sleep fragmentation and disrupted sleep. It is difficult to differentiate the causes of sleep disorders that are induced by medication or the neurologic condition itself, since there is little information on sleep in patients with untreated PD who are in an advanced stage of the disease. Therefore, we employed an animal model of PD by chronically subcutaneously infusing rotenone to determine the subsequent sleep architecture. Rotenone, an inhibitor of the neuronal mitochondrial complex I, produces specific dopaminergic cell degeneration accompanied by formation of α-synuclein of cytoplasmic inclusions, which are similar to those found in patients with PD.

The anatomic basis for the sleep disturbance in PD is still not clear. However, as the disease progresses, the neurodegeneration of dopaminergic neurons in the substantia nigra may cause the sleep alteration. Dopamine has been regarded as a wake promoter. Administration of dopaminergic analogues leads to increased activation of cortical electromyograph (EEG) and behavioral excitation in normal animals. The inhibitory output, mediated by γ-aminobutyric acid (GABA), from the internal segment of the globus pallidus (GPI) to the thalamus is subsequently enhanced as the PD progresses. It is well known that GABA-containing neurons in the hypothalamus and basal forebrain, which give rise to cortical projections, mediate the induction or maintenance of...
slow-wave sleep (SWS). It has also been demonstrated that the firing rate of GABAergic neurons in the globus pallidus increases during wakefulness, as compared with SWS, and that blockade of thalamic GABA 

receptor decreases EEG slow waves. Therefore, the involvement of dopaminergic and GABAergic neurons in rotenone-induced sleep alteration was investigated in this study.

Microglial activation and the subsequently increased release of inflammatory cytokines, such as interleukin (IL)-1β, IL-2, IL-4, IL-6 and tumor necrosis factor-alpha (TNF-α), in the nigrostriatal regions and in cerebrospinal fluid have been hypothesized to be the pathogenesis of PD. Several lines of evidence implicate the effects of TNF-α and IL-1 in physiologic sleep regulation. In this study, we then determined whether the expressions of IL-1β and TNF-α in 5 distinct brain regions, including the hypothalamus, hippocampus, brain stem, striatum, and cortex, had been altered and demonstrated the involvement of somnogenic cytokines in this PD model.

**MATERIALS AND METHODS**

**Substances**

Stock solutions of dopamine hydrochloride (Sigma, St. Louis, MO), bicuculline methobromide (Tocris, Bristol, UK), 2-hydroxysaclofen (Tocris), and human recombinant IL-1 receptor antagonist (IL-1ra) (Bachem, Torrance, CA) were dissolved in pyrogen-free saline (PFS). The rotenone (Sigma) was dissolved in 2 mL of a mixture of 50% dimethyl sulfoxide (DMSO) and 50% polyethylene glycol (PEG) (Sigma). These stock solutions were stored at -20°C until use. The doses of the substances used in these experiments were as follows: for dopamine, 30 and 100 nmol (5.7 and 19.0 μg, respectively); for bicuculline, 0.5, 1.0, and 2.5 μg; for 2-hydroxysaclofen, 0.5, 1.0, and 2.5 μg; for human IL-1ra, 0.1 and 0.2 μg. Rotenone dissolved in 2 mL of 1:1 DMSO:PEG were filled into an Alzet pump (model 2ML4; Durect Corp, Cupertino, CA), and the pump was implanted under the skin on the back of each rat. The rotenone was subcutaneously released at the rate of 3 mg/kg per day.

**Animals**

Male Sprague-Dawley rats (250 - 300 g; National Laboratory Animal Breeding and Research Center, Taiwan) were used in these experiments. These animals were anesthetized (ketamine/xylazine; 87/13 mg/kg), and injected with an analgesic (butorphanol tartrate) and a broad-spectrum antibiotic (penicillin G benzathine). The rats were surgically implanted with EEG screw electrodes, a guide cannula directed into the lateral ventricle, and a calibrated 30-kΩ thermistor (Model #44008; Omega Engineering, Stamford, CT) to monitor brain temperature at the surface of the cortex, as has been previously described. Insulated leads from the EEG electrodes and thermistor were routed to a Teflon pedestal (Plastics One, Roanoke, VA). The Teflon pedestal was then cemented to the skull with dental acrylic (Cranioplastic cement and Cyanoacrylate gel, Plastics One). The incision was treated topically with polysporin (polymixin B sulfate – bacitracin zinc), and the animals were allowed to recover for 7 days prior to the initiation of the experiments. The rats were housed in individual recording cages, 2 cages in each environmentally controlled chamber (COCONO model # LE-539; Ron-Fong Technology Corporation, HsinChu, Taiwan). The chambers were maintained at 23°C ± 1°C with a 12:12 hour light:dark cycle (20 Watt × 6 tubes illumination), and food and water were available ad libitum. All procedures performed in these studies were approved by the China Medical University Hospital Animal Care and Use Committee.

On the second postsurgical day, the rats were connected to the recording apparatus (see later) via a flexible tether. Three days after surgery, the patency and free drainage of the intracerebroventricular (ICV) cannulae were assessed by administering 200 to 400 ng angiotensin II [human angiotensin II octapeptide; Tocris Cookson, Inc.]; angiotensin elicited a drinking response mediated by structures in the preoptic area. At the end of each experimental protocol, all rats were again injected with angiotensin; only data from those rats that exhibited a positive drinking response were included in the subsequent analyses. The animals were habituated by daily handling and ICV injections of PFS timed to coincide with scheduled experimental administrations.

**Apparatus and Recording**

Signals from the EEG electrodes were fed into an amplifier (Colbourn Instruments, Lehigh Valley, Penn; model V75-01). The EEG was amplified (factor of 5000) and analog bandpass filtered between 0.1 and 40 Hz (frequency response: ± 3 dB; filter frequency roll off: 12 dB/octave). Signals from the thermistors were fed into an amplifier (Axon Instruments, Union City, CA; model: CyberAmp 380). Gross body movements were detected by custom-made infrared-based motion detectors (Biosobserve GmbH, Germany), and the movement activity was converted to a voltage output, which was digitized and integrated into 1-second bins. These conditioned signals (EEGs, brain temperature, gross body movements) were subjected to analog-to-digital conversion with 16-bit precision at a sampling rate of 128 Hz (NI PCI-6033E; National Instruments, Austin, Tex). The digitized EEG waveform and integrated values for body movement were stored as binary computer files until subsequent analyses.

