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Orexinergic projections to the cat midbrain mediate alternation of emotional behavioural states from locomotion to cataplexy

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Orexinergic projections to the midbrain mediate alternation of emotional behavioral states from locomotion to cataplexy.

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Abstract

Orexinergic neurons in the perifornical lateral hypothalamus project to structures of the midbrain, including the substantia nigra and the mesopontine tegmentum. The areas contain the mesencephalic locomotor region (MLR), and the pedunculopontine and laterodorsal tegmental nuclei (PPN/LDT) which regulate atonia during rapid eye movement (REM) sleep. Deficiencies of the orexinergic system result in narcolepsy, suggesting that these projections are concerned with switching between locomotor movements and muscular atonia. The present study characterizes the role of these orexinergic projections to the midbrain. In decerebrate cats, injecting orexin-A (60 μM to 1.0 mM, 0.20 to 0.25 μl) into the MLR reduced the intensity of the electrical stimulation required to induce locomotion on a treadmill (4 cats) or even elicit locomotor movements without electrical stimulation (2 cats). On the other hand, when orexin was injected into either the PPN (8 cats) or the substantia nigra pars reticulata (SNr, 4 cats), an increased stimulus intensity at the PPN was required to induce muscle atonia. The effects of orexin on the PPN and the SNr were reversed by subsequently injecting bicuculline (5 mM, 0.20 to 0.25 μl), a $GABA_A$ receptor antagonist, into the PPN. These findings indicate that excitatory orexinergic drive could maintain a higher level of locomotor activity by increasing the excitability of neurons in the MLR, while enhancing GABAergic effects on presumably cholinergic PPN neurons, to suppress muscle atonia. We conclude that orexinergic projections from the hypothalamus to the midbrain play an important role in regulating motor behavior and controlling postural muscle tone and locomotor movements when awake and during sleep. Furthermore, as the excitability is attenuated in the absence of orexin, signals to the midbrain may induce locomotor behavior when the orexinergic system functions normally but elicit atonia or narcolepsy when the orexinergic function is disturbed.

Introduction

Orexinergic neurons are located in the perifornical lateral hypothalamus and project to most central nervous system areas. In addition, the orexinergic projection to the brainstem monoaminergic and cholinergic neurons mediates sleep-wakefulness regulation (Chemelli et al., 1999; Lin et al., 1999; Nambu et al., 1999; Peyron et al., 1998, Saper et al., 2001; Taheri et al., 2002). It has been reported that FOS immunohistochemical studies and neural recording studies indicate that the orexinergic neurons increase their activity during waking (Alam et al., 2002; Estabrooke et al., 2001; Koyama et al., 2003; Lee et al., 2005; Mileykovskiy et al., 2005). When waking, orexin release increased markedly during periods of increased motor activity compared to its release during quiet, alert waking (Kiyashchenko et al., 2002). These findings indicate that the orexinergic system contributes to regulation of the state of vigilance and somatomotor control (Nishino, 2003; Sakurai, 2002; Siegel, 2004). It has also been considered that deficiencies in the orexinergic system result in narcolepsy (Chemelli et al., 1999; Lin et al., 1999). However the pathophysiological mechanisms by which the orexinergic system suppresses narcolepsy remain unclear.

One of the major orexinergic projections is present in the structures of the midbrain, including the substantia nigra and the mesopontine tegmentum (Nambu et al., 1999; Peyron et al., 1998). The latter contains the mesencephalic locomotor region (MLR) (Grillner et al., 1997; Takakusaki et al., 2003a), and the pedunculopontine and the laterodorsal tegmental nuclei (PPN/LDT) which regulate rapid eye movement (REM) sleep (Datta and Siwek, 1997; Koyama and Sakai, 2000; McCarley et al., 1995). Under normal conditions emotional stimuli induce alert responses which produce an increase in muscle tone and/or locomotor behavior (Garcia-Rill et al., 2004; Skinner et al., 2004). However, humans and animals with narcolepsy may experience cataplexy; a sudden loss of muscle tone induced by emotional stimuli (Nishino, 2003; Nishino and Mignot, 1997). It is possible therefore that orexinergic

projections to these areas may be involved in both locomotion and a loss of muscle tone.

Other investigations have reported that the activation of neurons in the MLR of acute decerebrate cats induced locomotion, and activation of neurons in the ventrolateral PPN-induced muscular atonia that was associated with REM (Takakusaki et al., 2003a, 2004b). The PPN-induced REM and atonia was associated with activation of cholinergic neurons. Moreover, an activation of neurons in the substantia nigra pars reticulata (SNr) prevented PPN-induced REM with atonia via GABAergic projections to the PPN (Takakusaki et al., 2004b). Because the orexinergic system is abnormal in the context of narcolepsy, we hypothesized that orexinergic projections to these midbrain structures may regulate the switching of emotional motor behavior. Emotional signals elicit locomotor behavior in the presence of orexins and induce cataplexy in the absence of orexins. Consequently, the goal of the present study was to characterize the role of orexinergic projections to the midbrain in the control of locomotion and postural muscle tone. For this purpose we employed acute decerebrate cats in which the cerebral hemispheres, including the hypothalamus, were removed. We then examined how MLR-induced locomotion, and PPN-induced REM and atonia, were altered after injections of orexin-A into each of the MLR, the PPN, and the SNr. The preliminary results have been published as abstracts (Takakusaki et al., 2004c).

Materials and methods

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). Every effort was made to minimize animal suffering and to reduce the number of animals required for these experiments.

Animal preparation

The experiments were performed with 24 cats, with a weight from 2.1 to 3.4 kg, from the animal facility at Asahikawa Medical College. Each cat was surgically decerebrated at the precollicular-postmammillary level while under halothane (Halothane, Otsuka, Osaka, Japan; 0.5–3.0%) and nitrous oxide gas (0.5–1.0 L/min) anesthesia with oxygen (3.0–5.0 L/min). The anesthesia was then discontinued. The trachea was intubated, and a catheter was placed in the femoral artery to monitor blood pressure. Another catheter was placed in the cephalic vein to administer epinephrine (Bosmin, Daiichi Co., Osaka, Japan). The head was fixed in a stereotaxic apparatus, and a rigid spinal frame secured the cat by clamping the dorsal processes of the first three thoracic vertebrae. The limbs rested on a static surface, or on the surface of a treadmill, and a rubber hammock supported the body. The animal's rectal temperature was maintained at 36–37°C by using radiant heat lamps. The mean blood pressure of each cat was maintained greater than 100 mmHg by an intravenous infusion of epinephrine (0.1–0.3 mg/kg, infusion rate of 0.01 mg/min), and the end tidal CO_2 was maintained between 4% and 6%.

Brainstem stimulation and EMG recording

Each stimulating electrode consisted of a glass micropipette filled with Wood's

metal. The tip of the micropipette was replaced with a carbon fiber (diameter, 7 μm; resistance, 0.2–0.5 MΩ; Takakusaki et al., 2003a, 2004b). The experimental design is schematically illustrated in Figure 2. A stimulating electrode was inserted into the mesopontine tegmentum (A 1.0 – P 3.0, LR 2.0 – 5.0, H +1.0 – -5.0). To evoke locomotion, repetitive stimuli with a constant pulse (10–50 μA, and 0.2 ms duration at 50 Hz) were delivered for 5–30 seconds while the treadmill belt was advanced at a speed of 0.3 m/s. The same electrode was used for mesopontine stimulation (10–50 μA, and 0.2 ms duration at 50 Hz, lasting for 5–10 seconds) to evoke REM with atonia while the animal's limbs rested on a stationary surface (Takakusaki et al., 2003a, 2004b). The stimulation was applied by moving the stimulating electrode in an interval of 0.5–1.0 mm in the dorsoventral, mediolateral, and rostrocaudal directions so that an optimal site for evoking locomotion, or REM with atonia could be identified in each animal. The optimal stimulus sites for evoking locomotion, so-called midbrain locomotor region (MLR), were mainly located in the cuneiform nucleus (CNF). The sites for evoking muscle tone suppression were located in the ventral and ventrolateral parts of the PPN (Figure 1).

Short train pulses of stimuli (3 trains, 5 ms intervals and 40 μA) were also delivered to each of the CNF, the locus coeruleus (LC), the medial pontine reticular formation (PRF), and the PPN, so that the excitatory and inhibitory effects from each site on muscle tone could be examined (Figure 8). The changes in the electromyographic activity of the left soleus muscles which were evoked from each site were rectified, integrated, and averaged, for 20 sweeps.

A micropipette which was filled with orexin A (60 μM–1.0 mM) was inserted into the mesopontine tegmentum (A 1.0 – P 3.0, LR 2.0 – 5.0, H +1.0 – -5.0) so that orexin could be injected into the CNF and PPN. In previous investigations we have shown that either electrical or chemical stimulation of the lateral part of the SNr inhibited the PPN-induced

REM and atonia (Takakusaki et al., 2004b). Consequently, an identical type of micropipette was also inserted into the caudal diencephalon (A 2.0 – A 5.0, LR 3.0–7.0, H +2.0 – -5.0) so that orexin could be injected into the SNr. By using an oil-driven microinjection system 0.25 μl of orexin A was injected into these midbrain areas at a rate of approximately 0.01 μl/sec. A Wilcoxon signed rank test was performed with the use of StatView statistical software (Abacus Concepts, Berkeley, CA.) to determine any significant difference in the stimulus intensity before and after the orexin injections (Figures. 3 and 5).

A pair of stainless steel wires (2 mm apart) were inserted into the left soleus (Sol) muscles to record the electromyogram activity (EMG). All of the EMGs were processed with a low pass filter of 5 Hz and a high pass filter of 100 Hz with a time constant of 0.03 seconds. The electrooculograms (EOG) were recorded with a bipolar electrode placed 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 seconds.

Histological control

At the end of an experiment, the stimulus sites were marked by passing a DC current of 30 μA through an electrode for 30 seconds. The injection sites were also marked with 10% fast green, using the same amount as the substances that had been previously injected. Each cat was then sacrificed with an overdose of sodium pentobarbital (60 mg/kg, i.p.) anesthesia. The brainstem was removed and fixed in 10% formalin. Frozen coronal or parasagittal sections (50 μm) were cut and stained with neutral red. The location of the microlesions and diffusion areas of the fast green were identified with the assistance of the stereotaxic atlases of Berman (1968) and Snider and Niemer (1961).

Choline acetyltransferase (ChAT) immunohistochemistry was performed to identify the boundaries of the PPN so that we could elucidate whether the effective stimulus

sites for evoking REM and atonia were located within the PPN. Six animals were deeply anaesthetized with Nembutal and transcardially perfused with 0.9% saline followed by a solution of 3.0% paraformaldehyde and 0.01% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brain of each cat was removed, saturated with a cold solution of 30% sucrose, and 50 μm frozen sections were prepared. Following this, ChAT immunohistochemistry was performed by using the peroxidase-antiperoxidase method combined with diaminobenzidine (Lai et al., 1993; Mitani et al., 1988; Takakusaki et al., 2003a, 2004a). Monoclonal anti-ChAT antibody (Boehringer Mannheim, Germany) was used for these preparations.

Results

Locomotor region and muscle tone inhibitory region in the midbrain

Before examination of the effects of the orexin injections into the mesopontine tegmentum, we confirmed the stimulus effects of the locomotor region and muscle tone inhibitory region in the mesopontine tegmentum, as described in a previous report (Takakusaki et al., 2003a). The findings shown in Figure 1A illustrate that repetitive electrical stimulation applied to the CNF induced locomotion on the moving treadmill (Figure 1Ab). On the other hand, stimulation of the ventral part of the PPN resulted in suppression of postural muscle tone and generation of REM (REM and atonia). The distribution of the optimal stimulus sites for evoking locomotion, and the muscle tone inhibitory region, are shown on parasagittal (Figure 1Ba) and coronal (Figure 1Bb) planes of the brainstem. It was confirmed that the locomotor region was mainly located in the CNF and partly included the dorsal region of the PPN. The muscle tone inhibitory region was located in the ventrolateral region of the PPN. The distribution of the cholinergic neurons, which were labeled by ChAT immunohistochemistry on a coronal section of the brainstem, is shown in Figure 1C. ChAT- positive, cholinergic neurons were located in the LDT and the PPN (Figure 1Ca). The PPN was defined by loosely arranged cholinergic neurons that surrounded the superior cerebellar peduncle (SCP; Figure 1Ca). The cholinergic neurons were preferentially distributed in an area corresponding to the inhibitory region, rather than the locomotor region.

