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Abbreviations:

A, anterior
ACh, acetylcholine
ARAS, ascending reticular activation system
CNF, cuneiform nucleus
CP, cerebral peduncle
EEG, electroencephalograms
EMG, electromyograms
EOG, electrooculograms
GABA, γ-amino butyric acid
H, horizontal
IC, inferior colliculus
IPSPs, inhibitory postsynaptic potentials
LDT, laterodorsal tegmental nucleus
L, left
LR, left or right
MLR, mesencephalic locomotor region
Mm, mammillary body
MRF, medullary reticular formation
NRPo, nucleus reticularis pontis oralis
PAG, periaquaductal grey
PGO, ponto-geniculo-occipital
PRF, pontine reticular formation
P, posterior
PPN, pedunculopontine tegmental nucleus
R, right
RBD, Rapid eye movement sleep behavioral disorder
REM, rapid eye movement
PMRF, pontomedullary reticular formation
RN, red nucleus
SC, superior colliculus
SNc, substantial nigra pars compacta
SNr, substantia nigra pars reticulata
SCP, superior cerebellar peduncle
Sol, soleus
STN, subthalamic nucleus
III, oculomotor nerve

Abstract

The present study was to determine how afferents from the substantia nigra pars reticulata (SNr) of the basal ganglia to the pedunculopontine tegmental nucleus (PPN) in the brainstem could contribute to the control of behavioral states. We used anesthetized and acutely decerebrated cats (n=22). Repetitive electrical stimulation (10-100 Hz, 20-50 μA, for 4-20 s) to the ventrolateral part of the PPN produced rapid eye movement (REM) associated with a suppression of postural muscle tone (REM with atonia). Although repetitive electrical stimuli (10-200 Hz, 10-60 μA, for 5-20 s) delivered to the dorsolateral part of the SNr did not evoke eye movements or muscular tonus in baseline conditions, it altered the PPN-induced REM with atonia. The following three types of effects were induced: (1) attenuation of the REM with atonia; (2) attenuation of muscular atonia without changes in REM (REM without atonia); and (3) attenuation of only REM. The optimal stimulus sites for these effects were intermingled within the lateral part of the SNr. The PPN-induced REM with atonia was abolished by an injection into the PPN of muscimol (1-15 mM, 0.1-0.25 μl), a γ-amino butyric acid $(GABA)_A$ receptor agonist, but not altered by an injection of baclofen (1-10 mM, 0.1-0.25 μl), a $GABA_B$ receptor agonist. Moreover, an injection of bicuculline (1-15 mM, 0.1-0.25 μl), a $GABA_A$ receptor antagonist, into the PPN, resulted in REM with atonia. On the other hand, an injection of muscimol into the dorsolateral part of the SNr (1-15 mM, 0.1-0.25 μl) induced REM with atonia, which was in turn eliminated by a further injection of muscimol into the PPN (5-10 mM, 0.2-0.25 μl).

These results suggest that a GABAergic projection from the SNr to the PPN could be involved in the control of REM with atonia, signs which indicate REM sleep. An excessive GABAergic output from the basal ganglia to the PPN in parkinsonian patients may induce sleep disturbances, including a reduction of REM sleep periods and REM sleep behavioral disorders (REM without atonia).

Key words

- Substantia nigra pars reticulata
- GABAergic projection
- Pedunculopontine tegmental nucleus
- Parkinson’s disease
- Decerebrate preparation
- REM sleep

Running title: The basal ganglia in the regulation of REM and atonia.

Patients with Parkinson's disease experience a number of sleep disorders, including insomnia, a high frequency of sleep fragmentation, and a wide range of parasomnias and daytime somnolence, specifically excessive daytime sleepiness, and sleep attacks (Ferini-Strambi, 2000; Larsen and Tandberg, 2001). Rapid eye movement (REM) sleep behavioral disorder (RBD) is also observed in parkinsonian patients (Bliwise et al., 2000; Boeve et al., 2001; Eisensehr et al., 2001; Rye et al., 1999). Patients with a lesion of the dorsolateral mesopontine tegmentum have clinically presented with REM without atonia which accompanies RBD (Culebras et al., 1989). The pathophysiological mechanism of RBD in Parkinson's disease is considered to be based not only on a dysfunction of a brainstem mechanism, but also on an impairment of mesopontine dopaminergic neurons (Albin et al., 2001). On the basis of clinical examinations of RBD in juvenile Parkinson's disease patients, Rye et al. (1999) have emphasized a potential role for dopamine and basal ganglia circuits in the modulation of behavioral states (e.g., wakefulness, and REM sleep).

It has been shown that mesopontine cholinergic neurons in the pedunculopontine tegmental nucleus (PPN) and laterodorsal tegmental nucleus (LDT) are involved in the generation of REM sleep (Datta, 2002; Datta and Siwek, 2002; Inglis and Winn, 1995; Jones, 1991; Koyama and Sakai, 2000; Maloney et al., 1999; Rye, 1997; Steriade et al., 1990; Vanni-Mercier et al., 1989; Webster et al., 1988). Major ascending cholinergic PPN projections into the thalamus, including the lateral geniculate nucleus, may provide ponto-geniculo-occipital (PGO) waves and desynchronization of electroencephalograms (McCormick and Bal, 1997; Steriade, 2001). Descending projections through the dorsolateral pontine tegmentum (Lai et al., 1993; Mitani et al., 1988; Semba, 1993; Takakusaki et al., 1996) and into the medial medullary reticular formation (Shiromani et al., 1990) are thought to be involved in the sensorimotor inhibition via reticulospinal systems (Chase and Morales, 1990; Takakusaki et al., 1994). In addition, projections to the

caudoventral pontine tegmentum possibly contribute to the generation of both REM and PGO waves (Vanni-Mercier and Debilly, 1998).

Neuroanatomically, the PPN receives a dense GABAergic input mostly from the substantia nigra pars reticulata (SNr), one of the output nuclei of the basal ganglia, in rats (Beckstead et al., 1979; Rye et al., 1987; Span and Grofova, 1991, 1992) and cats (Moriizumi et al., 1988). Nonetheless it has not yet been substantiated whether the pathway from the basal ganglia to the brainstem affects the generation of REM sleep. The present study was, accordingly, designed to elucidate how GABAergic nigrotegmental (SNr-PPN) projections could contribute to generate muscular atonia and REM. The muscular atonia associated with REM which is induced by chemical activation of the pontomedullary reticular formation in decerebrate preparations is a useful experimental model for understanding the neuronal mechanisms of REM sleep (Lai and Siegel, 1990; Morales et al., 1987; Takakusaki et al., 1993, 1994, 2001, 2003).

In the present experiments, we used acute decerebrate cats in which the striatum, thalamus, and cerebral cortex were removed, but the SNr was preserved. Because of the complexity of the mesopontine tegmentum a chemical stimulation technique was utilized, in addition to electrical stimulation, so that the neuronal activity could be selectively altered. In the first part of the investigation we examined whether electrical stimulation applied to the PPN could induce REM which was associated with muscular atonia. We then tested whether electrical stimulation applied to the SNr could affect the level of the muscle tone and the eye movements which were induced by the stimulation of the PPN. Finally, GABAergic neuroactive substances were injected into either the PPN or the SNr so that we could determine whether activation, and inactivation, of the GABAergic nigrotegmental projection altered the level of the muscle tone and the generation of REM. The present findings are discussed in relation to the sleep disturbances in parkinsonian patients. The preliminary results of the investigation have been reported in an abstract form (Takakusaki et al., 2002).

Experimental procedures

The experiments were performed with 23 cats from the animal facility at Asahikawa Medical College which weighed from 2.2 to 3.8 kg. All of the experimental procedures were approved by the Animal Studies Committee of Asahikawa Medical College and were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Guide, revised 1996). During the investigation every effort was made to minimize animal suffering and to reduce the number of animals which were used.

Surgical procedures

Surgical procedures were performed under halothane (Halothane, Hoechst, 0.5-–3.0%) and nitrous oxide gas (0.5–1.0 l/min) anesthesia with oxygen (3.0–5.0 l/min). Cats were surgically decerebrated either at a precollicular-postmammillary level. Particular attention was given to avoid injury to the cranial motor nerves for ocular movements, such as the oculomotor, trochlear and abducens nerves. The trachea was intubated, and a catheter was placed in a common carotid artery to monitor the blood pressure. After the surgery anesthesia was discontinued. The animal's head was fixed in a stereotaxic apparatus and a rigid spinal frame securely held the cat by clamping the dorsal processes of the three upper thoracic vertebrate. The limbs rested on a static surface while the body was supported by a rubber hammock. An animal’s rectal temperature was maintained at 36–37°C by using radiant heat lamps. Each cat’s mean blood pressure was maintained at greater than 100 mmHg, and the end tidal CO_2 was maintained between 4 and 6%.

Stimulation and recording

A glass micropipette filled with Wood’s metal, and with the tip replaced by a carbon fiber (diameter 6–7 μm, resistance 0.2–0.5 MΩ; Takakusaki et al., 2003) was inserted into the mesopontine tegmentum including the PPN, according to the

Horsley-Clark coordinates (anterior (A) 1.0 – posterior (P) 3.0; left or right (LR) 2.0 – 5.0; horizontal (H) +1.0 – -5.0). Constant pulse trains of repetitive electrical stimuli (10–60 μA and 0.2 ms duration at 10–100 Hz) were delivered for 4–20 seconds to the mesopontine tegmentum. To identify the optimal site for evoking muscle atonia and eye movements, the stimuli were delivered at 0.5–1.0 mm intervals in the dorsoventral, mediolateral, and rostrocaudal directions (Fig. 1). An identical type of electrode was also inserted into the caudal diencephalon (A 2.0 – A 5.0, LR 3.0 – 7.0, H +2.0 – -5.0). The SNr was stimulated with constant pulse trains (10–60 μA for 0.2 ms duration at 10–200 Hz). The stimuli were delivered at 0.5–1.0 mm intervals in the dorsoventral, mediolateral, and rostrocaudal, directions so that the optimal stimulus sites for inhibiting the PPN-induced effects could be identified (Fig. 5).

A micropipette which was filled with a solution of each of the following neuroactive substances was inserted into the mesopontine tegmentum (P 1.0–3.0, LR 3.5–4.5, H -1.0 – -3.5): muscimol (1.0–15 mM), a γ-amino butyric acid $(GABA)_A$ receptor agonist or baclofen (2.0–10 mM), a $GABA_B$ receptor agonist, or alternatively bicuculline (5.0–10.0 mM) or picrotoxin (2.0–10 mM), $GABA_A$ receptor agonists. In addition, a micropipette of the same type but filled with muscimol (1.0–15 mM) was also inserted into the SNr area (A 3.0–A 5.0, LR 3.0–6.0, H -2.0 – -4.0). Each neuroactive substance was injected into the PPN or SNr areas by using an oil-driven microinjection system (Takakusaki et al., 2003). The volume of each injection was invariably 0.1– 0.25 μl (in most cases 0.25 μl) and the injection rate was 0.01– 0.02 μl/second. All of the substances were dissolved in Ringer solution with the pH adjusted to 7.4. Any injection which did not alter the level of muscle tone or the eye movements within 20 minutes was judged as ineffective. Several injections were performed in each animal and an interval of more than 2 hours was allowed between each injection in order to examine either the reproducibility or dose-dependency of effects of injecting the neuroactive substances (Fig. 8).

