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# *Suppressive Effect of Morphine on Single-unit Activity of Cells in Rexed Lamina VII*

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**Using an extracellular microelectrode recording technique, the effects of intravenously administered morphine sulfate upon the single-unit activities of cells in Rexed lamina VII of the lumbar spinal cord were studied in cats following decerebration and spinal cord transection at L1. These neurons responded principally to high-threshold mechanical and thermal stimuli applied to a receptive field in the ipsilateral hind limbs. Morphine sulfate, 0.25, 0.5, and 1.0 mg/kg, caused dose-related suppression of the spontaneous activities of these neurons. The firing rates at maximum suppression, observed 5–10 min after administration of morphine, were 63.9 ± 9.2 (mean ± 1 SE), 43.0 ± 5.4, and 26.5 ± 6.0 per cent of the control values, respectively. Since these cells have been shown by others to be associated with the spinothalamic and spinoreticular pathways, the results suggest that the analgesic state may result from the action of morphine on the cells of origin of these major ascending pathways in the spinal cord. (Key words: Analgesics, narcotic, morphine; Spinal cord, Rexed laminae.)**

THE CYTOARCHITECTONIC INVESTIGATIONS of Rexed[1] established that neurons in the feline spinal cord are arranged in a series of clearly defined laminae. Electrophysiologic investigation in recent years has demonstrated some physiologic correlates of the dorsal horn lamination (*i.e.*, laminae I–VI).[2–4] It has also been demonstrated that many anesthetics and narcotics suppress the activity of dorsal horn neurons that are associated with nociception.[2,5–10] Compared with dorsal horn neurons, lamina VII neurons, which are located in the dorsal part of the ventral horn, have not been as well investigated.

Recent studies[11] from this laboratory have shown a potent suppressive effect of halothane on single-unit activity of Rexed lamina VII cells. The experiment described here was undertaken to study the effect of morphine sulfate on the spontaneous activity of single units in Rexed lamina VII, considered the major cells of origin of the spinothalamic and spinoreticular tracts,[12–14] which convey information from nociceptive afferents.

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## Methods

Forty-one cats of both sexes, 2.5 to 4 kg in weight, were used. Under halothane, nitrous oxide and oxygen anesthesia, animals were prepared with tracheostomy and carotid artery and jugular vein cannulations. Both carotid arteries were ligated distal to the cannulation and electrolytic lesions were made stereotaxically in the midbrain reticular formation for midcollicular decerebration. The spinal cord was transected at the level of L1 and lumbar and sacral cord segments were exposed and covered with a paraffin and mineral oil mixture maintained at 37 C. Anesthesia was then discontinued and the animals were artificially ventilated with pure oxygen. End-tidal $CO_2$ was held at 30–36 torr as measured by an infrared gas analyzer. Mean arterial blood pressure was maintained above 80 torr by an intravenous infusion of dextrose, 5 per cent, in half-physiologic saline solution with gallamine triethiodide, 0.1 per cent, at a rate of 5 to 7 ml/kg/hr, and rectal temperature was maintained at 37 ± 1 C with an infrared heating lamp. A glass-rod–platinum-sheathed Transidyne "Microtrode" microelectrode with a 1–2 $\mu$m exposed tip was then inserted by a hydraulic micromanipulator into the lumbar spinal cord near the L7 root entry zone. The input signals were acquired through a differential AC preamplifier, displayed on an oscilloscope, and recorded on magnetic tape. The dorsal horn lamination was identified by the depth of the electrode from cord dorsum, and by the spontaneous firing pattern of the unitary activity and the characteristic evoked responses to peripheral stimuli of the following types[8]: noxious mechanical (clamping, deep squeezing by hand and pinching), noxious thermal (radiant heat by a Hardy-Wolff-Goodell dolorimeter, application of ethyl chloride), and non-noxious mechanical (air puff, light stroking of hair or skin with a camel's hair brush, touch, pressure and joint movement).