A framework for this study

Figure 2 shows a framework for this study. The MLR and muscle tone inhibitory region in the PPN are in close proximity to each other in the lateral part of the midbrain (Takakusaki et al., 2003a, 2004a, 2004b). Activation of the MLR induces locomotor

movements via activation of central pattern generators in the spinal cord through the medullary reticulospinal tract (Rossignol, 1996). Activation of the MLR may also activate muscle tone excitatory systems, including the coerulospinal and raphespinal tracts (Mori et al. 1987; White and Fung, 1989). In contrast, activation of the PPN neurons induces REM and atonia. The PPN-induced muscular atonia is mediated through the pontomedullary reticulospinal tract (inhibitory system; Habaguchi et al., 2002; Takakusaki et al., 2003a, 2004a, 2004b). It is suggested that an interconnection between the mesopontine cholinergic nuclei and the caudoventral PRF could operate as a common generator of REM and ponto-geniculo-occipital waves (Datta and Hobson, 1994; Sakai and Jouvet, 1980; Vanni-Mercier and Debilly, 1998). The PPN-induced REM can be thus attributed to an activation of the REM generator in the PPN and the caudal PRF (Takakusaki et al., 2004b). Electrophysiological (Saitoh et al., 2003) and neuroanatomical (Grofova and Zhou, 1998) studies have suggested that GABAergic neurons in the SNr monosynaptically inhibit the activity of cholinergic PPN neurons. We have demonstrated that the PPN effects were under the control of GABAergic inhibitory projections from the SNr (Takakusaki et al., 2003a, 2004b).

Effects of injections of orexin A into the midbrain areas

Orexinergic neurons project to the mesopontine tegmentum, including the MLR, the PPN and the SNr. Consequently, orexin A (0.1–0.25 μl, 60 μM–1 mM) was injected into each of these areas to characterize how MLR/PPN-induced locomotion and REM and atonia were altered by these orexinergic projections. First, we examined the effects of an orexin injection into the MLR (Figure 3). Electrical stimulation (30 μA) which was applied to the lateral part of the CNF (indicated by a filled arrow in Figure 3A) elicited locomotion on the moving treadmill. However stimuli with a strength of 20 μA did not evoke locomotion

(Figure 3Ba). Next, orexin A with a concentration of 200 μM and a volume of 0.25 μl was injected into the region adjacent to the locomotor region (indicated by an open arrow in Figure 3A). Ten minutes after this injection stimulation with a strength of 20 μA evoked locomotion. Thirty minutes after the injection locomotion was elicited on the treadmill belt (indicated by an open line under the EMG records) without electrical stimulation (Figure 3Bc). In another cat (Figure 3C) stimulation of the CNF with a strength of 40 μA elicited locomotion (Figure 3Ca). Twenty minutes after an injection of orexin (60 μM, 0.25 μl) into the CNF a stimulus strength of 20 μA was enough to evoke locomotion (Figure 3Cb). Even 60 minutes after the injection locomotion was still evoked by stimuli with a strength of 30 μA (Figure 3Cc). The complete effects of the orexin upon locomotion were examined in 10 trials in 6 cats. In each trial the threshold current to elicit locomotion was reduced (Figure 3D). Moreover, injections of orexin with higher concentrations (200 and 500 μl) spontaneously induced locomotion without electrical stimulation in 2 animals.

Next, the effect of orexin injections into the PPN was examined. In the cat illustrated in Figure 4A, stimulation of the caudal part of the PPN (indicated by a filled arrow in Figure 4Aa) induced REM and atonia (Figure 4Ab). Orexin A was then injected into the PPN adjacent to the stimulus site (indicated by an open arrow in Figure 4Aa). It was generally observed that an orexin injection into the PPN alone did not change the level of muscle tone. However, 30 minutes after the injection of orexin, REM and atonia were abolished when the PPN was stimulated with the same intensity (Figure 4Ac). In 8 cats, orexin injections into the PPN either abolished the PPN effects, even when stimuli with an intensity of 50 μA were delivered (3 trials in 3 cats), or attenuated the PPN effects (8 trials in 5 cats). Figure 4B illustrates that the threshold stimulus strength which was required to elicit the PPN effect was increased in each trial after the orexin injections. These findings suggest that the orexinergic projection to the PPN suppresses the excitability of PPN neurons that are

involved in the generation of REM and atonia.

Because an activation of cholinergic neurons in the PPN induces REM and atonia (Takakusaki et al., 2003a, 2004a, 2004b), it is possible that orexin inhibits cholinergic neurons in the PPN. However, orexin excites cholinergic neurons located in the LDT (Burlet et al., 2002; Takahashi et al., 2002). Because non-cholinergic neurons, in particular GABAergic neurons, are located in the PPN (Ford et al., 1995; Kosaka et al., 1988; Ottersen and Storm-Mathisen, 1984), we attempted to examine whether an orexin injection indirectly inhibited cholinergic PPN neurons via local GABAergic interneurons in the PPN. The results that are shown in Figure 4C illustrate that an injection of bicuculline into the PPN after an orexin injection restored the PPN stimulus effects that were disturbed by the orexin. Essentially the same results were obtained from 2 other animals. This suggests that orexin inhibits cholinergic PPN neurons via GABAergic effects.

Further attempts were made to test whether orexinergic projections to the SNr could affect the PPN-induced REM and atonia via the GABAergic nigrotegmental projection (see Figure 2). The results are shown in Figure 5. After confirming REM and atonia (Figure 5Ab), which was induced by the PPN stimulation (indicated by a filled arrow in Figure 5Aa), orexin A was injected into the dorsolateral part of the SNr (indicated by an open arrow in Figure 5Aa). Although the orexin injection into the SNr did not alter the level of the muscle tone it did result in complete inhibition of the PPN-induced REM and atonia (Figure 5Ac). In 8 trials of 4 animals, orexin injections increased the stimulus strength that was required to produce the PPN-induced REM and atonia (Figure 5B). In another cat PPN-induced muscular atonia (Figure 5Ca) was blocked by an orexin injection into the SNr (Figure 5Cb). To further determine whether the effect of a nigral orexin injection was mediated through GABAergic projections to the PPN, bicuculline was injected into the PPN. It was observed that the PPN-induced muscular atonia was re-established 5 minutes after the injection of bicuculline

(Figure 5Cc). These results indicate that PPN-induced REM and atonia is inhibited by the GABAergic nigrotegmental projection.

Orexin injection sites and time course of the orexin effects

Fast green was used to identify the injection sites and to measure the spread of the infusions, which for each injection was limited to an area of approximately 1.0–1.5 mm in diameter. Figure 6 illustrates the locations of the injection sites on coronal (Figure 6A-C) and parasagittal planes (Figure 6D) of the brainstem. Ten injection sites which either facilitated MLR-induced locomotion or spontaneously elicited locomotion were located in an area corresponding to the CNF and adjacent region, including the dorsal part of the PPN (Figure 6A and D). Injection sites which inhibited the PPN effects were located in a region corresponding to the PPN (n=11, Figure 6B and D) and the lateral part of the SNr (n=8, Figure 6C and D). Two injections, which are indicated by an asterisk in Figure 6D, not only facilitated MLR-induced locomotion but also inhibited PPN-induced REM and atonia.

The relationship between the time course of the effects and the concentration of the orexin injected into the CNF (6 trials), the PPN (4 trials) and the SNr (4 trials) is shown in Figure 7. The orexin effects were determined by the threshold current for evoking either the MLR-induced locomotion (Figure 7A) or the PPN-induced muscular atonia (Figure 7B and C). The orexin effects usually started to appear 10 minutes after an injection, reached a maximum level 30–40 minutes later, and lasted for more than 100 minutes. Moreover the effects were considered to be dose-dependent. For example, in the case of orexin injections into the PPN (Figure 7B) stronger effects were induced with higher concentrations (200 and 500 μM) than with lower concentrations (60 and 100 μM). Figure 7D shows the time course of the effects of a bicuculline injection into the PPN. The bicuculline was injected 20–30 minutes after the orexin into either the PPN (filled and open triangles) or the SNr (filled and

hatched open squares). The bicuculline produced a decrease in the threshold current required for evoking the PPN effects which, in any trials, appeared within 5 minutes..

Modulation of descending excitatory and inhibitory effects upon muscle tone

Finally, we elucidated how orexin injections into the PPN modulate the descending excitatory and inhibitory effects on muscle tone (Figure 8). We first stimulated each of the CNF, the LC, the PRF, the dorsal PPN and the ventral PPN to examine the stimulus effects on soleus muscle activity (Figure 8B). We then compared these effects with the results obtained by the same stimulus procedures, but after an injection of orexin (Figure 8C and D). Short trains of stimuli which were applied to the CNF and the LC (indicated by open circles in Figure 8A) induced a mixture of excitatory and inhibitory effects on the muscle tone (1st and 2nd recordings in Figure 8B). In contrast, stimuli applied to the medial PRF and the dorsal and ventral PPN areas (indicated by filled circles in Figure 8A) induced prominent inhibitory effects (3rd and 5th recordings in Figure 8B). The stimuli which were applied to each site approximately 30–60 minutes after an orexin injection into the left PPN (Figure 8A) resulted in an increased excitatory effect on the muscle tone from the CNF and the LC. This excitatory effect was accompanied by a prominent decrease in the inhibitory effects from the PRF and the dorsal and ventral PPN (Figure 8C). Specifically, the duration and amplitude of the inhibitory effects were reduced, while those of the excitatory effects were increased. However, the effects of stimulating each site were not observed after 150–180 minutes (Figure 8D). These findings suggest that the orexinergic projection to the PPN facilitates the activities of descending excitatory systems from the CNF and the LC, and suppresses the descending inhibitory system arising from the PPN.

Discussion

Canine narcolepsy is used as a model for understanding human narcolepsy (Nishino and Mignot, 1997). Orexin knockout mice have been also used to examine the control of behavioral states by orexin and pathological mechanisms of narcolepsy (Chemelli, et al., 1999; Mochizuki et al., 2004; Willie et al., 2003). In the present study we used a decerebrated cat preparation in order to avoid endogenous orexinergic activity. In addition, we combined a chemical stimulation technique with electrical stimulation so that we could examine the effects of orexin on the target areas controlling postural muscle tone and locomotion. We have now shown that orexinergic projections to the midbrain regulate the level of postural muscle tone and generation of locomotor behavior. However, we need to clarify the limitations of the investigation and interpret the findings. For example, electrical stimulation may activate not only neuronal elements but also activate fibers. Chemical stimulation is suitable only for activation of neuronal elements and for supplementing any electrical stimulation. But we must consider that the effects of an injection of a drug depend on many factors including the receptor density of the cells at the injection site, the diffusion delay, and the time required for the recruitment of neurons (Takakusaki et al. 2003a, 2004b). Additionally, because the orexin system interferes with complex higher circuitry (Peyron et al., 1998) other than midbrain structures there is a need to integrate the present findings with previous results from studies of narcoleptic animals. In particular the present study could not examine emotional components unlike other experiments with narcoleptic animals.

Disturbances of neurotransmitter systems in narcolepsy and their regulation by orexin

Disturbances of the noradrenergic system have been repeatedly reported in human narcolepsy patients with respect to the induction of cataplexy. The reports have indicated therefore, that an enhancement of the noradrenergic system is powerful for the reduction of

cataplexy (Aldrich et al., 1994; Schwartz, 2005). Studies which have used a canine narcolepsy model have also reported that various neurotransmitter systems are affected, including the noradrenergic (Fruhstorfer et al, 1989), adrenergic (Mignot et al., 1993), dopaminergic (Kanbayashi et al., 2000; Nishino et al., 1991; Reid et al., 1996), serotonergic (Nishino et al., 1993) and cholinergic systems (Nishino et al., 1995, 1988; Reid et al., 1994a, 1994b). In particular, an increase in the activity of noradrenergic system ameliorated cataplexy (Fruhstorfer et al, 1989). But an activation of the cholinergic system caused the symptoms to worsen (Nishino et al., 1995, 1988; Reid et al., 1994a, 1994b). The deficiencies in these neurotransmitter systems were observed in both the brainstem (Reid et al, 1994a, 1994b; 1996) and in forebrain structures such as the amygdala (Guilleminault et al, 1998), the basal forebrain (Nishino et al., 1995, 1988) and the basal ganglia. In human narcolepsy patients, for example, an alteration of the dopaminergic system was observed in the basal ganglia (Eisensehr et al., 2003) and the amygdala (Aldrich et al., 1993).