Postacquisition determination of vigilance state was done by visual scoring of 12-second epochs using custom software (ICELUS, M. R. Opp) written in LabView for Windows (National Instruments). The animals’ behavior was classified as either SWS, rapid eye movement (REM) sleep, or waking based on previously defined criteria. Briefly, SWS is characterized by large-amplitude EEG slow waves, high power density values in the delta frequency band (0.5 - 4.0 Hz), lack of gross body movements, and declining brain temperature before and during entry. During REM sleep, the amplitude of the EEG is reduced, the predominant EEG power density occurs within the theta frequency (6.0 - 9.0 Hz), brain temperature increases rapidly at onset, and there are phasic body twitches. During waking, the rats are generally active, there are protracted body movements, brain temperature gradually increases, the amplitude of the EEG is similar to that observed during REM sleep, but power density values in the delta frequency band are generally greater than those in theta frequency band.

**Behavioral Tests**

Both the pole and the traction tests, which were modified from the evaluation of limb weakness on mice, were validated in...
rats. The pole test was performed to detect impairment of limb movement after rotenone administration. A cork ball (7.5-cm diameter) was fixed to the top of a vertical pole (50-cm length and 3-cm diameter). The pole was wrapped with a double layer of gauze to prevent slipping. The animal was placed on the cork ball. The times required for turning downward on the ball, for climbing down the upper half of the pole, and for climbing down the lower half of the pole were recorded and the performance was scored as 3 if within 3 seconds, 2 if within 6 seconds, and 1 if over 6 seconds, and the results were expressed as the total score. Limb impairment was also assessed by a traction test, hanging a rat from a horizontal wire by its forepaws. The rat was scored as 3 for gripping the wire with both hind paws, 2 for gripping the wire with 1 hind paw, and 1 for not gripping the wire with either hind paws. The results were expressed as the total score.

**Tyrosine Hydroxylase Immunocytochemistry**

A 40-μm slice of 4% paraformaldehyde-fixed brain was prepared for staining with a monoclonal rat reactive tyrosine hydroxylase (TH) antibody (Chemicon International, Inc., CA) and ABC staining kit (Santa Cruz Biotechnology, Inc., CA). After being rinsed in phosphate buffered saline (PBS), the sections were incubated in PBS with 5% normal goat serum, which served as a blocking solution, for 1 hour at ambient temperature. Then, these sections were further incubated with primary TH antibody (1:2000) in blocking solution (3% normal goat serum, 1% Triton X-100 in PBS) for 24 hours at 4°C. The slices were washed 3 times with PBS after incubation. The secondary antibody was biotinylated goat anti-mouse immunoglobulin G (IgG) (Santa Cruz Biotechnology), diluted at 1:250 in a blocking solution (3% normal goat serum, 0.1% Triton X-100 in PBS). Avidin-biotin-peroxidase complex and 3,3′-diaminobenzidine tetrachloride (DAB, Sigma) were used to visualize TH-immunopositive neurons in the SNpc. The TH-staining cells were counted bilaterally in the SNpc, as shown in a rat brain atlas from bregma coordinates -4.8 mm to -6.4 mm by an individual blinded to the experimental treatment. The counts of neurons were averaged at least over 3 sections for each rat.

**Experimental Protocol**

A total of 92 Sprague-Dawley rats was used and divided into 12 groups in this study. All rats were implanted subcutaneously with an Alzet minipump filled with PFS, 1:1 DMSO/PEG, or rotenone in 1:1 DMSO/PEG. The release rate of rotenone is 3 mg/kg per day for a total of 28 days in the following experiments. Sixteen rats received chronic infusion of PFS, 28 rats were given 1:1 DMSO/PEG, and 40 rats received rotenone (groups 1-11). An additional 8 rats served as control without implantation of the minipump (group 12). Those rats that received PFS and 1:1 DMSO/PEG all survived after a 28-day infusion; however 4 rats died after a 28-day infusion of rotenone. The following sample size described included those that survived after treatment. Rats in group 1 (n = 8) were subcutaneously implanted with the minipump filled with PFS; those in group 2 (n = 8) were infused with 1:1 DMSO/PEG. Rats in group 3 (n = 8) were implanted with a minipump filled with rotenone. The locomotion activity, pole test, and traction test were assessed in these 3 groups before and after infusion. Then, these rats were sacrificed and perfused with 4% paraformaldehyde for fixation and 40-μm thick brain slices, used for the TH immunocytochemistry (as described above), were sliced coronally by cryotome at the same brain level. Rats in groups 4 (n = 8), 5 (n = 8), 6 (n = 8), and 7 (n = 8) were surgically implanted with EEG electrodes and an ICV cannula, as previously described. After recovery, a 24-hour baseline recording and the recording after ICV administration of PFS were obtained. Then, an Alzet osmotic minipump was subcutaneously implanted; rats in group 4 received PFS, those in group 5 received 1:1 DMSO/PEG, and rats in both group 6 and 7 received rotenone. Subsequently, ICV administration of PFS was given 20 minutes prior to the beginning of the dark period, and the 24-hour sleep-wake activity was recorded from the dark period at the 28th day after chronic infusion. The rats in group 6 were then ICV administered 2 doses (30 and 100 nmol) of dopamine on the 29th and 30th day; the order of receiving these doses was randomized. During the 31st day, rats were ICV administered with PFS, and another 24-hour sleep-wake activity was recorded. Our results found that the sleep-wake activity recorded from the 31st day did not differ from that recorded from the 28th day (data not shown), which indicates that the effect of dopamine had been washed out. Then, these rats were randomly ICV administered 2 doses (100 and 200 ng) of IL-1ra in the subsequent 2 days (the 32nd and 33rd day). Rats in group 7 were in the similar protocol as those in group 6, except that they randomly received 3 doses (0.5, 1.0, and 2.5 μg) of bicuculline on the 29th, 30th, and 31st days and received 3 doses of 2-hydroxyascolafen (0.5, 1.0, and 2.5 μg) on the 32nd, 34th, and 35th days. Another ICV PFS recording was obtained on the 32nd day, and it also revealed no difference from that recorded from the 28th day (data not shown). The choice of the doses of dopamine, bicuculline, 2-hydroxyascolafen, and IL-1ra used in current experiment was primarily based on our previous experience. Rats in group 8 (n = 4) chronically received 1:1 DMSO/PEG, and those in group 9 (n = 4) given rotenone were decapitated at the 28th day at the beginning of the dark period, and the 5 distinct brain regions (ie, hypothalamus, hippocampus, cortex, brainstem, and striatum) were dissected and frozen at -80°C until used. These tissues were used for the Western blot of IL-1β and TNF-α, as described in following text. Rats in groups 10 (n = 8) and 11 (n = 8) received 1:1 DMSO/PEG and rotenone, respectively, and were also decapitated at the 28th day at the beginning of the dark period, and the 5 distinct brain regions were dissected. These tissues were used for the IL-1β ELISA. Rats in group 12 (n = 8) were implanted with EEG electrodes and ICV cannulae and served as control normal rats to assess the effects of ICV administration of dopamine, bicuculline, or 2-hydroxyascolafen prior to the dark period on spontaneous sleep-wake activity. The summary of group division and the experimental protocol are depicted as Figure 1.