A pair of stainless steel wires 2 mm apart was bilaterally inserted into the soleus (Sol) muscles to record the electromyograms (EMG), which were processed with a 5 Hz (low pass) and 100 Hz (high pass) filter with a time constant of 0.03 second. The electrooculograms (EOG) were recorded with a bipolar electrode screwed into the lateral part of the anterior wall of the bilateral frontal sinus. The EOG activity was recorded with a low pass filter of 0.5 Hz and a high pass filter of 200 Hz with a time constant of 0.03 second.

Histological controls

At the end of each experiment the stimulus sites were marked by passing a DC current of 30 μA through an electrode for 30 seconds. The injection sites and any spreading of the infusates were marked with an infusion of an equal volume of 10% fast green. Each cat was sacrificed with an overdose of Nembutal anesthesia and the brainstem was then removed. The brainstem was fixed in 10% formalin and later 50 μm frozen sections were cut in the coronal or parasagittal planes. Serial sections of brainstem tissue were examined to verify the injection sites, the location of the microlesions, and the diffusion injection sites that were marked with the fast green. The sites were identified with assistance from stereotaxic atlases (Berman, 1968; Snider and Niemer, 1961) which were used as references.

Results

A description of REM with atonia evoked by stimulating the PPN

We first examined the effects of stimulating the mesopontine tegmentum upon muscle tone and eye movements. Typical results are illustrated in Fig. 1. An electrical microstimulation of 40 μA applied to the ventrolateral part of the PPN (Fig. 1Ab, 1Bb) with a frequency of 50 Hz bilaterally abolished the soleus muscle tone and induced REM with a frequency of approximately 2 Hz. The eye movements were directed toward the stimulus side. The suppression of the muscle tone lasted for several seconds even after termination of the stimulation but the eye movements were evoked only during stimulation. However, the muscular atonia was not evoked by stimuli applied either dorsal or ventral to the sites (Fig. 1Aa, c) where eye movements were elicited (Fig. 1Ba, c). For example, stimulation of the subcoerular region (Fig. 1Ac) did not reduce muscle tone but evoked rhythmic eye movements with a frequency of approximately 1 Hz (Fig. 1Bc). In contrast, stimulation of the nucleus reticularis pontis oralis (NRPo) suppressed muscle tone (Fig. 1Ad) but did not induce eye movements (Fig. 1Bd). As shown in the examples of Fig. 1C, the latency of both the muscular atonia and the REM was reduced and the frequency of the REM was increased as the stimulus intensity was increased from 20 μA to 40 μA. Table 1A shows the characteristics of the PPN-induced REM with atonia which were observed in 8 animals. Although the mean latency of the atonia was variable (approximately 0.7–3.0 s) in each animal, the frequency of the REM was relatively constant at 2.0–2.5 Hz if the stimulus parameters were fixed (50Hz and 40 μA). It was a common observation that the frequency of the REM was increased, and the latency of the REM with atonia was reduced, as either the stimulus intensity was increased or the stimulus frequency was increased (Table 1B). In this animal, effective sites for evoking both REM and atonia were located in the ventrolateral part of the PPN (filled circles in Fig. 1D). Stimulus sites for evoking REM (filled and open circles) were located in both the dorsal and ventral part of the PPN. The stimulus sites for evoking

muscular atonia (filled circles and squares) were located in the PPN and the dorsolateral part of the NRPo. Three types of effects were thus induced by the mesopontine stimulation: REM, muscular atonia, and REM with atonia.

Figure 2 shows the distribution of the effective stimulus sites in 8 animals for evoking each effect on representative coronal (upper) and parasagittal (lower) planes of the brainstem. Sites for evoking REM (n=22) were located in the lateral part of the midbrain including the PPN (REM zone, Fig.2A). This zone was dorsoventrally extended for approximately 2.5 mm. The sites for induction of muscular atonia (n=42) were found in the PPN and the dorsolateral pontine reticular formation (PRF), corresponding to the NRPo (atonia zone, Fig.2B). Ten of the sites in each zone were overlapped, i.e., REM with atonia was induced from 10 sites which were preferentially located in the ventrolateral part of the PPN (REM with atonia zone, Fig. 2C). As can be seen in parasagittal planes, each zone was rostrocaudally extended for 2.0-2.5 mm.

From these findings we have proposed a framework, shown in Fig. 3, for the control of REM with atonia by the basal ganglia output from the SNr. An activation of PPN neurons is possibly involved in the generation of REM and muscular atonia through a REM generating system. This generating system is considered to be located in the caudoventral PRF (Vanni-Mercier and Debilly, 1998). A muscle tone inhibitory system descends from the pontomedullary reticular formation (Chase and Morales, 1990; Takakusaki et al., 1994). In addition, the PPN receives a dense GABAergic input from the SNr, a basal ganglia output nucleus (Beckstead et al., 1979; Moriizumi et al., 1988; Rye et al., 1987; Span and Grofova, 1991, 1992). On the basis of this framework, we investigated (as described in the next two sections) how GABAergic inhibitory input from the SNr to the PPN could regulate the generation of REM and muscular atonia.

<u>Involvement of SNr efferents to the PPN in the generation of REM and muscular atonia.</u>

We tested how stimulation of the SNr altered REM with atonia which was induced by stimulating the PPN. We found that a certain region in the SNr contributed to the elimination of the PPN-induced REM with atonia. Representative results are shown in Figure 4. Stimulation (100 Hz and 60 μA) of the lateral part of the SNr (an open arrow in Fig. 4B) did not induce any changes in muscle tone and eye movements (Fig. 4Ca). Stimulation of the PPN (a filled arrow in Fig, 4B) induced REM with atonia (Fig. 4Cb). Conditioning stimulation of the SNr with a relatively lower current (40 μA) attenuated PPN-induced REM with atonia (Fig. 4Cc). When stronger stimuli (60 μA) were applied to the SNr the PPN effects were eventually abolished (Fig. 4Cd). We delivered the SNr stimulation with frequencies between 10 and 200 Hz. The effects of the SNr stimuli in blocking the PPN effects were prominent when the stimulus frequency was between 50 and 100 Hz. Similar findings were observed in another 5 animals.

We further examined whether the SNr could have a functional topography in the control of PPN-induced REM and muscular atonia (Fig. 5). The procedure involved moving the stimulating electrode within and around the SNr so that the effective sites for changing the PPN-effects could be determined (Fig. 5A). The following three types of effects were induced by conditioning stimulation applied to the SNr: (1) attenuation of the REM with atonia; (2) attenuation of muscular atonia without changes in REM (REM without atonia); and (3) attenuation of only REM. The examples shown in Fig. 5D and E illustrate where conditioning stimuli were delivered at each site, together with vertical axes at A 4.0 and L5.5 (Fig. 5D) and at A 4.0 and L5.0 (Fig. 5E). Although conditioning stimuli to the SNr (indicated by an open arrow in Fig. 5A) alone did not affect the level of muscle tone or the eye movements (Fig. 5C), the stimuli eliminated most of the PPN-induced REM with atonia (Fig. 5B, Db and Dc). Stimuli at either dorsal or ventral sites at the axis of A 4.0 and L5.5 were less effective (Fig. 5Da and Dd). At the axis of A 4.0 and L5.0 (Fig. 5E), conditioning stimuli applied to a site dorsal to the SNr abolished the PPN-induced REM but the PPN-induced muscular atonia was less affected (Fig. 5Ea).

On the other hand, stimuli applied to the SNr did not block REM but attenuated the muscular atonia, i.e., REM without atonia was also induced by stimulation of the SNr (Fig. 5Eb and c).

In this animal, stimulus sites for eliminating the PPN-induced REM with atonia were located in the lateral part of the SNr (filled circles in Fig. 5A), while those for attenuating the PPN-induced atonia were located rather medially (open circles in Fig. 5A). These nigral stimulus effects were carefully examined in 4 animals. The results are summarized in Fig. 5F. The optimal SNr stimulus sites for eliminating both the PPN-induced REM and atonia are marked by filled circles (n=10). The stimulus sites for inhibiting only REM (n=6) and those for eliminating only muscular atonia (REM without atonia, n=8) are indicated by hatched squares and open circles, respectively. Generally, stimuli applied to the medial part of the SNr were less effective. Consequently, the following findings were obtained. First, the lateral part of the SNr was involved in the control of PPN-induced REM and muscular atonia. Second, a clear functional topography was not revealed within the lateral SNr, because the effective stimulus sites for evoking each effect were intermingled.

Contribution of nigral GABAergic afferents to the PPN to the control of REM and atonia

We further attempted to elucidate whether GABAergic afferents from the SNr to the PPN controlled the REM with atonia. For this, a chemical stimulation technique was utilized in combination with electrical stimulation. We first injected muscimol (a $GABA_A$ receptor agonist, 10 mM, 0.25 μl) into the left PPN where the electrical stimulation had evoked REM with atonia (Fig. 6Ba). It was observed that the muscimol injection attenuated (Fig. 6Bb) and finally abolished the PPN effects (Fig. 6Bc). When the PPN was stimulated two hours after the muscimol injection, the PPN effects had recovered (Fig. 6Bd). In the same preparation, a $GABA_B$ receptor agonist, baclofen (10 mM, 0.25

μl), was also injected into the right PPN. However a baclofen injection did not block the PPN effects (Fig. 6C). Similar findings were obtained in another 3 animals.

Bicuculline, one of the $GABA_A$ receptor antagonists, was injected into the ventrolateral PPN where the stimulation had evoked the REM with atonia (Fig. 7A). An injection of bicuculline into the left PPN (Fig. 7Ab) induced REM which started more than 1 minute after the injection (Fig. 7Ba). The tone of the bilateral soleus muscles was decreased and was noticeably diminished at approximately 3-4 minutes after the injection. In this animal the REM with atonia continued for more than 80 minutes. An injection of picrotoxin, another $GABA_A$ receptor antagonist, also induced REM with atonia. The injection sites for evoking REM with atonia in 6 animals are summarized in Fig. 8A. The optimal injection sites were located in the ventrolateral part of the PPN. In 4 animals the effects were induced by bicuculline (gray circles) and in 2 animals the effects were induced by picrotoxin (black circles). These findings suggest that the PPN-induced REM with atonia is produced by a release from tonic GABAergic inhibitory effects on PPN neurons via $GABA_A$ receptors. On the other hand, locomotor movements were evoked when these $GABA_A$ receptor antagonists were injected into the dorsal part of the PPN and/or the cuneiform nucleus (CNF). These areas corresponded to the mesencephalic locomotor region (MLR). Essentially the same findings have been reported previously (Garcia-Rill et al., 1985; Takakusaki et al., 2003).

