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## Neurotoxic lesions at the ventral mesopontine junction change sleep time and muscle activity during sleep: An animal model of motor disorders in sleep

Yuan-Yang Lai<sup>1</sup>, Kung-Chiao Hsieh<sup>1</sup>, Darian Nguyen<sup>1</sup>, John Peever<sup>2</sup>, and Jerome M. Siegel<sup>1</sup>

<sup>1</sup>Department of Psychiatry and Biobehavioral Science, David Geffen Sch Med, UCLA and VAGLAHS Sepulveda, North Hills, CA 91343.

<sup>2</sup>Systems Neurobiology Laboratory, Departments of Physiology and Cell & Systems Biology, University of Toronto, 25 Harbord Street, Toronto, ON, M5S 3G5, Canada

### Abstract

There is no adequate animal model of restless legs syndrome (RLS) and periodic leg movements disorder (PLMD), disorders affecting 10% of the population. Similarly, there is no model of rapid eye movement (REM) sleep behavior disorder (RBD) that explains its symptoms and its link to Parkinsonism. We previously reported that the motor inhibitory system in the brainstem extends from the medulla to the ventral mesopontine junction (VMPJ). We now examine the effects of damage to the VMPJ in the cat. Based on the lesion sites and the changes in sleep pattern and behavior, we saw three distinct syndromes resulting from such lesions; the rostromedial, rostromedial and caudal VMPJ syndromes. The change in sleep pattern was dependent on the lesion site, but was not significantly correlated with the number of dopaminergic neurons lost. An increase in wakefulness and a decrease in slow wave sleep (SWS) and REM sleep were seen in the rostromedial VMPJ lesioned animals. In contrast, the sleep pattern was not significantly changed after lesion in rostromedial and caudal VMPJ lesioned animals. All 3 groups of animals showed a significant increase in periodic and isolated leg movements in SWS and increased tonic muscle activity in REM sleep. Beyond these common symptoms, an increase in phasic motor activity in REM sleep, resembling that seen in human RBD, was found in the caudal VMPJ lesioned animals. In contrast, the increase in motor activity in SWS in rostral VMPJ lesioned animals is similar to that seen in human RLS/PLMD patients. The proximity of the VMPJ region to the substantia nigra suggests that the link between RLS/PLMD and Parkinsonism, as well as the progression from RBD to Parkinsonism may be mediated by the spread of damage from the regions identified here into the substantia nigra.

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Corresponding author: Y.Y. Lai, Ph.D., Neurobiology Research (151A3), VAGLAHS Sepulveda, 16111 Plummer Street, North Hills, CA 91343, Phone: (818) 891-7711x7569, Fax: (818) 895-9575, E-mail: yylai@ucla.edu.  
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## Keywords

periodic leg movements; REM sleep behavior disorder; Parkinsonism; pons; retrorubral nucleus; substantia nigra

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## Introduction

Periodic leg movement disorder (PLMD), is a symptom common in the restless legs syndrome (RLS; Montplaisir et al., 1992) and in rapid eye movement (REM) sleep behavior disorder (RBD; Schenck and Mahowald, 1990). PLMD is an increase in phasic motor activity in slow wave sleep (SWS). RLS, PLMD, and RBD are also commonly seen in Parkinsonism (Schenck et al., 1996; Wetter et al., 2000; Gagnon et al., 2002; Ondo et al., 2002; Boeve et al., 2004; Gatto et al., 2007). It is well known that Parkinsonian patients have damage in the substantia nigra (SN; Gibb et al., 1989; McRitchie et al., 1999; Yekhlief et al., 2003). However, the neuropathology underlying RLS, PLMD and RBD is uncertain.

In normal animals and humans, muscle tone in the postural muscles is low in SWS and absent in REM sleep. The brain mechanisms regulating muscle tone in SWS remain unclear. In contrast, the neural mechanisms responsible for the suppression of muscle tone in REM sleep are fairly well understood. It has been shown that the pontine inhibitory area (PIA) and the medial medulla, including the nuclei gigantocellularis, magnocellularis (NMC), and paramedianus, participate in the regulation of muscle tone during REM sleep (Kanamori et al., 1980; Lai and Siegel, 1988, 1990, 1991, 1999; Siegel et al., 1991; Kodama et al., 1992, 1998, 2003; Lai et al., 1993, 1999a, 2001; Yamuy et al., 1993). Activation of the PIA and medial medulla produces generalized skeletal muscle atonia in chronic and in decerebrate animals (Magoun and Rhines, 1946; George et al., 1964; Lai et al., 1987; Lai and Siegel, 1988; Hajnik et al., 2000). Damage to the PIA or NMC generates REM sleep without atonia in the cat (Henley and Morrison, 1974; Schenkel and Siegel, 1989; Shouse and Siegel, 1992; Holmes and Jones, 1994) and rat (Sanford et al., 2001). However, it has not been reported that phasic muscle activity during SWS is altered by PIA or NMC lesions. Because an increase in leg movement in SWS is seen in RBD patients (Schenck and Mahowald, 1990; Montplaisir et al., 1992), and because there has been no convincing evidence of damage to the PIA or medial medulla in such patients, damage to these areas does not appear to be a likely cause of human RBD.

Our previous studies demonstrated that the ventral mesopontine junction (VMPJ), including the caudal portion of the dopaminergic retrorubral nucleus (RRN), SN and ventral tegmental area (VTA), as well as the rostroventral paralemniscal tegmental field of the pons, is involved in the regulation of motor activity (Lai and Siegel, 1990). Short train stimulation in the VMPJ generates global inhibition of muscle tone during stimulation, and rhythmic activity appears during the inter-stimulus interval in the decerebrate cat (Lai and Siegel, 1990). Neurotoxic N-methyl-D-aspartic acid (NMDA) lesions in the VMPJ produce an increase in spontaneous or tactile stimulation induced rhythmic stepping-like activity or myoclonic jerks in the decerebrate animal (Lai and Siegel, 1997a). We hypothesized that the VMPJ may be involved in the control of muscle activity during sleep.

## Experimental Procedures

### Surgical preparation

All procedures were approved by the Animal Research Committee of the University of California, Los Angeles and of the VA Greater Los Angeles Healthcare System. Eight female and one male cat (University of California, Davis, California) weighing 2.5–3.5 kg were used in the study. Implantation of electrodes for sleep recording was described in a previous paper (Lai et al., 1999b). Briefly, cats were anesthetized with isoflurane (1.5%) for stereotaxic electrode implantation. Jewelers' screws were implanted over the sensorimotor cortex (A27, L4, L8 and L10) for recording cortical EEG, and into the orbital bone for the recording of eye movement (electrooculogram; EOG). Flexible multi-stranded stainless steel wires (7935, A-M Systems, Inc., Carlsborg, WA) were inserted into nuchal and forelimb musculature bilaterally for electromyogram (EMG) recording. Seven animals had EMG electrodes implanted in both the neck and limb musculature, whereas the remaining two cats (LC11 and LC12) had EMG electrodes implanted only in the neck muscles. Stainless steel tripolar electrodes were implanted into the lateral geniculate nuclei (A6, L10, H2) to record ponto-geniculo-occipital (PGO) spikes. Guide cannulae (19 G) were implanted 5 mm dorsal to the VMPJ for NMDA injection, bilaterally.

### Sleep recording and ventral brainstem lesion

Sleep recording was performed 7 days after electrode implantation, by which time the animal had recovered from surgery. After 3 consecutive 24-h baseline sleep recordings, a 25 G cannula connecting with a one microliter Hamilton microsyringe (Model 7001) was inserted into the VMPJ through the guide cannula. One half microliter of 0.5 M NMDA (Sigma, St. Louis) was injected into the VMPJ over a period of 5 min. The cannula was retained at its position for another 20 min. Sleep recordings were resumed on day 2 post-NMDA injection every day for 14 days. Then, a 3-day sleep recording was performed once every two weeks over a period of 4-months. In our previous study, we found that unilateral lesion of the VMPJ generated motor hyperactivity in sleep in the cat (Lai et al., 1999b; cat number: LC3). Thus, we wanted to determine whether unilateral and bilateral lesions produce motor hyperactivity in sleep. Among 9 cats in the present study, 2 (LC12 and FO5) had bilateral NMDA injection into the VMPJ simultaneously, 2 (FO4 and VJ4) had NMDA bilateral injections with the two injections given 4-months apart, and the remaining five cats had unilateral VMPJ lesions.

### Data analysis

Physiological variables were amplified with a Grass polygraph (Model 78D) and digitized by a CED 1401 Spike2 system (Cambridge Electronic, UK). Five sleep-waking states were scored, active waking, quiet waking, SWS stage 1, SWS stage 2 and REM sleep, according to Ursin and Serman (1981).