**Western Blotting for IL-1β and TNF-α**

Tissue lysates were separated on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The 43-KD β-actin was used as an internal control. After the run, the proteins were transferred onto polyvinylidene fluoride (PVDF) membrane (Immobilon™-P transfer membrane, Cat. No. IPVH00010, Millipore, Bedford, MA) followed by blocking of the membrane overnight at 4°C with 5% (w/v) nonfat dried milk in tris-buffered saline with Tween 20 (TBST), pH 7.6 (0.02 M Tris-HCl, 0.14 M NaCl, and 0.1% Tween 20; Sigma-Aldrich). The membranes were

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incubated with anti-IL-1β or anti-TNF-α monoclonal primary antibody (R&D System, Inc; 1:500) in TBST containing 5% (w/v) nonfat dried milk for 1 hour at room temperature, and then washed several times in TBST, followed by incubation with horse radish peroxidase (HRP) conjugated anti-mouse IgG antibody (R&D System, Inc; 1:2000) for 1 hour at room temperature, and washed as above. Protein signals were detected using an enhanced chemiluminescent kit (Chemiluminescence Reagent Plus, Cat. No. NEL104, NEL105, PerkinElmer Life Sciences Inc., Boston, MA). Band density was quantified using the Kodak 1D software (Eastman Kodak, Rochester, NY).

ELISA for IL-1β

The rat IL-1β ELISA kits were obtained from the Pierce Biotechnology, Inc. (Rockford, IL), and the procedure was according to the standard instruction provided by the manufacturer. The absorbance was measured by an ELISA plate reader (Multiskan EX, Thermo Electron Corp., Waltham, MA) set wavelength at 450 nm and 550 nm. The sensitivity is < 12 pg/mL, and the assay range is between 25.5 and approximately 2500 pg/mL. Intraassay precisions of variation are between 2.9% and 11.6%, and interassay precisions of variation are between 4.5–11.5%. The rat IL-1β ELISA kits were obtained from the Pierce Biotechnology, Inc. (Rockford, IL), and the procedure was according to the standard instruction provided by the manufacturer. The absorbance was measured by an ELISA plate reader (Multiskan EX, Thermo Electron Corp., Waltham, MA) set wavelength at 450 nm and 550 nm. The sensitivity is < 12 pg/mL, and the assay range is between 25.5 and approximately 2500 pg/mL. Intraassay precisions of variation are between 2.9% and 11.6%, and interassay precisions of variation are between

Figure 2—Rotenone-induced loss of tyrosine hydroxylase (TH)-immunoreactive neurons in the substantia nigra pars compacta (SNpc) (in groups 1-3). Coronal brain sections obtained from a 28-day subcutaneous infusion of pyrogen free saline (PFS) (A), 1:1 of 50% dimethyl sulfoxide and 50% polyethylene glycol (DMSO/PEG) (A) and rotenone in 1:1 DMSO/PEG (B). Slices demonstrated in the PFS-treated and 1:1 DMSO/PEG-treated rats were from the right side of the substantia nigra, and the top 2 slices in B were from the left side, and the bottom 2 slices were obtained from the right side in rats received 3 mg/kg per day of rotenone for 28 days. The cell number of TH-immunoreactive (TH-ir) neurons in the substantia nigra pars compacta (SNpc) are shown in C. Open bar: PFS; hatched bar: rotenone in 1:1 DMSO/PEG. Arrows indicate the areas used for cell counting. VTA refers to the ventral tegmental area.
The lesion of dopaminergic neurons in substantia nigra after a 28-day rotenone treatment was confirmed by in situ immunocytochemistry for TH. Since the rotenone was dissolved in 1:1 DMSO/PEG, we compared the density of TH-immunopositive cells in the SNpc among the rats that received PFS (group 1), 1:1 DMSO/PEG (group 2), and rotenone (group 3). In Figure 2A, we show 2 slices of the right-side SNpc from the PFS- and 1:1 DMSO/PEG-treated rats, respectively. We found that there was significant difference between control and manipulation. The statistical evaluation for the TH-immunopositive cell, pole test, traction test, and ELISA of IL-1β used an unpaired student t-test comparing the averages between the groups analyzed.

### RESULTS

#### Rotenone Successfully Induces Parkinsonism in Rats

The lesion of dopaminergic neurons in substantia nigra after a 28-day rotenone treatment was confirmed by in situ immunocytochemistry for TH. Since the rotenone was dissolved in 1:1 DMSO/PEG, we compared the density of TH-immunopositive cells in the SNpc among the rats that received PFS (group 1), 1:1 DMSO/PEG (group 2), and rotenone (group 3). In Figure 2A, we show 2 slices of the right-side SNpc from the PFS- and 1:1 DMSO/PEG-treated rats, respectively. We found that there was

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**Figure 3**—Effects of chronic subcutaneous administration of rotenone on sleep-wake activity (A) and the influence of intracerebroventricular (ICV) dopamine on rotenone-induced sleep-wake alteration (B) in group 6. Individual data points depict the mean ± SEM values. A & B: The open circles indicate the control values, in which the rats received ICV pyrogen free saline (PFS) before the subcutaneous infusion of rotenone. The closed circles represent the data obtained from the same group of rats, which received ICV PFS after a 28-day chronic administration of rotenone. ICV administration was given 20 minutes prior to the beginning of the dark period. Open triangles denote the values obtained after ICV administration of 100 nmol dopamine in the chronic rotenone-treated rats. ICV PFS + rotenone; closed circle, 30 nmol ICV dopamine + rotenone; open triangle, 100 nmol ICV dopamine + rotenone. The inset depicts the mean ± SEM of the body movement (mV) during the 24-h recording period. The Y axis is the amount of body movement (mV). From left to right: open bar, ICV PFS; closed bar, ICV PFS + rotenone; hatched bars, ICV 30 & 100 nmol dopamine + rotenone. D: Locomotion activity. Open circle, ICV PFS; shaded area, ICV PFS + rotenone; closed circle, ICV 30 & 100 nmol dopamine + rotenone. *Represents statistically significant difference between the values obtained after ICV administration of 100 nmol dopamine in the chronic rotenone-treated rats. C: The summary bar graph from data in A & B. The left 4 bars indicate the values obtained during the dark period and the right 4 bars were obtained from the light period. From left to right: open bar, ICV PFS; closed bar, ICV PFS + rotenone; hatched bars, ICV 30 & 100 nmol dopamine + rotenone. The inset depicts the mean ± SEM of the body movement (mV) during the 24-h recording period. The Y axis is the amount of body movement (mV). From left to right: open bar, ICV PFS; closed bar, ICV PFS + rotenone; hatched bars, ICV 30 & 100 nmol dopamine + rotenone. *Represents statistically significant difference between the values obtained from the same group of rats, which received ICV PFS after a 28-day chronic administration of rotenone. The closed circles represent the data obtained from the same group of rats, which received ICV PFS after a 28-day chronic administration of rotenone. ICV administration was given 20 minutes prior to the beginning of the dark period. Open triangles denote the values obtained after ICV administration of 100 nmol dopamine in the chronic rotenone-treated rats. ICV PFS + rotenone; closed circle, 30 nmol ICV dopamine + rotenone; open triangle, 100 nmol ICV dopamine + rotenone. The inset depicts the mean ± SEM of the body movement (mV) during the 24-h recording period. The Y axis is the amount of body movement (mV). From left to right: open bar, ICV PFS; closed bar, ICV PFS + rotenone; hatched bars, ICV 30 & 100 nmol dopamine + rotenone. *Represents statistically significant difference between the values obtained from the control and rotenone treatment. *Denotes the statistically significant difference between the values obtained from the ICV 100 nmol dopamine + rotenone and those from the ICV PFS + rotenone. The dark and open portions of horizontal bars represent the dark and light periods of the 12:12 hours light:dark cycle. WAKE refers to wakefulness; REMS, rapid eye movement sleep; SWS, slow-wave sleep.