Orexin neurons in the perifornical hypothalamus project to various regions in the nervous system (Peyron et al., 1998). Although most of the anatomical studies were performed in rats similar orexinergic projections have been reported in cats (Zhang et al., 2002, 2004). Monoaminergic neurons are major targets of the orexinergic system. In particular, a direct orexinergic projection to the LC may be in a position to enhance arousal and modulate plasticity in higher brain centers. These effects could occur through the developing noradrenergic neurons, which play an important role in modulating arousal, a vigilance state, selective attention, and memory (Horvath et al., 1999b; Soffin et al. 2002; van den Pol et al., 2002). The orexinergic system also excites dopaminergic (Korotkova et al., 2002), serotonergic (Liu et al., 2002; Soffin et al. 2004; Takahashi et al., 2005) and cholinergic (Burlet et al., 2002; Fadel, 2005; Takahashi et al., 2002; Wu et al., 2004) neurons. Moreover the orexinergic system exerts excitatory actions on glutamatergic (Li et al, 2002),

peptidergic (Horvath et al., 1999a) and GABAergic neurons in various brain regions (Korotkova et al., 2002; Wu et al., 2002). Orexin neurons, in turn, receive either excitatory or inhibitory effects from these neurotransmitter systems (Fu et al., 2004; Li and van den Pol, 2005; Yamanaka et al., 2005). A loss of orexin may thus lead to a massive imbalance in these systems, resulting in the dysregulation of vigilance states.

It has been shown that canine narcolepsy is caused by exon skipping mutations of the Orexin-receptor-2 gene (Hungs et al., 2001; Lin et al., 1999; Willie et al., 2003). Orexin-2 receptor mRNA has been observed in the cerebral cortex, hippocampus, medial thalamic groups, hypothalamic nuclei, and brainstem regions including the raphe nuclei, the SNr and the PPN (Marcus et al., 2001). Orexin-2 receptors could therefore act to maintain a normal level of muscle tone. Because orexin-A activates both orexin-1 and orexin-2 receptors (Willie et al., 2001), in the present study the effects of an injection of orexin-A could be due to activation of orexin-2 receptors in the midbrain regions.

Orexinergic modulation of REM sleep and postural muscle tone

Orexin neurons project to the LDT and the PPN (Nambu et al., 1999, Peyron et al., 2002) where both cholinergic neurons (Armstrong et al., 1983; Rye et al., 1987; Span and Grofova, 1992, Takakusaki et al., 1996) and non-cholinergic neurons, including glutamatergic, GABAergic (Kosaka et al., 1988; Ottersen and Storm-Mathisen, 1984) and peptidergic (Vincent et al., 1983) neurons are located. In the present study orexin injections into the PPN or the SNr suppressed PPN-induced REM and atonia. The effects were eliminated however, by subsequent injections of bicuculline into each area (Figures. 4 and 5). One interpretation of the above findings is that the orexin effects are mediated by local GABAergic neurons in the PPN and GABAergic projection neurons arising from the SNr. This possibility is supported by the following evidence. First, orexin injections into the rat

PPN increase the release of GABA in the PPN (Koyama et al., 2004). Second, GABAergic neurons in some brain areas are excited by orexin (Korotkova et al., 2002, 2003; Wu et al., 2002). Therefore the orexin effects can be mediated by the activation of GABAergic neurons, which in turn inhibit cholinergic PPN neurons (Torterolo et al., 2002; Pal and Mallick, 2004), resulting in the suppression of REM and atonia. Alternatively, orexin could stimulate presynaptic inhibitory inputs to the cholinergic neurons in the PPN, as has been shown in the LDT (Burlet et al., 2002). It was also demonstrated that orexins increase the frequency of GABAergic-mIPSCs in the neurons of the hypothalamus (Van den Pol et al., 1998) and hippocampus (Wu et al. 2002). Accordingly, orexin may act on presynaptic terminals of either local GABAergic interneurons in the PPN, or GABAergic neurons arising from the SNr, to facilitate the release of GABA. Immunohistochemical studies would be necessary to identify the orexinergic projections to the GABAergic neurons.

Several types of cholinergic neurons which are related to the sleep-awake cycle are located in the PPN/LDT. These neurons include those that are active during waking and REM sleep (W/REM-on neurons), and those that are specifically active during REM sleep (REM-on neurons). Desynchronization of the EEG and the regulation of wakefulness via ascending projections to the thalamus or cortex could be properties of W/REM-on neurons. A direct activation by orexin of W/REM-on neurons may therefore induce and maintain wakefulness. In contrast, REM-on neurons are thought to induce EEG desynchronization via ascending projections to forebrain structures, and muscular atonia during REM sleep via a descending projection to the PRF. We have demonstrated that non-cholinergic REM-on neurons in the PRF, which are excited by a cholinergic agonist, project to the medulla (Sakai and Koyama, 1996). An activation of cholinergic PPN neurons may thus excite the REM-on neurons in the PRF to suppress muscle tone via the pontomedullary reticulospinal tract (Takakusaki et al., 1994, 2001, 2003b). Because presumably the cholinergic REM-on

neurons in the mesopontine tegmentum were excited by bicuculline, REM-on neurons in the PPN/LDT could be inhibited through $GABA_A$ receptors during waking (Sakai and Koyama, 1996). Ulloor et al. (2004) demonstrated that $GABA_B$ receptors on PPN cholinergic neurons were also involved in the regulation of REM sleep. It is therefore highly probable that, when orexin excites GABAergic neurons in the PPN/LDT, REM-on neurons are more selectively inhibited by GABA than W/REM-on neurons. This would result in suppression of REM sleep and muscular atonia. It has been reported by Xi et al., (2001) that an injection of orexin into the LDT facilitated wakefulness and suppressed REM sleep. The former effect may be attributed to a direct excitatory effect of orexin on the cholinergic neurons (Burlet et al., 2002; Takahashi et al., 2002), while the latter may be mediated through orexin-induced activity of GABAergic neurons.

Orexin injections into the PPN not only suppressed inhibitory effects from the PPN and the PRF but also enhanced excitatory effects from the MLR and the LC (Figure 8). Descending monoaminergic systems, such as the coerulospinal and the raphespinal tracts, are muscle tone excitatory systems (Fung and Barnes, 1981; Sakai et al., 2000). There are also direct noradrenergic (Semba and Fibiger, 1992) and serotonergic projections to the PPN/LDT (Honda and Semba, 1994) and to the medial PRF (Semba 1993). The noradrenergic projection inhibits the mesopontine cholinergic neurons (Koyama and Kayama, 1993; Leonald and Llinás, 1994). The serotonergic projection reduces the activity of the inhibitory system arising from the medial PRF (Takakusaki et al., 1993, 1994). Lai et al. (2001) have reported that there was a reduced release of norepinephrine and serotonin in the spinal cord during muscular atonia which was induced by electrical or chemical stimulation applied to the medial PRF. They indicated that the activity of the coerulospinal and raphespinal tracts was inhibited by projections from the medial PRF to the LC and the raphe nuclei. Consequently, there are interconnections between the excitatory and inhibitory

systems. The orexinergic projections to the midbrain therefore may control the level of muscle tone by counterbalancing these systems (see Takakusaki et al., 2004d).

Orexinergic control of locomotor behavior

The MLR excites a spinal stepping generator to evoke locomotion via the medullary reticulospinal tract (Grillner, 2003; Rossignol, 1996). Signals from the MLR may also activate monoaminergic excitatory systems (see Mori, 1987). The lateral hypothalamus is known to be involved in the control of locomotion, especially of appetitive locomotion, while the medial hypothalamus probably controls defensive behavior associated with darting locomotion (Grillner et al., 1997, Sinnamon, 1993). This emotional locomotor behavior could be evoked through the projections from the hypothalamus to the midbrain, including the MLR and the medullary reticular formation (MRF) (Grillner et al., 1997). Torterolo et al. (2003) reported that orexinergic neurons expressed FOS only when somatomotor activity was present. The release of orexin in the lateral hypothalamus was higher during wakefulness than during non-REM sleep (Kiyashchenko et al., 2002). It was shown by Matsuzaki et al. (2002) that a central administration of orexins in rats significantly increased locomotor activity and induced changes in behavior. Because an orexin injection into the MLR induced or facilitated locomotion (Figure 3), an orexinergic projection to the MLR may be crucial for maintenance of the background excitability of the locomotor system. It follows that orexinergic projections to the midbrain cholinergic system, in addition to those to the dopaminergic and serotonergic systems, play a crucial role in the expression of emotional locomotor behavior (Matsuzaki et al., 2002). It has been reported that during the initial 10 – 20 minutes in a novel environment orexin knockout mice displayed a smaller increase in locomotor activity than wild mice even though their wakefulness was normal (Mochizuki et al., 2004). The mesopontine tegmentum integrates the limbic and motor output systems, and

concomitant sympathetic adjustments are likely to occur during complex behavioral changes (Inglis and Winn, 1995; Smith and DeVito, 1984; Winn et al., 1997). Krout et al. (2003) have shown that a considerable number of single orexinergic neurons in the lateral hypothalamus, and single cholinergic neurons in the PPN, directly or indirectly project to both the primary motor cortex and the stellate ganglion. This suggests that orexinergic and cholinergic neurons may integrate somatomotor and autonomic functions, and affect different types of behavior, such as arousal and sleep, and/or locomotion. All of these results suggest that an orexinergic system may separately control arousal systems and locomotor systems, and may link emotional stimuli to eliciting motivated locomotor behavior.

Role of orexinergic projections to the midbrain in the pathogenesis of narcolepsy

Our interest was how the orexinergic projections to the midbrain contribute to the pathogenesis of narcolepsy. Muscular atonia has been induced by injections of orexins into the medial PRF (Kiyashchenko et al, 2001; Xi et al., 2002, 2003) and the MRF (Mileykovskiy et al., 2002). Under some circumstances orexinergic projections to the above regions may induce muscular atonia. In contrast, the present study revealed that orexinergic projections to the midbrain inhibited REM and atonia. The inhibition could be due to postsynaptic and/or presynaptic effects upon the soma and terminals of GABAergic neurons in the PPN and the SNr which facilitate the release of GABA, as discussed above. A sustained release of GABA can not be maintained in the absence of orexin effects. These effects may increase the background excitability of systems generating REM and muscular atonia, and predispose affected individuals to attacks of cataplexy in narcolepsy. Therefore a higher sensitivity to orexin of the GABAergic neurons in the PPN and the SNr than of the REM sleep-related cholinergic neurons may underlie the pathogenesis of cataplexy. In this manner, cataplexy could occur by a disinhibition of the REM sleep generating system. In

addition, the orexinergic projection may increase the level of muscle tone and facilitate locomotor behavior (Figure 9A).

Based on these considerations we have proposed a model for the orexinergic control of emotional motor behavior and the disturbances that result in cataplexy in the narcoleptic state. In the normal waking state (Figure 9B), the excitability of the locomotor system and the muscle tone excitatory system are maintained by tonic orexinergic input. The excitability of the REM sleep generating system could be suppressed by the GABAergic inhibition from the SNr or in the PPN. Emotional signals that reach the midbrain via the limbic and hypothalamic structures (Derryberry and Tucker, 1992; Smith and Devito, 1984) may increase muscle tone and/or induce emotional locomotor behavior during wakefulness (Garcia-Rill et al., 2004; Shaikh et al., 1984; Skinner et al., 2004). However, in the narcoleptic state the excitability of both the locomotor system and the muscle tone excitatory system may be reduced because the orexinergic system is disturbed (Figure 9C). But the orexin deficiency may result in an increase in background excitability of the REM sleep generating system via disinhibition from GABAergic inputs to PPN cholinergic neurons. Cataplexy in the narcoleptic state could be induced by a decrease in the activity of the descending excitatory systems (Siegel, 2004) as well as by an enhancement of the atonia mediating system. Consequently, emotional signals could suddenly induce muscular atonia and this would result in cataplexy.

