

Anesthesiology

82:144-152, 1995

© 1995 American Society of Anesthesiologists, Inc.

J. B. Lippincott Company, Philadelphia

Effects of Opioid Microinjections in the Nucleus of the Solitary Tract on the Sleep-Wakefulness Cycle States in Cats

Francisco Reinoso-Barbero, M.D., Ph.D.,* Isabel de Andrés, Ph.D.†

Background: Previous studies have shown that the region of the nucleus of the solitary tract (NST) is involved in the control of electrocortical activity and in sleep mechanisms. It also is well known that this region contains the highest concentration of opioid receptors within the medullary brainstem. The involvement of the NST opioid system in sleep-wakefulness states was evaluated.

Methods: Ten cats were implanted with electrodes for chronic polygraphic recordings of their sleep-wakefulness states and provided with an implanted guide cannula stereotaxically aimed at the NST region. Microinjections of saline, morphine sulfate, morphiceptin (specific μ agonist), D-pen-2-D-pen-5-enkephalin (δ agonist), and U-50488H (κ agonist) were given to the freely moving animals (doses 0.8 – 2.4×10^{-9} M, in a volume of $0.05 \mu\text{l}$ of saline). After microinjections, sleep-wakefulness recordings were obtained for 8 h.

Results: Morphine microinjections in NST provoked a dose-dependent enhancement of all the polygraphic and behavioral manifestations of slow wave sleep. This effect was blocked by the prior intraperitoneal administration of naloxone. The μ and δ agonists also produced a hypnotic effect by enhancing slow wave sleep. By contrast, the κ agonist caused no changes in sleep-wakefulness states.

Conclusions: These results indicate that endogenous opioids could be involved in controlling electrocortical activity generated by NST and that activation of μ and δ NST opioid receptors enhanced the electroencephalographic synchronization associated with behavioral slow wave sleep in cats. (**Key words:** Brain, brainstem: nucleus of the solitary tract. Cat. Analgesics, opioid: δ -receptor agonist; κ -receptor agonist; morphine-mediated depression in basal

phine; μ -receptor agonist. Sleep: electroencephalogram; slow wave sleep.)

NATURALLY occurring opioids have been used for many thousands of years for their ability to depress the level of consciousness. Their strong analgesic effects were recognized much later. Although the central nervous system (CNS) structures and the opioid receptors involved in this analgesic effect have been identified, the sites of action and the opioid receptors that mediate other CNS effects of opioids, such as behavioral changes and variations in the electroencephalogram (EEG) and in the sleep-wakefulness cycle (SWC) remain unclear. The need to understand the mechanisms of opioid actions in the CNS in relation to the latter actions is important in anesthesiology given the EEG and sleep disturbances produced by opioids during intraoperative and postoperative periods.^{1,2} Additionally, the problem of intraoperative recall and awareness under general anesthesia often has been reported,³ and the drowsiness and decreased awareness commonly observed after opioid administration are considered adverse effects in pain treatment.⁴ Furthermore, the incidence of perioperative cardiopulmonary complications associated with the sleep state in patients who have received opioids is also significant.⁵

The behavioral effects provoked by opioids are typically complex because excitatory and inhibitory behavioral patterns coexist in humans and in different animal species. Systemic administration of morphine suppresses both slow wave sleep (SWS) and paradoxical sleep (PS)⁶⁻⁸; however, animal studies during the insomnia period have documented that opioids cause a typical EEG/behavioral dissociation, *i.e.*, high-voltage slow-frequency waves are present in the EEG while the animals remain awake exhibiting stupor^{7,9,10} or other more active behavior.^{8,11}

A number of experimental findings have indicated that the interaction of morphine with the forebrain has an excitatory effect on behavior¹² that is associated with

* Staff Anesthesiologist. Current position: Departamento de Anestesiología, Hospital Infantil "La Paz," Madrid, Spain.

† Full Professor, Departamento de Morfología, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain.

Received from the Departamento de Morfología, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain. Submitted for publication January 31, 1994. Accepted for publication August 23, 1994. Supported by 149/90 and 142/91 Comunidad Autónoma de Madrid grants. Presented at the 22nd annual meeting of the Society of Neuroscience, Anaheim, California, October 25-30, 1992.

Address reprint requests to Dr. Reinoso-Barbero: Departamento de Anestesiología, Hospital Infantil "La Paz", Paseo de la Castellana 261, Madrid 28046, Spain.

EEG desynchronization,^{12,13} this in contrast to morphine's effects in the whole lower brainstem, where, with a dose-dependent decrease of PS,¹⁴ it produces behavioral signs of SWS¹⁴ and EEG synchronization.¹² Recent experiments using microinjection techniques¹⁵ have indicated that local administration of morphine in the medial pontine reticular formation inhibits both SWS and PS. However, little is known about other specific brainstem structures and receptors that could be implicated in the excitatory and/or inhibitory behavioral and EEG effects produced by opioids.

The density of opioid receptors¹⁶⁻¹⁸ as well as neuronal enkephalinergic endings and somata¹⁹ in the region that Ramon y Cajal²⁰ called complex of the solitary tract, located in the floor of the fourth ventricle, is very high. The visceral sensitive information from the 7th, 9th, and 10th cranial nerves is organized in the region of the nucleus of the solitary tract (NST), which mediates the most important respiratory²¹ and cardiovascular²² reflexes. In addition, it has long been recognized that electrical stimulation of the NST produces EEG synchronization,²³ and more recent experimental data confirmed the important hypnogenic and EEG synchronizing role of this region.²⁴⁻²⁷

These facts led us to evaluate the possible involvement of NST opioid receptors in both EEG synchronization and behavioral depression. To test this hypothesis, morphine sulfate and highly specific agonists of μ , δ , and κ opioid receptors were unilaterally microinjected into the NST of intact, unanesthetized cats and, after the microinjections, their SWC states were monitored. The results revealed that administration of morphine, or of either of the specific μ and δ agonists in the NST, enhanced EEG synchronization associated with SWS behavior.