6.2% and 12.3% (manufacturer’s specifications). This ELISA kit is specific for the measurement of natural and recombinant rat IL-1β and does not cross-react with rat IFNγ, IL-1α, IL-2, IL-4, IL-6, IL-10, MCP-1, MIP-1α, or RANTES.

**Statistical Analyses for Experiment Protocol**

All values were presented as the mean ± SEM for the indicated sample sizes. One-way analyses of variance (ANOVA) for the duration of each vigilance state (SWS, REM sleep, WAKE) and for sleep-architecture parameters were performed, comparing before and after manipulation within subjects, across the two 12-hour time blocks. If statistically significant differences were detected, posthoc comparisons were made to determine which hourly intervals during experimental conditions deviated from values obtained from the same animals during control conditions. An α level of p ≤ .05 was taken as indicating a statistically significant difference between control and manipulation. The statistical evaluation for the TH-immunopositive cell, pole test, traction test, and ELISA of IL-1β used an unpaired student t-test comparing the averages between the groups analyzed.
Table 1—Effects of Long-Term Treatment of Rotenone, ICV IL-1ra 200 ng, and ICV Dopamine 100 nmol On Sleep-Wake Architecture Parameters of Rats

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>Hour</th>
<th>L:D Cycle</th>
<th>Number of boutsa</th>
<th>Bout durationb</th>
<th>Transitions</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>WAKEc</td>
<td>SWSd</td>
<td>REMc</td>
</tr>
<tr>
<td>ICV PFS (control)</td>
<td>1-12</td>
<td>D</td>
<td>4.7 ± 0.5</td>
<td>6.2 ± 0.5</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>+ ICV PFS SC rotenone</td>
<td>1-12</td>
<td>D</td>
<td>4.6 ± 0.5</td>
<td>7.3 ± 0.7</td>
<td>3.0 ± 0.8*</td>
</tr>
<tr>
<td>+ ICV IL-1ra SC rotenone</td>
<td>1-12</td>
<td>D</td>
<td>4.2 ± 0.3</td>
<td>5.0 ± 0.5#</td>
<td>1.3 ± 0.4#</td>
</tr>
<tr>
<td>+ ICV dopamine ICV PFS (control)</td>
<td>13-23</td>
<td>L</td>
<td>5.4 ± 0.8</td>
<td>7.0 ± 1.0</td>
<td>2.1 ± 0.6</td>
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<tr>
<td>SC rotenone</td>
<td></td>
<td></td>
<td>6.7 ± 0.3</td>
<td>9.9 ± 0.6</td>
<td>1.5 ± 0.5</td>
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<tr>
<td>+ ICV PFS SC rotenone</td>
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<td>6.8 ± 0.5</td>
<td>9.3 ± 0.7</td>
<td>1.0 ± 0.3</td>
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<tr>
<td>+ ICV IL-1ra SC rotenone</td>
<td>13-23</td>
<td>L</td>
<td>5.8 ± 1.4</td>
<td>7.7 ± 1.9</td>
<td>0.9 ± 0.3</td>
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<tr>
<td>+ ICV dopamine ICV PFS (control)</td>
<td>13-23</td>
<td>L</td>
<td>7.0 ± 0.7</td>
<td>9.0 ± 1.0</td>
<td>0.7 ± 0.3</td>
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Data are presented as mean ± SEM. Differences were detected by 1-way analyses of variance within the indicated time blocks. * denotes a statistically significant difference between values obtained during intracerebroventricular (ICV) pyrogen-free saline (PFS; control) and those obtained after long-term treatment of rotenone (3 mg/kg per day for 28 days) with ICV PFS. # denotes a statistically significant difference between values obtained after the ICV PFS with rotenone and those obtained after ICV IL-1ra (200 ng) or ICV dopamine (100 nmol) with rotenone.

aNumber of bouts per hour (mean ± SEM) for each vigilance state.
bMean (± SEM) bout duration (in minutes) for each vigilance state.
cNumber of transitions from one behavioral state to another (mean ± SEM) per hour.
dExperimental manipulation.

eManipulation time blocks.

Table 1—Effects of Long-Term Treatment of Rotenone, ICV IL-1ra 200 ng, and ICV Dopamine 100 nmol On Sleep-Wake Architecture Parameters of Rats

- **No significant change in cell numbers of the TH-immunopositive cells in SNpc between rats that received PFS and those that received 1:1 DMSO/PEG (Figure 2C).** However, a chronic subcutaneous infusion of rotenone induces a symmetrically marked loss of density of the TH-immunopositive neurons in the SNpc, as shown in Figure 2B (n = 8; p < .05 comparing values obtained after rotenone treatment [group 3] with those obtained from 1:1 DMSO/PEG treatment [group 2], unpaired student t-test). The locomotor activity was significantly reduced from 222.9 ± 2.7 mV, obtained before rotenone administration, to 210.6 ± 2.9 mV, obtained at the 28th day after chronic rotenone infusion (Figure 3D, n = 8, p < .05, 1-way ANOVA comparing data obtained before and after rotenone treatment within subjects). However, long-term administration of 1:1 DMSO/PEG did not alter locomotor activity in our observation. In addition, the reduced scores from the pole test and traction test in rats treated with rotenone implicate an impairment in limb strength. The difference between the scores of the pole test obtained from rotenone-treated rats and from the PFS-treated rats achieved statistical significance, which were 1.5 ± 0.2 and 2.8 ± 0.2 (n = 8, p < 0.05, unpaired student t-test comparing groups 1 and 3), respectively. The values of the traction test were statistically decreased from 2.8 ± 0.2 obtained from the control rats to 1.5 ± 0.2 after treated with rotenone (n = 8, p < 0.05, unpaired student t-test comparing between groups 1 & 3). Administration of 1:1 DMSO/PEG did not alter any aspect in the pole test and the traction test when compared with those obtained in group 1.