However, the above model may not be consistent with the previous results. For example, according to this model which has an absence of forebrain structures, a lack of orexin input may result in an increase in the background excitability of the REM sleep generating system. However, an increase in REM sleep has not been consistently observed in human narcolepsy (Aldrich, 1992), or in canine (Mitler and Dement, 1977) and mice (Mochizuki et al., 2004) narcoleptic models. Furthermore, a canine study has demonstrated

that the cyclicity of the normal REM sleep interval is not disturbed in the affected animals (Nishino, et al., 1997). Such an inconsistency could be derived from the lack of a contribution by forebrain structures in this model, because orexins modulate various neurotransmitter systems in forebrain structures and the brainstem (Selbach et al., 2004). These neurotransmitter systems are altered during a narcoleptic state, as described above.

It is therefore critical to consider how forebrain structures contribute to the pathogenesis in narcolepsy. In fact, activity of cerebral cortex was altered in human narcolepsy patients (Oliviero et al., 2005). Mesopontine tegmentum receives volitional signals from the cerebral cortex and emotional signals from limbic structures such as the hippocampus and the amygdala (see Takakusaki et al., 2004d). Because the basal ganglia receive afferents from these two structures, the mesopontine tegmentum may play key roles for initiation, integration, selection, or switching of volitionally-guided and emotionally-triggered motor behavior (Grillner et al., 1997; Jordan, 1998; Takakusaki et al., 2004d). We propose that descending and ascending systems from the brainstem may mediate changes of activity in the cerebral cortex, basal ganglia, limbic structures, and the brainstem, which would result in the generation of narcoleptic symptoms.

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Figure legends

Figure 1. Locomotion, and REM and atonia induced by midbrain stimulation.

A. (a) The stimulus sites for evoking locomotion (open circles), and REM and atonia (filled circles), are indicated on a parasagittal plane (L 4.0). (b) Locomotion on the moving treadmill elicited by stimulation (40 μA) of the CNF. (c) REM and atonia induced by stimulation (30 μA) of the PPN. In (b) and (c) 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 period is indicated by lines under each recording.

B. Optimal stimulus sites for evoking locomotion (open circles) and REM and atonia (filled circles) on parasagittal (a) and coronal planes (b) of the brainstem. Locomotor evoking sites were mainly located in the CNF, and inhibitory sites were located in the ventrolateral part of the PPN. A location of the PPN where cholinergic neurons were distributed is indicated by the grey area.

C. (a) A microphotographic presentation of cholinergic neurons, which were labeled by ChAT immunohistochemistry, in the mesopontine tegmentum. The area corresponds to the area enclosed by a square in B (b). (b) Higher magnification of the area enclosed by a square in (a).

Abbreviations: IC, inferior colliculus; CNF, cuneiform nucleus; L, lateral; LDT, laterodorsal tegmental nucleus; MLR; midbrain locomotor region; NRPo, nucleus reticularis pontis oralis; P, posterior; PPN, pedunculopontine tegmental nucleus; REM, rapid eye movements; SC, superior colliculus; SCP, superior cerebellar peduncle; SNr, substantia nigra pars reticulata;

Figure 2. A framework for the present study

See text for explanation.

Figure 3. Orexin controls locomotor movements

A. A stimulus site (a filled arrow) and an orexin injection site (an open arrow) on coronal sections of the midbrain.

B. Upper and lower traces are electromyographic activity obtained from the left (L) and right (R) soleus (Sol) muscles. (a) Before an orexin injection stimulation of the CNF with an intensity of 20 μA did not evoke locomotor movements on the moving treadmill. (b) Locomotor movements were evoked 10 minutes after an orexin injection (200 μM, 0.25 μl) into the CNF. In (a) and (b) the treadmill speed was 0.3 m/s throughout the period of each trial. (c) Locomotor movements were observed without electrical stimulation and 30 minutes after the orexin injection. The treadmill speed was 0.3 m/s. The open bar under the EMG recording indicates the period of moving treadmill.

C. (a) Locomotion elicited by stimulating the CNF with an intensity of 40 μA. (b) Twenty minutes after an orexin injection (60 μM, 0.25 μl) into the CNF, stimuli with an intensity of 20 μA elicited locomotion. (c) CNF stimulation with an intensity of 30 μA elicited locomotion 60 minutes after the injection. In each trial the treadmill speed was 0.3m/s.

D. Changes in threshold stimulus intensity for evoking locomotion. The threshold current of any trial was reduced after the orexin injections. For the trials indicated by the symbols, the concentrations of the injected orexin were: open circles, 60 μM; open squares, 100 μM; filled squares, 200 μM; and filled circles, 500 μM. The threshold currents for evoking locomotion before (mean $\pm$ standard deviation = 31.2 $\pm$ 7.5 μA, median = 30 μA,) and after (mean $\pm$ standard deviation = 12.5 $\pm$ 7.5 μA, media = 15 μA) the orexin injections were significantly different ($p=0.005$).

Abbreviation: PRF, pontine reticular formation.

Figure 4. Orexin controls PPN-induced REM and atonia

A. The effects of an orexin injection into the PPN on PPN-stimulus effects. (a) A stimulus site (a filled arrow) and an orexin injection site (an open arrow) in the PPN area are shown on coronal sections of the mesopontine tegmentum. (b) From upper to lower: an EOG and EMGs of the left and right soleus muscles. Stimulation (30 μA and 50 Hz) of the PPN induced REM and muscular atonia. The REM was induced only during the period of the stimulation, which is denoted by a line below the recording. (c) An orexin injection (200 μM, 0.25 μl) into the PPN abolished the PPN-induced REM and atonia.

B. The threshold stimulus strength which was required to elicit the PPN effect was increased in each trial after the orexin injections. For the trials indicated by the symbols, the concentrations of the injected orexin were: open circles, 60 μM; open squares, 100 μM; filled squares, 200 μM; filled circles, 500 μM; and filled triangles, 1000 μM. The stimulus strength which was required to elicit muscular atonia was compared in 8 trials in 5 cats. The threshold currents for eliciting muscular atonia before (23.8 $\pm$ 5.2 μA, 25 μA) and after (42.5 $\pm$ 4.6 μA, 45 μA) the orexin injections were significantly different ($p=0.003$).

C. The effects of bicuculline injection into the PPN. (a) Muscular atonia induced by PPN stimulation (25 μA and 50 Hz). (b) Twenty minutes after the injection of orexin (1000 μM, 0.25 μl) into the PPN, the PPN-induced muscular atonia was abolished. (c) A bicuculline injection (1 mM, 0.25 μl) into the PPN, 20 minutes after the orexin injection, reversed the PPN effect. A line below each recording indicates the period of PPN stimulation.

Figure 5. Orexinergic input to the SNr controls PPN-induced REM and atonia.

A. The effects of an orexin injection into the SNr on the PPN effects. (a) The sites of the stimulus (a filled arrow) in the PPN, and the injection of orexin (an open arrow) in the SNr, on coronal sections of the midbrain. (b) From upper to lower: an EOG and EMGs obtained

from the left and right soleus muscles. REM and atonia induced by PPN stimulation (30 μA and 50 Hz). (b) An orexin injection (200 μM, 0.25 μl) into the SNr abolished the PPN effects.

B. The strength of the threshold stimulus which was required to elicit the PPN effect was increased in each trial (8 trials in 4 cats) after the orexin injections. The trials and concentrations of orexin injected were: open squares, 100 μM; filled squares, 200 μM; filled circles, 500 μM; and filled triangles, 1000 μM.

The stimulus strength which was required to elicit muscular atonia was compared in 7 trials with 4 cats. The threshold currents for eliciting muscular atonia before (23.8 $\pm$ 4.9 μA, 25μA) and after (39.3 $\pm$ 4.5 μA, 40 μA) the orexin injections were significantly different ($p=0.011$).

C. The effects of bicuculline injection into the PPN. (a) Muscular atonia induced by PPN stimulation (30 μA and 50 Hz). (b) Thirty minutes after an orexin injection (1000 μM, 0.25 μl) into the SNr, the PPN-induced muscular atonia was not observed. (c) A subsequent bicuculline injection (1 mM, 0.25 μl) into the PPN, 30 minutes after the orexin injection, reversed the PPN-effect. A line below each recording indicates the period of PPN stimulation.

Figure 6 Effective injection sites for orexin-A.

A. Most of the sites of injections which facilitated MLR-induced locomotion (n=10) were located in the CNF and an adjacent area, including the dorsal part of the PPN.

B. The sites of injections which inhibited PPN-induced REM and atonia (n=11) were located in the ventrolateral part of the PPN.

In A and B, the effective sites are plotted on coronal planes of the mesopontine tegmentum at the levels of AP0, P1, and P2.

C. The sites of injections which inhibited PPN-induced REM and atonia (n=8) are illustrated

on coronal planes of the caudal diencephalon at the levels of A3 and A4. The sites covered an area corresponding to the dorsolateral part of the SNr.

D. The injection sites are illustrated on a parasagittal plane of the brainstem at the level of LR4–5. There was a clear functional topography in the orexinergic control of locomotion and postural muscle tone. Two injections, which are indicated by an asterisk, not only facilitated MLR-induced locomotion but inhibited PPN-induced REM and atonia.

Abbreviations: A, anterior; AP, anterior-posterior; CP; cerebral peduncle; LR, left and right.

Figure 7. The time course of the orexin-effects.

A–C. The time course of the effects of the orexin injections into the (A) CNF, (B) PPN and (C) SNr are shown with respect to the different concentrations of orexin.

A. An orexin injection reduced the threshold current for evoking MLR-induced locomotion. In 5 of 6 trials the orexin-effect was observed within 10 minutes of the injection. Locomotor movement induced by the moving treadmill without stimulation was observed when higher concentrations (200 μM and 500 μM) of orexin were injected into the CNF.

B. Orexin injections into the PPN increased the threshold current required for evoking PPN atonia. The latency of the orexin effect was 10–20 minutes. In two cats stimulation of the PPN with a maximum intensity of 50 μA did not abolish muscle tone when higher concentrations of orexin (200–500 μM) were injected into the PPN.

C. Orexin injections into the SNr also increased the threshold current required for PPN atonia. The effects were observed within 20 minutes.

In A–C, the trials used the following concentrations of orexin: open circles, 60 μM; open squares, 100 μM; hatched squares, 200 μM; and filled circles, 500 μM. Higher concentrations of orexin produced stronger effects in each area. The effects continued for more than 100 minutes.

D. The effects of injections of bicuculline into the PPN after injections into the PPN (filled and open triangles) and the SNr (filled and open squares) of orexins. Bicuculline (1 mM, 0.25 μl) was injected 20–30 minutes after the orexin injections, its effects were observed within 5 minutes, and lasted for more than 30 minutes. The filled and open symbols indicate orexin injections with concentrations of 1000 μM and 200 μM, respectively.