Phasic muscle activities occurring in the neck and limb during SWS were divided into two categories, periodic neck/leg movement and isolated neck/leg movement. Periodic motor activities were scored based on the following criteria for humans (Zucconi et al., 2006). 1) Duration of muscle activity was between 0.5 to 5 sec. 2) Inter-jerk intervals ranged between

5 to 80 sec. 3) Amplitude of muscle twitches were at least double that of the existing background EMG. 4) At least 4 consecutive jerks fulfilled criteria 1–3. Phasic muscle activities which meet the above criteria 1–3 but not 4 were described as isolated activities. The periodic neck movement index (PNMI) and periodic leg movement index (PLMI) were defined as the number of periodic neck and limb muscle activities in SWS in 24-h recording (total number of periodic muscle activities/total SWS time in 24-h), respectively. The INMI and ILMI were the number of isolated motor activities in the neck and leg per 24-h of SWS time (total number of isolated muscle activities/total SWS time in 24-h), respectively. Muscle tone (REM sleep without atonia) and phasic muscle activity during REM sleep were analyzed to evaluate changes in muscle activity in REM sleep after VMPJ lesion. Changes in percent of REM sleep without atonia in REM sleep, which was the number of total duration of REM sleep without atonia divided by the total REM sleep in 24-h, after VMPJ lesion, were used to quantify the effect of VMPJ lesions on muscle tone in REM sleep. The baseline level of sleep states and phasic motor activities in each animal was taken from an average of 2 days of recording, and an average of 2 days of recording was used for the analysis of sleep states and motor activity in sleep after lesion.

## Histology

Cats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.v.) at the end of the experiment. Then, animals were perfused intracardially with cold (4° C) saline followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer saline (PBS) solution at pH 7.4. The brainstem was removed and kept in 4% paraformaldehyde solution at 4° C for 2 hours, then, stored in 30% sucrose in 0.1 M PBS at 4° C.

40  $\mu$  serial coronal sections of the brainstem were obtained. Brain sections were processed with immunohistochemistry for tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT) to determine the magnitude of the loss of dopaminergic neurons in the RRN and cholinergic neurons in the pedunculopontine nucleus (PPN). For TH and ChAT immunohistochemistry, tissue sections were rinsed with 0.1 M PBS three times and then incubated in 10% normal goat serum for 2 hours. Then, tissue sections were sequentially incubated with primary antibody against TH (1:1,000, MAB5280, Chemicon, Temecula, CA) or ChAT (1:1,000, MAB5270, Chemicon), both were raised from mouse, for 72 hours, biotinylated goat anti-mouse IgG (BA-1000, Vector, Burlingame, CA) for one hour and Vectastain *Elite* ABC kit (PK-6102, Vector) for another hour. Tissue sections were rinsed with 0.1 M PBS three times between incubations. The final products of TH and ChAT were visualized with 3,3'-diaminobenzidine (DAB, Sigma) solution containing 0.05% DAB and 0.02% hydrogen peroxide in 0.1 M PBS. Alternative tissue sections were also processed with neutral red after immunohistochemical processing to identify the lesion area and to estimate the total number of neurons lost (Lai et al., 1999b). Tissue sections processed with TH, ChAT, and neutral red of 2 cats from our previous anatomical studies (Lai et al., 1999a) served as control.

Tissue sections were observed under a Nikon microscope. The lesion sites were mapped with a NeuroLucida microscope system (MBF Bioscience, Williston, Vermont).

## Results

### The lesion sites in the brainstem

NMDA microinjections killed all neuronal phenotypes (Fig. 1). Although injections were aimed at the VMPJ, the precise lesion locus varied across animals. Animals could be divided into 3 groups, rostral-medial (Group R-M), rostral-lateral (Group R-L), and caudal (C) lesions (Fig. 1). The rostral VMPJ lesioned groups (Groups R-M and R-L) had lesions extending from the caudal red nucleus at their rostral limit to the trochlear nucleus at their caudal limit (Berman, 1968). The lesioned areas in the Group R-M cats (LC11, LC12, and FO5) included the RRN, the ventral portion of the mesencephalic reticular formation (MRF) and the caudal portion of the VTA, whereas, the Group R-L cats (FO4 and VJ3) had lesions in the RRN, MRF, and the caudal portion of the SN. The other 4 cats had lesions in the caudal VMPJ (Group C) including the caudal RRN in the midbrain and the rostral-ventral paralemniscal tegmental field in the pons. Table 1 shows the estimated number of neuron lost in brainstem structures across animals.

Immunohistochemical staining showed that one cat (MO1) had a lesion extending into the PPN. However, we estimated that less than 1% of cholinergic neurons in the PPN were removed by the NMDA injection. No PPN lesion was found in the remaining 8 cats. Loss of dopaminergic neurons in the RRN (Fig. 2) was found in all cats to different degrees (Table 2). With the exception of cat FO4, no cat lost more than 30% of the dopaminergic neurons in the VMPJ.

### Behavior responses to NMDA-VMPJ lesion

Motor activities in waking appeared normal in all animals after NMDA-VMPJ lesions. On day 2 post-NMDA injection, cats were able to stand and walk. Reflex activities, blinking and head turning appeared normal. Eating and drinking behaviors were not affected by NMDA injections.

### Change in sleep after neurotoxic lesion in the VMPJ

Visually scored 24-hour sleep recordings showed a change in sleep architecture after VMPJ lesions. Regression analysis between dopaminergic cell loss in the VMPJ and changes in sleep at day 7, 30, 60, 90 and 120 post-lesion indicated that the change in waking, SWS, and REM sleep after VMPJ lesion was not correlated with the degree of dopaminergic neuron loss in the VMPJ ( $p > 0.2$ , Table 3). In contrast, the duration of sleep-wake stages differed as a function of lesion location (ANOVA,  $df=4$ ,  $p < 0.05$ ). Although the animals in both of Groups R-M and C showed an increase in SWS and REM sleep after lesion, the change in sleep pattern was not significantly different from the baseline control (Fig. 3, Group R-M: ANOVA,  $df=21$ ,  $p > 0.05$ ; Group C: ANOVA,  $df=30$ ,  $p > 0.05$ ). In contrast to Groups R-M and C, a significant increase in wakefulness with reduced SWS and REM sleep was found in cats after rostralateral VMPJ lesions (Fig. 3, ANOVA,  $df=14$ ,  $p < 0.05$ ). Thus, a unilateral VMPJ lesion was sufficient to change sleep architecture.

### Motor activity in SWS in the NMDA-VMPJ lesioned animals

Basal muscle tone was low during SWS in all cats before VMPJ lesions. Basal muscle tone in waking and SWS was unaltered after VMPJ lesions. However, an increase in phasic muscle activity in SWS was seen after lesions in the VMPJ in all animals (Fig. 4; Table 4). The time course of changes in phasic motor activity in SWS could be segregated into 2 phases, early (first week after lesion) and late (after the first week). In the early phase, phasic motor activity appeared as irregular and non-periodic, isolated leg/neck movements. Then, phasic muscle activity in the leg and neck gradually consolidated into regular and periodic leg/neck movements. Thus, the number of isolated leg and neck phasic activities in SWS gradually decreased to a low level by 60 days after VMPJ lesion, whereas, periodic leg and neck phasic activities appeared in the late phase and continued throughout the entire 4-month period of observation (Fig. 5). Periodic leg movements occurred unilaterally and bilaterally. In some cases, these were accompanied by periodic neck muscle activity. Periodic motor events were accompanied by EEG desynchronization in most of cases in the VMPJ lesioned cat (Fig. 4).

### Motor activity in REM sleep in the NMDA-VMPJ lesioned animals

The major change in muscle activity during REM sleep after NMDA-VMPJ lesion was an increase in tonic activity, i.e. REM sleep without atonia (Fig. 6). Although the lesion area differed between animals, an increase in tonic muscle activity in REM sleep was found in all animals in our VMPJ lesion series. REM sleep without atonia appeared in the neck and/or limb musculatures, unilaterally and/or bilaterally. The percent of REM sleep time without atonia gradually increased beginning 7 days after NMDA injection into the VMPJ and reaching a plateau level by 60 days after lesion (Fig. 6).

Abnormal phasic motor activity in REM sleep was found in cats that had lesions of the caudal VMPJ (Group C; Table 5). Periodic phasic muscle activities and leg twitching not only appeared in SWS but also occurred in REM sleep (Fig. 7). High amplitude muscle activity abruptly appeared in all recorded muscles in REM sleep, while the EEG remained desynchronized and the EOG recording showed rapid eye movements (Fig. 8). Behavioral activities observed during this episode of REM sleep appeared as jerking, kicking and extending of the leg; raising and moving the head; and lifting of the body. This abnormal REM sleep behavior, which resembled RBD seen in humans, lasted for 20 – 62 seconds with an average of  $43 \pm 18$  seconds. Animals went into REM sleep immediately after the episodes of RBD-like activity (Fig. 8).

### Effect of second lesion contralateral to the side of first lesion on motor activity in sleep

Two cats (FO4 and VJ4) had a second lesion on the side contralateral to the first lesion. The second lesion failed to alter motor activities in sleep, periodic leg/neck movements, isolated leg/neck movements, REM sleep without atonia and RBD, from the level observed 4 months after the first lesion (Table 4 and Table 5). As was the case with the change in sleep pattern, unilateral lesion of the VMPJ was sufficient to cause motor hyperactivity in sleep.