Material and Methods

After institutional approval was obtained, ten adult cats were anesthetized with sodium pentobarbital (35 mg·kg⁻¹ intraperitoneally) and, under sterile conditions, implanted with electrodes for chronic polygraphic recordings of the SWC states. These electrodes recorded EEG, electroculogram, electromyogram, and ponto-geniculo-occipital waves from the lateral geniculate bodies of the thalamus. As part of the same surgery, a 20-G cannula provided with a stylet was implanted, stereotaxically aimed at the NST according to the coordinates of the Reinoso-Suarez' atlas²⁸ (AP - 10.0, V 3.5, and L 3.0, $\theta = 18^\circ$ from vertical). The tip

of the cannula was left 4.0 mm above the target site. All the electrodes were terminated in an Amphenol connector. The electrodes and the cannula were secured permanently with acrylic cement to the skull of the animal.

Eight to 10 days after surgery, each cat was acclimatized for 2-3 days to the recording conditions. The animal was placed in a sound-attenuated ventilated recording chamber, with food and water *ad libitum*, where it could be observed through a one-way mirror. After becoming familiar with the chamber, all cats had a baseline polygraphic recording before starting the treatments with opioid microinjections in the NST area. When the cat was awake, but gently restrained in a loosely fitting bag, morphine sulfate, morphiceptin (specific μ agonist²⁹), D-pen-2-D-pen-5-enkephalin (DPDPE, a highly selective δ agonist³⁰) or U-50488-H (κ agonist³¹) in a volume of 0.05 μ l of saline was injected into the NST using a 0.5 μ l Hamilton syringe inserted through the stereotaxically implanted cannula. The tip of the needle protruded 4 mm from the cannula. All cats received single equimolar microinjections of each opioid agonist separated by an interval of at least a week from the next microinjection. Six cats received a dose of 1.7×10^{-9} M of each opioid agonist (three of them received additional treatments with the lowest dose 0.8×10^{-9} M of each agonist); the remaining four cats received the high dose (2.4×10^{-9} M) of each opioid agonist. In all cats, control data were collected using identical procedures after microinjecting 0.05 μ l of saline in the NST area. Without strictly following a Latin square design, opioid and saline microinjections were given in a quasirandomized order. Finally, in six cats in which opioids had been administered previously, microinjections of morphine sulfate (2.4×10^{-9} M in three cats and 1.7×10^{-9} M in the remaining three) were repeated 10 min after administering intraperitoneal naloxone 2 mg/kg. These combined morphine-naloxone experiments were done at the end of the series of opioid treatments in four animals and in middle of the series in the remaining two animals. After receiving the microinjections, the cats were released in the sound-attenuated chamber, and the polygraphic recordings were immediately begun. Baseline and experimental polygraphic recordings lasted at least 8 h. Behavioral observations and videotape recordings were made during the first 2 h of the recordings.

Since behavioral observations indicated that none of the cats presented any EEG/behavioral dissociation after opioid delivery in the NST region, polygraphic re-

cordings were scored visually in 1-min epochs according to the polygraphic criteria of wakefulness, drowsiness, SWS, and PS of the cat.^{11,32} Wakefulness is characterized by desynchronized EEG with low voltage/fast activity, well sustained electromyogram, and frequent eye movements; drowsiness by synchronized EEG with bursts of high voltage waves superimposed over low voltage/fast activity, well sustained electromyogram and occasional eye movements; SWS by synchronized EEG with continuous high voltage/slow activity, diminished electromyogram activity and no eye movements; and PS by desynchronized EEG with low voltage/fast activity, ponto-geniculo-occipital waves, muscle atonia and rapid eye movements. The sleep recordings were blocked in 1-h intervals. The percentage of time spent in each SWC state and the mean duration and the number of its episodes were calculated for each 1-h interval. All statistical analyses were based on the values of the 1-h blocks after baseline and saline controls and the different opioid treatments.

According to the dose and the location of the microinjections, the cats were classified into three groups (Results). In each group of cats, changes in wakefulness, drowsiness, SWS, and PS among baseline, saline, and the different opioid treatments during either the entire 8 h or the first 4 h of recordings were assessed by one-way analysis of variance for repeated measures. Further multiple pair contrasts were made using Fisher's pairwise comparison test. Statistical significance was set at $P \leq 0.05$.

After the experiments were finished, the cats were deeply anesthetized with sodium pentobarbital. Brains were perfused by cannulating the aorta and infusing saline and a 10% formalin solution. Brains were frozen, sectioned at 50 μm thickness, and stained with Nissl stain. Each frontal section containing the needle tract was projected on sagittal planes of the cat's brain.

Results

Behavioral observations during the polygraphic recordings indicated that there was a strict correlation between behavior and the polygraphic events that characterize the different SWC states of the normal intact cat in all our experiments. Local microinjections of morphine and the other opioids delivered in the lower brainstem did not produce the typical EEG/behavioral dissociation observed after systemic administration of low doses of morphine in intact cats^{8,11} and in other species.^{7,9}

The photomicrograph of figure 1 shows the injection site in one animal whose microinjections were located in the NST region, and figure 2 shows the sequential organization of the SWC states in representative experiments in the same cat during the first 2 h of recordings. After a saline microinjection in the NST (saline-NST, fig. 2) the different states of the SWC alternated one after another. However, when morphine was microinjected in the NST (morphine-NST, fig. 2), the cat exhibited a long episode of SWS with all its behavioral and polygraphic characteristics. Further facilitation of SWS episodes also took place in the second hour of the recording. However, this SWS enhancement did not occur when intraperitoneal naloxone was administered before the microinjection of morphine into the NST (intraperitoneal naloxone plus NST-morphine, fig. 2).