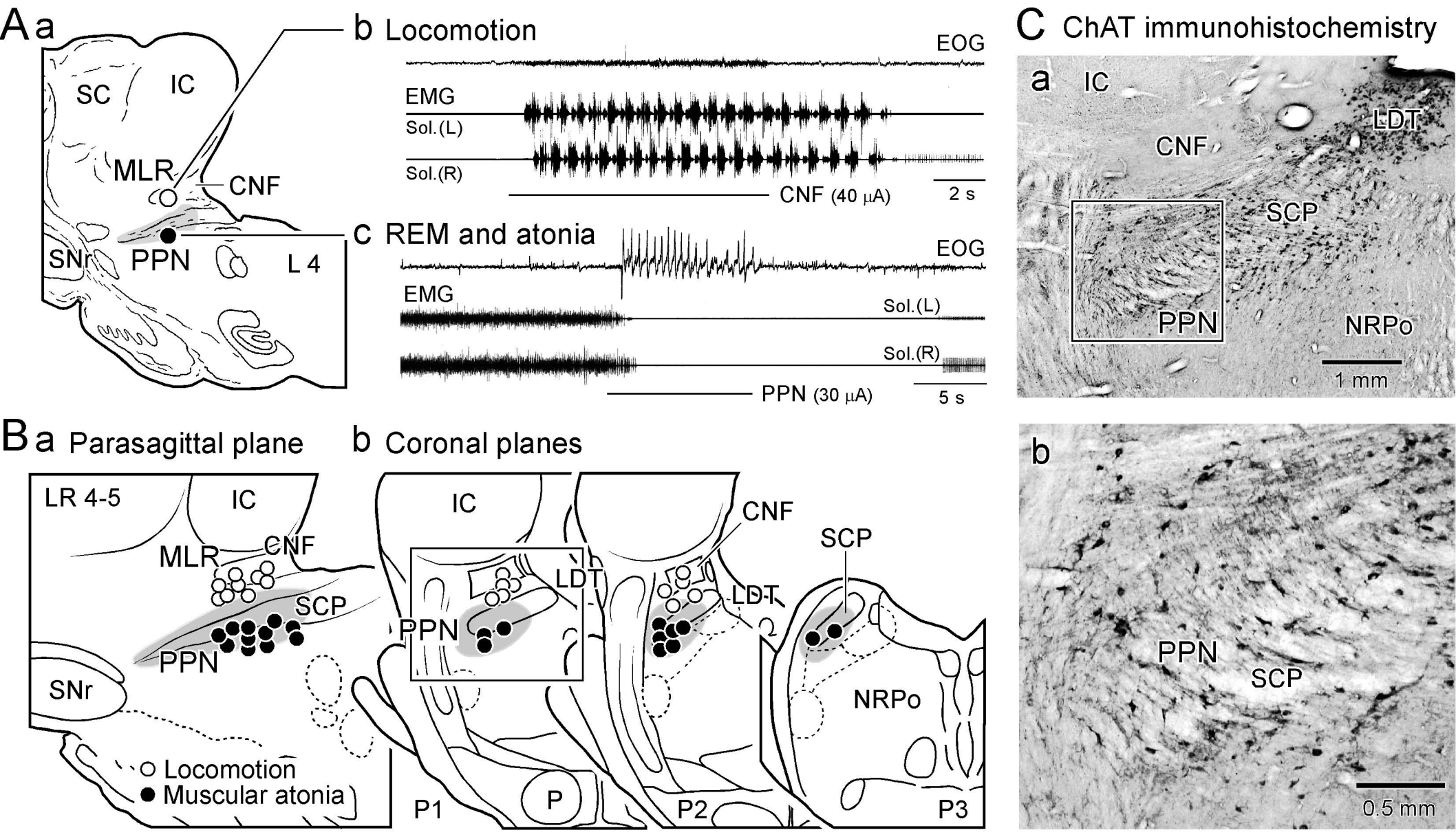
Figure 8. Orexinergic modulation of descending pathways from the brainstem.
A. An orexin injection site (an open arrow) and stimulus sites (open and filled circles) on a coronal section of the mesopontine tegmentum.
B. From upper to lower: EMG activities induced by stimulating the MLR, locus coeruleus (LC), PRF, dorsal and ventral PPN. Stimulation of the MLR and the LC induced a mixture of excitatory and inhibitory effects. Stimulation of the PRF and the PPN suppressed EMG activities.
C and D. The effects of an orexin injection into the PPN. EMG activities were recorded 30–60 minutes (C) and 150–180 minutes (D) after the injection of orexin. The EMG activity from the left soleus (Sol) muscles was rectified, integrated and averaged for 20 sweeps. Short train pulses of stimuli (3 trains, 5 ms intervals and 40 μA) were delivered to each site.

Figure 9. Role of orexinergic projections to the midbrain in the regulation of the locomotor system, REM and atonia systems, and possible mechanisms of induction of cataplexy in narcolepsy.
A. A summary of the present results. Orexin excited the locomotor system and the SNr. The REM sleep generating (REM and atonia) system was inhibited by orexin through activation of GABAergic input, possibly from either local interneurons or SNr neurons.
B. In a normal waking state orexin maintained an excitability of the locomotor system and

suppressed the REM sleep generating system. Thus, emotional signals may elicit locomotor behavior that is accompanied by muscle tone augmentation.

C. In narcolepsy an orexin deficit may decrease the excitability of the locomotor system, whereas the excitability of the REM sleep generating system could be increased by a release from the inhibitory effects of orexin and nigral GABAergic input. Emotional signals may thus produce cataplexy.

"+" and "– " signs indicate excitatory and inhibitory effects, respectively.