## Discussion

The major finding of the present study is that lesions in the VMPJ induce changes in sleep patterns, as well as in the duration and nature of motor activity during sleep. Changes in sleep patterns were site-dependent with a significant increase in wakefulness in rostral-lateral VMPJ lesioned animals. Dopaminergic mechanisms have been reported to be involved in the regulation of sleep (Gerashchenko et al., 2006; Monti and Monti, 2007), however, our present study found that changes in sleep pattern after VMPJ lesions were not correlated with the number of caudal midbrain dopaminergic neurons lost. Although all VMPJ lesioned animals developed PLMD and REM sleep without atonia, RBD-like behaviors were only seen in cats with caudal VMPJ lesions. We also found that unilateral lesion of the VMPJ was sufficient to elicit motor hyperactivity in sleep and a change in the sleep pattern. A clinical study found that RBD can be induced by a unilateral tumor located at the pontocerebellar angle (Zambelis and Soldatos, 2002).

In RBD patients, motor hyperactivity is not only seen in REM sleep but also found in SWS. An increase in periodic and isolated leg movements during SWS is seen in 60% and 40% of RBD patients, respectively (Schenck and Mahowald, 1990). Periodic leg movements persist in REM sleep in RBD patients (Lapierre and Montplaisir, 1992). Although loss of muscle atonia in REM sleep is a symptom of RBD, these patients experience periods of atonia intermixed with persistent muscle tone (Schenck et al., 1992; Fantini et al., 2003). Sleep organization is reported to be either changed or unaltered in idiopathic RBD. Schenck et al. (1993) and Massicotte-Marquez et al. (2005) reported that more than 80% of idiopathic RBD patients show an increase in SWS compared with age-matched normal subjects. In contrast, Iranzo et al. (2002) found that SWS is not significantly altered in idiopathic RBD. REM sleep was also reported to be either increased in 43% of RBD patients (Schenck and Mahowald, 1990) or unaltered (Iranzo et al., 2002), as we see in the current study. The decrease in sleep after rostral-lateral VMPJ lesions in the present study is consistent with Gerashchenko et al.'s findings (2006). They found that rats with lesions in the SN but not in the VTA developed insomnia. The decrease in sleep seen in the rostral-lateral VMPJ lesioned cat may mimic that seen in Parkinson's disease patients.

The physiological role of VMPJ in sleep regulation remains unclear. Fos-expressing neurons have been found in the VMPJ in the phase of REM sleep rebound after REM sleep deprivation (Verret et al., 2006) indicating the VMPJ may be involved in the regulation of REM sleep. Anatomical studies have shown that neurons in the VMPJ project to sleep-related areas including basal forebrain (Sawchenko et al., 1983; Willoughby and Blessing, 1987; Vertes, 1988; Jones and Cuello, 1989), PIA (Lai et al., 1993) and NMC (Luppi et al., 1988; Lai et al., 1999a). Glutamatergic projections from the VMPJ to the PIA and NMC (Lai et al., 1993; 1999a) may be involved in the generation of REM sleep (Onoe and Sakai, 1995; Kodama et al., 1998). Neurons in the rostral-lateral VMPJ, perhaps containing  $\gamma$ -aminobutyric acid (GABA), also project to locus coeruleus (LC; Verret et al., 2006). GABA has been shown to inhibit LC neuronal activity (Gervasoni et al., 1998), and its levels in the LC are increased during SWS and REM sleep (Nitz and Siegel, 1997). The dorsal raphe nucleus has been found to project to the medial portion of the VMPJ (Vertes and Kocsis, 1994). However, the role of raphe projections to the VMPJ remains unclear.

The effect of VMPJ lesion on muscle activity in sleep may be mediated through the caudal brainstem and cerebellum. We demonstrated that motor hyperactivity induced by VMPJ lesions can be attenuated or blocked by glutamate injection into the NMC in the decerebrate cat (Lai and Siegel, 1997b). Anatomically, the VMPJ is one of the major sources of projections to the caudal brainstem muscle inhibitory areas, the NMC (Luppi et al., 1988; Lai et al., 1999a) and the PIA (Lai et al., 1993). Axonal fibers from the VMPJ were also found to innervate the LC (Verret et al., 2006), which has been shown to have a role in motor facilitation (Fenik et al., 2005a,b,c; Fung and Barnes, 1981, 1987; Lai et al., 1989; Liu et al., 1995; Wu et al., 1999). Activation of the VMPJ may thus activate neuronal activity in the PIA and NMC via glutamatergic mechanism and suppress LC neuronal activity through GABAergic projections. We hypothesize that the effects of VMPJ on PIA, NMC and LC may contribute to the motor inhibition in sleep.

The PIA has been demonstrated to be involved in the regulation of muscle tone in REM sleep and has been hypothesized to participate in the generation of RBD (Morrison, 1988). Although electrolytic lesion in the PIA generates motor hyperactivity, orienting, walking, and attacking in REM sleep (Morrison et al., 1981), chemical lesions in the PIA in the cat induce REM sleep without atonia but fail to elicit the elaborate motor behavior characteristic of RBD seen with electrolytic lesions (Webster and Jones, 1988; Shouse and Siegel, 1992). Work in the rat reported that REM sleep atonia is preserved during inactivation of PIA neuronal activity by tetrodotoxin injection (Sanford et al., 2005), although electrolytic lesions of the same site induces REM sleep without atonia (Sanford et al., 2001). Therefore, the behavioral activation in REM sleep seen in the PIA-electrolytic lesioned cat may result from damage to passing fibers. Indeed, clinical studies have shown that the PIA appears normal in RBD patients (Schenck and Mahowald, 1996; Mazza et al., 2006), however, dysfunction of the ventral pons has been reported in human RBD (Schenck and Mahowald, 1990; Mazza et al., 2006). Severe neural degeneration in the ventral pons is reported in olivopontocerebellar atrophy patients, who also develop REM sleep without atonia and RBD (Salva and Guilleminault, 1986; Schenck et al., 1993; Tachibana et al., 1995). We found in the present study that the integrity of the rostroventral pons is critical for preventing RBD, whereas, the caudal midbrain is involved in the control of phasic motor activity in SWS and tonic activity in REM sleep. Clinically, dopaminergic and benzodiazepine-related agonists are the most potent drugs in the treatment in RLS/PLMD (Montplaisir et al., 1986; Earley and Allen, 1996) and RBD (Schenck et al., 1986). Our present finding that caudo-ventral midbrain lesions generate PLMD and that the rostroventral pontine lesions generate RBD may be underlie the differences in the effective pharmacological treatment between PLMD and RBD.

RLS and PLMD are closely associated with Parkinsonism (Wetter et al., 2000; Ondo et al., 2002; Gomez-Esteban et al., 2007). REM sleep without atonia (Wetter et al., 2001; Gagnon et al., 2002) and RBD (Schenck et al., 1996; Montplaisir et al., 1997; Boeve et al., 1998; Wetter et al., 2001; Gagnon et al., 2002; Askenasy, 2003) are commonly seen in Parkinsonism. Recent studies found that patients may be diagnosed simultaneously with Parkinson's disease and RBD, or diagnosed with Parkinson's disease and then develop RBD, or vice versa (Schenck et al., 1996; Olson et al., 2000; Eisensehr et al., 2001). However, neuronal degeneration is not found in the SN in idiopathic RBD (Boeve et al.,



2007), but it is found in RLS patients (Allen et al., 2001), 85% of which have PLMD (Montplaisir et al., 1997). The association between Parkinson's disease and RLS/PLMD/RBD has not been explained. The findings of the current and our previous studies (Lai et al., 1999b) suggest that there might be an anatomical link between Parkinson's disease and RLS/PLMD/RBD (Fig. 9). Our present study showed that REM sleep without atonia and RBD-like behavior can be seen in cats with lesions in the rostral (B in Fig. 9) and caudal (C in Fig. 9) VMPJ, respectively. In conjunction with our previous findings that lesions in the rostroventral midbrain of the SN (A in Fig. 9), which is located rostral and adjacent to the VMPJ, induce sleep fragmentation and insomnia (Lai et al., 1999b), we hypothesize that neuronal degeneration can be generated in either part of the ventral brainstem, the rostral ventral midbrain and the VMPJ, and progressively extend to the caudal or rostral part of the brainstem (Lai and Siegel, 2003). Parkinson's disease will develop first if the lesion starts in the rostral ventral midbrain, whereas REM sleep without atonia and/or RBD will be seen first if neuronal degeneration begins in the VMPJ (Lai and Siegel, 2003). The loss of dopaminergic neurons in the rostral ventral midbrain would be expected to cause further sleep disruption (Gerashchenko et al., 2006).

Our present study found that neurotoxic lesion in the rostral and caudal VMPJ produced PLMD and REM sleep without atonia and RBD-like activity in the cat. Investigation of the neuronal activity and neurochemistry of the cell groups responsible for these effects will further our understanding of both RBD and Parkinsonism and may suggest more effective treatments.