Histologic analyses revealed that the microinjections were located within the NST region in seven cats, whereas in the three remaining cats, the microinjections were situated in neighboring lower brainstem structures. The location of all the microinjection sites is summarized schematically in figure 3. The cats were divided into three groups depending on the location of the microinjection and the doses of the opioids: group A, four cats with NST microinjections of which received the highest (2.4×10^{-9} M) opioid dose; group B, three cats with NST microinjections of 1.7×10^{-9} M of opioids (one animal on this group also had the treatments with the lowest opioid dose (0.8×10^{-9} M)); group C: the remaining three cats with 1.7×10^{-9} M



Fig. 1. Photomicrograph of a Nissl stained cross-section of the medulla oblongata in one cat with the injection site within the NST (arrow). NST = nucleus of the solitary tract; ST = solitary tract; X = dorsal motor nucleus of vagus nerve.

SLEEP AND OPIOIDS IN NUCLEUS TRACTUS SOLITARIUS

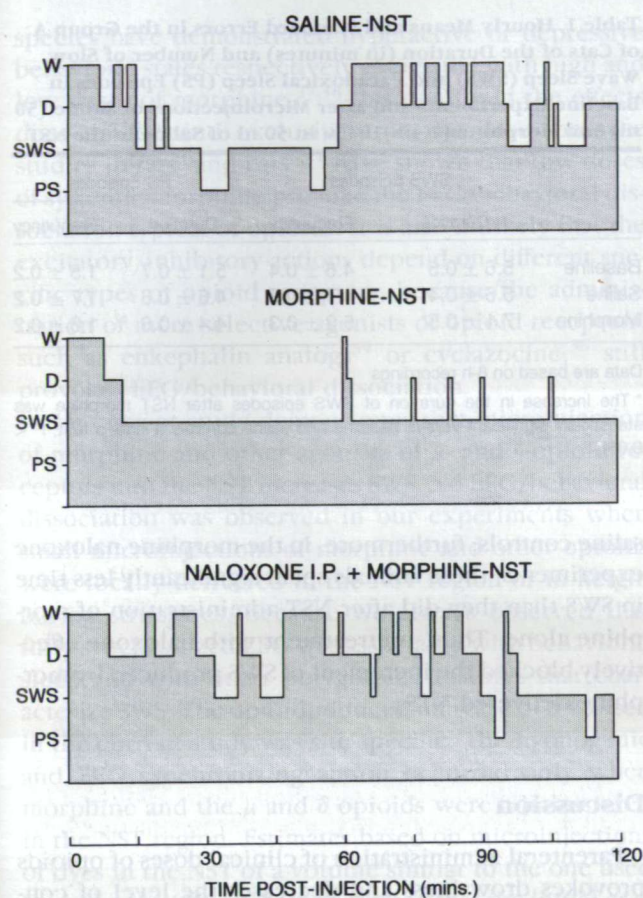


Fig. 2. Representative 2-h hypnogram in experiments in a single cat whose microinjection site in the nucleus of the solitary tract region is shown in figure 1. D = drowsiness; PS = paradoxical sleep; SWS = slow wave sleep; W = wakefulness.

of opioid microinjections located outside the NST region (two animals of this group also had microinjections at the 0.8×10^{-9} dose). Because analyses of variance for repeated measures were used in three groups to determine statistically significant changes in each state of the SWC, data from the 0.8×10^{-9} M experiments were excluded from groups B and C.

In group A, analyses of variance showed that there were significant variations among treatments in the amount of time spent in both SWS ($F_{5,155} = 9.127$, $P < 0.001$) and wakefulness ($F_{5,155} = 4.158$, $P < 0.001$) during the entire 8 h of recordings; changes in drowsiness and PS did not reach statistically significant values (time for drowsiness, $F_{5,155} = 2.271$, $P < 0.10$; for PS, $F_{5,155} = 1.839$, $P < 0.20$). Pairwise comparisons (fig. 4) indicated that none of the SWC states presented sta-

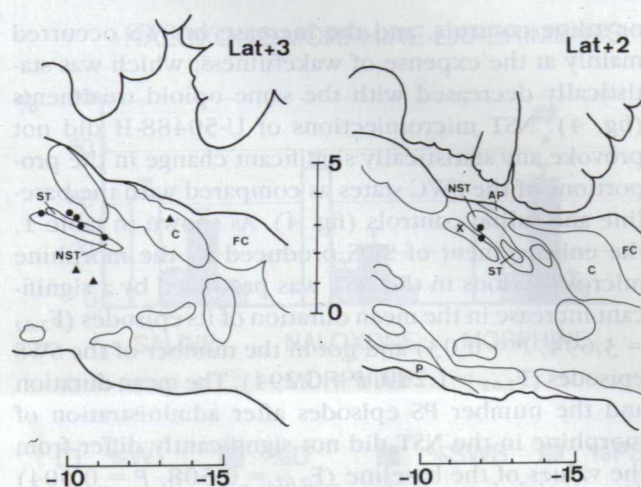


Fig. 3. Reconstruction from the Reinoso-Suárez cat atlas of the sagittal planes of the brainstem 2 and 3 mm from the middle line, with location of the injection sites in the different cats used in the current study. Full circles = the seven cases with microinjections located in the NST region; triangles = the three cases with microinjections situated in structures neighboring on the NST. AP = area postrema; C = cuneate nucleus; FC = cuneate fascicle; NST = nucleus of the solitary tract; P = pyramidal tract; ST = solitary tract; X = dorsal motor nucleus of vagus nerve.