A
a
SC
IC
MLR
CNF
SNr
PPN
L 4
b Locomotion
EOG
EMG
Sol.(L)
Sol.(R)
CNF (40 μA)
2 s
c REM and atonia
EOG
EMG
Sol.(L)
Sol.(R)
PPN (30 μA)
5 s
B
a Parasagittal plane
LR 4-5
IC
MLR
CNF
SCP
PPN
SNr
Locomotion
Muscular atonia
b Coronal planes
IC
CNF
SCP
LDT
PPN
LDT
NRPo
P1
P
P2
P3
C ChAT immunohistochemistry
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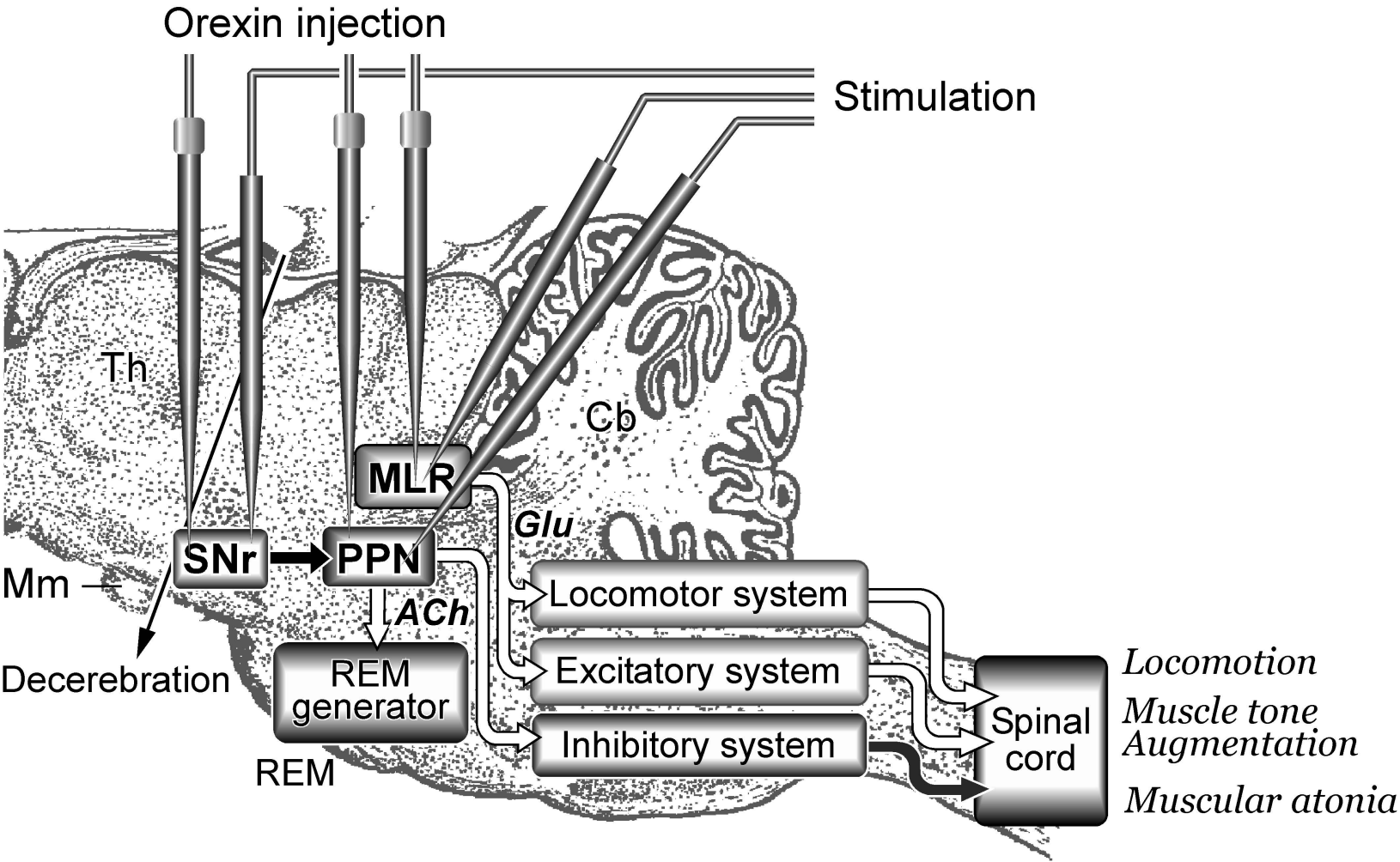
IC
LDT
CNF
SCP
PPN
NRPo
1 mm
b
PPN
SCP
0.5 mm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Stimulation
Th
Cb
MLR
Glu
SNr
PPN
Mm
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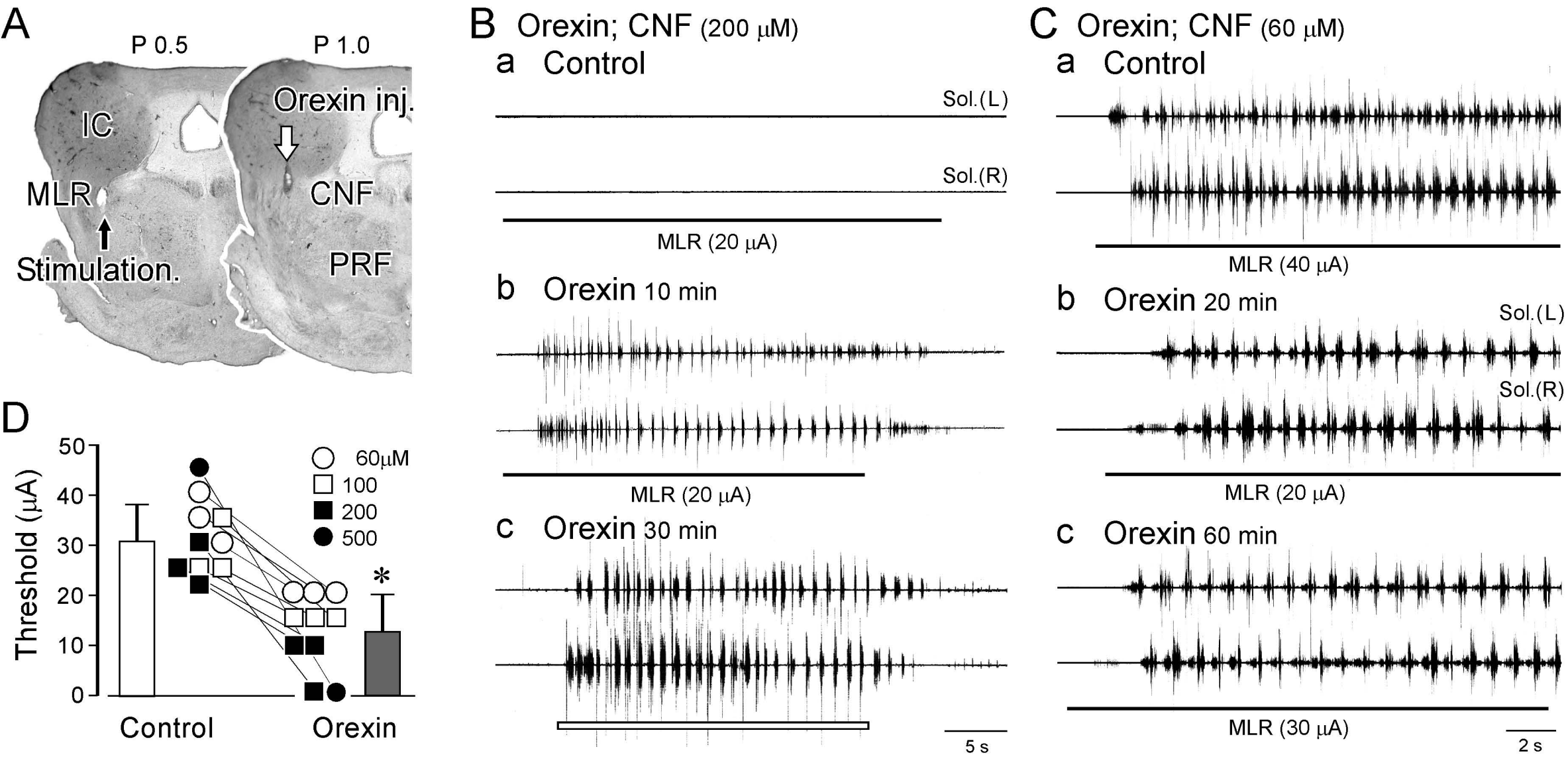
Decerebration
REM generator
REM
Locomotor system
Excitatory system
Inhibitory system
Spinal cord
Locomotion
Muscle tone Augmentation
Muscular atonia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P 1.0
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Orexin inj.
MLR
CNF
PRF
Stimulation.
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Sol.(L)
Sol.(R)
MLR (20 μA)
b Orexin 10 min
MLR (20 μA)
c Orexin 30 min
5 s
C Orexin; CNF (60 μM)
a Control
MLR (40 μA)
b Orexin 20 min
Sol.(L)
Sol.(R)
MLR (20 μA)
c Orexin 60 min
MLR (30 μA)
2 s
D
Threshold (μA)
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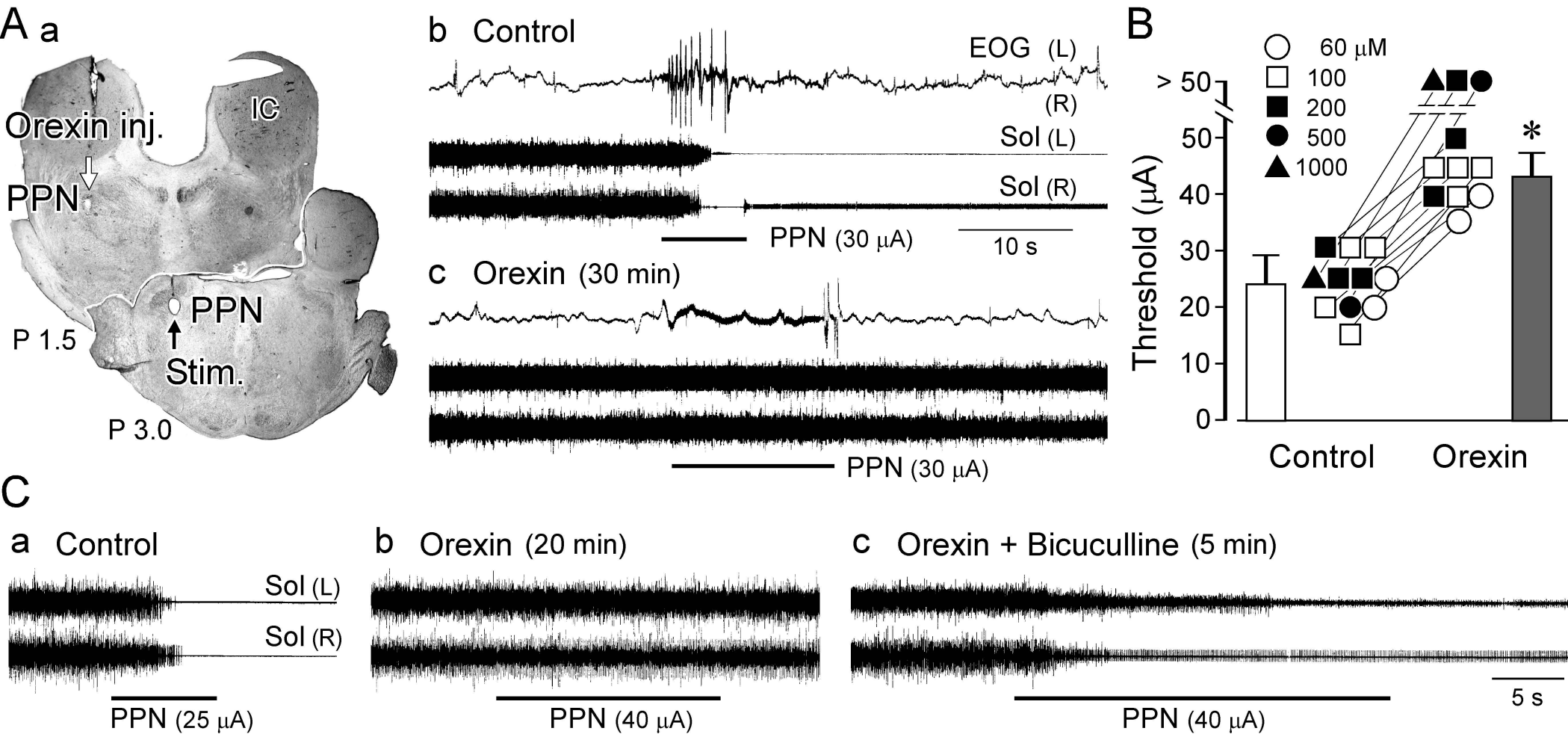
40
30
20
10
0
60μM
100
200
500
*
Control
Orexin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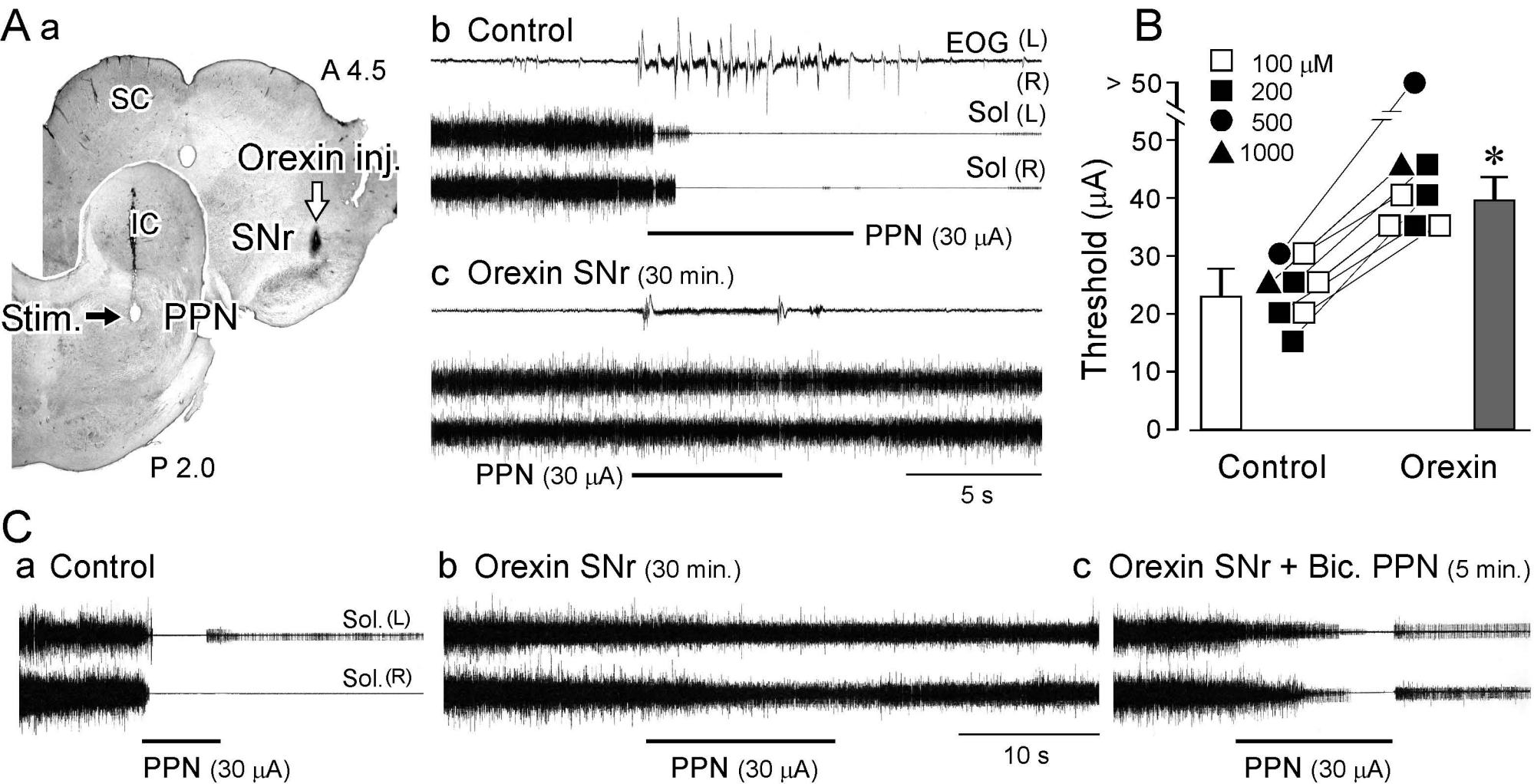
Orexin inj.
IC
PPN
PPN
P 1.5
Stim.
P 3.0
b Control
EOG (L)
(R)
Sol (L)
Sol (R)
PPN (30 μA)
10 s
c Orexin (30 min)
PPN (30 μA)
B
60 μM
100
200
500
1000
> 50
50
40
30
20
10
0
Threshold (μA)
*
Control
Orexin
C
a Control
Sol (L)
Sol (R)
PPN (25 μA)
b Orexin (20 min)
PPN (40 μA)
c Orexin + Bicuculline (5 min)
PPN (40 μA)
5 s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SC
Orexin inj.
IC
SNr
Stim.
PPN
P 2.0
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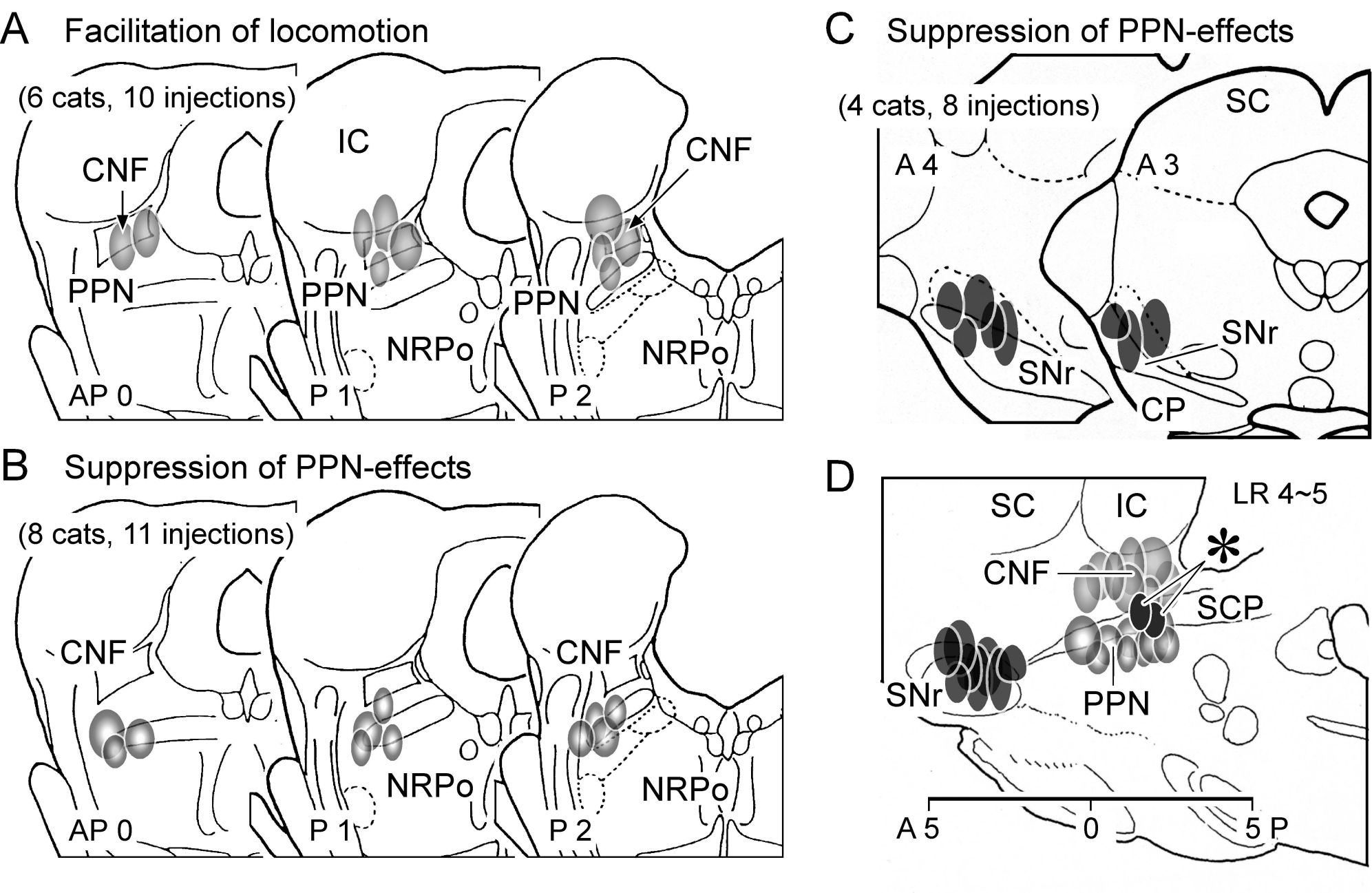
EOG (L)
(R)
Sol (L)
Sol (R)
PPN (30 μA)
c Orexin SNr (30 min.)
PPN (30 μA)
5 s
B
100 μM
200
500
1000
> 50
50
40
30
20
10
0
Threshold (μA)
*
Control
Orexin
C
a Control
Sol. (L)
Sol. (R)
PPN (30 μA)
b Orexin SNr (30 min.)
PPN (30 μA)
10 s
c Orexin SNr + Bic. PPN (5 min.)
PPN (30 μA)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(6 cats, 10 injections)
CNF
IC
CNF
PPN
PPN
PPN
NRPo
NRPo
AP 0
P 1
P 2
B
Suppression of PPN-effects
(8 cats, 11 injections)
CNF
CNF
NRPo
NRPo
AP 0
P 1
P 2
C
Suppression of PPN-effects
(4 cats, 8 injections)
SC
A 4
A 3
SNr
SNr
CP
D
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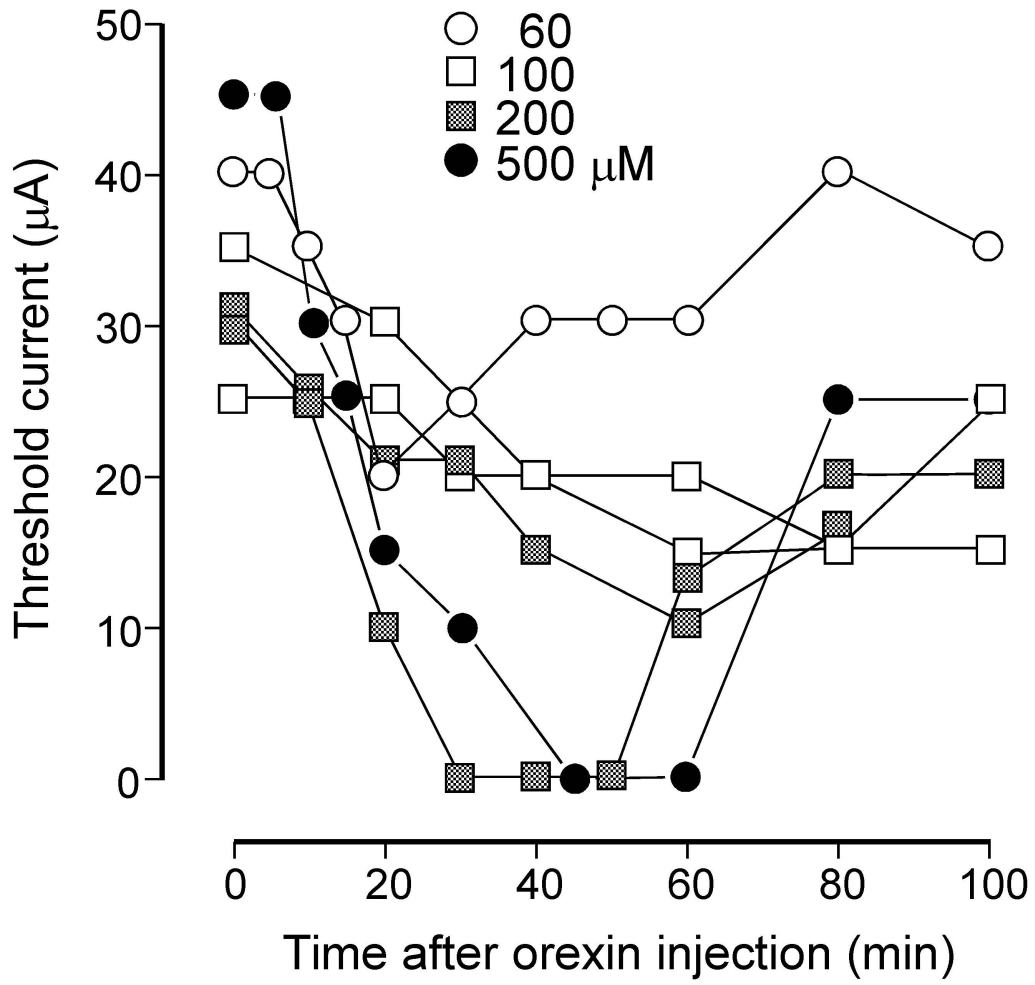
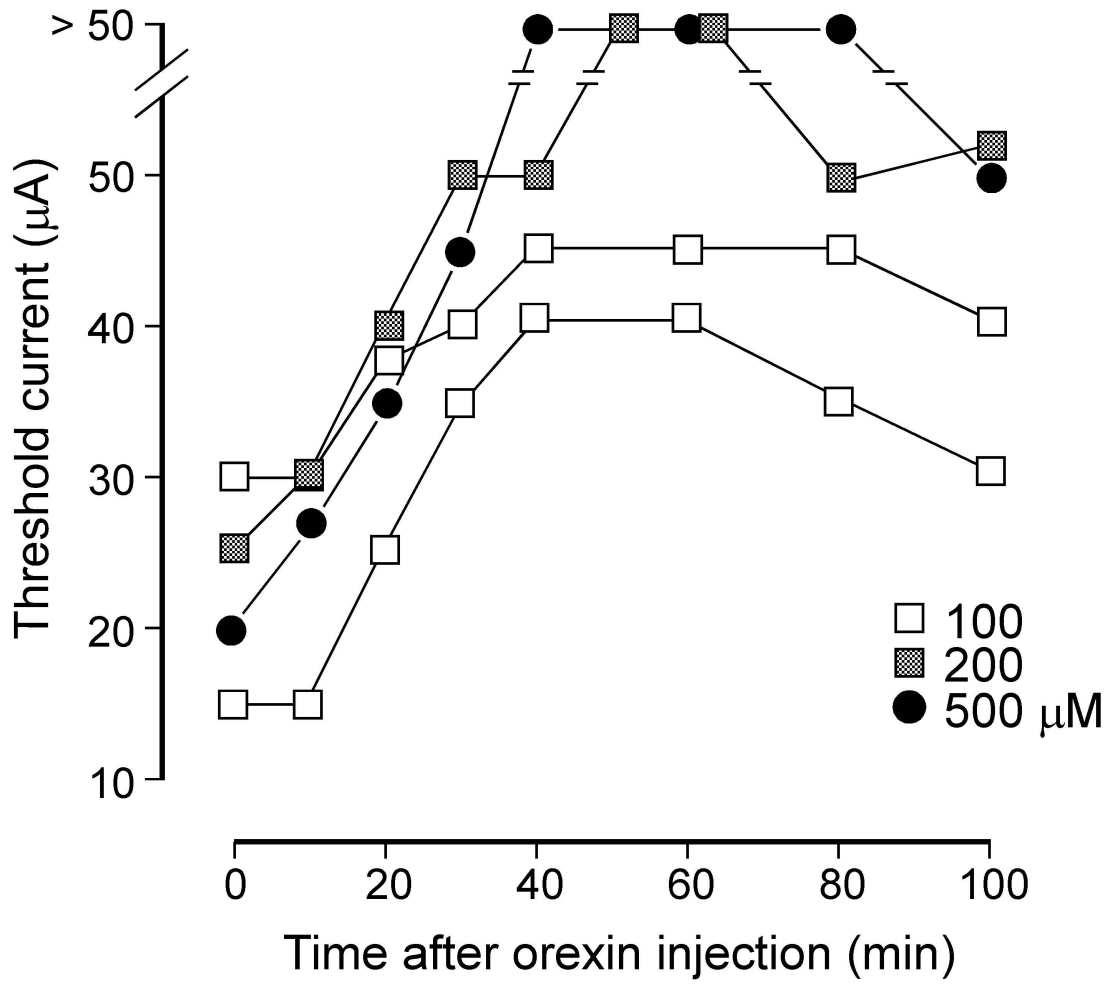
LR 4~5
CNF
SCP
SNr
PPN
A 5
0
5 P