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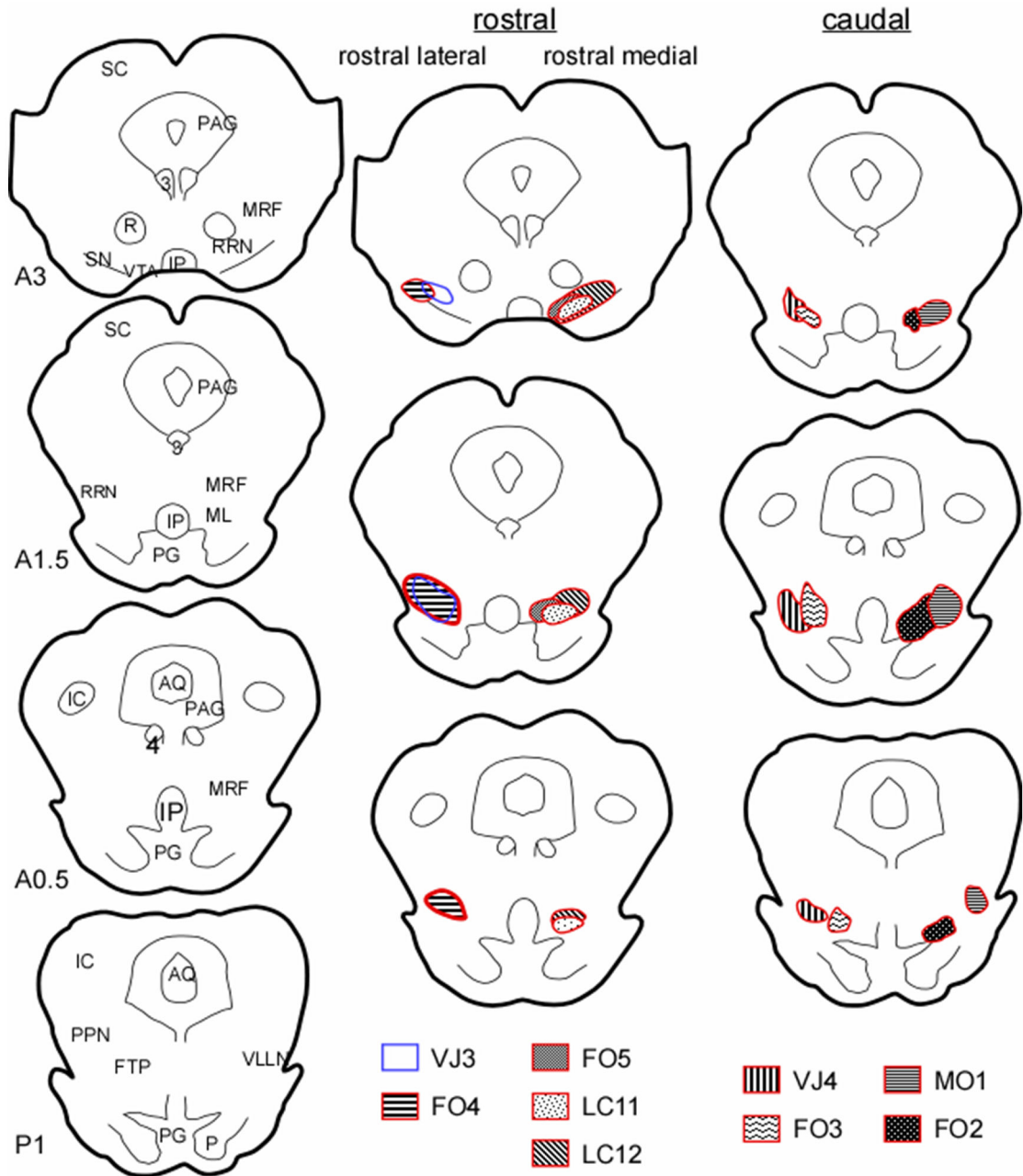
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## Abbreviations

<b>AQ</b>	aqueduct
<b>ChAT</b>	choline acetyltransferase
<b>CS</b>	superior central nucleus
<b>DAB</b>	3,3'-diaminobenzidine
<b>EMG</b>	electromyogram
<b>EOG</b>	electrooculogram
<b>FTP</b>	paralemniscal tegmental field
<b>GABA</b>	$\gamma$ -aminobutyric acid

<b>Hip</b>	hippocampal theta
<b>IC</b>	inferior colliculus
<b>ILMI</b>	isolated leg movement index
<b>INMI</b>	isolated neck movement index
<b>IP</b>	interpeduncular nucleus
<b>LC</b>	locus coeruleus
<b>LGN</b>	lateral geniculate nucleus
<b>LL</b>	left limb muscle EMG
<b>LR</b>	right limb muscle EMG
<b>ML</b>	medial lemniscus
<b>MRF</b>	mesencephalic reticular formation
<b>NL</b>	left neck muscle EMG
<b>NMC</b>	nucleus magnocellularis
<b>NMDA</b>	N-methyl-D-aspartic acid
<b>NR</b>	right neck muscle EMG
<b>P</b>	pyramidal tract
<b>PAG</b>	periaqueductal gray
<b>PBS</b>	phosphate buffer saline
<b>PG</b>	pontine gray
<b>PGO</b>	ponto-geniculo-occipital
<b>PIA</b>	pontine inhibitory area
<b>PLMD</b>	periodic leg movements disorder
<b>PLMI</b>	periodic leg movement index
<b>PNMI</b>	periodic neck movement index
<b>PPN</b>	pedunculopontine nucleus
<b>R</b>	red nucleus
<b>R/A</b>	REM sleep without atonia
<b>RBD</b>	REM sleep behavior disorder
<b>REM</b>	rapid eye movement
<b>RLS</b>	restless legs syndrome
<b>RRN</b>	retrosubthalamic nucleus
<b>SC</b>	superior colliculus

<b>SN</b>	substantia nigra
<b>SWS</b>	slow wave sleep
<b>TH</b>	tyrosine hydroxylase
<b>THA</b>	thalamus
<b>TRP</b>	tegmental reticular nucleus, peripheral division
<b>VLLN</b>	ventral nucleus of the lateral lemniscus
<b>VMPJ</b>	ventral mesopontine junction
<b>VTA</b>	ventral tegmental area
<b>3</b>	oculomotor nucleus
<b>4</b>	trochlear nucleus

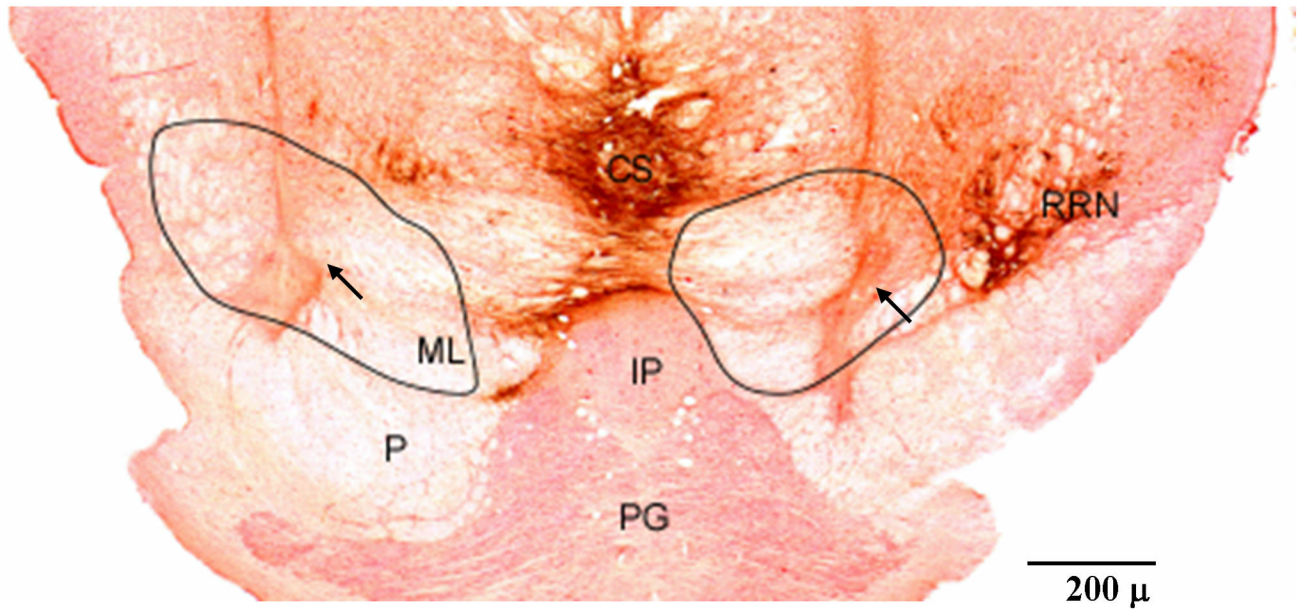


**Figure 1.**

Reconstruction of lesion sites. Two animals had bilateral lesions (LC12 and FO5) and the other 7 had unilateral lesion in the VMPJ. Among the 7 unilateral VMPJ lesioned animals, 2 (FO4 and VJ4) had an additional lesion on the side contralateral to the first lesion 4 months after the first lesion. AQ: aqueduct, FTP: paralemniscal tegmental field, IC: inferior colliculus, IP: interpeduncular nucleus, ML: medial lemniscus, MRF: mesencephalic reticular formation, PAG: periaqueductal gray, PG: pontine gray, PPN: pedunculopontine nucleus, R: red nucleus, RRN: retrobulbar nucleus, SC: superior colliculus, SN: substantia