Finally we investigated whether the GABAergic afferents from the SNr to the PPN could generate REM with atonia. We injected muscimol, a $GABA_A$ receptor agonist, into the lateral part of the SNr (Fig. 7B). The injection was at the location where electrical stimulation had blocked the effects of the PPN-induced REM with atonia. The muscimol injection site (arrow) and stimulation site (open arrowhead) in the left SNr are shown in Fig. 7Bc. Following the injection of muscimol into the SNr eye movements started at approximately 2 minutes, and muscle tone was diminished at approximately 3 minutes. After 20 minutes muscimol was also injected into the ventral PPN so that the

PPN neurons could be GABAergically inactivated. This injection reduced the frequency of the REM, and the muscle tone started to be restored. Several minutes after the muscimol injection into the PPN the eye movements were diminished and the normal level of the muscle tone was re-established (Fig. 7Bb). On the other hand, a muscimol injection into the medial part of the SNr induced rhythmic alternation of hindlimb movements (Fig. 7Cb). This type of locomotor activity started 12 minutes after the injection and lasted for about 30 minutes.

In all, muscimol was injected into the SNr area in 12 animals (Fig. 8). In 7 cats REM with atonia was induced, and the injection sites were mainly located in the dorsolateral part of the SNr (filled areas). Locomotor activity was induced in 2 cats (hatched areas) when muscimol was injected into the medial part of the SNr. However, muscimol injections into either a more medial part of the SNr (1 cat) or dorsal to the SNr (2 cats) were ineffective.

The spread of an infusion of fast green was usually used to identify the injection sites and to a measure the spread of the injections. As can be seen in Fig. 7, each injection of 0.2–0.25 μl was limited to an area of approximately 2.0 mm diameter in the dorsoventral direction and approximately 1.0 mm diameter in the mediolateral directions. The graphs in Fig. 8C and D illustrate the dose-dependency of REM with atonia induced with bicuculline injections into the PPN (3 animals) and that with muscimol injections into the SNr (3 animals), respectively. Injections of physiological saline into each area were not effective. Injections into the same sites with an increased concentration of each substance in the same volume (0.25 μl) obviously reduced the latency of the REM with atonia. All of the results indicate, in the decerebrate animal, that the GABAergic projection from the lateral part of the SNr to the PPN is involved in the regulation of REM with atonia via $GABA_A$ receptors of the PPN neurons.

Discussion

The present study has provided important evidence that the basal ganglia afferents from the SNr to the PPN are involved in the regulation of REM with atonia. The results indicate that the basal ganglia possibly contribute to the control of a behavioral state, i.e., the generation of REM sleep. The neuronal mechanisms of generating the PPN-induced REM with atonia are discussed with respect to the pathogenesis of sleep deficiencies in basal ganglia disorders such as Parkinson's disease.

Neuronal mechanisms of PPN-induced REM and atonia

Stimulation of the ventrolateral part of the PPN induced REM with atonia. The stimulus strength was relatively small (less than 60 μA), and the current spread was estimated to be less than 0.5 mm because displacement of the stimulating electrode by 0.5 mm usually evoked different effects. However the electrical stimuli possibly activated both the neuronal elements and fibers of passage. Thus neuroactive substances were injected into the target region so that we could alter the activity of only the neuronal elements. As a result, injections of $GABA_A$ receptor antagonists into the ventral PPN area also produced REM with atonia. It has been shown that injections of a glutamatergic agonist (kainic acid) into the PPN of rats induced REM sleep (Datta, 2002). Neurotoxic lesions of the PPN area resulted in a prominent reduction of a period of REM sleep which was significantly correlated with a decrease in the number of cholinergic neurons (Webster and Jones, 1988). Moreover, a percentage of the period of REM sleep was positively correlated with tegmental cholinergic c-*fos* cells (Maloney et al., 1999). These findings suggest that an activation of cholinergic PPN neurons critically contributes to the generation of REM sleep, and that both the $GABA_A$ receptors and non-NMDA receptors on the PPN neurons are involved in this process. The latency of the REM with atonia was reduced by increasing either the intensity of the electrical stimulation or the concentration of the bicuculline injection applied to the PPN. The phenomena could be

the result of a recruitment of PPN neurons. There are not only cholinergic (Armstrong et al., 1983; Rye et al., 1987; Span and Grofova 1992) neurons but also non-cholinergic neurons, such as GABAergic (Kosaka et al., 1988; Ottersen and Storm-Mathisen, 1984), glutamatergic (Clements et al., 1991) and peptidergic (Vincent et al., 1983) neurons in the PPN. Recent studies have revealed a contribution of GABAergic neurons in the mesopontine tegmentum to the induction of REM sleep (Maloney et al., 1999; Torterolo et al., 2001). Xi et al. (1999, 2001) reported that injections of $GABA_A$ receptor antagonists into the PRF also induced REM sleep, and suggested that the motor inhibitory system during REM sleep is tonically inhibited by GABAergic effects on the PRF neurons.

With respect to the functional topography in the mesopontine tegmentum, although the “REM zone” and “atonia zone” partly overlapped, each zone was roughly segregated (Figs. 1 and 2). The REM zone coincided mostly with the locations of neurons that were closely linked to the generation of the PGO waves (PGO-on neurons, Datta and Hobson, 1994; Koyama and Sakai, 2000). On the other hand, the atonia zone corresponded to the ventrolateral part of the PPN and extended to the dorsolateral part of the NRPo. Electrical stimulation applied to each area has induced muscular atonia in acute decerebrate cats (Lai and Siegel, 1990; Takakusaki et al., 1994, 2003; Oka et al., 1993). These findings suggest that there is a functional organization with respect to the generation of REM and muscular atonia. It can be postulated that subpopulations of neurons responsible for either REM or muscular atonia are intermingled within the REM with atonia zone. Alternatively, a particular population of neurons in the “REM with atonia zone” could be capable of evoking both REM and atonia.

Both REM and PGO waves are prominent phasic events of REM sleep which occur in conjunction. It has been reported that PGO-on neurons are recorded at discrete regions around the brachium conjunctivum including the PPN (Datta and Hobson, 1994; Koyama and Sakai, 2000; McCarley et al., 1978; Sakai and Jouvet, 1980) and in the

caudal PRF (Pivik et al., 1977). Vanni-Mercier and Debilly (1998) have provided evidence for a parallel organization of the oculomotor and PGO wave systems. An injection of carbachol into the caudoventral PRF, a key structure, produced a long burst of PGO waves during waking, and an injection of atropine sulfate decreased the PGO burst that associated eye saccades during REM sleep. They suggested that an interconnection between the mesopontine cholinergic nuclei and the caudoventral PRF could operate as a common generator of REM and PGO waves. In addition, a projection from the superior colliculus (SC) to the paramedian PRF is involved in the saccadic eye movements (Hikosaka et al., 2000). Because the projection from the PPN to the SC is thought to induce express saccades (Kobayashi et al., 2001), a contribution of polysynaptic pathways from the PPN to the paramedian PRF via the SC to the generation of REM should also be considered.

Stimulation of the PPN has evoked inhibitory postsynaptic potentials (IPSPs) in hindlimb motoneurons (Takakusaki et al., 1997a). The nature of the IPSPs resembled those evoked by stimulating the PMRF (Chase et al., 1986; Fung et al., 1982) during REM sleep in the chronic cat preparation. Accordingly, PPN stimulation possibly activated the PMRF that is responsible for motor inhibition during REM sleep (Chase and Morales, 1990; Takakusaki et al., 1994, 2001). It has been demonstrated that certain lesions of the dorsal PRF (Hendrics et al, 1982; Stanford et al., 1994) and the medial MRF (Schenkel and Siegel 1989) in cats eliminated the muscle atonia during REM sleep (REM without atonia) and elaborated motor behavior during REM episodes. These findings support the evidence that projections from the PPN to the PMRF are involved in the PPN-induced muscular atonia.

Mechanisms of GABAergic SNr efferents to the PPN in the control of REM and atonia

We have previously demonstrated a functional topography in the GABAergic nigrotegmental projections, i.e., projections from the medial SNr to the MLR and from

the lateral SNr to the ventrolateral PPN control locomotion and muscle tone, respectively (Takakusaki et al., 2003). The above results were further supported by the present findings that injections of muscimol into the lateral and medial SNr induced muscular atonia and locomotor activities, respectively (Fig. 7). However, a clear functional topography was not observed in the SNr with respect to the control of REM and muscular atonia. The following effects were induced from the SNr: (1) attenuation of the REM with atonia; (2) attenuation of muscular atonia without changes in REM (REM without atonia); and (3) attenuation of only REM. The stimulus sites for evoking each of these effects were intermingled within the lateral part of the SNr (Fig. 5).

Then how were these various effects induced by stimulation of the SNr? One of the explanations is that SNr stimulation excited not only SNr neurons but also passing fibers arising from the subthalamic nucleus (STN). Because fibers from the STN project to both the SNr and the PPN (Carpenter et al., 1981), stimulation of the SNr may activate not only nigrotegmental fibers but also subthalamotegmental fibers. The nigrotegmental fibers may induce inhibitory effects on PPN neurons (Granata and Kitai, 1989; Saitoh et al., 2003: Takakusaki et al., 1997b) whereas the subthalamotegmental fibers may induce excitatory effects on PPN neurons (Granata and Kitai, 1989; Takakusaki et al., 1997b). Accordingly, SNr stimulation may induce either excitatory or inhibitory effects or a mixture of both, on the tegmental neurons and evoke various effects. An alternative explanation is as follows. Because the “REM zone” and “atonia zone” were partly segregated in the mesopontine tegmentum, each may receive GABAergic inputs from different populations of SNr neurons. Specifically, functionally segregated nigrotegmental systems which regulate either eye movements or muscle tone may exist. Accordingly, SNr stimulation might activate each nigrotegmental projection alone, or the nigrotegmental projections together, to induce various effects. On the other hand muscimol injections into the lateral SNr mostly induced REM with atonia (Fig.7B). This

could be a result from the simultaneous inactivation of the functionally segregated nigrotegmental neurons.