tistically significant variations in baseline and saline control experiments, however, the effects of NST administration of 2.4×10^{-9} M morphine, morphiceptin or D-pen-2-D-pen-5-enkephalin (DPDPE) significantly increased the amount of SWS as compared to baseline

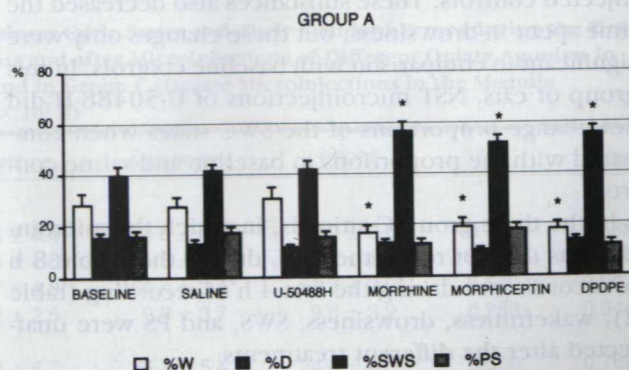


Fig. 4. Group A. Hourly mean percentages of the different sleep-wakefulness cycle states and their standard errors during the 8 h of recordings in control baseline and saline experiments and after NST microinjections of 2.4×10^{-9} M of different opioid agonists. Asterisks indicate statistically significant differences (Fisher's test, $P < 0.05$) in pairwise comparisons between the different opioid treatments and the saline control values.

or saline controls, and the increase in SWS occurred mainly at the expense of wakefulness, which was statistically decreased with the same opioid treatments (fig. 4). NST microinjections of U-50488-H did not provoke any statistically significant change in the proportions of the SWC states as compared with the baseline and saline controls (fig. 4). As shown in table 1, the enhancement of SWS produced by the morphine microinjections in the NST was produced by a significant increase in the mean duration of its episodes ($F_{2,62} = 3.694$, $P = 0.03$) and not in the number of the SWS episodes ($F_{2,62} = 1.249$, $P = 0.294$). The mean duration and the number PS episodes after administration of morphine in the NST did not significantly differ from the values of the baseline ($F_{2,62} = 0.508$, $P = 0.604$) or saline ($F_{2,62} = 0.379$, $P = 0.686$) control recordings.

In group B, changes in the proportions of wakefulness, drowsiness, SWS, and PS did not reach statistically significant levels for the entire 8 h of recordings, as shown by the corresponding analyses of variance (time for wakefulness, $F_{5,115} = 1.315$, $P < 0.30$; for drowsiness, $F_{5,115} = 1.281$, $P < 0.30$; for SWS, $F_{5,115} = 2.184$, $P < 0.10$; and for PS $F_{5,115} = 1.219$, $P < 0.40$). However, the different treatments produced significant variations in the amount of time spent in SWS and drowsiness during the first 4 h of recordings (table 2). Changes in wakefulness and PS did not reach statistically significant differences (table 2). Pairwise comparisons indicated that the effects of the administration of 1.7×10^{-9} M of morphine, morphiceptin, or DPDPE in the NST significantly increased the time spent in SWS during the first 4 h as compared with baseline or saline-injected controls. These substances also decreased the time spent in drowsiness, but these changes only were significant in comparison with baseline controls. In this group of cats, NST microinjections of U-50488-H did not change proportions of the SWC states when compared with the proportions in baseline and saline controls.

In the three group C animals, in which the microinjections did not reach the NST, during the whole 8 h of recording or during the first 4 h of recording (table 2), wakefulness, drowsiness, SWS, and PS were unaffected after the different treatments.

Figure 5 shows the amount of time spent in SWS during the first hour of recordings in combined experiments of naloxone intraperitoneally administered 10 min before morphine delivery in the NST in three cats from group A. In these experiments, the amount of SWS did not significantly differ from that found in the

Table 1. Hourly Means and Standard Errors in the Group A of Cats of the Duration (in minutes) and Number of Slow Wave Sleep (SWS) and Paradoxical Sleep (PS) Episodes in Baseline Experiments and after Microinjections of Saline (50 nl) and Morphine (2.4×10^{-9} M in 50 nl of Saline in the NST

	SWS Episodes		PS Episodes	
	Duration	Frequency	Duration	Frequency
Baseline	5.6 ± 0.5	4.6 ± 0.4	5.1 ± 0.7	1.5 ± 0.2
Saline	5.6 ± 0.4	4.7 ± 0.3	4.6 ± 0.6	1.7 ± 0.2
Morphine	7.1 ± 0.5*	5.2 ± 0.3	4.4 ± 0.6	1.6 ± 0.2

Data are based on 8-h recordings

* The increase in the duration of SWS episodes after NST morphine was statistically significant versus baseline and saline controls (Fisher's test, $P < 0.05$).

saline controls; furthermore, in the morphine-naloxone experiments, these animals spent significantly less time in SWS than they did after NST administration of morphine alone. Thus, pretreatment with naloxone effectively blocked the increment of SWS produced by morphine-delivered NTS.