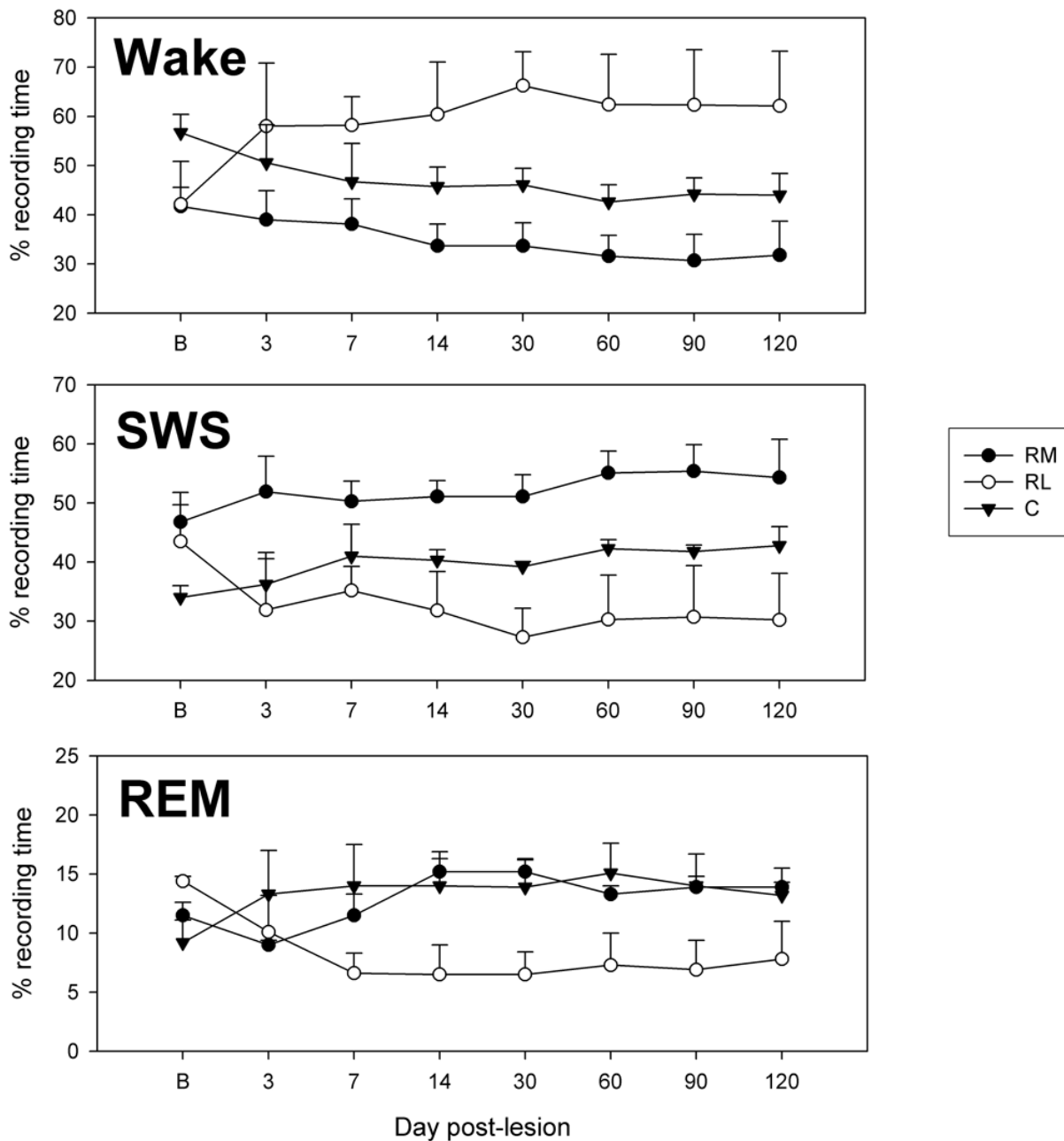


nigra, VLLN: ventral nucleus of the lateral lemniscus, VTA: ventral tegmental area. 3: oculomotor nucleus, 4: trochlear nucleus. A and P represent rostral and caudal to the interaural zero.

