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Do neurotoxic lesions in rostral medullary nuclei induce/accentuate hypoventilation during NREM sleep?

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Abstract

Experimentally induced neuronal dysfunction in respiratory regions of the rostral medulla decrease breathing more in anesthetized mammals than in awake mammals. Sleep is similar to anesthesia in that excitatory inputs to respiratory neurons are reduced compared to the awake state; thus, we hypothesized that neurotoxic lesions in rostral medullary nuclei would, relative to wakefulness (WK), induce and/or accentuate hypoventilation during non-rapid eye movement (NREM) sleep. To test the hypothesis, goats were studied between 21:00 h and 03:00 h: (1) before and 30 days after chronically implanting microtubules bilaterally into the rostral medulla and, (2) 9–15 h and 2–17 days after unilateral injections of 100 nl to 1 μl, 50 mM ibotenic acid into the vestibular, gigantocellularis reticularis, or facial nuclei, or the retrotrapezoid nucleus/parapyramidal region. Arterial blood was repeatedly sampled in all studies during WK, and NREM and rapid eye movement (REM) sleep states. There was no significant ($P > 0.10$) change in Pa_{CO_2} between WK and NREM sleep (and REM sleep when sufficient data were obtained) before or after implantation of microtubules and in studies after creating the neurotoxic lesions. Breathing frequency also did not significantly ($P > 0.10$) differ between states in any of the studies. The data thus did not support the hypothesis. We speculate that in goats efficient compensatory mechanisms maintain Pa_{CO_2} homeostasis during normal sleep and the same and/or other mechanisms maintain homeostasis when excitatory drive is further reduced by lesions in rostral medullary nuclei.
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Keywords: Brainstem; rostral medulla; neurotoxic lesions; pattern of breathing; hypoventilation; Sleep

1. Introduction

Cooling the rostral ventrolateral medulla (RVLM) under anesthesia results in sustained apnea (Forster et al., 1997; Ohtake et al., 1995; Schlaefke et al., 1970). However, cooling the RVLM during wakefulness (WK) causes only a mild reduction in breathing and there is only a

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slight, non-significantly greater effect of cooling during non-rapid eye movement (NREM) sleep (Forster et al., 1997; Ohtake et al., 1996). Similarly neurotoxic or electrolytic lesions in the RVLM under anesthesia cause terminal or near terminal apneas (Nattie and Li, 1990; Nattie et al., 1988, 1991), but neurotoxic lesions in awake animals do not result in persistent apneas. There is, however, a modest hypoventilation and significantly attenuated CO_2 sensitivity (Akilesh et al., 1997; Forster et al., 1998; Wenninger et al., 2001) for 2–3 weeks after creating lesions in rostral medullary nuclei. The greater effect of cooling and lesioning in anesthetized versus the awake state is thought to reflect the relatively fewer compensatory mechanisms available in the anesthetized state when any single excitatory stimulus is attenuated (Forster et al., 1997, 1998).

Similar to anesthesia, absence of the excitatory WK stimuli during NREM sleep results in reduced breathing and hypoventilation in many mammalian species (Dempsey et al., 1991; Guazzi and Freis, 1969; Morrell et al., 1990; Phillipson, 1978; Phillipson and Bowes, 1986). It would seem that the reduced ventilatory drive during NREM sleep, would result in a reduction of mechanisms available to compensate for the attenuation of excitatory drive caused by a neurotoxic lesion. We therefore postulated that neurotoxic lesions in rostral medullary nuclei would relative to WK, induce/accentuate hypoventilation during NREM sleep. The purpose of this study was to test this hypothesis.

2. Methods

The study protocol and animal care was reviewed and approved by the Animal Care Committee at the Medical College of Wisconsin prior to the initiation of any studies.

We have already published data (from most of the same goats as studied herein) showing that during WK, acute and chronic dysfunction in the retrotrapezoid nucleus/parapyramidal region (RTN/P_{PY}), or facial (FN), or gigantocellularis reticularis (RGN) nuclei altered eupneic breathing, CO_2 sensitivity, and the exercise hyperpnea (Wenninger et al., 2001). We have also reported that altered neuronal activity in the FN and RGN change the coordination/interaction of breathing and swallowing (Feroah et al., 2002), and that perturbations in the RTN/P_{PY}, FN, and vestibular nucleus (VN) induce or increase the frequency of fractionated breaths (Feroah et al., 2003). We have studied goats primarily because we can complete comprehensive protocols under physiologic conditions; thus we have a large database on the effects of neuronal dysfunction in anesthetized, awake, and asleep goats (Feroah et al., 2003; Forster et al., 1997, 1998; Ohtake et al., 1995, 1996; Wenninger et al., 2001).

2.1. Surgical procedures

The surgical procedures have been previously published (Feroah et al., 2002; Wenninger et al., 2001). Briefly, an initial surgery was performed to elevate the carotid arteries. We also implanted electrodes to obtain electroencephalograms (EEG), electrooculograms (EOG), and diaphragm and upper airway muscle electromyograms (EMG). At least 3 weeks later, surgery was performed for chronic bilateral implantation of microtubules into the medulla. The microtubules (made of PE-50 tubing) were not always implanted into the same nucleus on each side (see below). A 25-gauge stainless steel wire was inserted into the tubule before the tubule was advanced through the cerebellum into the medullary nuclei with the use of a manual micromanipulator.

At least for the initial 24 h after microtubule placement, laboratory personnel continuously monitored the goats. To minimize brain edema and infection, goats were medicated three times per day with a previously published medication regime (Feroah et al., 2002; Wenninger et al., 2001). Buprenorphine was administered 3 and 12 h after implantation to minimize pain.

2.2. Experimental design

Five groups of goats were studied. *Group 1:* Since it had not been established whether goats hypoventilate during NREM and rapid eye movement (REM) sleep (Parisi et al., 1987, 1992) eight

goats were studied one night during the normal sleep period prior to microtubule implantation. These goats were also studied for 15–30 min during the day before and after the night study. *Group 2:* About 30 days after implanting microtubules, ten goats (three from group 1) were studied during the day for 15–30 min before and 5 h after unilateral injection of mock cerebrospinal fluid (mCSF) into one of the microtubules. To determine whether implantation of the microtubules altered breathing during sleep, the goats were also studied for 6 h the same night and they were studied again for at least 15–30 min the following day. *Group 3:* Usually 1 day later, 12 goats (ten from group 2) were studied during the day for 15–30 min before and for 5 h after unilateral injection of 100 nl to 1 μl of 50 mM ibotenic acid into the RTN/P_{PY} ($n = 3$), FN ($n = 4$), RGN ($n = 4$), or VN ($n = 1$). These goats were also studied for 6 h the same night and again for 15–30 min the following day. *Group 4:* Three to ten days later, eight of the 12 goats in group 3 were similarly studied after unilateral injection of ibotenic acid into the RTN/P_{PY} ($n = 2$), FN ($n = 2$), or RGN ($n = 4$). This second injection was always contralateral to the side of the first injection. Technical problems prevented a second injection study in the other four goats of group 3. Accordingly, four goats were lesioned at only one site while eight were lesioned at two sites (see Fig. 1). *Group 5:* Two to seventeen days after the last ibotenic acid injection 11 goats (all but one from group 3) were studied for about 15–30 min during the day before and after a 6-h night study.