A Orexin (CNF)



B Orexin (PPN)



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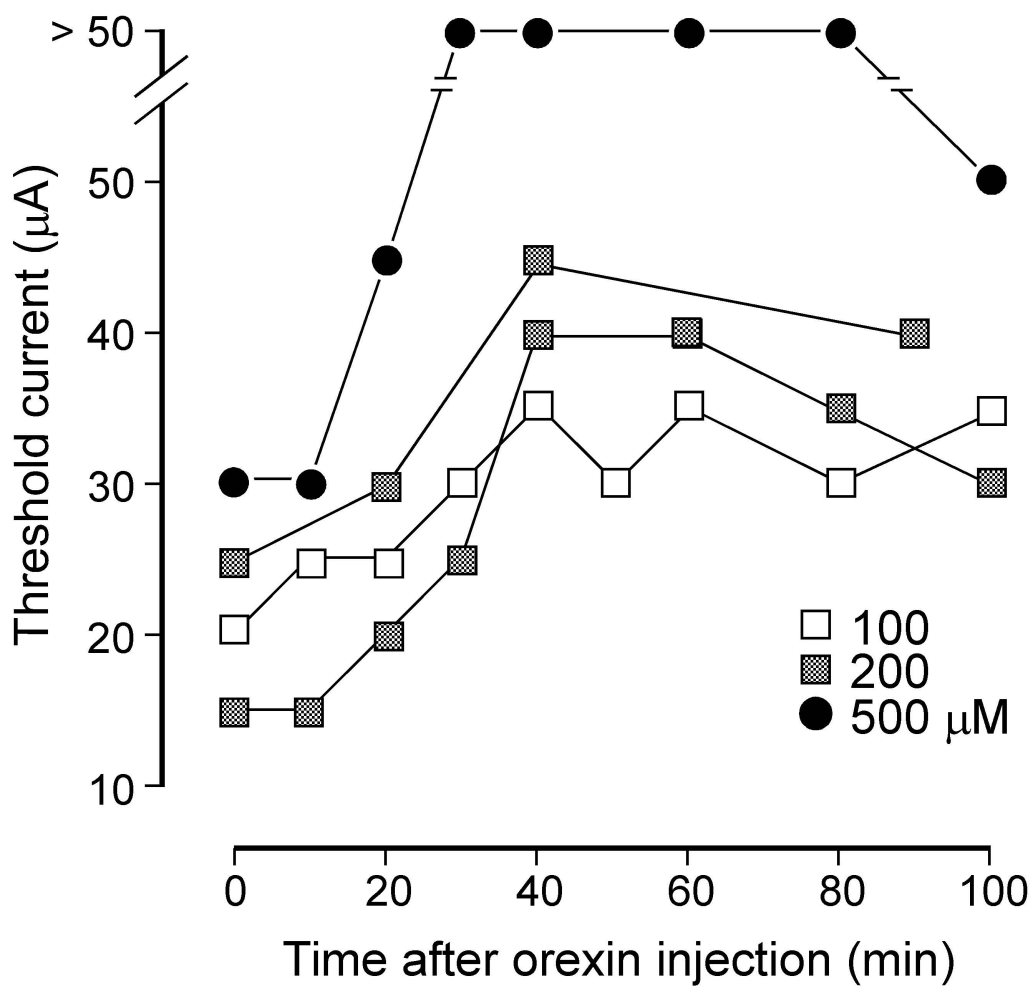