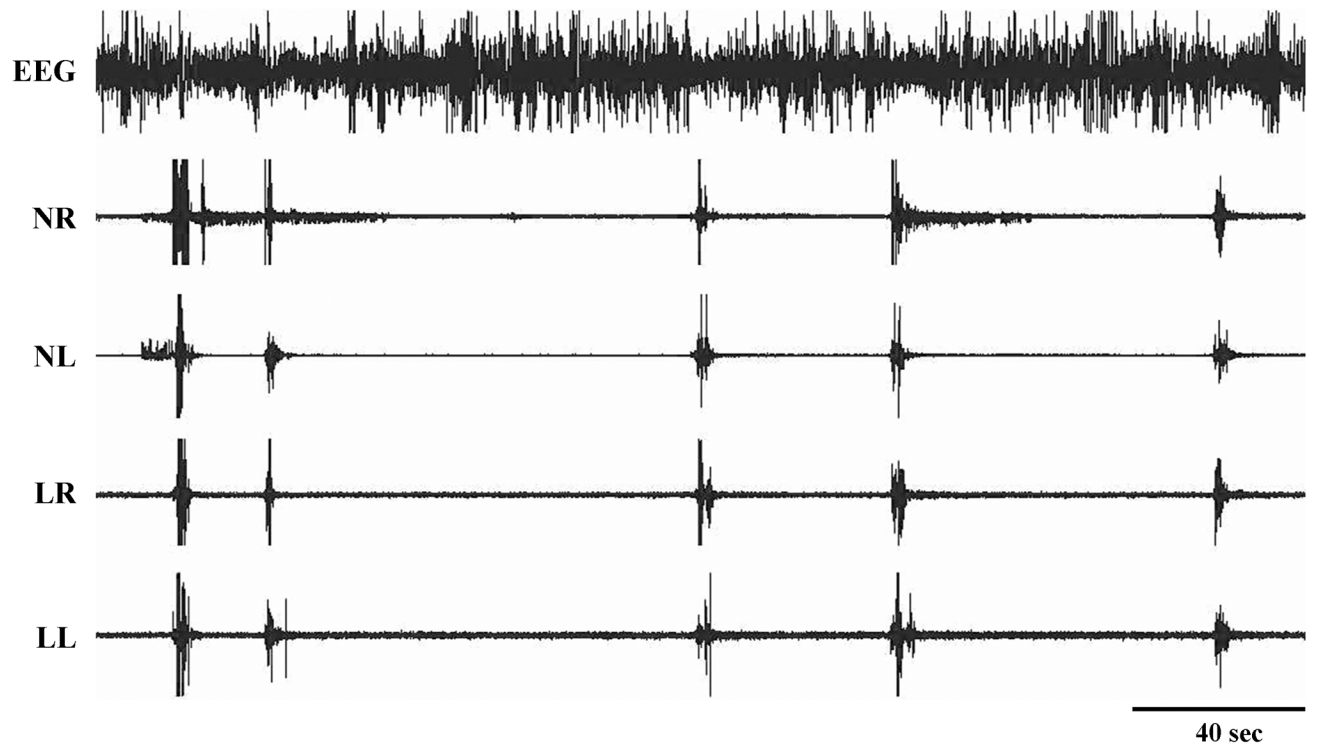


**Figure 2.**

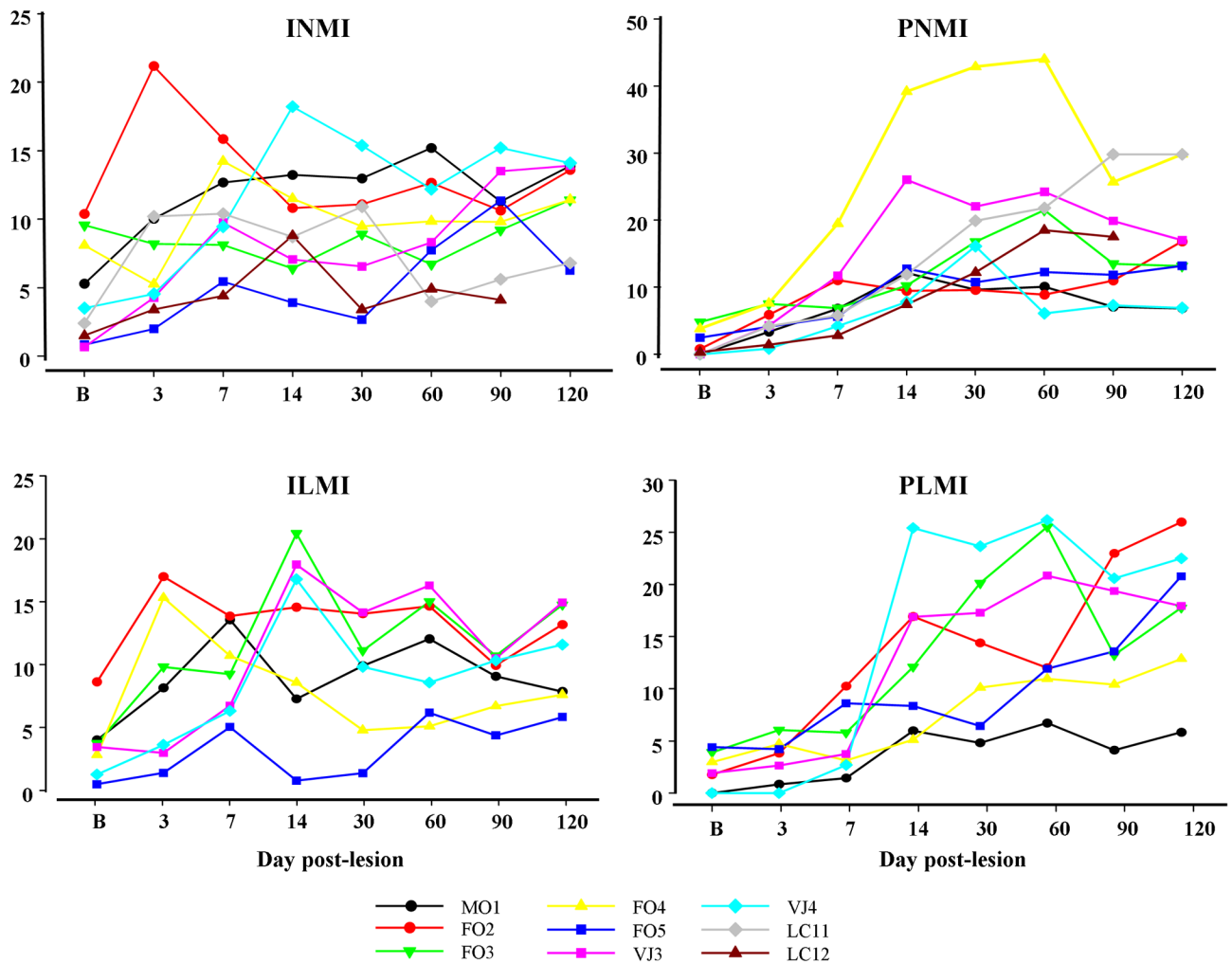
Example of NMDA lesion at the ventral mesopontine junction (VMPJ). Microphotograph was taken from cat FO4. Tissue section was processed with immunohistochemistry for tyrosine hydroxylase (dark brown) and then with neutral red. The first and second lesion sites were in the rostralateral and rostromedial VMPJ. Some dopaminergic neurons were found in the lateral (right) and medial (left) retrotrubral nucleus (RRN). The arrows indicate the injection sites. The areas within the line represent the lesioned areas. CS: superior central nucleus, IP: interpeduncle nucleus, ML: medial lemniscus, P: pyramidal tract, PG: pontine gray, RRN: retrotrubral nucleus.



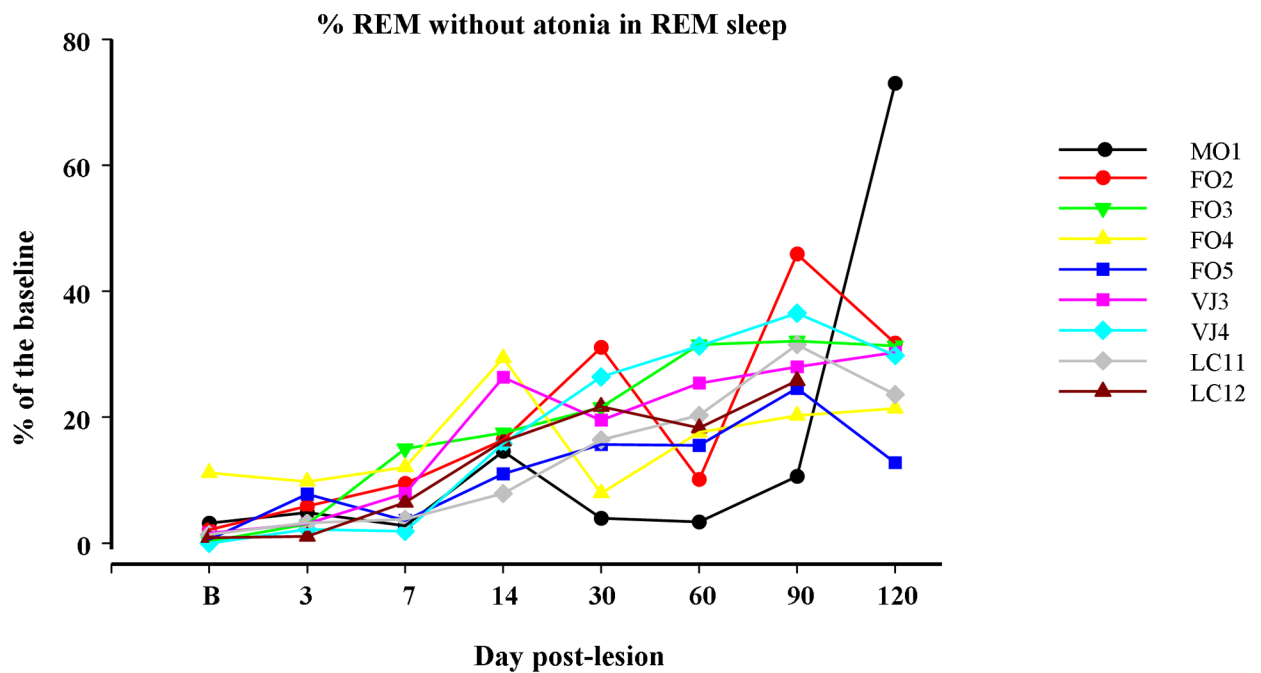
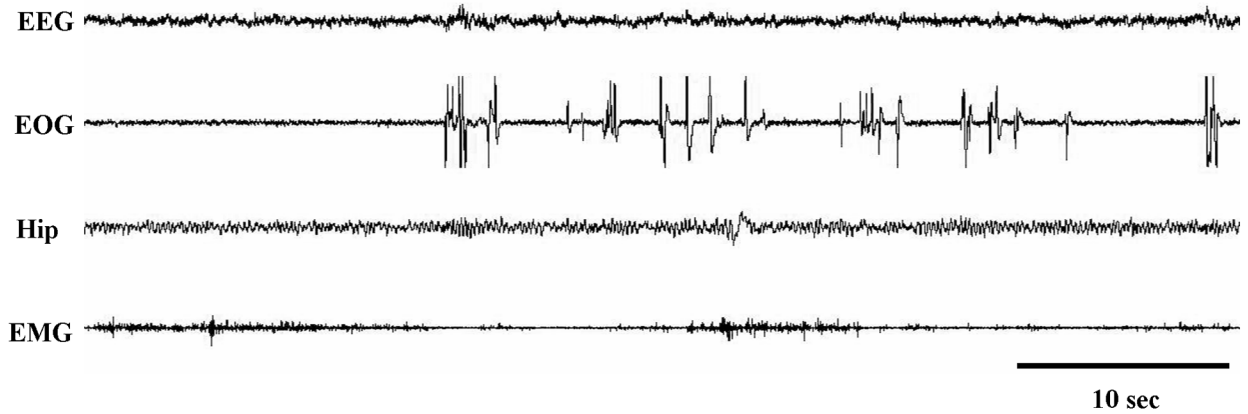
**Figure 3.** Change in the duration of wake, slow wave sleep (SWS) and REM sleep after VMPJ lesion. A significant increase in wakefulness and decrease in SWS and REM sleep were found in the rostral-lateral VMPJ lesioned cats (Group RL, ANOVA,  $p < 0.05$ ,  $n = 2$ ). On the other hand, the increase in SWS and REM sleep observed in the rostral-medial (Group RM, ANOVA,  $p > 0.05$ ,  $n = 3$ ) and caudal (Group C, ANOVA,  $p > 0.05$ ,  $n = 4$ ) VMPJ lesioned cats, was not significantly different from the baseline sleep pattern. B: baseline.