2.3. Protocols and procedures for day studies

Day studies during WK usually began about 08:30 h. A fitted mask was taped to the snout and a breathing valve was attached to the mask. The inspired port of the valve was connected to a Hans Rudolf pneumotach. The breathing signal was conditioned and amplified by a Validyne demodulator and pressure transducer, which was connected to a Grass model 7D oscillograph recorder and a Citus 486 computer. The expired port of the valve was connected to a Tissot spirometer for collection of expired air that was analyzed for O_2 and CO_2 concentration and used to calculate metabolic rate ($\dot{V}_{O_2}$). The elevated carotid artery was chronically catheterized to monitor arterial blood pressure and to obtain blood samples for pH, arterial P_{CO_2} (Pa_{CO_2}), and arterial P_{O_2} determination (model 278, Ciba-Corning). Rectal temperature (T_{re}) was measured after each blood sample was taken. Breathing, heart rate (HR), mean arterial blood pressure (MABP), and $\dot{V}_{O_2}$ were monitored continuously for about 30 min except HR and MABP monitoring was interrupted for two, 3-min intervals for withdrawal of arterial blood. For 1 and 5 of the experimental design, no further daytime studies were completed. For 2, 3, and 4 of the design, the control periods were followed by an injection of mCSF or ibotenic acid. A 31-gauge stainless steel tube of the same length as the microtubule implants was connected to a 500 nl or 1 μl Hamilton syringe, which was loaded with mCSF or the neurotoxin, and then 100 nl to 1 μl was manually injected into the medullary nuclei. Physiologic variables were then monitored over the subsequent 5 h just as during the pre-injection control period.

2.4. Protocols and procedures for night studies

Prior to initiating these studies, the goats were trained during the day to stand and during the night to lay and sleep in a stanchion with a mask taped to the snout, and recording electrodes attached. In an attempt to consolidate sleep at night, the goats were not allowed to lay down until the start of the night study. Three to five hours prior to the night study, the goats had free access to feed and water. At about 20:00 h the EMG, EEG, and EOG electrodes were connected and in some goats a mask was placed on the snout to prevent chewing of the instrument lines. At about 21:00 h the lights were turned off and the goats were allowed to lie down. EEG, EOG, EMG signals were recorded continuously until about 03:00 h the next morning. HR and MABP were also monitored continuously except for periods when arterial blood was withdrawn, which occurred every 30 min or as needed to establish blood gases during the different states. Total ventilation was not recorded because we had

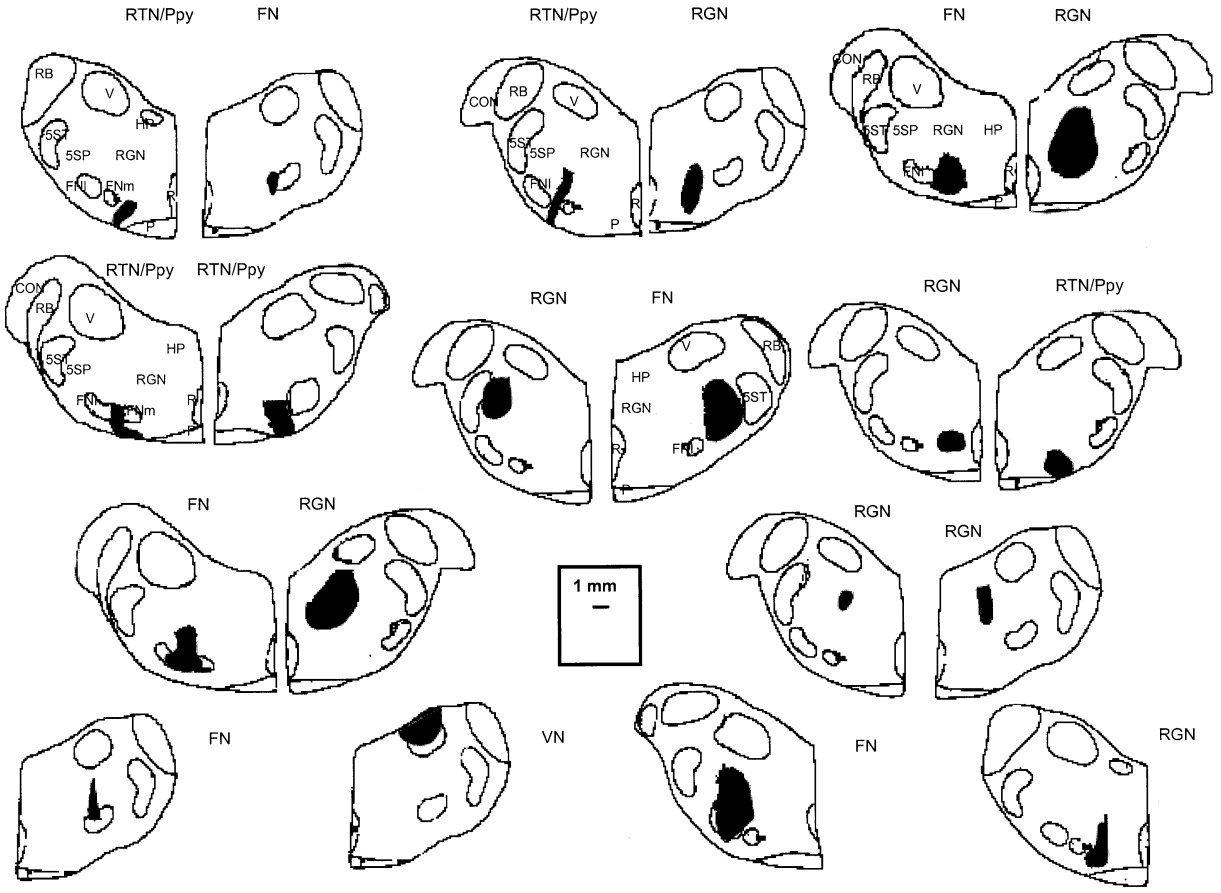

Fig. 1. Drawings of transverse medullary hemisections depicting areas (in 2 dimensions) of ibotenic acid lesions in each goat. Note that eight goats (top three rows) had bilateral lesions while four goats (bottom row) had unilateral lesions. The abbreviations RTN/P_{PY}, FN, RGN, or VN next to each hemisection indicate that the overall lesion (in all 3 dimensions) was primarily (but not exclusively) in the retrotrapezoid nucleus/parapyramidal region, facial nucleus, RGN nucleus, or the VN, respectively. Additional abbreviations are as follows: R, raphe nucleus; HP, hypoglossal propositus; RB, restiform body; 5ST, spinal trigemimal tract; P, pyramids; CON, cochlear nucleus. For clarity, all nuclei are not labeled on every hemisection. For the top three rows, the two hemisections for each goat are in some case at slightly different rostral distance from obex.

previously found that the complete recording instrumentation reduces or eliminates sleep in some goats (Ohtake et al., 1996). Accordingly our primary objective was to determine whether there was hypoventilation during sleep, which was assessed by Pa_{CO_2} measurements. Breathing frequency (f) was monitored via diaphragm EMG. For these and all studies the goat was in a room isolated from the investigator and all recording equipment. The recording cables and the blood pressure and sampling line exited the room through conduits in the wall.

2.4.1. Histological studies

The details of our histological analysis have been previously described (Wenninger et al., 2001). Briefly, the medullas were sectioned transversely at 20 μM, stained with hematoxylin and eosin, and analyzed under a microscope to locate the end of the implanted microtubules, and to identify the

area of dead neurons (eosinophilic and absence of a nucleus). The total number of living and dead neurons were counted in the area from the end of the microtubule to the most distal site of dead neurons (rostral–caudal, medial–lateral, and dorsal–ventral directions).

2.4.2. Sleep staging

Sleep was assessed via standard EEG/EOG (Feroah et al., 2001). The awake state was defined as low voltage, mixed frequency EEG. NREM was defined as a synchronized low-frequency EEG (2 Hz or less) with an amplitude two to three times greater than found during awake, and a concurrent absence of REMs. REM sleep was defined as a desynchronization of the EEG with relatively low voltage, mixed frequency EEG with frequent REMs and reduced upper airway muscle activity. REMs were distinguished from eye blinks as they were defined as sharp waves with an amplitude $>$ 30 μV from baseline. Three experienced investigators independently analyzed these data by visual inspection.

2.5. Statistical analyses

For each goat and each night of study, the total time, the time awake and the time in NREM and REM sleep were computed. The percent of the total nighttime in each state was then computed. ANOVA was used to determine whether the percent time differed ($P < 0.05$) between WK, NREM, and REM within each of the five experimental conditions. ANOVA was also used to determine whether the percent time in each state differed ($P < 0.05$) between the five conditions of the experimental design. In addition, ANOVA was used to determine whether the percent time in each state differed between injections in the RTN/P_{PY}, FN, and RGN, and whether for each nucleus the percent time differed between the three states.