Although the PPN receives mostly GABAergic basal ganglia afferents from the SNr, the PPN also receives basal ganglia efferents from either the internal segment of the globus pallidus (Nauta and Mehler, 1966) or the entopeduncular nucleus (Grofova and Zhou, 1998; Moriizumi and Hattori, 1992; Spann and Grofova 1991). There are also efferents from the nucleus accumbens (Groenewegen et al., 1993) and the substantia innominata (Semba and Fibiger, 1992; Swanson et al., 1984). It is thus necessary to consider whether these GABAergic afferents to the PPN are involved in the regulation of the REM with atonia. Moreover, as demonstrated by Maloney et al. (1999), a contribution of local GABAergic neurons in the PPN cannot be disregarded to the regulation of REM sleep. Because PPN-induced REM with atonia was not blocked by an injection of baclofen, but was completely abolished by an injection of muscimol into the PPN, the $GABA_A$ receptors of the PPN neurons could be more important than the $GABA_B$ receptors. This suggestion is supported by a recent investigation of Saitoh et al. (2003). Their investigation, which used *in vitro* rat slice preparations, demonstrated that stimulation of the SNr induced monosynaptic IPSPs in cholinergic PPN neurons. The IPSPs were completely diminished after application of $GABA_A$ receptor antagonists, but not diminished with $GABA_B$ receptor antagonists. Based on these observations, together with the present results, we support the evidence that cholinergic PPN neurons, which are responsible for the induction of REM and atonia, can be under the control of a sustained GABAergic inhibition from the SNr. That is, the GABAergic SNr-PPN projection is possibly involved in the generation of REM sleep.

Datta et al. (1991) have demonstrated that a group of SNr neurons projecting to the PPN area increased their firing rate during REM sleep. Maloney et al. (2002) have shown that a c-*fos* expression of GABAergic SNr neurons during REM sleep was higher than during non-REM sleep and when awake. These studies suggest that GABAergic SNr

neurons do not necessarily contribute to the induction of REM sleep in normal animals. An interaction between the cholinergic and monoaminergic systems (Hobson et al., 1986; Koyama and Sakai, 2000; Maloney et al., 1999; Takakusaki et al., 1994) in the mesopontine tegmentum can play a more important role for the generation of REM sleep than the GABAergic SNr-PPN projection. Based on the above considerations, we consider that the GABAergic projection system may affect the generation of REM sleep if the excitability of SNr neurons is significantly disturbed.

Involvement of GABAergic mechanisms in the generation of sleep disturbances in basal ganglia disorders

Since the classical study of Moruzzi and Magoun (1949), the pontomesencephalic reticular formation has been known to comprise the ascending reticular activation system (ARAS). The PPN has been considered as a part of the ARAS (Garcia-Rill, 1997; Inglis and Winn, 1995; Jones, 1991; Steriade, 1996). The SNr has a direct projection to the thalamic nuclei (Hendry et. al., 1979; Parent et al., 1983; Paré et al., 1990) in addition to the PPN. Consequently, our idea is that basal ganglia output from the SNr may affect awake-sleep cycles by modulating the activity of the ARAS through dual systems. One system is through a direct nigrothalamic projection, and the other, which is considered in this study, is by indirect connections via the PPN.

Patients with Parkinson's disease experience a number of sleep disorders, including RBD (Ferini-Strambi, 2000; Larsen and Tandberg, 2001). Rye et al. (1999) have described an unmedicated juvenile Parkinsonian patient with RBD, and emphasized the potential role of dopamine and basal ganglia circuits in the modulation of behavioral states. In Parkinson’s disease the GABAergic output from the basal ganglia can be excessive because of a deficiency of the nigrostriatal dopaminergic systems (Delong, 1990; Wichmann and Delong, 1996).

Based on all of the considerations described so far, we have proposed a model, shown in Fig. 9, where we emphasize the role of a SNr-PPN system in the regulation of REM sleep. The GABAergic basal ganglia output may affect, via the PPN, the activity of the REM generator and the muscle tone inhibitory system, which together are responsible for the production of REM and muscular atonia during REM sleep (Fig. 9A). In Parkinson's disease excessive GABAergic effects upon the PPN could reduce the activity of PPN neurons. If PPN neurons in both the "REM zone" and the "atonia zone" are equally suppressed by excessive GABAergic inputs, the activity of both the REM generating system and the muscle tone inhibitory system could be reduced. This reduction would result in a decrease in the period of REM sleep (Fig. 9Ba). On the other hand, if the excessive nigral GABAergic inhibition acts on only the "atonia zone", activity of the muscle tone inhibitory system could alone be reduced, i.e., a state of REM without atonia could be induced (Fig. 9Bb). This may support the proposition that a decrease in basal ganglia dopaminergic activity could be involved in the reduction of REM sleep and in RBD (Albin et al., 2000; Inglis and Winn, 1995; Rye et al., 1999). We consider that this model may assist understanding the pathophysiological mechanisms of sleep disturbances in Parkinson's disease. Nevertheless, because the results were obtained from decerebrate animals, there is a need to elucidate whether the basal ganglia afferents to the PPN contribute to an expression of all REM sleep signs in normally behaving animals.

Conclusions

The present study provided evidence that the GABAergic SNr-PPN projection possibly regulates a generation of REM sleep. The dysfunction of this system may produce sleep disturbances observed in Parkinson disease.

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Table 1. Characteristics of the PPN induced REM with atonia in cats.

A Fixed parameters (50 Hz, 40 μA)

	Stimulus parameters		Events	Frequency (Hz)	Latency to REM with atonia (s)	
Cat 1	50 Hz	40 μA	(n=84)	2.54 ± 0.37	0.72 ± 0.14	(8 trials)
Cat 2	50 Hz	40 μA	(n=72)	2.36 ± 0.96	2.83 ± 0.56	(6 trials)
Cat 3	50 Hz	40 μA	(n=76)	2.48 ± 0.57	1.98 ± 0.27	(6 trials)
Cat 4	50 Hz	40 μA	(n=66)	2.22 ± 1.12	1.82 ± 0.27	(7 trials)
Cat 5	50 Hz	40 μA	(n=54)	2.17 ± 0.55	1.12 ± 0.23	(6 trials)
Cat 6	50 Hz	40 μA	(n=42)	2.07 ± 0.50	2.50 ± 0.43	(5 trials)
Cat 7	50 Hz	40 μA	(n=48)	2.29 ± 0.59	1.96 ± 0.31	(5 trials)
Cat 8	50 Hz	40 μA	(n=66)	2.38 ± 0.42	1.68 ± 0.45	(7 trials)

B Changes in stimulus parameters

	Stimulus parameters		Events	Frequency (Hz)	Latency to REM with atonia (s)	
Cat 1	50 Hz	20 μA	(n=46)	1.47 ± 0.46	4.12 ± 0.64	(6 trials)
		30 μA	(n=66)	1.79 ± 0.42	2.58 ± 0.24	(8 trials)
		40 μA	(n=84)	2.54 ± 0.37	0.72 ± 0.14	(8 trials)
Cat 2	50 Hz	20 μA	(n=48)	1.80 ± 0.16	4.41 ± 0.64	(5 trials)
		40 μA	(n=55)	2.36 ± 0.96	2.83 ± 0.56	(5 trials)
		60 μA	(n=72)	2.72 ± 1.15	0.78 ± 0.20	(6 trials)
Cat 3	40 μA	20 Hz	(n=53)	2.03 ± 0.56	3.10 ± 0.30	(6 trials)
		50 Hz	(n=76)	2.48 ± 0.57	1.98 ± 0.27	(6 trials)
		67 Hz	(n=72)	3.28 ± 0.52	1.33 ± 0.26	(5 trials)
		100 Hz	(n=78)	3.92 ± 0.78	1.02 ± 0.38	(5 trials)
Cat 4	40 μA	20 Hz	(n=48)	1.67 ± 0.20	4.14 ± 0.25	(6 trials)
		50 Hz	(n=66)	2.22 ± 1.12	1.82 ± 0.27	(7 trials)
		100 Hz	(n=69)	2.46 ± 1.28	1.18 ± 0.22	(6 trials)

A. The REM with atonia was obtained from 8 animals. Even when the stimulus parameter was fixed for each animal, the latency to the onset of the REM with atonia and the amplitude of the REM were varied. In contrast, the frequency of the REM was relatively constant for each animal at approximately 2–2.5 Hz. B. The effects of changes in the PPN stimulus parameters on the REM with atonia. Generally, the latency to the REM with atonia was reduced when either the stimulus intensity was increased (up to 60 μA, cat 1 and 2) or the stimulus frequency was increased (up to 100 Hz, cat 3 and 4).

Figure legends

Figure 1. Mesopontine tegmental areas subserving rapid eye movements and muscular atonia.

A. Stimulus sites in the mesopontine tegmentum on a coronal plane at P 2.0. B. Effects on muscle tone following stimulation at each site in A. The upper recording is an EOG, the middle and lower recordings are EMGs from the left (L) and right (R) soleus (Sol) muscles. The stimulus parameters were: intensity, 40 μA; frequency, 50 Hz; duration, 4–8 s. The stimulus period is indicated by lines under each recording. C. REM with atonia depends on the PPN stimulus intensity. The stimulus intensity was increased from the records (a) to (c) as follows: (a), 20 μA; (b), 30 μA; (c), 40 μA. The latency to the onset of the muscular atonia and to the REM was decreased as the stimulating intensity was increased. Moreover, the frequency of the eye movements were increased as the stimulus intensity was increased. The mean frequency of the REM was increased from 1.5 Hz (a) to 2.5 Hz (c). The stimulus period is indicated by lines under each recording. D. Effective stimulus sites from which REM with atonia (filled circles), REM (open circles) and muscular atonia (filled squares) were evoked. An appropriate location of the PPN is indicated by the grey area. IC, inferior colliculus; CNF, cuneiform nucleus; PPN, pedunculopontine tegmental nucleus; NRPo, nucleus reticularis pontis oralis.

Figure 2. Effective sites for evoking REM and muscular atonia.

A. Stimulus sites for evoking REM (n=22). B. Stimulus sites for evoking muscular atonia (n=42). C. Stimulus sites for evoking REM with atonia (n=10). Note that there is gross functional organization in the mesopontine tegmentum. SCP, superior cerebellar peduncle.

Figure 3. A framework for the basal ganglia (SNr) control of REM with atonia.

The PPN mostly receive GABAergic input from the SNr. A cholinergic projection from the PPN to the PMRF (Lai et al., 1993; Mintani et al., 1988; Shiromani et al., 1990) activates a REM generator and a muscle tone inhibitory system, which induce REM and muscular atonia, respectively. GABA, γ-aminobutylic acid; Mm, mammillary body; SC, superior colliculus; SNr, substantia nigra pars reticulata; ACh, acetylcholine.

Figure 4. The effects of stimulation of the SNr upon the PPN-induced REM with atonia. A. An experimental diagram. B. Stimulus sites in the SNr and the PPN on frontal sections of the brainstem. The stimulus sites were marked by electrical microlesions. C. The effects of stimulation of the SNr upon the PPN-induced REM with atonia. (a) Stimulation of the SNr with an intensity of 60 μA and a frequency of 100 Hz alone neither affected the level of muscle tone nor the eye movements. (b) Stimulation of the PPN (40 μA, 50 Hz) induced REM with atonia. (c) A conditioning stimulation of the SNr (40 μA, 100 Hz) partly blocked the REM and attenuated the muscle tone suppression that was induced by the PPN stimulation. (d) By increasing the intensity of the conditioning stimulation (60 μA) the PPN effects were completely diminished. The period of the PPN and the SNr stimuli is respectively indicated by black and dashed lines under each recording. CP, cerebral peduncle; PAG, periaquaductal grey; RN, red nucleus; III, oculomotor nerve.