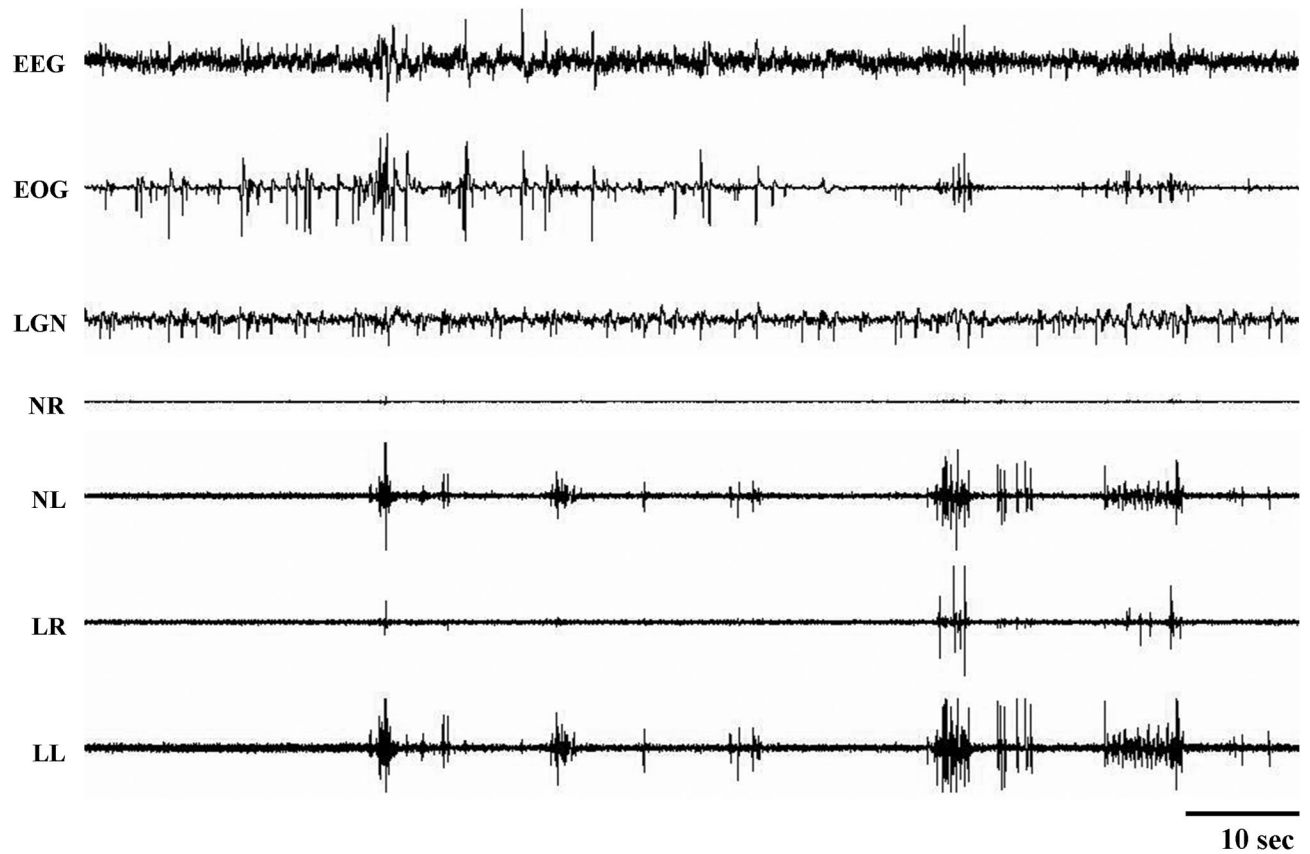
**Figure 4.** Periodic movements in the neck and leg during SWS after VMPJ lesion. NL and NR: left and right neck muscle EMG; LL and LR: left and right limb muscle EMG.



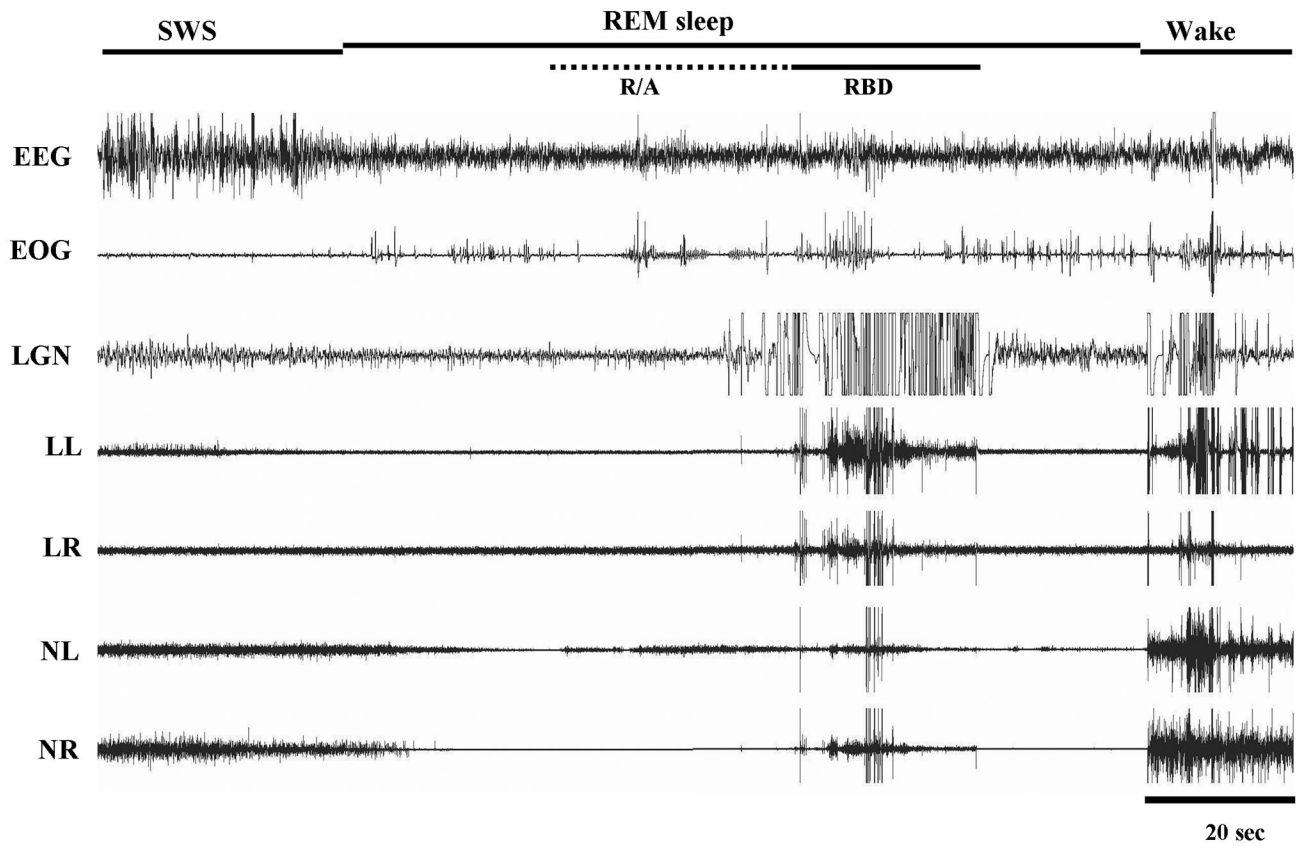
**Figure 5.** Changes in periodic and isolated leg and neck muscle activity in SWS after VMPJ lesion. An increase in periodic and isolated leg and neck muscle activity in SWS was seen by day 7 and day 3 after VMPJ lesion and continued for the entire 4-month period. The change in the index of periodic leg (PLMI, bottom right) and neck (PNMI, top right) muscle activity, as well as isolated leg (ILMI, bottom left) and neck (INMI, top left) muscle activity reached a plateau level at 14 days after VMPJ lesion. The Y-axis represents the index of INM, PNM, ILM, and PLM.



**Figure 6.** REM sleep with and without atonia (upper panel) and change in percent of REM sleep without atonia in REM sleep (bottom panel) after VMPJ lesion. EEG desynchronization, rapid eye movement, and hippocampal theta (Hip) waves with high muscle tone were recorded during REM sleep after VMPJ lesion. Bottom: percent of REM sleep without atonia in REM sleep was gradually increased after VMPJ lesion in all animals. EOG: electrooculograph.



**Figure 7.** Periodic leg movements in REM sleep after caudal VMPJ lesion. Periodic leg movements during REM sleep was not seen under baseline conditions. LGN: lateral geniculate nucleus activity.