The first step in the statistical analysis of the Pa_{CO_2} values obtained at night was to group the individual Pa_{CO_2} values for awake, NREM, and REM states into three intervals (9 p.m. to 11 p.m., 11 p.m. to 1 a.m., and 1 a.m. to 3 a.m.). Complete data were available for the awake and NREM states; thus, ANOVA was used to establish for these two states, for each of the five conditions, whether Pa_{CO_2} significantly varied throughout the night. Insufficient data were obtained for the same analysis for REM. Since there was no significant ($P > 0.10$) variation throughout the night for either WK and NREM states for each condition, the individual Pa_{CO_2} values for each state for each condition were averaged for each goat. When sufficient data were available for REM sleep, ANOVA was then used to determine whether Pa_{CO_2} differed between states for each of the five conditions and also for each nucleus of injection. When insufficient REM data was available (after second injection and after injections into the FN and RTN), a paired t-test was used to determine whether Pa_{CO_2} differed between awake and NREM sleep states. ANOVA or a paired t-test was also used to determine whether Pa_{CO_2} in each state varied significantly between conditions and injection nuclei.

Minute average values were computed (day or night studies) for pulmonary ventilation ($\dot{V}_I$), breathing frequency (f), tidal volume (V_T), HR, and MABP, and $\dot{V}_{O_2}$ was computed for 5-min epochs. The ANOVA (one- and/or two-way) or paired t-test were used to determine whether physiologic variables changed significantly ($P < 0.05$) throughout the day or night studies and to determine whether there were differences between day and night studies. The Bonferroni post hoc test was used to establish differences when $P < 0.05$ (by ANOVA).

3. Results

3.1. Location of injections and lesions

As previously reported, disrupted and presumably fibrotic tissue and dead neurons were found up to 0.3–0.5 mm lateral to the microtubule and up to about 1.5 mm in all directions from the tip of the microtubule. The percentage of dead neurons decreased with distance from the microtubule from a maximum of 20–30% to less than 3% 1.5 mm in any direction from the microtubule tip. The neuronal death was usually greatest in one nucleus which provided the basis for grouping physiologic

data to single nuclei, but in most goats neuronal death was not confined to the one nucleus.

3.2. Acute effects of ibotenic acid injection during the day

During the 5 h following unilateral injection of ibotenic acid into the FN and RTN/P_{PY}, there was no significant ($P > 0.10$) change in $\dot{V}_I$, f, and V_T. However, in many goats there was an initial increase in $\dot{V}_I$ (Figs. 2 and 3), which is consistent with the known excitatory effects of ibotenic acid on glutamate receptors. In addition, over the 5 h after the injection, there were periods of elevated $\dot{V}_I$ interspersed with periods of normal or reduced $\dot{V}_I$ (Figs. 2 and 3). Similar oscillations were also evident in metabolic rate and HR. These oscillations were generally greater than the preceding control period and greater than the oscillations that occurred over 5 h after injections of mCSF. The oscillations did not all occur at the same points in time for all goats (compare Figs. 2 and 3), which accounts for the absence of significant changes with FN and RTN/P_{PY} injections. However, about 2.5 h after injection of the neurotoxin into the RGN, $\dot{V}_I$, $\dot{V}_{O_2}$, and HR were significantly ($P < 0.05$) increased above values after the injection of mCSF (Fig. 4). Pa_{CO_2} did not change significantly ($P > 0.10$) over the 5 h after the injection of mCSF or after the injections of ibotenic acid into the RTN/P_{PY}, FN, or RGN (Fig. 5).

3.3. Effects on sleep before and after ibotenic acid injection

For the night studies of each of the five conditions (Fig. 6) and for each site/nucleus of injection, the percent of time awake was greater ($P < 0.01$) than the two sleep states and the NREM percent time was greater ($P < 0.01$) than the REM percent time. The percent of time spent awake differed ($P > 0.01$) between pre-microtubule implantation, 30 days post-implantation, 9–15 h after the first and second neurotoxin injection, and 2–17 days after the second neurotoxin injection (Fig. 6). The post hoc test indicated that after the second ibotenic acid injection, the percent of time awake was greater than during the pre-implantation condition. There were no significant changes over the five conditions in the percent of time in NREM sleep, but there was a tendency for reduced NREM sleep after the second injection of ibotenic acid. The percent of time in REM sleep differed significantly ($P < 0.01$) over the five conditions, and specifically the pre-implantation value was greater than the post-implantation and after the first and second injections of ibotenic acid. The percent of time in each state did not vary significantly ($P > 0.10$) between the site/nucleus of ibotenic acid injection. Data on goats studied twice either before implantation or several days after neurotoxin injections indicated good reproducibility in night time sleep–awake periods (data not shown).

There were no significant ($P > 0.10$) changes in Pa_{CO_2} within a state (WK, NREM, and REM sleep) throughout any of the night studies; therefore, the individual values for each state were averaged to obtain a single value for each goat for each state for each condition. We also compared the awake night Pa_{CO_2} with the Pa_{CO_2} obtained while awake the day of and the day following the night studies. No significant ($P > 0.10$) differences were found for these comparisons for any condition (data not shown).

There were no significant differences in Pa_{CO_2} between WK, NREM sleep, and REM sleep during any of the night studies (Fig. 7). Prior to microtubule implantation, Pa_{CO_2} was 39.7 ± 0.7 mmHg, 40.3 ± 0.8 mmHg, and 41.2 ± 1.2 mmHg during WK, NREM sleep, and REM sleep, respectively. About 30 days after microtubule implantation, there was a non-significant tendency toward hyperventilation during REM sleep when Pa_{CO_2} was 39.4 ± 1.3 mmHg compared to 40.8 ± 1.1 mmHg and 40.9 ± 1.2 mmHg during WK and NREM sleep states. When the data were grouped irrespective of injection site, the average Pa_{CO_2} for WK, NREM, and REM states were 41.1 ± 1.0, 40.9 ± 0.9, and 42.3 ± 1.5, respectively, after the first injection. The Pa_{CO_2} values were 40.9 ± 0.8, 40.3 ± 0.8, and 41.4 for awake, NREM and REM states, respectively, after the second injection. There were no significant ($P > 0.10$) differences between states when grouped irrespective of injec-