Discussion

Parenteral administration of clinical doses of opioids provokes drowsiness and decreases the level of consciousness in humans. However, EEG and polygraphic studies after parenteral administration of morphine in humans contradictorily reveal both a decrease in SWS (stages III and IV) and PS and an increase in wakefulness and stage I sleep.⁶ Likewise, both excessive sedation or behavioral excitation, with different time courses, can be observed after intrathecal administration of morphine.³³ Studies in other species have confirmed the sleep-suppressing effects of systemic or intraventricular morphine^{7,8,11} and other opioids.³⁴⁻³⁶ In addition, these studies have indicated that, during this insomnia, there is an EEG/behavioral dissociation: the desynchronized EEG pattern typical of normal wakefulness is usually absent; instead, behavioral wakefulness is accompanied by a synchronized EEG with high-voltage slow waves resembling the pattern that appears during physiologic SWS.

Several factors might explain the complex excitatory/inhibitory actions evoked by opioids, namely: dose dependency, the stimulation of different types of opioid receptors, and different sites of interaction within the CNS. Although experimental studies in different animal

SLEEP AND OPIOIDS IN NUCLEUS TRACTUS SOLITARIUS

species have demonstrated hyperactive or depressive behavioral states respectively associated with high and low doses of morphine,^{37,38} this duality in the effects does not depend exclusively on the dose, because studies in rats⁷ and cats^{8,11} have shown that low doses of systemic morphine provoke the EEG/behavioral dissociation typical of opioids. It is also unlikely that the excitatory/inhibitory actions depend on different specific types of opioid receptors, because the administration of more selective agonists of opioid receptors, such as enkephalin analogs³⁴ or cyclazocine,³⁶ still provokes EEG/behavioral dissociation.

The current study demonstrates that microinjection of morphine and other agonists of μ - and δ -opioid receptors into the NST increases SWS. No EEG/behavioral dissociation was observed in our experiments when small microinjections of morphine and other opioids were locally delivered in the NST region or in neighboring structures, because we always observed that fully synchronized EEG was associated with behavioral sleep and with other polygraphic features that characterize SWS. The opioid-induced increase of SWS seen in the current study was site-specific. The hypnogenic and EEG synchronizing action occurred only when morphine and the μ and δ opioids were administered in the NST region. Estimates based on microinjections of dyes in the NST of a volume similar to the one used in our study (50 nl) have indicated that the initial area of diffusion has a long diameter, about 500 μm .³⁹ In our experiments, morphine and the μ and δ opioids

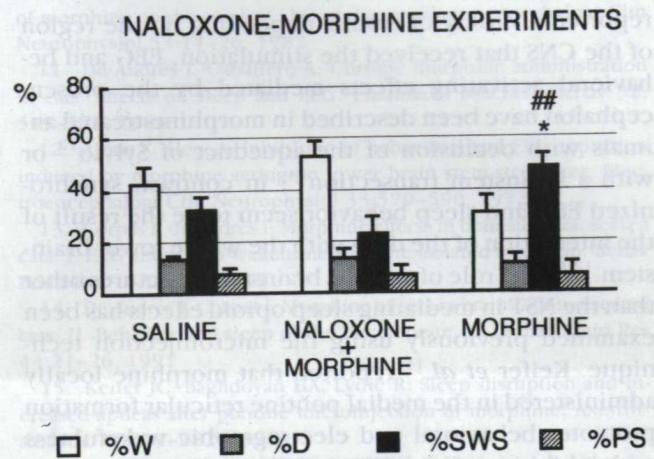


Fig. 5. Percentages of the different sleep-wakefulness cycle states and their standard errors during the first hour in saline experiments and after the delivery of 2.4×10^{-9} M in the nucleus of the solitary tract (NST) and with the previous intraperitoneal administration of naloxone in three cats from group A. Note that the slow wave sleep (SWS) increase produced by the NST morphine delivery was already noticeable in the first hour of the experiment ($*P < 0.05$, comparisons with saline values). Intraperitoneally administered naloxone blocked the SWS enhancement produced by the NST microinjection of morphine ($\#P < 0.05$, comparisons with the microinjection of morphine into the NST plus prior intraperitoneal naloxone).

were unable to increase SWS when microinjected into sites only 1 mm away from NST (fig. 3).

Previous studies dealing with the sites of action of morphine for generation of EEG and SWC effects have

Table 2. Hourly Mean Percentages of the Different Sleep-Wakefulness Cycle States and Their Standard Errors during the First 4 Hours of Recordings in Baseline and Saline Control Experiments and after Microinjections of Different Opiate Agonists in Group B (NST Opiate Microinjection at the Dose of 1.7×10^9 M) and in Group C (Opiate Microinjections in the Medulla Oblongata in the NST Neighboring Structures at the Dose of 1.7×10^9 M)

	Baseline	Saline	U-50488-H	Morphine	Morphiceptin	DPDPE	F _{5,55}	P Value
Group B								
W	19.0 ± 3.5	23.6 ± 8.2	20.5 ± 4.7	13.3 ± 4.4	14.0 ± 4.1	16.0 ± 3.6	0.756	0.5854
D	20.4 ± 1.9	16.8 ± 3.0	19.9 ± 2.5	10.8 ± 1.6	11.8 ± 3.4	8.2 ± 1.5	5.034	0.0007
SWS	49.6 ± 3.6	50.3 ± 7.0	50.7 ± 5.9	68.7 ± 4.8*	64.4 ± 3.4*	66.2 ± 2.9*	3.589	0.0070
PS	11.0 ± 3.1	9.3 ± 2.5	8.9 ± 2.5	7.2 ± 2.5	9.8 ± 3.7	9.6 ± 3.2	0.238	0.9440
Group C								
W	29.3 ± 6.0	28.5 ± 6.6	31.7 ± 5.6	29.0 ± 6.7	36.3 ± 5.4	24.4 ± 6.6	0.508	0.7691
D	17.5 ± 2.3	13.9 ± 1.6	14.5 ± 2.8	18.9 ± 3.7	15.7 ± 2.7	12.2 ± 2.2	0.902	0.4865
SWS	46.0 ± 5.7	43.1 ± 5.2	46.0 ± 5.8	40.2 ± 5.6	43.1 ± 4.9	50.7 ± 5.9	0.504	0.7722
PS	6.2 ± 2.3	14.5 ± 3.6	7.8 ± 2.2	11.9 ± 3.4	4.9 ± 1.9	13.3 ± 3.0	2.202	0.6710