Figure 5. Effective sites in the SNr where stimulation altered the PPN-induced REM and muscular atonia. A. A coronal plane at a level of A 4.0 shows effective stimulus sites for blocking PPN-induced REM (open circles), muscular atonia (a grey square) and REM with atonia (filled circles) in a single animal. A microlesion in the PPN at a coronal plane of P 3.0 shows an effective site for induction of REM with atonia. B. Stimulation of the PPN (40 μA, 50 Hz) induced REM with atonia. C. Stimulation of the SNr (60 μA, 100 Hz), by itself, neither affected the level of muscle tone nor the eye movements. D.

Changes in the PPN-effects following conditioning stimuli which were delivered to the SNr area from dorsal (a; H = -2.0) to ventral (e; H = –5.0) locations along with a vertical axis at A 4.0 and L 5.5. The stimulus sites are shown in A. SNr stimuli delivered at H = –3.0 abolished the PPN effect (b). However, stimuli delivered at H = –4.0 (c) attenuated the PPN effect. The stimuli at more dorsal (a) and ventral (d) areas less affected the PPN effect. E. Conditioning stimuli were delivered to the SNr areas along with a vertical axis at A 4.0 and L 5.0. Stimuli delivered at H = –2.0 only abolished the PPN-induced REM (a). However, stimuli delivered at H = –3.0 and -4.0 (c) blocked muscular atonia but did not remove REM. The period of the PPN and the conditioning SNr stimuli is indicated by black and dashed lines under each recording, respectively. F. Nigral stimulus sites for modification of the PPN-effects, which were examined in 4 animals. Sites for totally eliminating the PPN-effects are shown by filled circles (n=10). Sites for attenuating REM are indicated by grey squares (n=6) and those for attenuating muscular atonia are indicated by open circles (n=8). SNc, substantia nigra pars compacta.

Figure 6. GABAergic inhibition of the PPN-induced REM with atonia.
A. An experimental diagram. B. The effects of an injection of muscimol (10 mM, 0.25 μl) into the left PPN upon the PPN-induced REM with atonia. (a) Stimulation of the PPN (30 μA, 50 Hz) induced REM with atonia. (b) - (c) Muscimol injection attenuated (b) and abolished the PPN effects (c). (d) The PPN effects were re-established 120 minutes after the muscimol injection. C. The effects of an injection of baclofen (10 mM, 0.25 μl) into the right PPN upon the PPN-induced REM with atonia. (a) Stimulation of the right PPN (40 μA, 50 Hz) induced REM and atonia. (b) The PPN stimulation induced REM and atonia even after injection of baclofen. The period of stimulation is indicated by lines under each recording.

Figure 7. The GABAergic nigrotegmental projection controls REM and atonia.

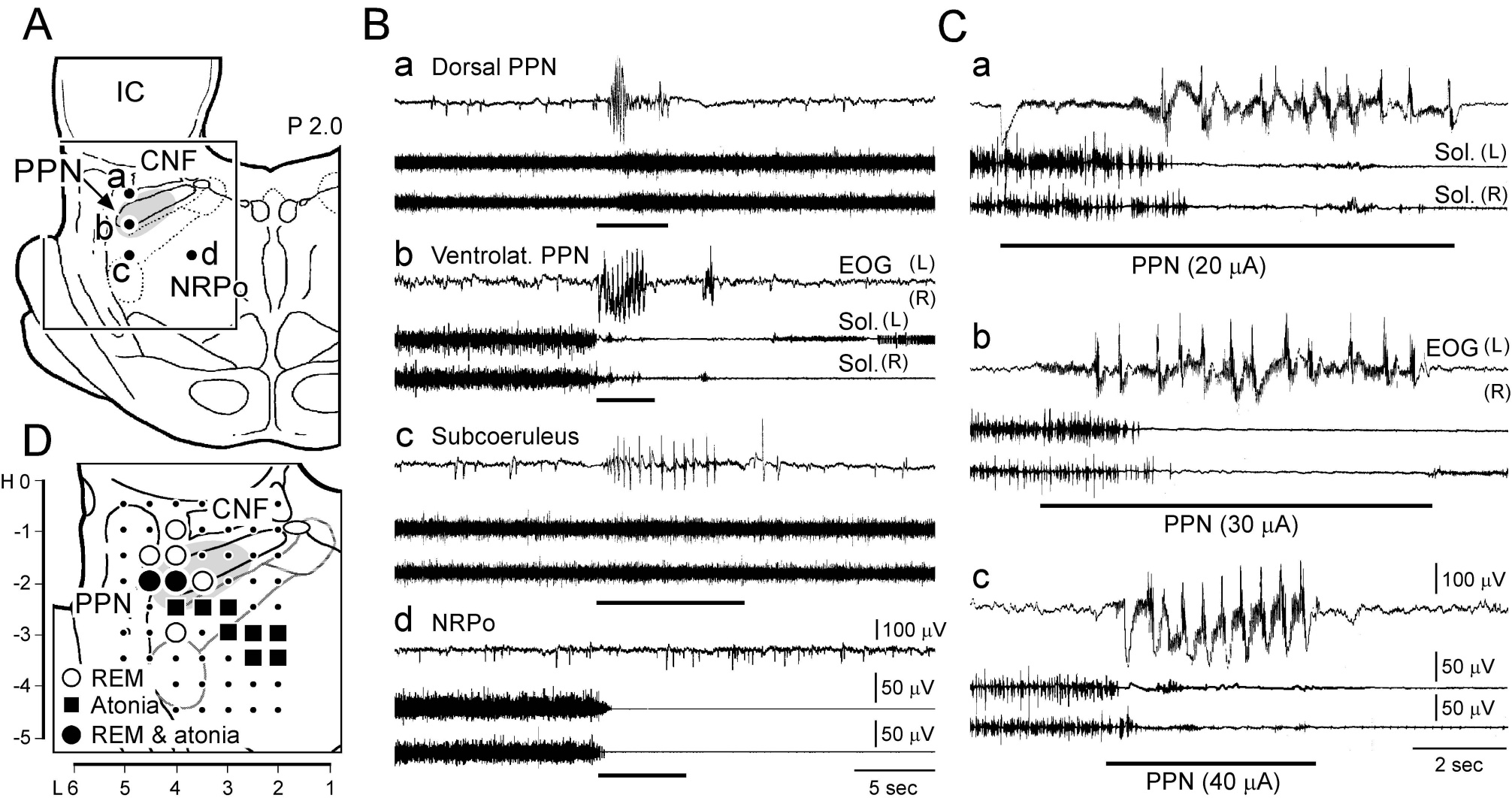
A. (a) REM with atonia induced by a microinjection of bicuculline into the left PPN. The REM started approximately 80 s after the injection and following this the muscle tone started to decrease. The muscular tonus was bilaterally diminished approximately 4 min after the injection. (b) A bicuculline injection site which was marked with fast green in a frontal section of the pons (see methods). B. (a) REM with atonia which was induced by a microinjection of muscimol into the left SNr. The REM started approximately 1 min 30 s after the injection and the muscle tone was abolished bilaterally approximately 2 min after the injection. (b) Approximately 20 min after the SNr muscimol injection, muscimol was injected into the left PPN. The REM was reduced and the muscle tone was re-established approximately 6 min after the PPN muscimol injection. (c) The muscimol injection site (indicated by an arrow), and the microstimulation site (indicated by an open arrowhead) in the dorsolateral part of the left SNr. The period of the injections in A and B are indicated above each recording. C. (a) Recordings of EOG and EMGs of bilateral soleus muscles before muscimol injection. (b) Locomotor activity, which was observed at 20 min after injection of muscimol into the medial part of the left SNr. (c) The muscimol injection site (indicated by an arrow). D. An experimental diagram.

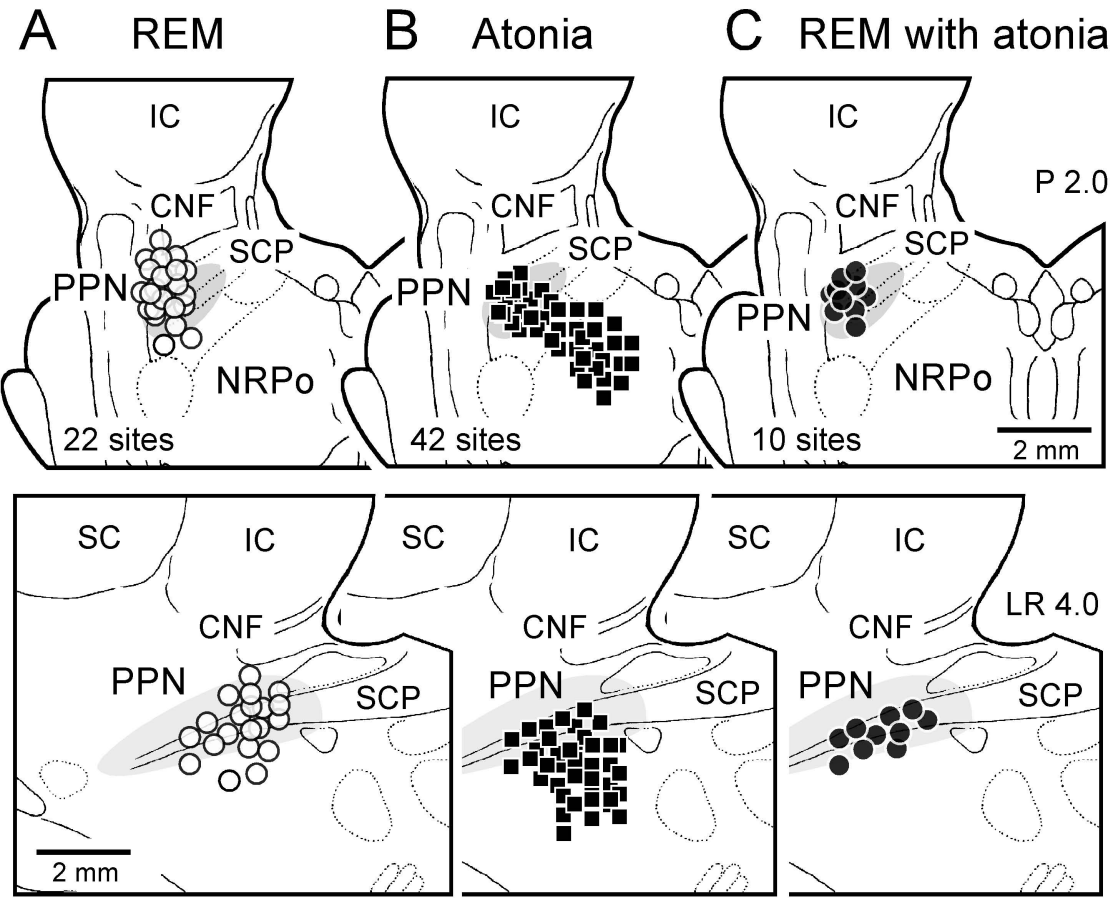
Figure 8. The optimal infusion sites in the PPN and the SNr for induction of REM with atonia, and dose-dependency of REM with atonia. A. Six injection sites in the PPN area from which REM with atonia was induced. The bicuculline and picrotoxin injection sites are indicated by black (2 cats) and gray (4 cats) circles, respectively. B. Effective and ineffective muscimol injection sites in the SNr area. REM with atonia was induced in 6 animals, and the injection sites are indicated by filled symbols. Two injections, which are indicated by hatched symbols, induced locomotor activities. Open symbols are ineffective injection sites. C and D. The dose-dependency of the chemically-induced REM with atonia. C. The dose-dependent effects of bicuculline injections into the PPN which were observed in 3 animals are shown. D. The dose-dependent effects of