**Rotenone-Induced Sleep-Wake Alteration**

Sleep-wake architecture was unchanged in rats that received a 28-day 1:1 infusion of DMSO/PEG, when compared with the architecture of rats that received a 28-day infusion of PFS. The amounts of time spent in SWS during the dark period obtained after PFS (group 4, n = 8) and 1:1 DMSO/PEG treatment (group 5, n = 8) were 21.6% ± 1.8% and 22.3% ± 2.7%, respectively. The percentages of REM sleep during the dark period were 4.1% ± 1.2% after PFS (group 4) and 3.9% ± 1.5% after 1:1 DMSO/PEG (group 5). In addition, there was also no significant alteration in SWS or REM sleep during the light period; the times spent in SWS and REM sleep during the light period after PFS were 45.3% ± 2.5% and 4.5% ± 1.8%, and those obtained after 1:1 DMSO/PEG were 44.7% ± 2.8% and 4.8% ± 2.5%, respectively. A chronic 28-day administration of rotenone at a dose of 3 mg/kg per day increased the total time spent in SWS during the 12-hour dark period from 20.0% ± 1.6%, the value obtained before rotenone was given, to 26.7% ± 1.7% (n = 8, p < .05, 1-way ANOVA comparing within subjects) but suppressed SWS from 44.3% ± 2.1% to 35.4% ± 1.8% (n = 8, p < .05, 1-way ANOVA comparing within subjects) during the subsequent 12-hour light period. The SWS amount during the 12-hour dark period in rats chronically treated with rotenone was statistically different from the value obtained in rats receiving 1:1 DMSO/PEG during the dark period (p < .05, unpaired student t-test comparing between group 6 and 5). However, the time spent in SWS during 24 hours was not significantly altered after rotenone, the values were 31.6% ± 2.4% obtained before rotenone and 30.9%...
± 1.4% after rotenone (Figure 3A). The amount of REM sleep was also increased from 3.8% ± 0.7% to 7.9% ± 1.0% during the dark period after rotenone treatment (n = 8, p < .05, 1-way ANOVA comparing within subjects). There was no significant change during the light period; the values obtained before and after rotenone were 4.3% ± 0.6% and 3.0% ± 0.6%, respectively. The times spent in REM sleep were 4.0% ± 1.2% before rotenone and 6.3% ± 1.8% after rotenone, which did not achieve statistically significant change when a 24-hour period was analyzed (Figure 3A). There was a mirror effect on wakefulness. The amount of wakefulness was suppressed from 76.3% ± 1.9% to 65.4% ± 2.5% (n = 8, p < .05, 1-way ANOVA comparing within subjects) during the 12-hour dark period and was increased from 51.4% ± 2.3% to 61.7% ± 2.0% (n = 8, p < .05, 1-way ANOVA comparing within subjects, Figure 3A) during the 12-hour light period, although the total time spent in wakefulness during 24 hours was not significantly altered. The increased SWS during the active (light) period and the reduced SWS during the rest (light) period in our rotenone animal model of PD mimic the phenomenon of excessive daytime somnolence and the insomnia during the nighttime in patients with PD.

Analyses of sleep-architecture parameters across hours 1 to 12 revealed that the increase in SWS during the dark period induced by rotenone was primarily due to an increase in SWS bout duration, whereas the enhancement in REM sleep during the dark period was due to a significant increase in the numbers of REM sleep bouts. In contrast, the suppression of SWS during the light period was primarily due to a decrease in SWS bout duration (Table 1). The number of transitions from one state of vigilance to another during the 12-hour dark period and the 12-hour light period was not altered, which indicates that sleep was not fragmented after rotenone treatment (Table 1). In addition to the alteration in sleep quantity and architecture, both the EEG slow-wave activities during the SWS in the light and the dark periods were not altered after administration of rotenone. Slow-wave activity (SWA) during SWS were 1221 ± 6 µV²/Hz and 1195 ± 8 µV²/Hz during the dark period and the light period, respectively, in rats before chronic rotenone infusion. Slow-wave activity during SWS obtained after a 28-day rotenone treatment were 1205 ± 6 µV²/Hz and 1208 ± 8 µV²/Hz during the dark and the light periods, respectively (Figure 4D, n = 8). This result suggests that administration of rotenone has no effect on sleep propensity because the EEG SWA during SWS is thought to reflect both sleep debt and intensity and is a characteristic homeostatic response to sleep deprivation and sleep fragmentation.21
The Involvement of Dopaminergic System in Rotenone-Induced Sleep Alteration

The cause of PD is primarily due to the deficiency of dopamine in the striatum, resulting from the loss of pigmented neurons in the SNpc. We determined the role of loss in the dopaminergic system on rotenone-induced sleep alteration. Our results demonstrated that ICV administration of 2 doses of dopamine, 30 and 100 nmol prior to the beginning of dark period, has no effect on the rotenone-induced increase of SWS during the dark period (Figures 3B and 3C), except for a significant reduction of rotenone-induced SWS increase during the period of hour 3 after administration of 100 nmol dopamine. The time spent in SWS during hour 3 decreased from 35.6% ± 1.8% to 25.5% ± 3.1% (n = 8, p < .05, 1-way ANOVA comparing within subjects, Figure 3B). The suppression of rotenone-induced SWS increase in hour 3 may be due to the enhancement of body movement at that same time point after giving dopamine at a dose of 100 nmol (Figure 3D). However, the decrease in body movement induced by rotenone was not altered after administration of dopamine either during the 12-hour dark period or during the 12-hour light period (Figure 3D). The percentage of REM sleep was not significantly altered after ICV administered dopamine, although it decreased from 7.9% ± 1.0% to 6.2% ± 0.9% (Figures 3B and 3C, n = 8, 1-way ANOVA comparing within subjects). ICV administration of dopamine prior to the dark period had no influence on either SWS or REM sleep during the subsequent 12-hour light period in rotenone-treated rats (Figures 3B and 3C). ICV-administered 30 or 100 nmol of dopamine 20 minutes prior to the beginning of the light period, respectively, decreased the total SWS by 5.6% ± 1.6% and 10.5 ± 2.6%, but did not decrease REM sleep, during the 12-hour light period. The suppression mainly occurred at hour 1 and 2 after the administration.