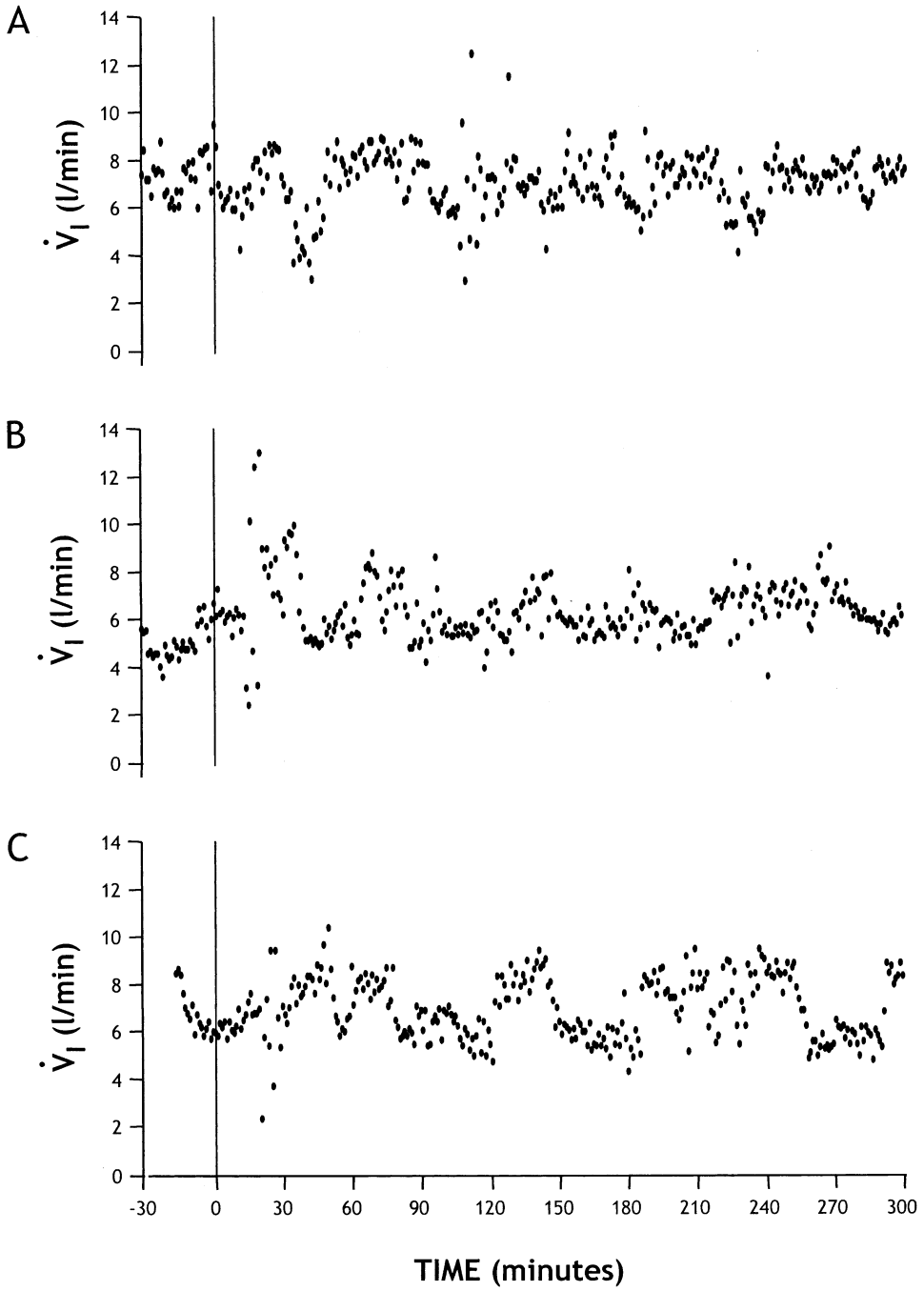

Fig. 2. Inspired pulmonary ventilation ($\dot{V}_I$) of one goat before and after microinjection of 100 nl mock cerebral spinal fluid (Panel A) or 100 nl 50 mM ibotenic acid into the left (Panel B) and 3 days later the right (Panel C) retrotrapezoid nucleus. One-minute average $\dot{V}_I$ values are presented. The vertical line in each panel indicates the time of the injection. Note the transient hyperpnea a few minute after the ibotenic acid microinjections (Panels B and C) and the periodic oscillation in $\dot{V}_I$ after ibotenic injections that tended to be greater than oscillation prior to the injections and during the 5 h after injection of mock cerebral spinal fluid.

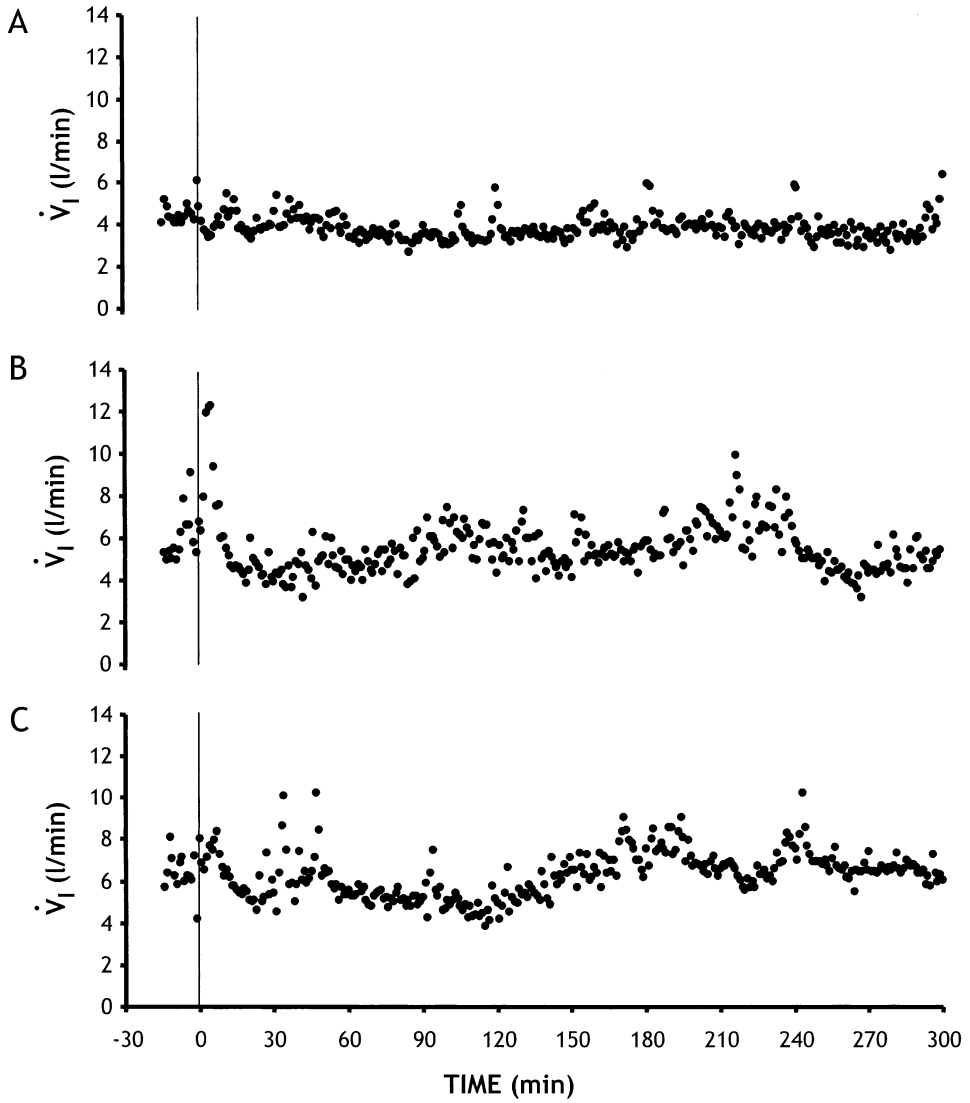

Fig. 3. Inspired pulmonary ventilation ($\dot{V}_I$) of one goat before and after microinjection of 100 ml mock cerebral spinal fluid (Panel A) or 100 nl 50 mM ibotenic acid into left retrotrapezoid nucleus (Panel B) and 3 days later the right RGN nucleus (Panel C). One minute average $\dot{V}_I$ values are presented. The vertical line in each panel indicates the time of the injection. Note the transient hyperpnea a few minutes after the ibotenic acid microinjections (Panels B and C) and the periodic oscillation in $\dot{V}_I$ after ibotenic injections that tended to be greater than oscillation prior to the injections and during the 5 h after injection of mock cerebral spinal fluid.

tion site (Fig. 7) or when grouped according to the site of injection (Fig. 8). Due to the elimination of REM sleep in several goats with the second injection, minimal data were obtained during REM sleep with this injection. Two to seventeen days after the second injection, Pa_{CO_2} was 40.5 ± 0.7, 40.7 ± 0.9, and 39.2 ± 0.7 during WK, NREM sleep, and REM sleep states, respectively (Fig. 7), and there were no significant ($P > 0.10$) differences between states. For each of state, there were no significant differences in Pa_{CO_2} between conditions or between site/nuclei of injection.