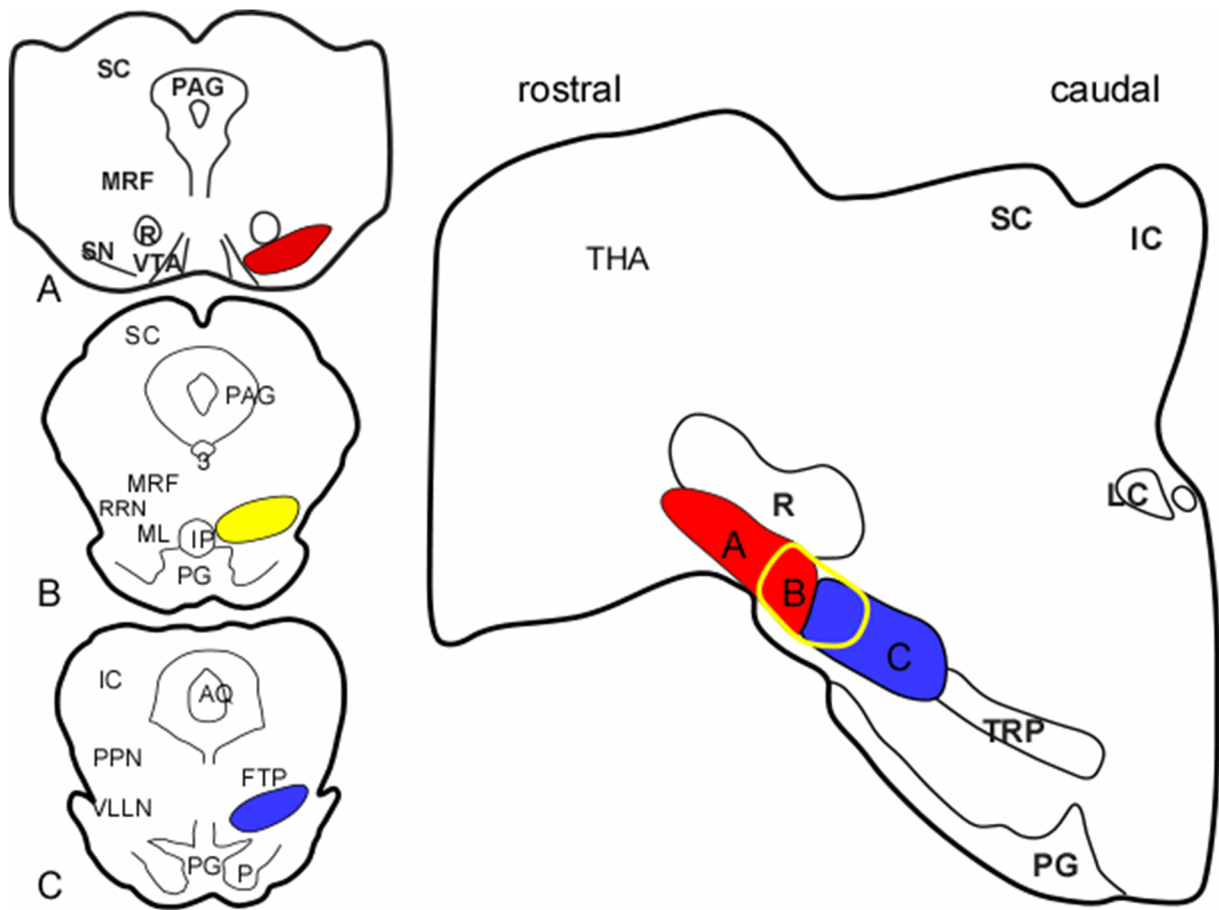


**Figure 8.**

REM sleep behavior disorder- (RBD) like activity. Muscle tone gradually increased and then, phasic muscle activity was seen in REM sleep. EEG desynchronization and rapid eye movements were seen during motor activity. Leg kicking was observed during the episode of RBD-like activity. Normal REM sleep returned after the episode of RBD-like activity.

R/A: REM sleep without atonia.





**Figure 9.**

Hypothetical anatomical link between motor disorders in sleep and Parkinsonism. The frontal sections (left) represent the corresponding areas shown on the sagittal section (right), and show the interface between the rostral midbrain and VMPJ. Neuronal degeneration in A (red area), which included substantia nigra, developed motor symptoms of Parkinsonism. Animals with neuronal degeneration in C (blue area), which is located in the caudal VMPJ, developed RBD. Whereas, neuronal degeneration in B (yellow circled), which is located in the rostral VMPJ generated PLMD and REM sleep without atonia. We hypothesize that neuronal degeneration may begin at either part of the ventral brainstem and then, extend to the rostral or caudal brainstem. IC: inferior colliculus, LC: locus coeruleus, PG: pontine gray, PPN: pedunculopontine nucleus, R: red nucleus, SC: superior colliculus, THA: thalamus, TRP: tegmental reticular nucleus, peripheral division.

**Table 1**

The total number of neurons lost\* after NMDA injection into the VMPI.

Cat	VLLN	FTP	PPN	MRF	RRN	VTA	SN	Total
VJ3	--	60	--	1508	1208	--	648	3424
FO4(1)	--	102	--	935	312	264	858	2471
FO4(2)	--	--	--	480	3756	--	2718	6954
FO5(1)	--	--	--	348	1032	--	1612	2992
FO5(2)	--	--	--	18	1425	--	1932	3375
LC11	--	--	--	1908	852	450	--	3210
LC12(1)	--	--	--	981	789	870	99	2739
LC12(2)	--	--	--	417	1936	726	--	3079
MO1	303	745	450	948	1390	--	--	3836
FO2	--	812	--	684	1616	--	--	3112
FO3	108	1298	--	856	610	--	--	2872
VJ4(1)	--	908	--	1034	566	--	--	2508
VJ4(2)	--	1218	--	534	552	--	--	2304

\* including the dopaminergic neurons in the RRN, SN and VTA, as well as the cholinergic neurons in the PPN.

-- No neurons were lost in the area.

FTP: paralemical tegmental field (including ventral part of the lateral lemniscus); MRF: mesencephalic reticular formation (including medial lemniscus); PPN: pedunculopontine nucleus, RRN: retrorubral nucleus; SN: substantia nigra; VLLN: nucleus of the lateral lemniscus, ventral portion; VTA: ventral tegmental nucleus.

**Table 2**

Percent of dopaminergic neurons lost in the caudal ventral midbrain

cat	LC11	LC12	FO4	FO5	VJ3	FO2	FO3	MO1	VJ4
%	14	27	32	22	17	7	5	22	13

**Table 3**

Relationship between dopaminergic neuron degeneration in the VMPJ and changes in sleep pattern. No significant relations were seen.

	D3	D7	D14	D30	D60	D90	D120
Wake							
R value	0.34	0.31	0.35	0.47	0.44	0.37	0.56
P	0.37	0.42	0.36	0.2	0.23	0.33	0.15
SWS							
R value	-0.1	-0.1	-0.28	-0.43	-0.43	-0.37	-0.56
P	0.81	0.8	0.46	0.25	0.25	0.32	0.15
REM sleep							
R value	-0.14	-0.21	-0.37	-0.48	-0.41	-0.33	-0.3
P	0.72	0.6	0.33	0.19	0.27	0.39	0.47

**Table 4**

Isolated and periodic movement index after second VMPJ lesion.

Cat	Index	B	D3	D7	D14	D30	D60	D90	D120
FO4	INMI	11.4	7.6	6.3	2.3	9.6	4.4	10.0	8.2
	ILMI	7.6	1.5	2.6	1.2	8.1	4.6	9.7	4.6
FO4	PNMI	29.8	19.8	31.3	27.5	30.8	25.4	28.7	24.2
	PLMI	12.9	10.8	11.4	11.2	12.6	13.1	15.7	10.1
V14	INMI	14.1	19.7	21.7	26.0	11.4	18.7	15.4	12.2
	ILMI	11.6	17.4	9.2	16.3	12.1	9.5	14.3	13.8
V14	PNMI	6.9	8.5	12.3	9.7	11.2	7.9	8.1	8.3
	PLMI	22.5	19.6	17.1	25.4	26.8	27.2	21.5	25.3

Baseline of the second lesion was taken from data taken 4-months after the first lesion.

INMI and ILMI: Isolated neck and leg movements index, respectively.

PNMI and PLMI: Periodic neck and leg movements index, respectively.

**Table 5**

Index of phasic motor activity in REM sleep before (B) and after VMPJ lesion.

Cat	B	D3	D7	D14	D30	D60	D90	D120
<i>Group R-M</i>								
LC11	5.4	4.4	5.7	12.6	18.9	11.7	5.9	7.3
LC12	9.6	8.2	4.7	18.2	23.4	14.1	7.9	N/A
FO5	2.0	8.1	16.8	3.6	5.9	6.6	2.7	3.9
<i>Group R-L</i>								
FO4(1)	91.8	97.1	192.9	99.4	78.8	46.4	65.6	80.8
FO4(2)	80.8	65.4	56.1	49.2	51.9	74.8	79.2	83.1
VJ3	3.7	5.2	8.4	5.0	4.9	4.6	3.3	4.4
<i>Group C</i>								
FO2	19.0	22.5	29.4	48.2	79.3	37.5	39.5	46.8
FO3	7.3	4.5	5.3	10.1	16.4	12.9	N/A	19.7
MO1	7.1	12.7	17.3	29.3	10.4	20.5	41.4	46.5
VJ4(1)	10.4	11.7	14.7	54.4	66.0	62.6	61.7	54.9
VJ4(2)	54.9	57.1	51.4	49.6	63.7	55.8	57.5	51.6

Baseline of the second lesion was taken from data of 4-month after first lesion.

(1) and (2): the first and second VMPJ lesions.

N/A: data were not available.