After identification of dorsal horn neurons,[8] the microelectrode was advanced 500–1,000 $\mu$m ventral to lamina VI to sample neurons responding principally to high threshold mechanical or thermal stimulation. Preliminary study in the animals not given morphine showed that the activity of neurons remained stable for several hours, and allowed us to ob-

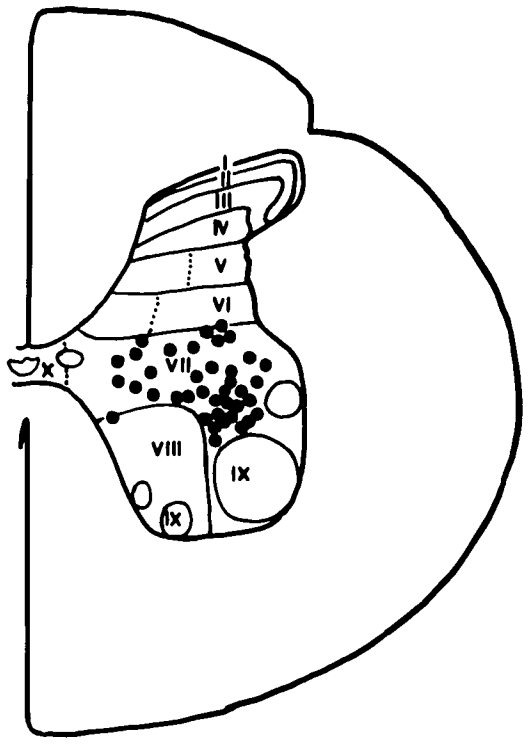


FIG. 1. Schematic representation of locations of cells recorded in lamina VII, verified histologically.

serve functional and pharmacologic properties of neurons for at least two to three hours. Following the recording of spontaneous firing of the unit during the 10–15-min control period, morphine sulfate, 0.25 mg/kg (six animals), was slowly administered intravenously. Neuronal activity recordings were continued in maximal depression and then recovery of the firing activity had occurred. The same experiment was repeated in other animals using morphine, 0.5 mg/kg (18 animals), and 1.0 mg/kg (15 animals). In two animals receiving 0.5 mg/kg and in five animals receiving 1.0 mg/kg morphine, in which complete recovery was not observed after two to three hours, naloxone, 0.01 mg/kg, was administered intravenously to observe its antagonistic effect to morphine. At the end of the experiment, an electrolytic lesion was made by applying DC current (50 microamperes for 60 sec) through the recording microelectrode. The animals were then sacrificed by administering pure nitrous oxide. The segment of spinal cord with the microelectrode track was fixed in 10 per cent formalin for later histologic examination. After fixation, frozen sections were cut to a thickness of 30 $\mu$m, stained with cresyl violet, and the lesions were verified histologically. Data recorded on magnetic tape were proc-