Throughout the night studies there were no significant time dependent changes in f, HR, or

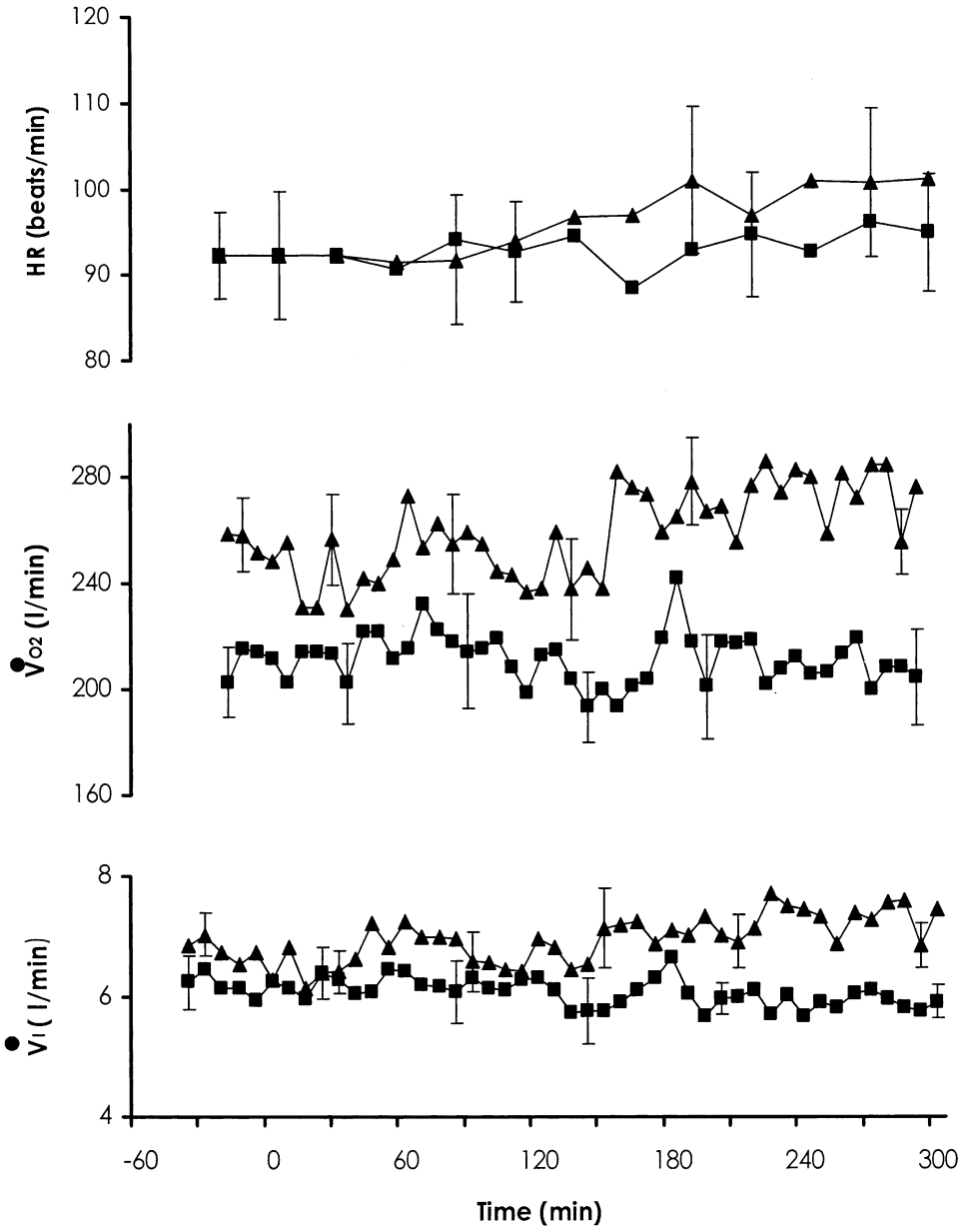

Fig. 4. Group average (±S.E.M.) HR, oxygen consumption ($\dot{V}_{O_2}$), and pulmonary ventilation ($\dot{V}_I$) of goats before and after unilateral injection of mCSF (squares) or 50 mM ibotenic acid (triangles) into the RGN nucleus of awake goats. The SEM were consistent across all HR, $\dot{V}_{O_2}$, and $\dot{V}_I$; thus, for clarity only a few are presented. Zero on the X-axis denotes the time of the injection. A two-way ANOVA indicated that $\dot{V}_E$, $\dot{V}_{O_2}$, and HR all differed ($P < 0.001$) between the two interventions and the Bonferroni post hoc test indicated these differences were significant ($P < 0.05$) for virtually all time points 150 min after the injections.

MABP within each state for any condition; thus ($P > 0.10$), average values for each state, goat and condition were computed. There were no significant changes in f and MABP between WK, NREM sleep, and REM sleep during any condition or with injection at any site (Table 1).

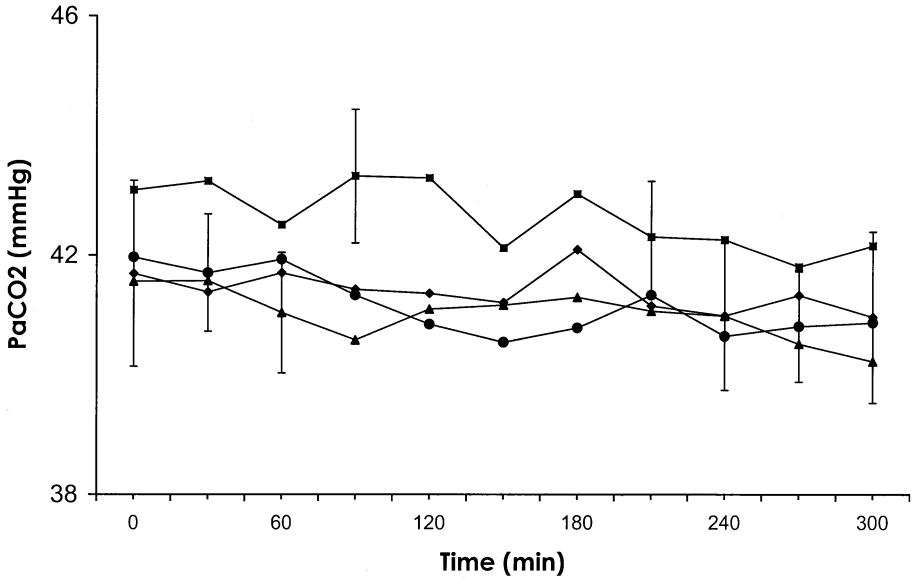

Fig. 5. Group average (±S.E.M.) Pa_{CO_2}, before and after injection of 50 mM ibotenic acid into the retrotrapezoid nucleus/parapyramidal region or the (◆), facial (●), or RGN (■) nuclei or injection of mock cerebrosperal fluid (▲) into one of these nuclei. The SEM are consistent for each data set; thus, for clarity only a few are presented. Zero on the *X*-axis denotes the time of the injection. Although there was a trend toward time-dependent hyperventilation with all injections, Pa_{CO_2} did not change significantly ($P > 0.10$) after any injection.

However, for most conditions and most injection sites HR was lower ($P < 0.05$) during NREM and REM sleep than during WK (Table 1). For each state, HR was higher ($P < 0.05$) after the implant and 9–15 h after each injection than it was prior to the implant and 2–17 days after the second injection. Finally for each state, MABP was higher ($P < 0.05$) prior to the microtubule implant than during all other conditions.

4. Discussion

The major finding of the present study was that goats do not, relative to WK, hypoventilate during NREM sleep prior to or 30 days after implantation of microtubules into the rostral medulla, nor do they hypoventilate during NREM sleep 9–15 h or 2–17 days after neurotoxin injection into the rostral medullary nuclei.