There was also no prominent change when analyzing sleep-architecture parameters across hours 1 to 12 of the dark period and hours 13 to 23 of the light period after ICV injection of dopamine (Table 1). Although the bout duration of SWS was significantly
The Role of the GABAergic System in Rotenone-Induced Sleep-Wake Alteration

Since the deficiency of dopamine in the striatum results in the decrease of GABAergic output from the striatum to the GPi and increases the GABAergic efferent from the GPi to the thalamus as a consequence of the direct pathway of basal ganglia circuits, herein we employed pharmacologic blockade to further elucidate the involvement of the GABAergic system. There are 2 types of GABA receptors, GABA_A and GABA_B. We applied either GABA_A receptor antagonist bicuculline or GABA_B blocker 2-

**Figure 6**—Effects of intracerebroventricular (ICV) administration of interleukin (IL)-1ra on rotenone-induced sleep-wake alteration in group 6. A: The closed circle is the value obtained after ICV pyrogen free saline (PFS) + rotenone, and the open triangle depicts the value from ICV 0.2 µg IL-1ra + rotenone. B: The bar graph from left to right indicates the values obtained after ICV PFS (open bar), ICV PFS + rotenone (closed bar), ICV 0.1 µg IL-1ra + rotenone (the first hatched bar), and ICV 0.2 µg IL-1ra + rotenone (the second hatched bar), respectively. C: Locomotion activity. The open circle represents the value obtained after ICV PFS, the shaded area is the value obtained after ICV PFS + rotenone, the open triangle depicts the value from ICV 0.1 µg IL-1ra + rotenone, and the closed circle indicates the values from ICV 0.2 µg IL-1ra + rotenone. D: The slow-wave activity (SWA) during slow-wave sleep (SWS). The open circle: ICV PFS; closed circle: ICV PFS + rotenone; open square: ICV 0.2 µg IL-1ra + rotenone; open triangle: ICV 100 nmol dopamine + rotenone. *Represents the statistical difference between the values obtained from the control and rotenone treatment. #Indicates the statistical difference between the values obtained from the ICV IL-1ra + rotenone and those from the ICV PFS + rotenone. REMS refers to rapid eye movement sleep.
The Expression of IL-1β and Its Function in Sleep-Wake Regulation in Rotenone-Treated Rats

The subsequent increase of IL-1β and TNF-α after microglial activation may have implication in the neuronal apoptosis of dopaminergic neurons in the substantia nigra in PD. We determined the expressions of IL-1β and TNF-α in 5 distinct brain regions, including the hypothalamus, hippocampus, cortex, brainstem and striatum, after chronically subcutaneously infusing rotenone. The results of Western blots showed that the expression of 17 KD IL-1β in the hypothalamus, but not in other brain structures, increased after rotenone treatment (Figure 5A, n = 4 from group 8 [1:1 DMSO/PEG] and n = 4 from group 9 [rotenone]). This result was further confirmed by the ELISA specific for IL-1β.

The concentrations of IL-1β in the hypothalamus significantly increased from 158.5 ± 53.8 pg/mg of total protein, obtained from group 10 (1:1 DMSO/PEG, n = 8), to 348.2 ± 59.6 pg/mg of total protein after a chronic rotenone treatment in group 11 (Figure 5B, n = 8, p < .05, unpaired student t-test comparing between groups). In contrast, we did not detect any change in the 17 KD TNF-α expression in all 5 regions from the results of Western blot (data not shown).

Our previous results had shown that IL-1ra 200 ng administered ICV at the beginning of the dark period did not alter sleep-wake activity during the dark period in the normal control rat.20 However, our current results indicate that ICV-administered IL-1ra 20 minutes prior to the beginning of the dark period successfully reversed rotenone-induced increases of SWS and REM sleep during the 12-hour dark period. IL-1ra at doses of 100 and 200 ng suppressed SWS from 26.7% ± 1.7% to 19.5% ± 2.5% and 16.3% ± 1.4% (n = 8, p < .05, 1-way ANOVA comparing within subjects), respectively, during the dark period in rotenone-treated rats in group 6 (Figures 6A and 6B). Only the 100-ng IL-1ra administered 20 minutes prior to the beginning of the dark period blocked the rotenone-induced decrease of SWS during the subsequent light period; the SWS was enhanced from 35.4% ± 1.8% to 45.0% ± 2.1% (Figure 6B, p < .05, 1-way ANOVA comparing within subjects). The increment in REM sleep induced by rotenone was also blocked after ICV administration of 100 and 200 ng IL-1ra 20 minutes prior to the beginning of the dark period; REM sleep significantly decreased from 7.9% ± 1.0%, obtained after ICV PFS, to 4.2% ± 0.8% and 3.8% ± 0.7%, respectively, in rotenone-treated rats (Figure 6B, n = 8, p < .05, 1-way ANOVA comparing within subjects).

Analyses of sleep-architecture parameters across hours 1 to 12 of the dark period revealed that the blockade of the rotenone-induced increase in SWS by the ICV administration of 200 ng of IL-1ra was primarily due to the decrease in both the SWS bout duration and the number of bouts (Table). The effect of 200 ng of IL-1ra on the blockade of REM sleep induced by rotenone was due to the decrease in the number of REM sleep bouts. The number of transitions from one state of vigilance to another during the 12-hour dark period was not altered, indicative of no sleep fragmentation (Table). The EEG SWA during SWS was also not altered by ICV administration of IL-1ra (Figure 6D, n =8) in rotenone-treated rats. In addition, 2 doses of IL-1ra had no effect on improving the rotenone-induced decrease of body movement; in contrast, 200 ng of IL-1ra exaggerated the impairment of locomotor activity induced by rotenone (Figure 6C).