Statistical analysis with the F value of ANOVA for repeated measures and corresponding statistical significance are shown for each SWC state in both groups of cats.

* Statistically significant differences (Fisher's test, $P < 0.05$) between the different opiate treatments and corresponding saline control values for each group of cats.

reported a variety of results depending on the region of the CNS that received the stimulation. EEG and behavioral activating effects mediated by the prosencephalon have been described in morphine-treated animals with occlusion of the aqueduct of Sylvio¹² or with a brainstem transection¹³; in contrast, synchronized EEG and sleep behavior seem to be the result of the interaction of the drug with the whole lower brainstem.^{12,14} The role of specific brainstem structures other than the NST in mediating sleep opioid effects has been examined previously using the microinjection technique. Keifer *et al.*¹⁵ reported that morphine locally administered in the medial pontine reticular formation promotes behavioral and electrographic wakefulness with inhibition of both SWS and PS; however, morphine microinjections in the dorsolateral pontine tegmentum at the level of the locus ceruleus increased SWS.⁴⁰ Therefore, it is evident that either activating or depressing actions can be obtained depending on the CNS loci at which the opioids act. All of these results emphasize the importance of determining the brain sites through which opioids alter EEG and behavioral arousal.

Interspecies differences between cats and humans partially limit the extrapolation of the results of the current study on behavioral effects and EEG changes associated with perioperative use of opioids to humans. However, the EEG and sleep responses of cats^{8,11} to relatively low doses of morphine are similar to those found in humans and other species. These findings, along with the wide use of the cat in the research of sleep mechanisms, support the validity of this species as an experimental model for the study of the neural mechanisms involved in EEG and sleep effects of opioids. Thus, the complex and often contradictory behavior observed in humans^{6,32} under systemic morphine, as well as the EEG/behavioral dissociation described in animals,^{7,8} could be explained by the simultaneous activation of both EEG-desynchronizing and EEG-synchronizing structures. Also, the perioperative hypnosis and EEG synchronization induced by small doses of opioids, when administered with other anesthetic agents,⁴¹ could be explained by the activation of synchronizing structures like the NST region and the inhibition of the CNS-activating mechanisms by the anesthetic drugs. What is more, the very high doses of μ -opioid agonist required for adequate intraoperative hypnosis^{42,43} could be explained by the need for predominant stimulation of μ receptors located in synchronizing structures. On the other hand, because

interaction of morphine with NST lengthens the SWS episodes, opioids also would strengthen the hypnotic effects of anesthetic drugs⁴⁴ by enhancing the duration of the SWS episodes once these were induced by the anesthetic agents. This would explain why the drowsiness after administration of parenteral opioids can be strongly exacerbated by the concomitant use of any hypnotic drug, even in small doses, or by the presence of a physiologic period of sleep, independently of adequate therapeutic plasmatic concentration of the opioid drug employed.⁴⁵

In our experiments, intraperitoneal pretreatment with naloxone effectively blocked the SWS increase produced by the NST morphine microinjection. Pretreatment with the antagonist blocked the sleep-suppressing effects of morphine in the medial pontine reticular formation.¹⁵ These findings are consistent with the hypothesis that the sleep effects of morphine interacting in distinct regions of the brainstem are opioid-receptor phenomena. Microdialysis studies performed in the medial pontine reticular formation have indicated that inhibition of acetylcholine release in this area could be a mechanism through which morphine may inhibit PS.⁴⁶ In addition to the well known EEG-synchronizing and SWS-enhancing effects mediated by the NST,^{23,24} this region has a role in PS mechanisms, because both the administration of cholinergic agonists into the NST²⁷ and destruction of its serotonergic innervation²⁶ produces enhancement of PS. Despite this and the fact that NTS contains muscarinic receptors,¹⁸ this study shows that PS was unchanged after NST administration of the different opioids. No significant changes were detected in the amount of PS or the duration and number of its episodes. It seems that μ and δ opioids in the NST cause an enhancement of SWS that mimics the results observed after serotonergic^{24,25,47} and not acetylcholine stimulation.

Concerning the identification of the opioid receptors that mediated the increase in SWS caused by the NST morphine injections in cats, the present study showed that the microinjection of morphiceptin and DPDPE (highly specific agonists of μ - and δ -opioid receptors, respectively) into the NST provoked an increase in SWS like that produced by morphine. These effects were also dose-dependent and site-specific. However, microinjection of U-50488-H, the most selective κ -opioid agonist,³¹ into the NST did not produce any significant changes in the SWC states. It therefore seems that the SWS enhancement caused by NST administration of morphine in cats is mediated by the μ - and δ -opioid

receptors. However, further studies using specific antagonists to block the effects of morphiceptin and DPDPE are needed to provide unequivocal evidence of the involvement of both μ and δ receptors in the enhancement of SWS produced by opioids in the NST.