4.1. Limitations of this study

For chronic animal studies, there is no totally satisfactory technique for identification of neurons affected by microinjections into the brain (Erlichman et al., 1998). We currently utilized the extent of neuronal death presumably caused by the injected neurotoxin. There was a decrease in dead neurons with distance from the injection site; thus it appears that ibotenic acid diffused passively to cause cell death in a volume that approximated 1.5 ml. We have calculated the approximate unilateral volume of the RTN/P_{PY}, FN, and RGN to be 3.5 ± 0.5, 5.8 ± 0.7, and 73.5 ± 0.2 ml, respectively, in a 35–50 kg goat. Accordingly, approximately 44, 26, and 2% of the RTN/P_{PY}, FN, and RGN, respectively, were destroyed by the neurotoxin. The absolute size of the lesions is larger than others have made in rats and cats (Akilesh et al., 1997; Nattie and Li, 1990), but it is relatively small considering that the medulla of a 35 kg goat is 160% of the size of the medulla in a 2

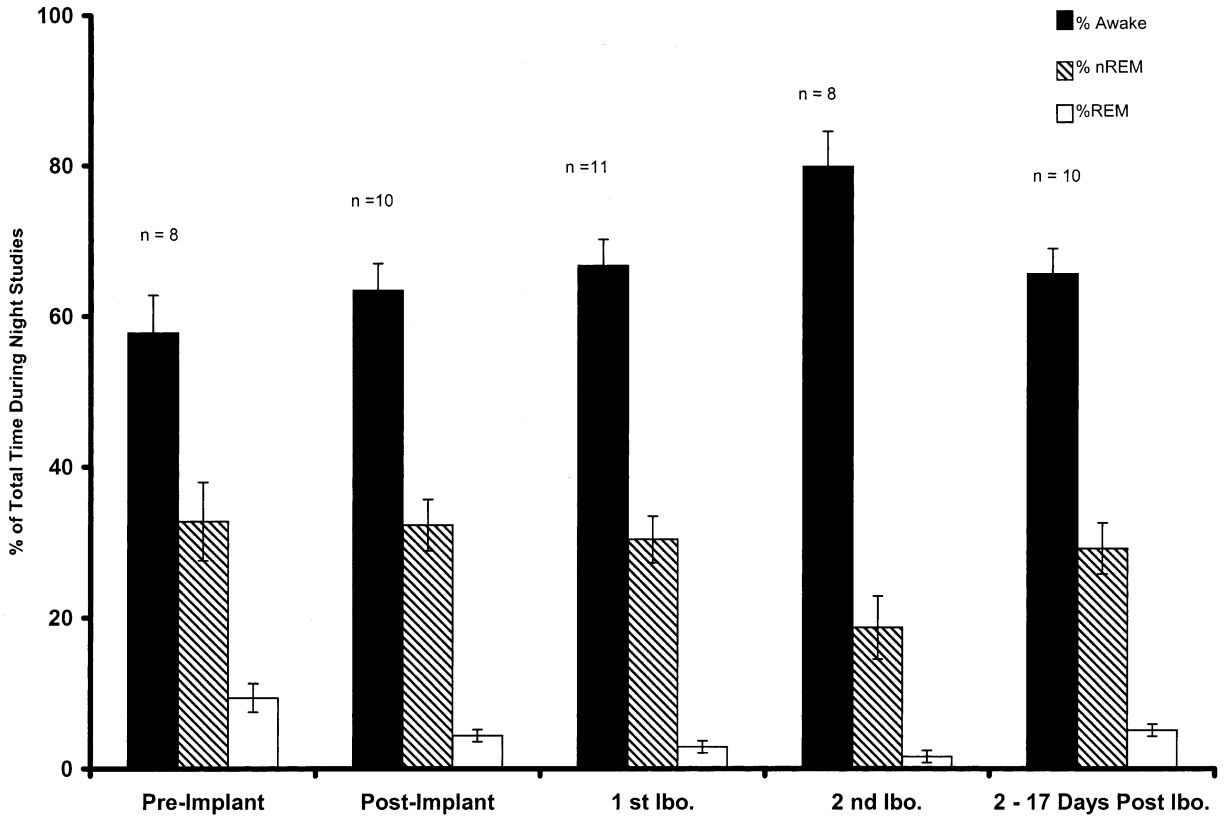

Fig. 6. The percent of the time at night that the goats were in the awake, NREM sleep, and REM sleep states. Data are presented for goats prior to (Pre-Implant) and 30 days after (Post-Implant) implantation of microtubules, 9–15 h after the first (1st IBO) and second (2nd IBO) ibotenic acid injection, and 2–17 days after the last IBO injection. The average absolute time (in minutes) in the awake state for each of the five conditions was respectively 255, 231, 239, 301, 237; for NREM sleep the times were 102, 119, 105, 71, and 102; for REM sleep the absolute times were 37, 16, 11, 6, and 19. For each condition, awake time was greater ($P < 0.01$) than NREM sleep which was greater ($P < 0.01$) than REM sleep. The percent time awake ($P < 0.015$) and the percent time in REM sleep ($P < 0.001$) differed over the five conditions. Specific differences were pre-implant versus second ibotenic for awake state and pre-implant versus each of the next four conditions for REM sleep. The percent of NREM sleep did not differ ($P > 0.10$) between the different conditions.

kg cat (Dean-Bernhoft et al., 1999). For most goats, the volume of dead neurons was primarily in a single nucleus, but dead neurons were usually found at the edge of an adjacent nucleus.

It was difficult to place the microtubules consistently at exactly the target sites. Others have also had difficulty in placing microtubules at precise locations in rats in which the anatomy is more defined and in whom with the smaller size, any error in surface coordinates results in less deviation in placement from the target site (Akilesh et al., 1997; Lu et al., 2000).

Since the neurotoxin did not induce or accentuate hypoventilation during sleep, it is important to emphasize several findings indicating that the neurotoxin did indeed have the intended effect. First, dead neurons were found only at the injection site. Second, over the initial minutes and the first 5 h after the injection, breathing was altered from normal (Figs. 3 and 4). Finally, as previously reported (Wenninger et al., 2001), breathing at rest, in the awake state, was near normal over 2 weeks after creating the lesions, but CO_2 sensitivity was attenuated by 25–75% in most goats (Wenninger et al., 2001). On average, 8–10 days after injections into the FN and RGN, CO_2 sensitivity was attenuated ($P < 0.05$) by 23 and 31%, respectively. These attenuations are only