DISCUSSION

This study demonstrates the underlying mechanism involved in sleep alteration induced by a PD animal model. We employed the chronic subcutaneous infusion of rotenone in a dose of 3 mg/kg per day for 28 days to mimic the clinical features of PD and successfully established an animal model of PD in rats with the dramatic loss of TH-immunopositive cells in the SNpc and the reduction of locomotor activity. There are 2 major pathways in the basal ganglia circuit, the direct and indirect pathways.22 The lesion in SNpc causes deficiency of dopamine in the striatum and an increase of GABA from the GPi to the thalamus, through the direct and the indirect pathways. The action of dopamine on the sleep-wake state is a biphasic response. Central administration of hydroxsalofen 20 minutes prior to the beginning of the dark period and recorded the subsequent 23-hour sleep-wake activity in normal rats and in rotenone-treated rats. Our results demonstrated that neither ICV administration of bicuculline nor injection of 2-hydroxsalofen 20 minutes prior to the beginning of the dark period altered any aspect of sleep parameters during the dark period in normal rats (group 12). The percentages of SWS during the dark period before and after receiving 2.5 µg of bicuculline were 20.3% ± 1.9% and 21.4% ± 2.5%, and those of REM sleep were 4.8% ± 1.2% and 5.2% ± 1.8% in rats without rotenone treatment (n = 8). SWS and REM sleep after ICV administration of 2.5 µg of 2-hydroxsalofen were 20.8% ± 1.5% and 4.5% ± 2.1% in the same group of normal rats. In chronic rotenone-treated rats, ICV administrations of bicuculline at doses of 0.5, 1.0, and 2.5 µg 20 minutes prior to the dark onset had no effect on rotenone-induced SWS increase (Figures 4A and 4B). The time spent in SWS obtained before rotenone treatment, from ICV administered PFS + rotenone, and from ICV administered 2.5 µg bicuculline + rotenone was 13.0% ± 1.1%, 24.1 ± 1.9% and 21.4% ± 1.6% (n = 8), respectively. REM sleep also increased after rotenone treatment in this group of rats (Figure 4A). High dose of 2.5 µg bicuculline, but not the doses of 0.5 and 1.0 µg, significantly blocked rotenone-induced increase of REM sleep from 10.5% ± 1.3% to 6.8% ± 0.9% (Figures 4A and 4B, n = 8, p < .05, 1-way ANOVA comparing within subjects). ICV administered bicuculline had more prominent action on improving the decrease in locomotor activity induced by rotenone. The locomotor activity significantly increased from 199.2 ± 1.2 mV after ICV administration of PFS to 205.5 ± 1.2 mV, 205.3 ± 2.2 mV, and 207.7 ± 2.4 mV obtained after ICV administration of bicuculline at doses of 0.5, 1.0, and 2.5 µg, respectively, in rotenone-treated rats (Figure 4C, n = 8, p < .05, 1-way ANOVA comparing within subjects). ICV administration of the GABA<sub>B</sub> receptor antagonist 2-hydroxsalofen had a similar influence on sleep-wake activity and body movement, although the prominent action for 2-hydroxsalofen to reverse the increase of REM sleep induced by rotenone was at doses of 0.5 and 1.0 µg (Figures 4D and 4E); the time spent in REM sleep significantly decreased from 10.5% ± 1.3% to 5.9% ± 0.9% and 6.7% ± 1.0% (n = 8, p < .05, 1-way ANOVA comparing within subjects), respectively. The GABA<sub>B</sub> antagonist 2-hydroxsalofen, at doses of 0.5, 1.0, and 2.5 µg, also statistically reversed the rotenone-induced decrease in body movement from 199.2 ± 1.2 mV to 211.5 ± 1.2 mV, 210.0 ± 2.6 mV, and 209.5 ± 1.6 mV (n = 8, p < .05, 1-way ANOVA comparing within subjects), respectively (Figure 4F).
dopamine analogues or receptor agonists increases wakefulness at the expense of SWS and REM sleep,\textsuperscript{23} in addition to facilitating locomotion and stereotypic movements, suggesting its wake-promoting effect. A low dose of dopaminomimetics promotes sleep via D\textsubscript{2}-like dopamine receptors,\textsuperscript{24} whereas the high dose increases waking and locomotor activity and suppresses SWS and REM sleep via D\textsubscript{1} receptors.\textsuperscript{25} Nevertheless, lesions of dopaminergic afferents from the SNpc to the striatum may contribute to sleep changes in rotenone-induced parkinsonism. Our results demonstrate that both SWS and REM sleep increase at the expense of wakefulness, and locomotion decreases during the dark period after rotenone infusion. In contrast, SWS decreases and waking increases during the light period, with no change in REM sleep. The total of SWS is slightly reduced, and REM sleep increases during the 24-hour light:dark period, although both changes do not achieve statistical significance. This observation is partially consistent with some of the sleep disturbance seen in patients with PD. For example, the reduction of total sleep time,\textsuperscript{26} excessive daytime sleepiness,\textsuperscript{27} a narcolepsy-like phenotype of an increased number of sleep-onset REM episodes,\textsuperscript{3,4} and nocturnal sleep disturbance\textsuperscript{27} have been described as the most common sleep syndromes in patients with PD. We found that dopamine had no effect on the time spent in different vigilance states during the 12-hour dark period in rotenone-treated rats, except for the decreased SWS and the increased locomotion during hour 3 after administration of 100 nmol of dopamine. There was no significant alteration in SWS during the first 6-hour block of the dark period when 100 nmol of dopamine was ICV administered into the rotenone-treated rats. We propose that the dopamine-induced suppression of SWS during hour 3 is primarily due to the coincident enhancement of body movement in rats, rather than its effect on sleep-wake activity per se. If sleep alteration were due to the deficiency of striatal dopamine, ICV dopamine administered prior to the dark period should immediately suppress the rotenone-induced SWS enhancement after injection, but should not occur at hour 3 while the locomotion is improved. In order to avoid the nonspecific waking promotion by central administration of dopamine, we ICV administered relative lower doses of dopamine in present study. Therefore, the overall negative results could be due to the lower doses of dopamine we used or to the property of rapid clearance of dopamine itself.