The NST has been reported to be basically enkephalinergic,¹⁶ but the NST neurons can produce dynorphins and endorphins.⁴⁸ Nevertheless, the opioid-receptor subtype distribution varies among the different species: Thus, μ - and κ -opioid receptors have been localized in the rat NST,¹⁷ whereas μ and δ but not κ receptors have been demonstrated in the NST of rabbits⁴⁹ and cats.^{16,18} Therefore, the lack of effect of the specific κ agonist U-50488-H in the current study would seem to be explained by the absence of κ receptors in the feline NST.

The authors thank Professor F. Reinoso-Suárez, for his support and advice; G. de la Fuente and M. T. Fernández Yuste, for their assistance with the research and illustrations, respectively; and J. Hernández-Claumarchirant, P. Romero, and R. M. Sánchez-Lozano, for their help with the histologic preparations. The U-50488-H was provided by Upjohn Laboratories.

References

- Smith NT, Benthuisen JL, Bickford RG, Sanford TJ, Blasco T, Duke PC, Head N, Dec Silver H: Seizures during opioid anesthetic induction are they opioid-induced rigidity? *ANESTHESIOLOGY* 71:852-862, 1989
- Knill RL, Moote CA, Skinner MI, Rose EA: Anesthesia with abdominal surgery leads to intense REM sleep during the first postoperative week. *ANESTHESIOLOGY* 73:52-61, 1990
- Ghoneim MM, Block RI: Learning and consciousness during general anesthesia. *ANESTHESIOLOGY* 76:279-305, 1992
- Etches RC, Sandler AN, Lawson SL: A comparison of the analgesic and respiratory effects of epidural nalbuphine or morphine in post-thoracotomy patients. *ANESTHESIOLOGY* 75:9-14, 1991
- Cately DM, Thornton C, Jordan C, Lehane JR, Royston D, Jones JG: Pronounced, episodic oxygen desaturation in the postoperative period: Its association with ventilatory pattern and analgesic regimen. *ANESTHESIOLOGY* 63:20-28, 1985
- Kay DC: Human sleep during chronic morphine intoxication. *Psychopharmacologia* 44:117-124, 1975
- Khazan N: The implication and significance of EEG and sleep-awake activity of experimental drug dependence on morphine, *Methods in Narcotics Research*. Edited by Ehrenpreis S, Neidle A. New York, Dekker 1975, pp 173-214
- De Andrés I, Villablanca JR, Burgess JW: Reassessing morphine effects in cats: II. Protracted effects on sleep-wakefulness and the EEG. *Pharmacol Biochem Behav* 21:923-928, 1984
- Pickworth WB, Sharpe LG: EEG-behavioral dissociation after morphine and cyclazocine-like drugs in the dogs: Further evidence for two opiate receptors. *Neuropharmacology* 18:617-622, 1979
- Bronzino JD, Kelly ML, Cordova C, Gudz M, Oley N, Stern WC, Morgane PJ: Amplitude and spectral quantification of the effects of morphine on the cortical EEG of the rat. *Electroencephalogr Clin Neurophysiol* 53:14-26, 1982
- De Andrés I, Caballero A: Chronic morphine administration in cats: Effects on sleep and EEG. *Pharmacol Biochem Behav* 32:519-526, 1989
- Albus K, Herz A: Inhibition of behavioral and EEG activation induced by morphine acting on lower brain stem structures. *Electroencephalogr Clin Neurophysiol* 33:579-590, 1972
- Corpas I, de Andrés I: Morphine effects in brainstem-transected cats: I. EEG and 'sleep-wakefulness' in the isolated forebrain. *Behav Brain Res* 44:11-19, 1991
- De Andrés I, Corpas I: Morphine effects in brainstem-transected cats: II. Behavior and sleep of the decerebrate cat. *Behav Brain Res* 44:21-26, 1991
- Keifer JC, Baghdoyan HA, Lydic R: Sleep disruption and increased apneas after pontine microinjection of morphine: *ANESTHESIOLOGY* 77:973-982, 1992
- Dashwood MR, Muddle JR, Spyer KM: Opiate receptor subtypes in the nucleus tractus solitarii of the cat: The effect of vagal section. *Eur J Pharmacol* 155:85-92, 1988
- Mansour A, Lewis ME, Khachaturian H, Akil H, Watson SJ: Pharmacological and anatomical evidence of selective mu, delta and kappa opioid receptor binding in rat brain. *Brain Res* 399:69-79, 1986
- Leslie RA, Murphy KM, Robertson HA: Nodose ganglionectomy selectively reduces muscarinic cholinergic and delta opioid binding sites in the dorsal vagal complex of the cat. *Neuroscience* 32:481-492, 1989
- Maley BE, Elde R: Immunohistochemical localization of putative neurotransmitters within the feline nucleus tractus solitarii. *Neuroscience* 7:2469-2490, 1982
- Ramón y Cajal S: *Textura del Sistema Nervioso Central del Hombre y de los Vertebrados*: Tomo II. Madrid, Moya N, 1899, pp 58-88
- Donnelly DF, Sica AL, Cohen MI, Zhang H: Dorsal medullary inspiratory neurons: Effects of superior laryngeal afferent stimulation. *Brain Res* 491:243-252, 1988
- Mifflin SW, Felder RB: An intracellular study of time-dependent cardiovascular afferent interactions in nucleus tractus solitarius. *J Neurophysiol* 59:1798-1813, 1988
- Magnes J, Moruzzi G, Pompeiano O: Synchronization of the EEG produced by low frequency electrical stimulation of the region of the solitary tract. *Arch Ital Biol* 99:33-61, 1961
- Laguzzi R, Reis DJ, Talman WT: Modulation of cardiovascular and electrocortical activity through serotonergic mechanism in the nucleus tractus solitarius of the rat. *Brain Res* 304:321-328, 1984
- Puizillout JJ: Noyau du fascicieu solitaire, serotonine et regulation de la vigilance. *Rev Electroencephalogr Neurophysiol Clin* 16:95-106, 1986
- Nosjean A, Arluison M, Laguzzi RF: Increase in paradoxical sleep after destruction of serotonergic innervation in the nucleus tractus solitarius of the rat. *Neuroscience* 23:469-481, 1987
- Danguir J, De Saint Hilarie Kafi S: Somatostatin antiserum blocks carbachol-induced increase of paradoxical sleep in the rat. *Brain Res Bull* 20:9-12, 1988
- Reinoso-Suárez F: *Topographischer Hirnatlas der Katze*. Darmstadt, Merck, 1961, pp 1-63
- Chang KJ, Killian A, Hazun E, Cuatrecasas P: Morphiceptin (NH₄-Tyr-Pro-Phe-Pro-CONH₂): A potent and specific agonist for morphine (μ) receptors. *Science* 212:75-77, 1981
- Kovács GL, Nyolczas N, Kriván M, Gulya K: Analgesic and tolerance-inducing effects of the highly selective δ opioid agonist (D-