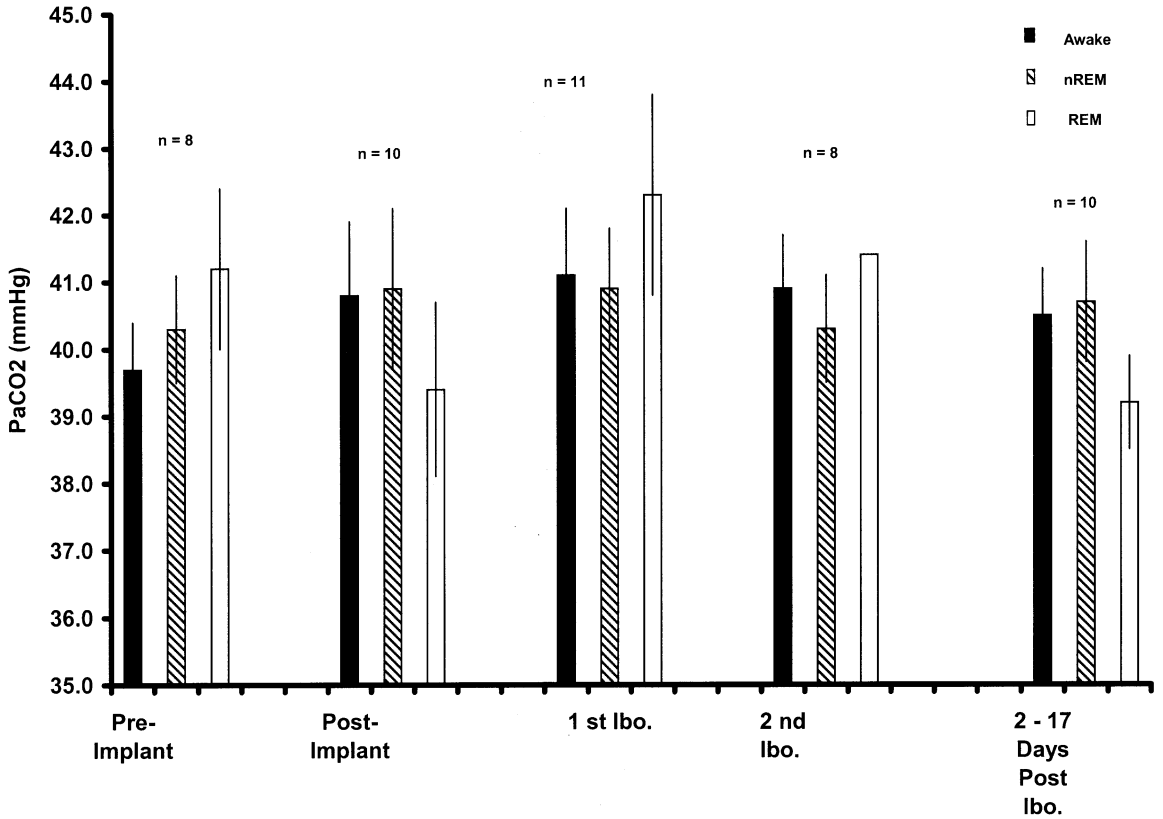

Fig. 7. The average (±S.E.M.) Pa_{CO_2} for awake, NREM sleep, and REM sleep states for goats prior to (Pre-Implant) and 30 days after (Post-Implant) implantation of microtubules, 9–15 h after the first (1st IBO) and second (2nd IBO) ibotenic acid injection, and 2–17 days after the last IBO injection. The average number of blood samples per goat for the five conditions was respectively 14.6, 13.4, 12.0, 18.3, and 14.2 for the awake state and 12.6, 9.8, 10.7, 9.4, and 9.1 for NREM sleep, and 8.0, 5.1, 4.9, 4.0, and 6.6 for REM sleep. There was no significant ($P > 0.10$) difference in Pa_{CO_2} between states for any of the five conditions and there was no significant difference in Pa_{CO_2} between conditions for any of the three states.

slightly less than the 39% attenuation 3 weeks after unilateral ibotenic acid lesions in the RTN of rats (Akilesh et al., 1997). In addition, lesions in the rostral medullary nuclei altered the coordination of breathing and swallowing (Feroah et al., 2002) and increased the incidence of fractionated breathing (Feroah et al., 2003). Accordingly, it is clear the neurotoxin affected the control of breathing; thus, in spite of limitations of the present study, the conclusion is warranted that neurotoxic lesions in these rostral medullary nuclei do not induce/accentuate hypoventilation during NREM sleep. However, we limit this conclusion to NREM sleep because we feel insufficient data were obtained throughout all conditions to warrant definitive conclusions regarding REM sleep.

4.2. Acute effect of the neurotoxin injections

Ibotenic acid binds to glutamate receptors to increase the firing rate of neurons, but eventually, the increased firing rate supposedly causes neuronal death. If the binding is to receptors on respiratory neurons, breathing in an anesthetized animal often will initially increase but subsequently decrease. This temporal pattern is usually observed within minutes after injection of a neurotoxin. To our knowledge, the only previous study which followed the temporal pattern of breathing over the first few hours after the injection of a neurotoxin in the awake state were those we completed in which ejections were made on the ventrolateral medullary surface (Forster et

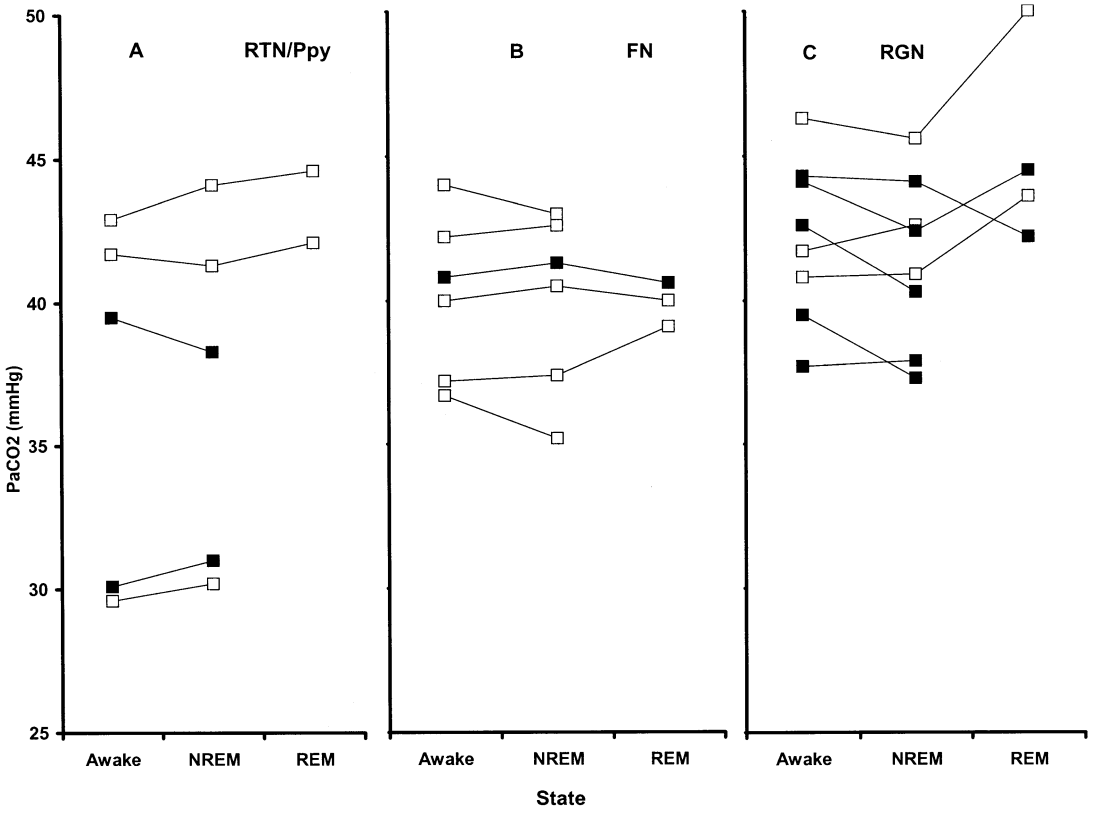

Fig. 8. Pa_{CO_2} of individual goats while awake and in NREM sleep and in REM sleep states during night studies 9–15 h after injection of 50 mM ibotenic acid into the retrotrapezoid nucleus/parapyramidal area (RTN/P_{PY}), facial (FN), or RGN nuclei. Closed symbols denote values obtained after the second unilateral injection of acid. There was no significant difference in Pa_{CO_2} between states for any nuclei.

al., 1998). These previous ejections likely affected RTN neurons, and as in the present study caused transient, or sustained increases in breathing but did not cause any apneic periods. We are unable to explain the difference in the effects between awake and anesthetized states. The gradual increase in $\dot{V}_I$, $\dot{V}_{O_2}$, and HR over 5 h after injection into the RGN suggest there was a prolonged net excitatory effect of the injection on multiple physiologic variables. However, ibotenic acid is metabolized to muscimol, a $GABA_A$ agonist which has an inhibitory influence on neurons (Aldinio et al., 1983; Curtis et al., 1979). Conceivably, breathing over a few hours after the injections reflected a changing balance between the direct excitatory effect of ibotenic acid and on inhibitory effect of muscimol which is why there were oscillations in breathing that tended to be greater than the normal physiologic variation in goats. It is likely that any excitatory or depressive effect at the injection site was compensated by respiratory neurons at other sites. This compensatory capacity might be greater or differ between awake and anesthetized states, which may contribute to the state dependent effects of ibotenic acid injections.