GABA contributes to SWS regulation through the afferents from thalamus and basal forebrain to the cerebral cortex.\textsuperscript{9} Insufficient dopamine in the striatum results in the enhancement of the GABAergic component from the GPi to the thalamus. The sleep-promoting effect has been demonstrated by in vivo microinjection of a GABA uptake blocker into the thalamus.\textsuperscript{28} In addition, the concentration of GABA increases during SWS, compared with that of wakefulness, in the ventroposterolateral nuclei of the thalamus; in contrast, GABA decreases during REM sleep.\textsuperscript{29} Therefore, we further determined whether or not the rotenone-induced SWS increase is due to enhancing GABA transmission from the GPi to the thalamus. Our results indicate that neither bicuculline nor 2-hydroxysaclofen alters the rotenone-induced SWS increase, suggesting that GABA may not be involved in SWS regulation in this PD animal model. However, our results indicate that ICV administration of bicuculline in a relative high dose significantly suppresses rotenone-induced increase in REM sleep. The low dose of 2-hydroxysaclofen also had similar effect on REM sleep. Both GABA\textsubscript{A} and GABA\textsubscript{B} antagonists improve the locomotion activity. Recent attention to the brainstem areas involved in the control of locomotion and the regulation of REM sleep has focused on the pedunculopontine tegmental nucleus (PPN). The pallidal projections terminate on the PPN.\textsuperscript{30} The other projections received from the pars reticular of substantia nigra also terminate mainly on the PPN neurons in rats.\textsuperscript{31} Both the pallidal and substantia nigral projections to the PPN are GABAergic.\textsuperscript{32} The significance of PPN on sleep-wake activity is REM sleep regulation, although the effects of GABA on REM sleep are still controversial.\textsuperscript{33-36} Inhibition of PPN by the GABA\textsubscript{A} agonist muscimol abolishes PPN-induced REM sleep and that disinhibition of PPN by the GABA\textsubscript{B} receptor antagonist bicuculline results in REM sleep.\textsuperscript{34} However, an opposite observation that microinjection of muscimol into the PPN increases REM sleep and bicuculline decreases REM sleep and SWS has been demonstrated, suggesting that the GABA\textsubscript{A} receptor within the PPN facilitates the generation of REM sleep.\textsuperscript{35} In addition, bilateral microinjection of picrotoxin, a GABA\textsubscript{A} receptor antagonist, into the PPN, which is an area rich in REM-ON neurons, reduces REM sleep.\textsuperscript{33} Pal and Mallick proposed that the suppression of REM sleep by a GABA\textsubscript{A} antagonist is not likely due to the disinhibitory effect on REM-ON cells per se but, rather, by blocking the presynaptic GABA receptors on non-GABAergic inhibitory terminals that originate from the wake-inducing areas and keep the PPN REM-ON neurons inhibited during waking and SWS.\textsuperscript{33} Furthermore, Torterolo et al have observed that both wake-related and REM-related neurons coexist within the PPN and hypothesized that a GABA agonist produces a stronger suppression of wake-related components of the PPN than of the REM-related components, so that the REM-related mechanisms exhibit a greater extent. The opposite effects arise as a result of disinhibition by bicuculline.\textsuperscript{35} Our results that bicuculline blocked rotenone-induced REM sleep enhancement favors the explanation proposed by Pal and Torterolo. Although wakefulness and SWS were not altered, the rotenone-induced suppression in locomotion was blocked by bicuculline, suggesting that the effect of improving locomotor activity is beyond the influence of sleep-wake activity. Administration of a GABA\textsubscript{B} antagonist into PPN also suppresses the neuronal firing of REM-ON cells.\textsuperscript{36} Our results have shown that a lower dose of 2-hydroxysaclofen blocks rotenone-induced REM sleep increase and locomotion enhancement with no influence on wakefulness and SWS. In contrast, a high dose of 2-hydroxysaclofen has no significant action on REM sleep. We hypothesized that the action of 2-hydroxysaclofen is not directly on postsynaptic cholinergic neurons, but may act on the presynaptic or extrasynaptic GABA\textsubscript{B} autoreceptors, because the REM-sleep suppression was only observed when low doses of 2-hydroxysaclofen were given. A high dose of 2-hydroxysaclofen may bind to the postsynaptic GABA\textsubscript{B} receptor instead. However, this mechanism needs to be further confirmed.

Alteration of dopamine and GABA levels affected after the degeneration of the dopaminergic afferent from the SNpc to the striatum may not mediate the rotenone-induced SWS enhancement during the dark period from our current observation. Recently, microglial activation has been associated with the pathogenesis of PD\textsuperscript{37} and other neurodegenerative diseases (i.e., Alzheimer disease and multiple sclerosis) in humans.\textsuperscript{37} The extensive microglial activation and increase of cytokine expression, such as IL-1\textbeta, IL-6, and TNF-\textalpha, that occurs in striatum and substantia nigra have been demonstrated.
in the rotenone-treated rats and from patients with PD. Rotenone, a common pesticide, has been reported to possess dual actions of neurotoxicity to the dopaminergic neurons; the direct toxicity on dopaminergic neurons and the microglial activation are deleterious to neurons. Our present results confirmed the loss of TH-immunopositive cells in SNpc and the increase of IL-1β in the hypothalamus after chronic infusion of rotenone, although there was no significant alteration of IL-1β expression in the areas of the striatum, brainstem, cortex, and hippocampus. The increase of IL-1β in the hypothalamus might be due to the consequence of damaging dopaminergic neurons in the hypothalamus after chronic rotenone treatment; however, we did not confirm the loss of dopaminergic neurons in the hypothalamus in this current study. Furthermore, our results in Figure 5 suggest that there was an increased tendency of IL-1β expression in the brainstem after rotenone treatment. Therefore, it is possible that IL-1β also increased in the SNpc but was not detected because the entire brainstem was assayed together. It will be worthwhile to determine the dopaminergic neuronal loss in the hypothalamus and the specific concentration changes of IL-1β in SNpc in future study. In addition, our results indicate that the concentration of TNF-α was not altered in these distinct brain regions after rotenone treatment in rats. The somnogenic property of IL-1 is well documented. Administration of IL-1 into rats during the active periods enhances SWS and reduces waking. Anti-IL-1β antibodies, the IL-1 receptor antagonist, and an IL-1 receptor fragment reduce spontaneous SWS. IL-1 mRNA expression in rat brain exhibits circadian fluctuations, with expression highest during the light period of the light:dark cycle, the period when rats sleep the most, and lowest during the dark period, when rats are most active. The present results demonstrate that ICV administration of IL-1ra 20 minutes prior to the dark onset blocked the SWS and REM-sleep enhancement during the dark period in the chronic rotenone-treated rats, without improving any parameter in the locomotor activity. IL-1ra exhibited long-lasting effect after administration. The subsequent rotenone-induced decrease of SWS during the light period was reversed by 100 ng of IL-1ra but not by 200 ng of IL-1ra. This result may imply that the decrease of SWS during the light period after chronic administration of rotenone is due to the compensatory effect of the enhancement of SWS during the dark period; however, we have no explanation of why this effect only occurred when a low dose of IL-1ra was given. Surprisingly, IL-1ra also reduced rotenone-induced REM sleep during the dark period, although previous observation demonstrates that administration of IL-1 enhances SWS but does not alter REM sleep in rats. This result may be simply due to the action of IL-1ra on the blockade of IL-1-induced increase of GABA concentrations in the hypothalamus and other brain regions, which is consistent with our observation of the GABA effect on REM sleep in our rotenone rat model. Furthermore, our results indicate that IL-1ra did not alter rotenone-induced decreases in body movement. A high dose of IL-1ra even exaggerated the loss of locomotion. Comparing the results obtained after ICV administration of dopamine and GABA antagonists suggests that 2 distinct mechanisms respectively control the motor and sleep disturbances in the current rotenone PD model. Dopaminergic and GABAergic systems contribute to the control of locomotor activity, whereas the sleep disruption may be mediated by IL-1β. In conclusion, the current results clearly indicate that the rotenone-induced PD rat model mimics the sleep disruption observed in human subjects with PD. We are also the first to demonstrate the involvement of IL-1β in rotenone-induced sleep alteration. This may provide a perspective on therapeutic direction for sleep disturbance in patients with PD.

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