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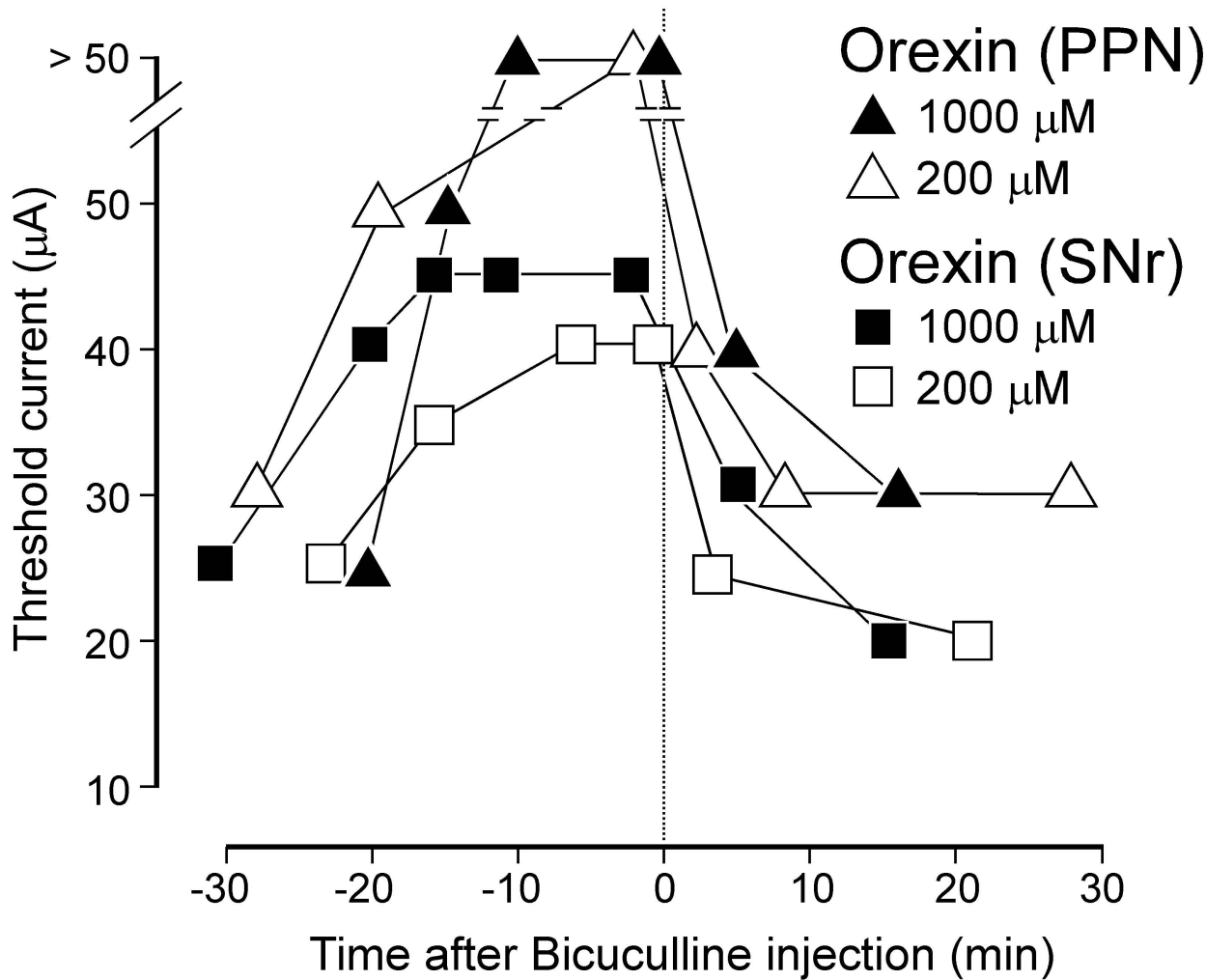
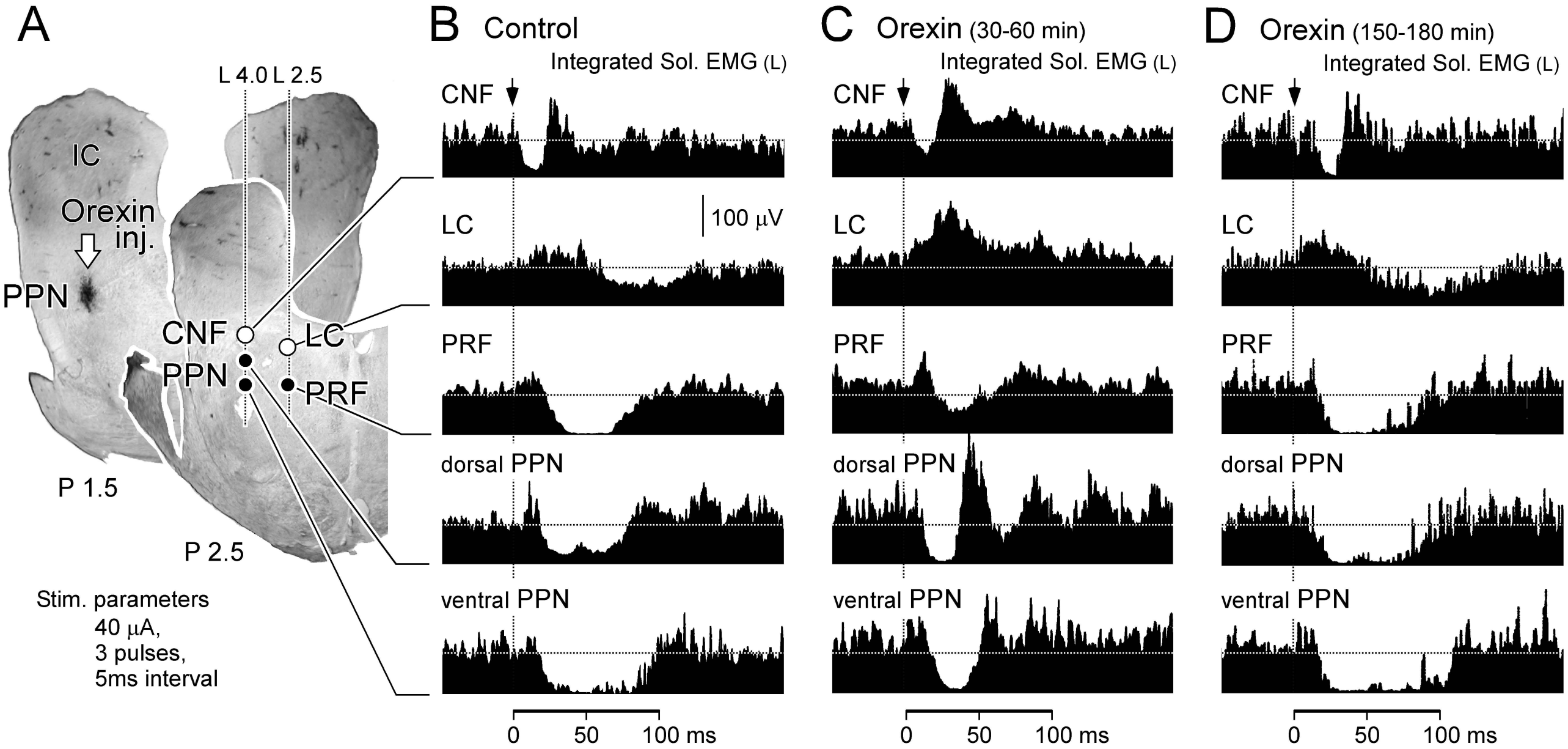
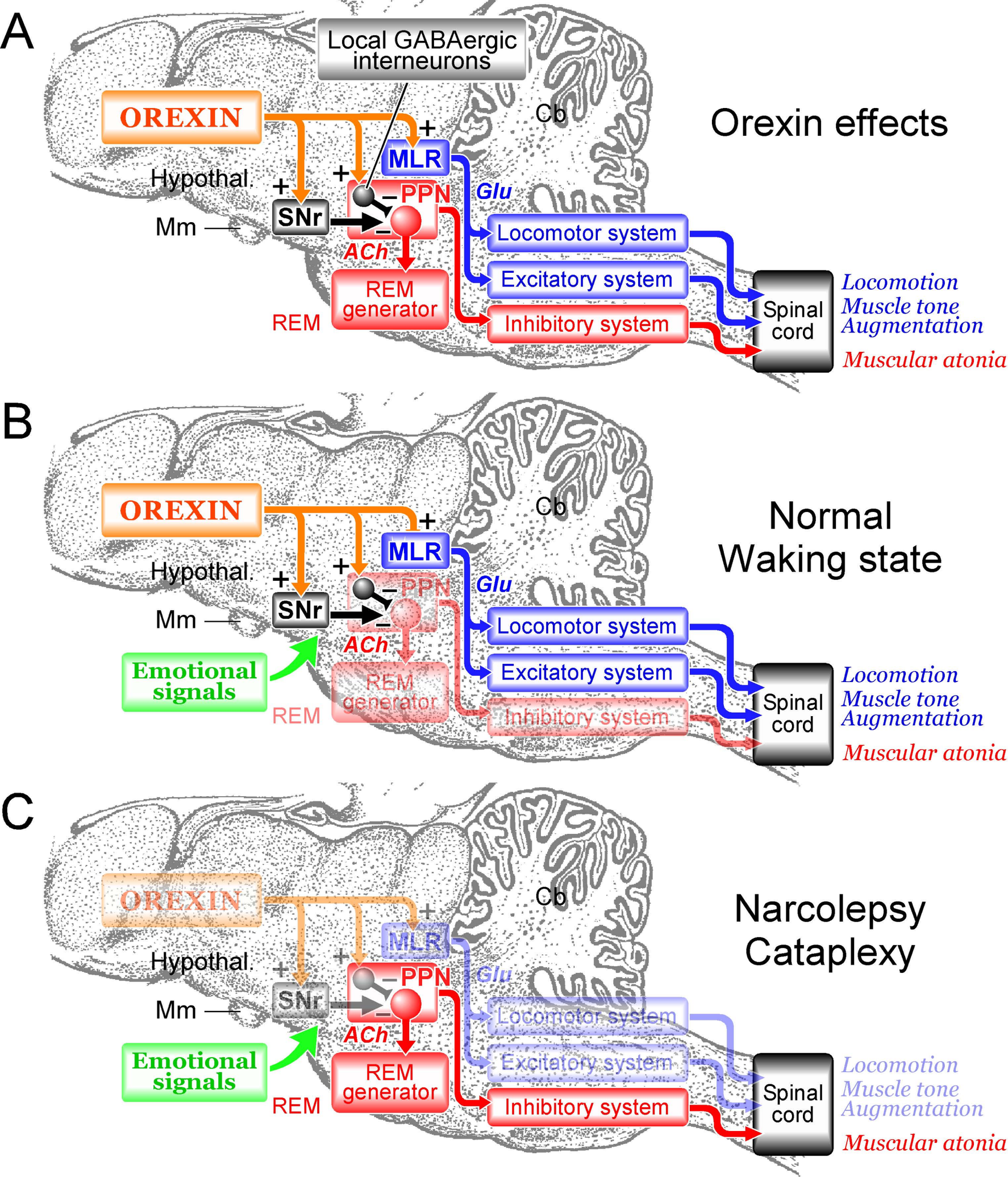



A
L 4.0 L 2.5
IC
Orexin
inj.
PPN
CNF
LC
PPN
PRF
P 1.5
P 2.5
Stim. parameters
40 μA,
3 pulses,
5ms interval
B Control
Integrated Sol. EMG (L)
CNF
LC
100 μV
PRF
dorsal PPN
ventral PPN
0
50
100 ms
C Orexin (30-60 min)
Integrated Sol. EMG (L)
CNF
LC
PRF
dorsal PPN
ventral PPN
0
50
100 ms
D Orexin (150-180 min)
Integrated Sol. EMG (L)
CNF
LC
PRF
dorsal PPN
ventral PPN
0
50
100 ms


A
Local GABAergic interneurons
OREXIN
Cb
Orexin effects
Hypothal.
MLR
PPN
Glu
SNr
Mm
Locomotor system
ACh
Excitatory system
REM generator
Spinal cord
Locomotion
Muscle tone
Augmentation
REM
Inhibitory system
Muscular atonia
B
OREXIN
Cb
Normal
Waking state
Hypothal.
MLR
PPN
Glu
SNr
Mm
Locomotor system
ACh
Excitatory system
Emotional signals
REM generator
Spinal cord
Locomotion
Muscle tone
Augmentation
REM
Inhibitory system
Muscular atonia
C
OREXIN
Cb
Narcolepsy
Cataplexy
Hypothal.
MLR
PPN
Glu
SNr
Mm
Locomotor system
ACh
Excitatory system
Emotional signals
REM generator
Spinal cord
Locomotion
Muscle tone
Augmentation
REM
Inhibitory system
Muscular atonia