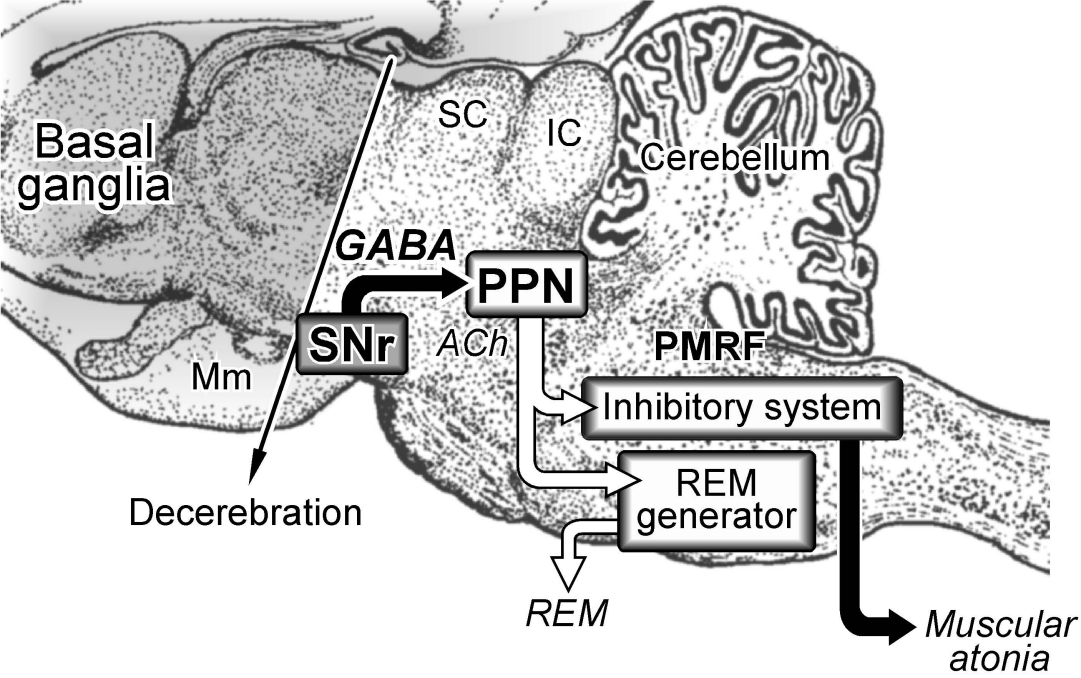
muscimol injections into the SNr in 3 animals are also illustrated. Although injections of saline did not have any effects in, an increase in the concentration of each of the neuroactive substances reduced the latency to the onset of the REM with atonia. The same volume of solution was stereotaxically injected into a specific site for each trial. Note that the data obtained from different animals are indicated by different symbols.

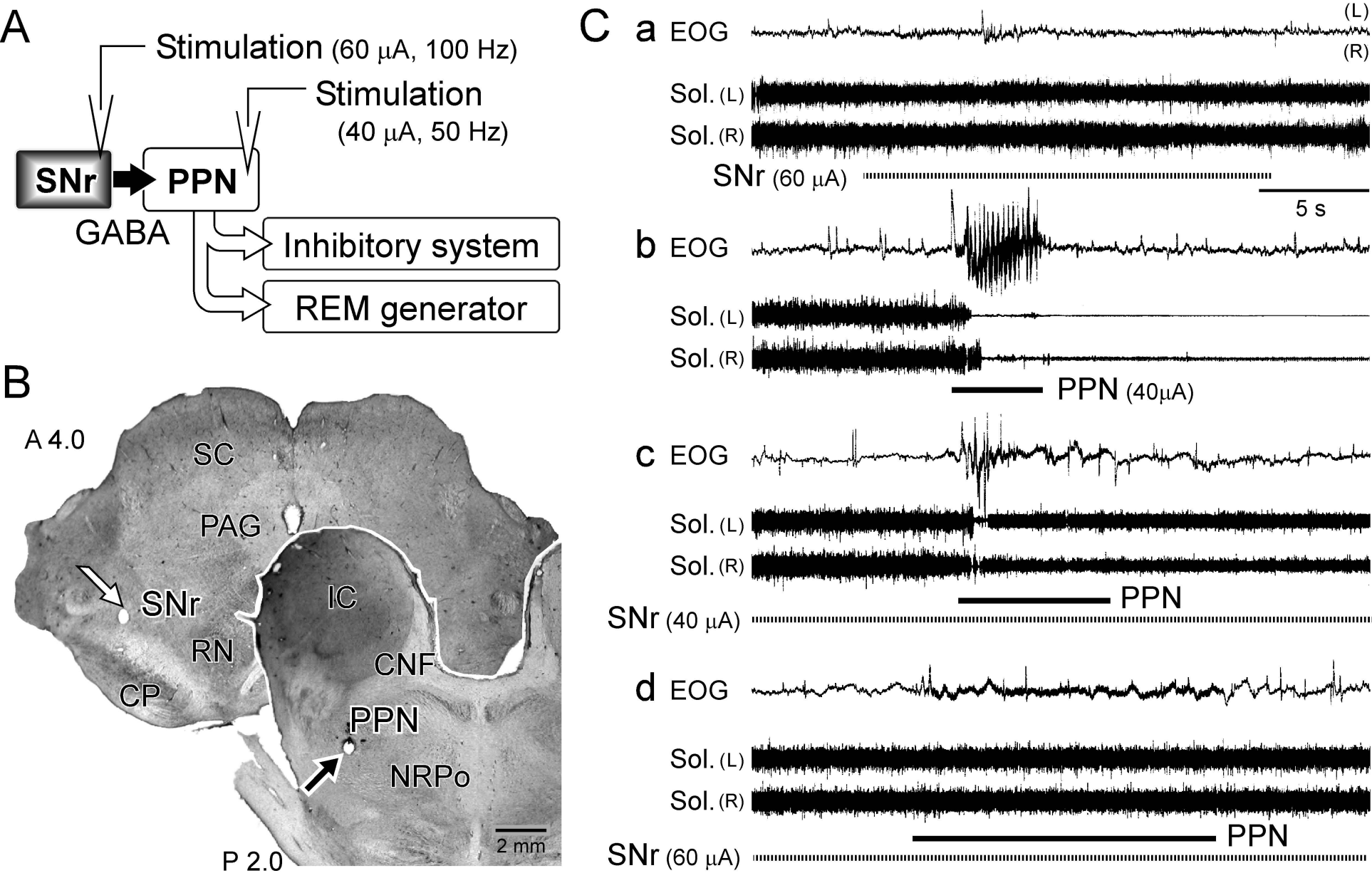
Figure 9. A summary of the results and a hypothetical model of sleep deficiency in a parkinsonian state.

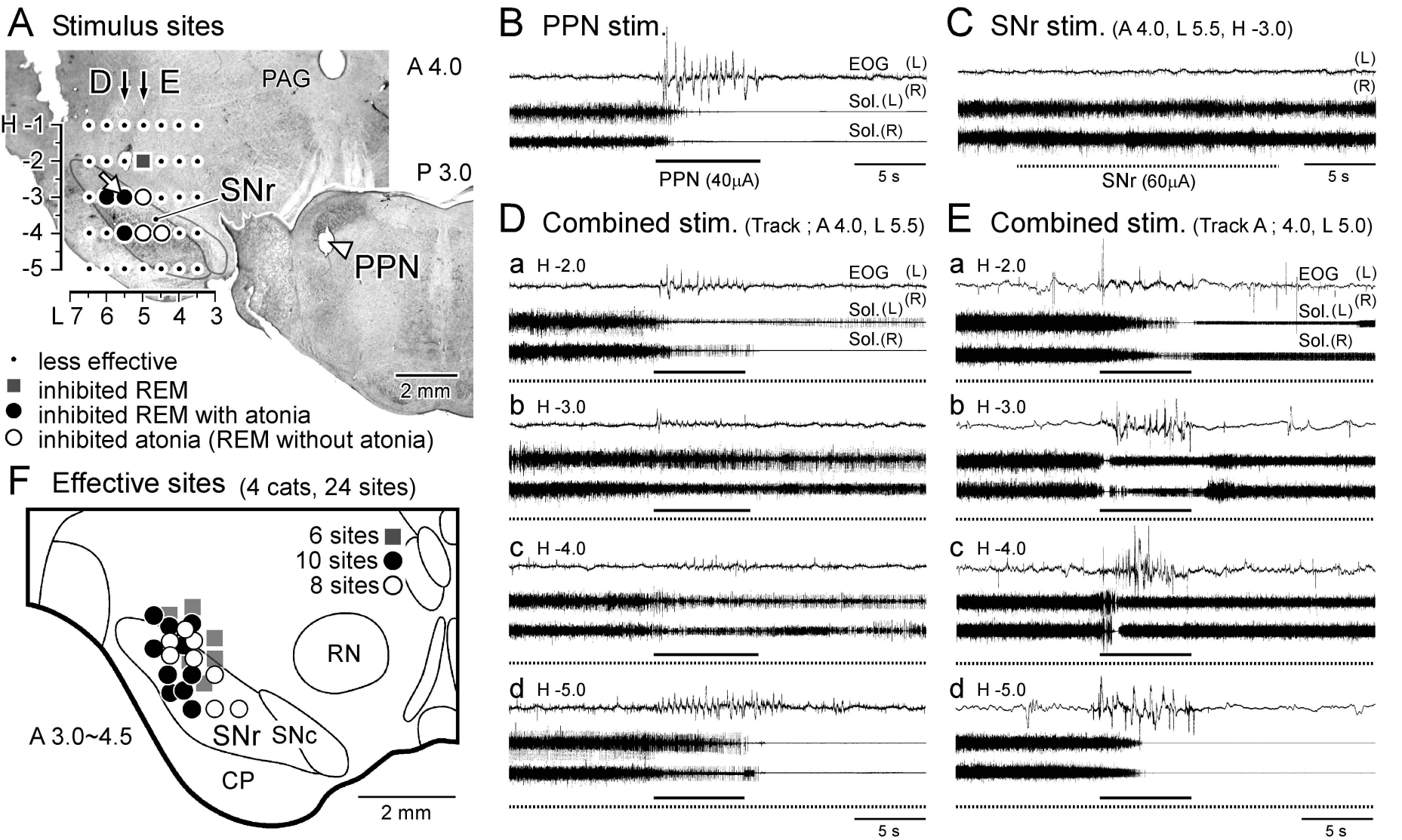
A. In a normal condition, where adequate dopamine is secreted from the substantia nigra pars compacta (SNc), GABAergic inhibition from the SNr to the PPN does not disturb REM and muscular atonia during a REM sleep period. B. REM Sleep disturbances in parkinsonian state. (a) Excessive GABAergic output from the SNr to both “REM zone” and “atonia zone” in the PPN area may reduce the activity of the REM generator and the muscle tone inhibitory system. Consequently, there would be a decrease of the REM and attenuation of the muscular atonia during the REM period. (b) If excessive GABAergic inhibition acts on only the “atonia zone” in the PPN area, a state of REM without atonia or RBD could be induced.