4.3. Effect of neurotoxic lesions on breathing during sleep

Electrolytic, neurotoxic, and cooling-induced neuronal dysfunction in rostral medullary nuclei decrease breathing more when animals are anesthetized than when awake (Akilesh et al., 1997; Forster et al., 1997, 1998; Nattie and Li, 1990;

Table 1
Breathing frequency (f), HR, and MABP in goats during nights while awake or in NREM or REM sleep

	Pre-implant ($n=8$)			Post-implantation ($n=8$)			1st IBO ($n=10$)		
	Awake	NREM	REM	Awake	NREM	REM	Awake	NREM	REM
f (br/min)	21.6	17.7	18.8	13.9	17.2	18.7	24.3	21.4	21.5
	1.6	1.1	1.7	2.4	1.5	4.4	4.1	3.5	3.0
HR (bts/min)	86.8	83.3	83.1	97.4*	88.8	89.5	107.8	101.7	97.6
	6.0	6.7	6.3	4.7	5.4	7.0	5.0	5.2	6.1
MABP (mmHg)	107.6	104.8	110.6	99.1	98.7	98.6	96	95.5	96.7
	5.3	6.0	5.3	4.6	4.4	4.4	3.6	3.1	3.1
	2nd IBO ($n=6$)			2–17 D.P. IBO ($n=8$)					
f (br/min)	19.2	19.0	17.2	19.1	16.9	18.2			
	2.4	1.1	2.8	1.6	1.3	1.8			
HR (bts/min)	106.3*	90.1	81.6	86.5*	79.3	75.3			
	9.2	9.9	11.9	3.1	3.7	3.4			
MABP (mm Hg)	96.3	94.2	93.6	97.8	95.6	97.5			
	5.5	5.8	3.0	3.5	4.0	5.0			
	RTN/P_{PY} ($n=5$)			FN ($n=5$)			RGN ($n=6$)		
f (br/min)	20.81	18	23	25.4	22.4	20.5	20.5	19.3	20.0
	–	–	–	6.7	5.7	5.5	2.2	1.3	2.9
HR (bts/min)	116.5	106.9	105.1	106.3*	97.9	80.8	100.3*	91.8	90.3
	6.3	3.6	5.5	8.2	8.0	8.7	7.9	9.8	11.5
MABP (mm Hg)	94.9	94.4	98.7	92.9	94.9	91.8	99,8	95.6	93.4
	6.3	2.5	5.0	6.4	6.7	3.2	3.1	4.1	4.5

Data are average and SEM for goats prior to implantation of microtubules, 30 days post-implantation, 9–15 h after 1st and 2nd injections of ibotenic acid (IBO) into the rostral medulla and 2–17 days (D) post-(P)ibotenic acid injection. The bottom three groups are subsets of goats included in the 1st and 2nd IBO groups, i.e. 9–15 h after injection of IBO into the retrotrapezoid nucleus/parapyramidal region (RTN/P_{PY}), or the facial (FN), or RGN nuclei.

* Denotes significant ($P<0.05$) difference between states.

Nattie et al., 1988, 1991; Schlaefke et al., 1970; Wenninger et al., 2001). We reasoned that with the reduced excitatory drive to breath during sleep (Dempsey et al., 1991, 1997; Orem et al., 1985; Pack, 1997), neuronal dysfunction during sleep would, like anesthesia, reduce breathing more than while awake (Forster et al., 1997; Ohtake et al., 1996). With cooling-induced dysfunction of the RVLM, we previously found a slight but not significantly greater reduction in breathing during sleep than while awake (Forster et al., 1997; Ohtake et al., 1996). However, after carotid body denervation, RVLM cooling during NREM sleep resulted in sustained apnea (Forster et al., 1997; Ohtake et al., 1996). We therefore hypothesized that neurotoxic lesions in the rostral medulla would relative to the awake state, induce/accentuate hypoventilation during NREM sleep. Our data do not support this hypothesis; the indexes of breathing, Pa_{CO_2} and f, did not differ after the neurotoxic lesions between WK and NREM sleep (and REM sleep when sufficient data were obtained). This absence of an effect was observed on the first night (9–15 h) after the injection during which there was a trend toward reduced NREM and a significantly reduced REM. The reduced sleep conceivably may have been due to a persistent excitatory phase of the neurotoxin, which may also have affected breathing. However, absence of an affect was also observed during nights 2–17

days after the injection when in most awake goats, CO_2 sensitivity was below normal (Wenninger et al., 2001).

Two previous studies have evaluated the effect on breathing during sleep of altered neuronal activity in rostral medullary nuclei. Curran et al. (2001) studied the effect on breathing in awake and asleep piglets of microdialyzing muscimol into the rostral–ventral medulla (RVM). They found that during room air conditions, breathing did not differ between WK and NREM sleep and that muscimol did not alter breathing either during WK or during sleep. However, muscimol did attenuate ventilatory CO_2 sensitivity ($\sim$35%) and this attenuation tended to be greater during sleep than during WK. In a second study, Nattie and Li in the RVM area of rats selectively lesioned neurons with neurokinin I receptors. With unilateral lesions, breathing was not significantly altered while awake or asleep; after bilateral lesions breathing was reduced in both states but the rats were hypoventilating only during the awake state. Accordingly, even though different species and techniques were used, the data of Curran et al. (2001) and Nattie and Li (2002), and from our study agree that presumed attenuation of RVM neuronal activity does not cause hypoventilation when changing from WK to sleep.

Many mammals hypoventilate during sleep (Dempsey et al., 1991; Guazzi and Freis, 1969; Phillipson, 1978). Goats are an exception as Parisi et al. (1987, 1992) in one of two studies and in our goats prior to neurotoxic lesions, Pa_{CO_2} did not differ between awake, NREM sleep and REM sleep. The hypoventilation during sleep in most species is thought to reflect reductions in excitatory drive for breathing and/or the effect of increased airway resistance associated with altered airway muscle activity (Dempsey et al., 1991; Horner et al., 1994; Morrell et al., 1990; Orem et al., 1985; Pack, 1997). We have previously shown in goats that excitatory drive to the diaphragm and to the pharyngeal constrictor and dilator muscles also decreases progressively from the awake, to NREM sleep, to REM sleep (Feroah et al., 2001). In this respect goats respond to sleep similar to other mammals. Apparently, the magnitude of these changes differ in goats from other species or in goats other factors contribute to the Pa_{CO_2} homeostasis over different states. Conceivably, this compensatory mechanism also prevented neurotoxic lesions from disrupting Pa_{CO_2} during sleep even when CO_2 sensitively was reduced. Whatever the mechanism, it seems clear that goats are capable of tight regulation of Pa_{CO_2} with changes in state.

4.4. Circadian rhythms and ventilatory control

Stephenson et al. (2001) recently found that breathing and metabolic rate of rats differed throughout the circadian cycle independent of sleep state. In the present study, we measured Pa_{CO_2} while awake at night and while awake during the day before and after the night study. We found no significant difference in Pa_{CO_2} over these sets of measurements; thus, we found no evidence for a circadian variation in eupneic Pa_{CO_2}. This finding provides additional evidence of potent mechanisms in goats for maintaining Pa_{CO_2} homeostasis.

4.5. Sleep in goats

Over a 6 h night prior to microtubule implantation, our goats were awake and in NREM and REM sleep nearly to the same extent as others have previously found (Klemm, 1966; Ruckebusch, 1972). Microtubule implantation and the neurotoxic lesions significantly reduced REM sleep and in some goats also reduced NREM sleep while particularly after the second ibotenic injection, the percent of the night the goats were awake was increased. These findings are similar to observations by: (1) Darnall et al. (2001), who found that microdialysis of muscimol into the RVM of newborn piglets abolished sleep cycling and/or decreased EEG delta power, and (2) Nattie and Li (2002) who found that selective lesioning of neurokinin I receptor neurons in the RTN/P_{PY} of rats promoted WK. Darnall et al. (2001) had hypothesized that the increased $GABA_A$ inhibition after the muscimol dialysis would reduce WK or increase EEG delta power. Since ibotenic acid is metabolized to muscimol (Aldinio et al., 1983; Curtis et al., 1979), it is conceivable that the effects

we observed on goats after ibotenic acid injection were in part due to muscimol, which would account for the similar findings by us and Darnall et al. (2001). These studies indicate that altered neuronal activity in the RVM could result in sleep deprivation and fragmentation. RVM sites are not usually considered as primary sites for initiating and terminating different states. However, Darnall et al. (2001) pointed out that these medullary sites are part of a neural circuit that includes the locus coeruleus, which is involved in vigilance and arousal. It is thus possible that neurotoxic lesions and muscimol dialysis affected 'groups of neurons involved in modulation of arousal, increasing the probability of EEG desynchronization and WK' (Darnall et al., 2001). The clinical significance of these findings deserve additional study.

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