Pen²,D-Pen⁵) enkephalin in mice. *Euro J Pharmacol* 150:347-353, 1988

31. Von Voigthlander PF, Lahti RA, Ludens JH: U-50488-H a selective and structurally novel non-mu (κ) opioid agonist. *J Pharmacol Exp Ther* 224:7-12, 1982

32. Ursin R, Sterman MB: *A Manual for Standardized Scoring of Sleep and Waking Patterns in the Adult Cat*. Los Angeles, Brain Information Service, Brain Research Institute, University of California, 1981, pp 1-103

33. Cousins MJ, Bridenbaugh PO: *Spinal opioids and pain relief in acute care, Acute Pain Management*. Edited by Cousins MJ, Phillips GD. New York, Churchill Livingstone, 1986, pp 151-185

34. Pacheco P, Erwin FR, Hodgkon JC: Effects of leucine⁵- and methionine⁵-b-endorphin on behavior and electroencephalogram in cats. *Life Sci* 32:181-190, 1983

35. King C, Masserano JM, Codd E, Byrne W: Effects of B-endorphin and morphine on the sleep-wakefulness behavior of cats. *Sleep* 4: 259-262, 1981

36. Calligaro D, Young GA, Khazan N: Dissociation of opioid and nonopioid effects of Cyclazocine. *Pharmacol Biochem Behav* 30:163-167, 1988

37. Carlsson C, Smith DS, Keykhah HM, Englebach I, Harp JR: The effects of high-dose fentanyl on cerebral circulation and metabolism in rats. *ANESTHESIOLOGY* 57:375-380, 1982

38. Schnur P, Raigoza VP: Effects of naloxone on morphine induced sedation and hyperactivity in the hamster. *Pharmacol Biochem Behav* 24:849-854, 1986

39. Narvaez JA, Aguirre JA, Fuxe K: Subpicomolar amounts of NPY(13-16) injected into the nucleus tractus solitarius of the rat counteract the cardiovascular responses to L-glutamate. *Neurosci Lett* 151:182-186, 1993

40. Garzón M, Tejero S, Benítez A, De Andrés I: Effect of sleep of opiate microinjections in the dorsolateral pontine tegmentum (abstract). *J Sleep Res* 1(suppl):79, 1992

41. Bowdle TA, Ward RJ: Induction of anesthesia with small doses of sufentanil or fentanyl: Dose *versus* EEG response, speed of onset, and thiopental requirement. *ANESTHESIOLOGY* 70:26-30, 1989

42. Sebel PS, Bovill JG, Wauquier A, Pieternella R: Effects of high-doses fentanyl anesthesia on the electroencephalogram. *ANESTHESIOLOGY* 55:203-211, 1981

43. Scott JC, Ponganis KV, Stanski DR: EEG quantification of narcotic effect: The comparative pharmacodynamics of fentanyl and alfentanil. *ANESTHESIOLOGY* 62:234-241, 1985

44. Pechnick RN, Terman GW: The role of opiate receptors in the potentiation of pentobarbital sleeping time by the acute and chronic administration of opiates. *Neuropharmacology* 26:1589-1593, 1987

45. Van Dercar DH, Martinez AP, De Lisser EA: Sleep apnea syndrome: A potential contraindication for patient controlled analgesia. *ANESTHESIOLOGY* 74:623-624, 1991

46. Lydic R, Keifer JC, Baghdoyan HA, Becker L: Microdialysis of the pontine reticular formation reveals inhibition of acetylcholine release by morphine. *ANESTHESIOLOGY* 79:1003-1012, 1993

47. Koella WP: Serotonin and sleep. *Sleep: Neurotransmitters and Neuromodulators*. Edited by Wauquier A. New York, Raven, 1985, pp 185-196

48. Van Giersbergen PLM, Palkovitz M, De Jong W: Involvement of neurotransmitters in the nucleus tractus solitarii in cardiovascular regulation. *Physiol Rev* 72:789-824, 1992

49. May CN, Dashwood MR, Whitehead CJ, Mathias CJ: Differential cardiovascular and respiratory responses to central administration of selective opioid agonists in conscious rabbits: Correlation with receptor distribution. *Br J Pharmacol* 98:903-913, 1989