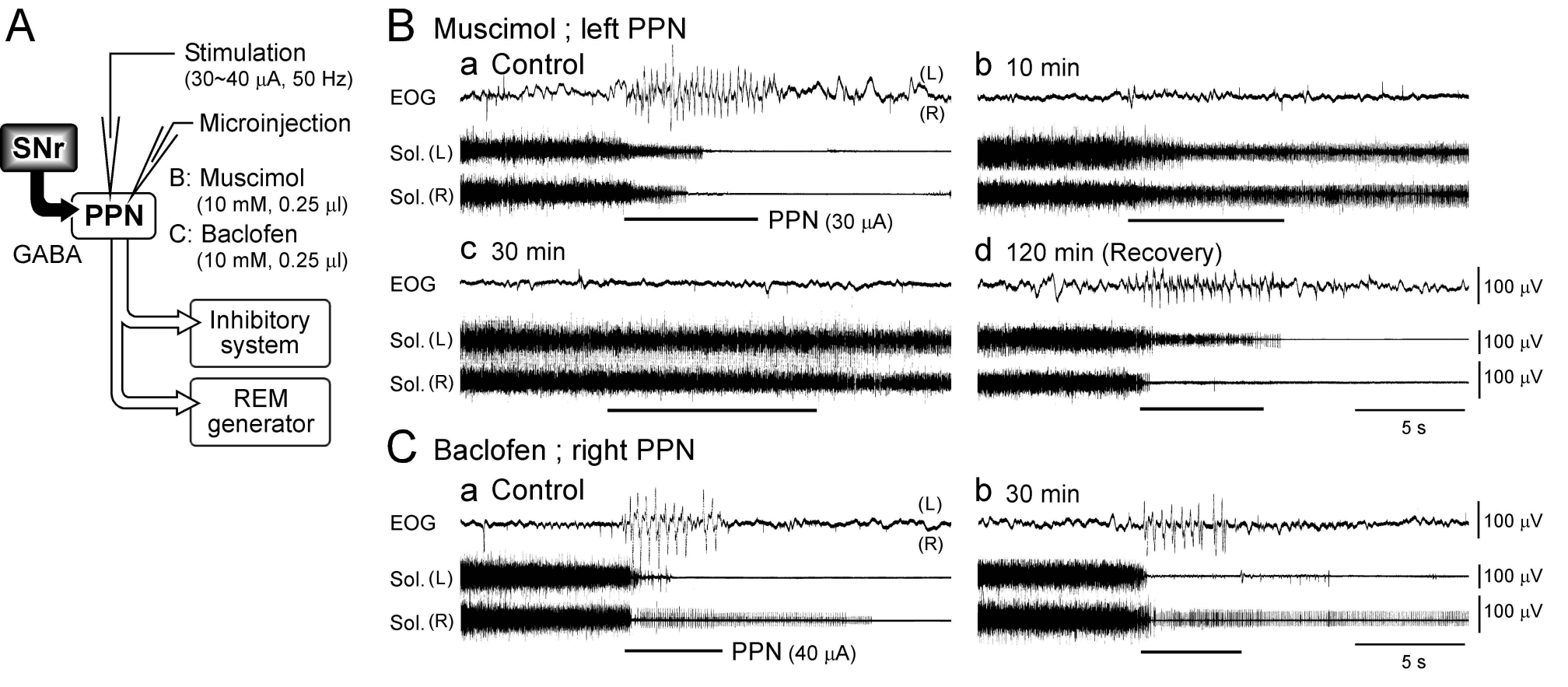

A
IC
P 2.0
PPN
CNF
a
b
c
d
NRPo
B
a Dorsal PPN
b Ventrolat. PPN
EOG (L)
(R)
Sol. (L)
Sol. (R)
c Subcoeruleus
d NRPo
100 μV
50 μV
50 μV
5 sec
C
a
Sol. (L)
Sol. (R)
PPN (20 μA)
b
EOG (L)
(R)
PPN (30 μA)
c
100 μV
50 μV
50 μV
2 sec
PPN (40 μA)
D
H 0
-1
-2
-3
-4
-5
CNF
PPN
REM
Atonia
REM & atonia
L 6
5
4
3
2
1

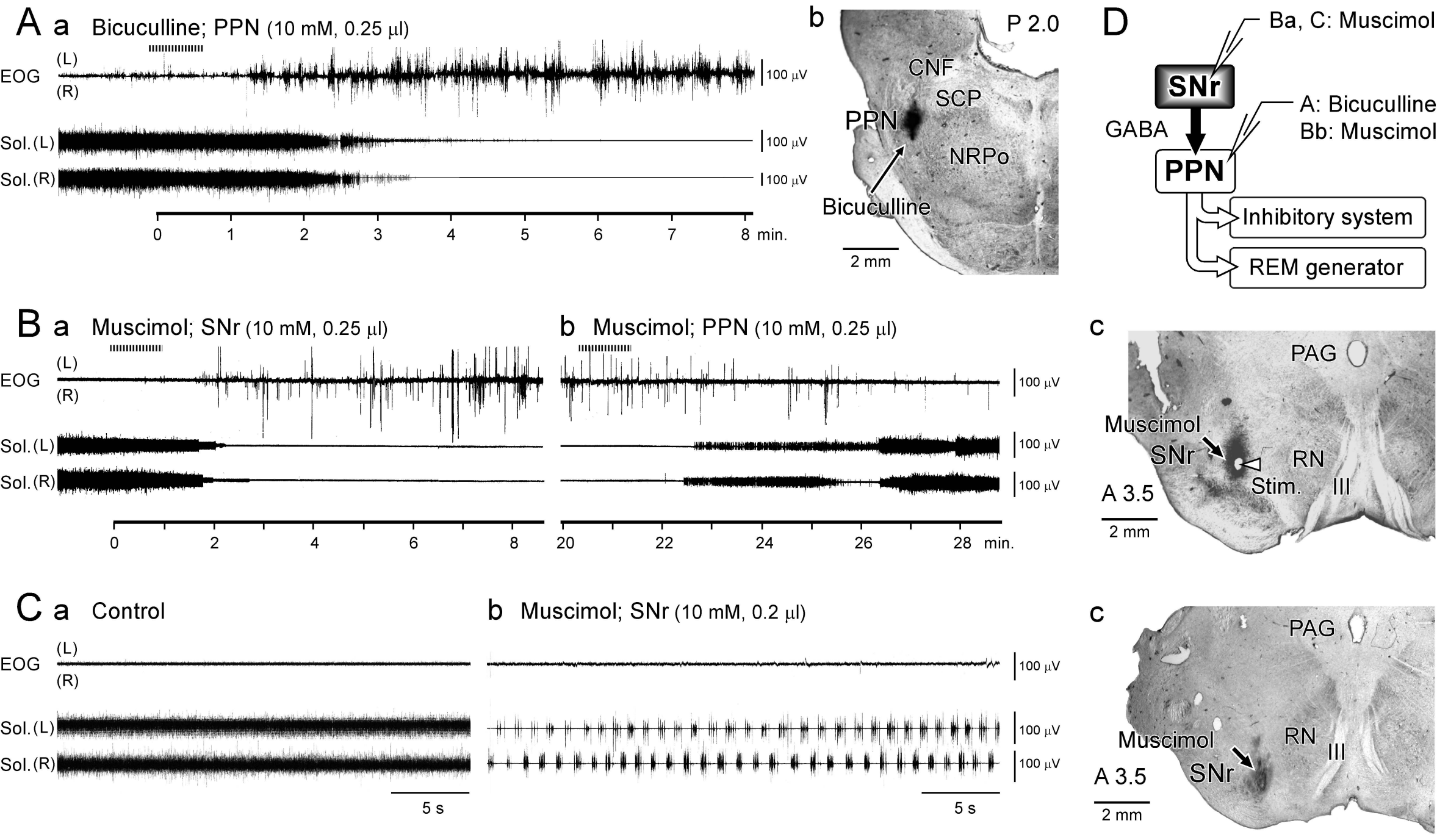

A
REM
B
Atonia
C
REM with atonia
IC
CNF
SCP
PPN
NRPo
P 2.0
22 sites
42 sites
10 sites
2 mm
SC
LR 4.0

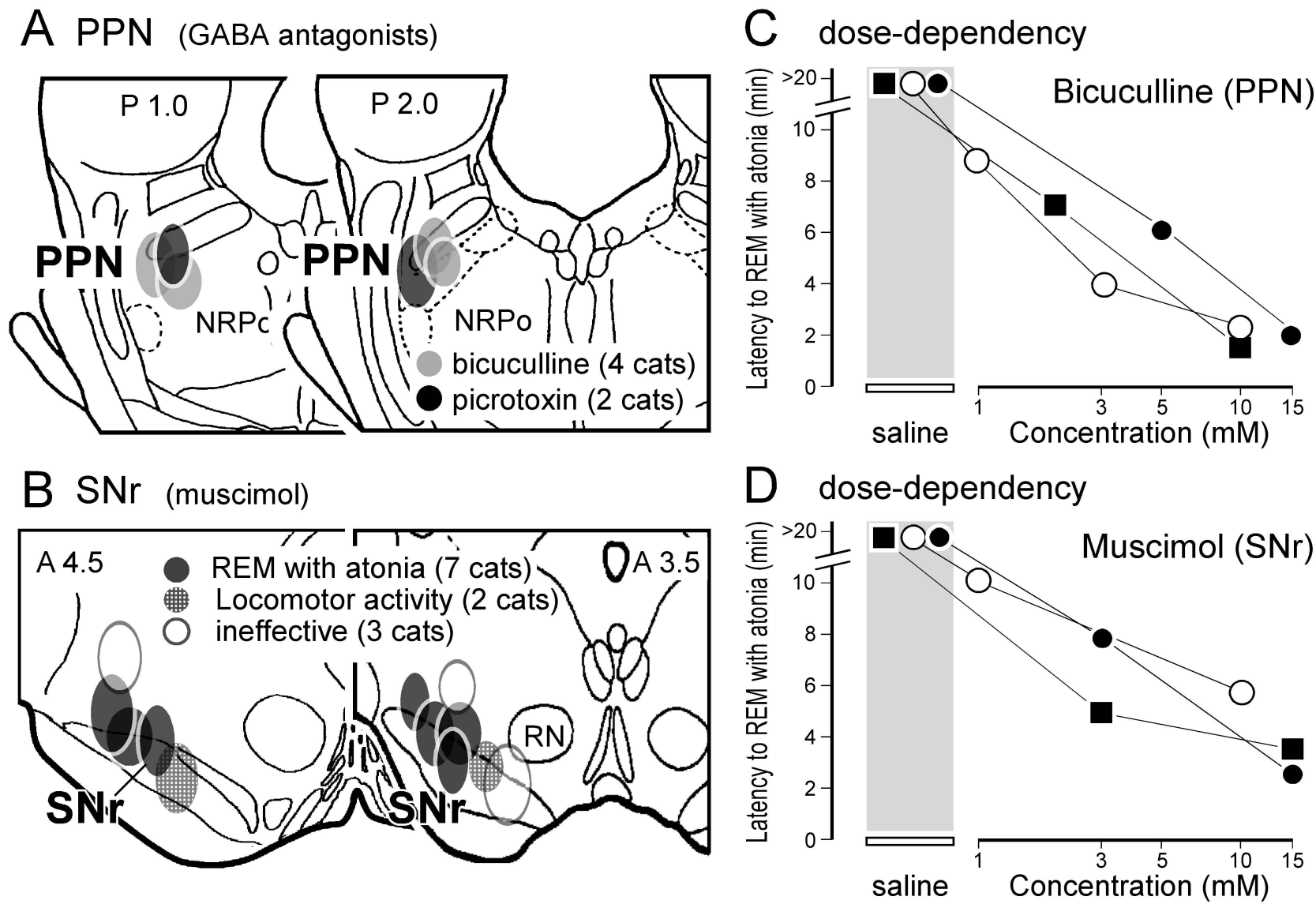

Basal ganglia
SC
IC
Cerebellum
GABA
PPN
SNr
ACh
PMRF
Mm
Inhibitory system
REM generator
Decerebration
REM
Muscular atonia

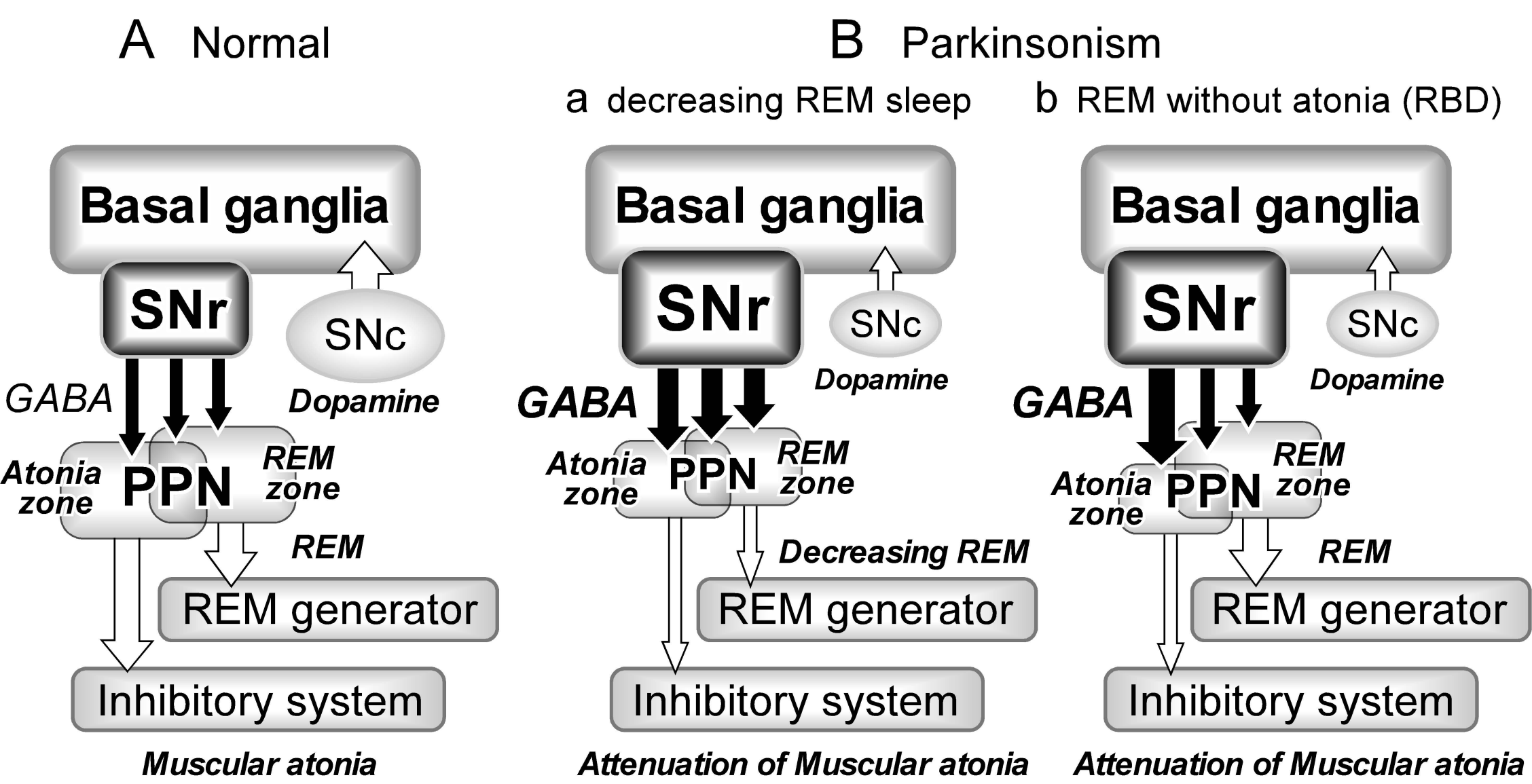

A
Stimulation (60 μA, 100 Hz)
Stimulation
(40 μA, 50 Hz)
SNr
PPN
GABA
Inhibitory system
REM generator
B
A 4.0
SC
PAG
SNr
IC
RN
CNF
CP
PPN
NRPo
2 mm
P 2.0
C
a EOG
(L)
(R)
Sol. (L)
Sol. (R)
SNr (60 μA)
5 s
b EOG
Sol. (L)
Sol. (R)
PPN (40μA)
c EOG
Sol. (L)
Sol. (R)
PPN
SNr (40 μA)
d EOG
Sol. (L)
Sol. (R)
PPN
SNr (60 μA)


A Stimulus sites
D I I E
PAG
A 4.0
P 3.0
H -1
-2
-3
-4
-5
SNr
PPN
L 7 6 5 4 3
2 mm
less effective
inhibited REM
inhibited REM with atonia
inhibited atonia (REM without atonia)
B PPN stim.
EOG (L)
Sol.(L) (R)
Sol.(R)
PPN (40μA)
5 s
C SNr stim. (A 4.0, L 5.5, H -3.0)
(L)
(R)
SNr (60μA)
5 s
D Combined stim. (Track ; A 4.0, L 5.5)
a H -2.0
b H -3.0
c H -4.0
d H -5.0
5 s
E Combined stim. (Track A ; 4.0, L 5.0)
a H -2.0
EOG (L)
Sol.(L) (R)
Sol.(R)
b H -3.0
c H -4.0
d H -5.0
5 s
F Effective sites (4 cats, 24 sites)
6 sites
10 sites
8 sites
RN
A 3.0~4.5
SNr
SNc
CP
2 mm


A
Stimulation
(30~40 μA, 50 Hz)
Microinjection
SNr
B: Muscimol
(10 mM, 0.25 μl)
PPN
C: Baclofen
(10 mM, 0.25 μl)
GABA
Inhibitory system
REM generator
B Muscimol ; left PPN
a Control
b 10 min
c 30 min
d 120 min (Recovery)
EOG
(L)
(R)
Sol. (L)
Sol. (R)
PPN (30 μA)
100 μV
5 s
C Baclofen ; right PPN
a Control
b 30 min
PPN (40 μA)


A a Bicuculline; PPN (10 mM, 0.25 μl)
EOG
(L)
(R)
Sol. (L)
Sol. (R)
100 μV
0 1 2 3 4 5 6 7 8 min.
b
P 2.0
CNF
SCP
PPN
NRPo
Bicuculline
2 mm
D
Ba, C: Muscimol
SNr
GABA
A: Bicuculline
Bb: Muscimol
PPN
Inhibitory system
REM generator
B a Muscimol; SNr (10 mM, 0.25 μl)
b Muscimol; PPN (10 mM, 0.25 μl)
0 2 4 6 8
20 22 24 26 28 min.
c
PAG
Muscimol
SNr
RN
Stim.
III
A 3.5
2 mm
C a Control
b Muscimol; SNr (10 mM, 0.2 μl)
5 s
c
PAG
Muscimol
RN
III
SNr
A 3.5
2 mm


A PPN (GABA antagonists)
P 1.0
P 2.0
PPN
PPN
NRPc
NRPo
bicuculline (4 cats)
picrotoxin (2 cats)
B SNr (muscimol)
A 4.5
A 3.5
REM with atonia (7 cats)
Locomotor activity (2 cats)
ineffective (3 cats)
RN
SNr
SNr
C dose-dependency
Bicuculline (PPN)
Latency to REM with atonia (min)
>20
10
8
6
4
2
0
saline
1
3
5
10
15
Concentration (mM)
D dose-dependency
Muscimol (SNr)
Latency to REM with atonia (min)
>20
10
8
6
4
2
0
saline
1
3
5
10
15
Concentration (mM)


A Normal
B Parkinsonism
a decreasing REM sleep
b REM without atonia (RBD)
Basal ganglia
SNr
SNc
GABA
Dopamine
Atonia zone
PPN
REM zone
REM
REM generator
Inhibitory system
Muscular atonia
Basal ganglia
SNr
SNc
GABA
Dopamine
Atonia zone
PPN
REM zone
Decreasing REM
REM generator
Inhibitory system
Attenuation of Muscular atonia
Basal ganglia
SNr
SNc
GABA
Dopamine
Atonia zone
PPN
REM zone
REM
REM generator
Inhibitory system
Attenuation of Muscular atonia