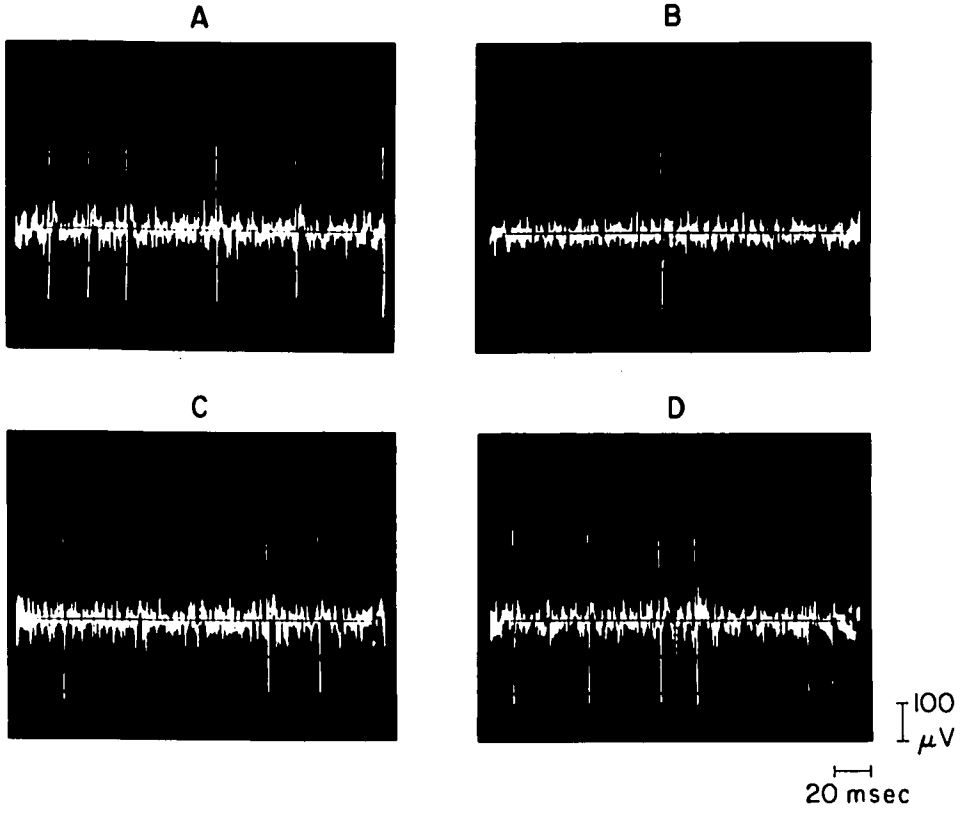


FIG. 2. Example of single-unit activity in Rexed lamina VII, displayed on oscilloscope. *A*, during control; *B*, *C*, and *D*, 10, 60, and 135 min after administration of morphine sulfate, 1 mg/kg, iv.

essed with a digital computer**,[8] and a rate interval analyzer.†† The significances of the mean values of firing rate during the control period, after administration of morphine, and during the recovery period were assessed by Student's t test.

## Results

Recordings were obtained from 39 single units of Rexed lamina VII, verified histologically, at depths of 3,000–4,200 $\mu$m from the dorsal surface of the spinal cord (fig. 1). The receptive fields were generally situated at the distal part of the hind limb. The ratio of nociceptive to non-nociceptive neurons in this area was about 1 to 1. The nociceptive neurons responded principally to clamping, to deep squeezing, to noxious radiant heat, and to cooling with the application of ethyl chloride. The receptive field sizes of the neurons studied ranged from several paw pads to entire ipsilateral foot. These neurons did not respond significantly to stimulation with a puff of air, to stroking with a brush, and to joint movements. The spontaneous activity of these neurons was suppressed with the stimulation of hair or of skin adjacent to the receptive fields. Low-intensity mechanical stimulation of the corresponding areas on the contralateral hind limb usually inhibited the activity of these neurons.

No significant change of blood pressure or pulse rate was observed with administration of morphine. Morphine had a consistent suppressive effect on the spontaneous activity of these neurons (fig. 2). The effects of morphine began within 3–5 min of injection and reached a maximum value in 5–10 min. There was dose-dependent depression of the unitary activity by morphine, and the times to recovery to control firing rate ranged from one to three hours, depending upon the dose of morphine (table 1, fig. 3). In some cases with the larger dose of morphine, recovery was not observed within four hours. In such cases data were excluded from the statistical analysis.

Naloxone reversed the suppression of neuronal activity produced by morphine in all cases tested, regardless of the dose of morphine. The spontaneous firing rate before administration of naloxone was 40 ± 9 (mean ± 1 SE) per cent of the control firing rate. The reversal occurred within 5 min after administration of naloxone, and by 10 min the firing rate averaged 139 ± 8 (mean ± 1 SE) per cent of the control firing rate ($P < 0.01$ *vs.* pre-naloxone firing rate).

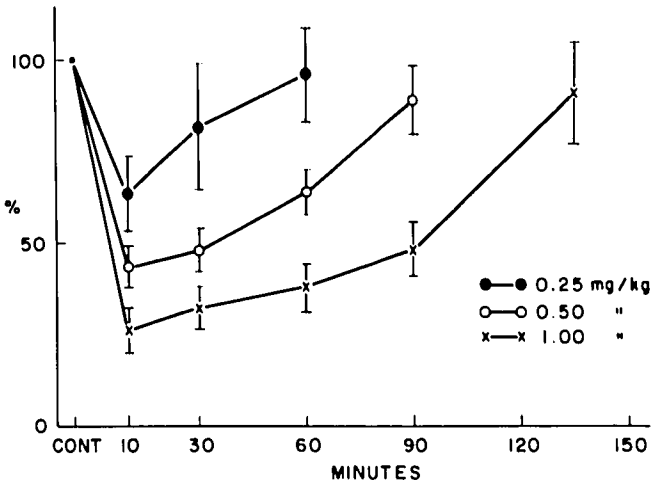


FIG. 3. Effects of morphine sulfate (0.25, 0.5 and 1.0 mg/kg, iv) on spontaneous single-unit activity of cells in lamina VII, expressed as percentages (mean ± 1 SE) of control values.

TABLE 1. Effects of Morphine Sulfate on Spontaneous Single-unit Activity of Cells in Rexed Lamina VII, Expressed as Percentages of Control Values (Mean ± 1 SE)

| Dose | Control (Impulses/Sec) | Per Cent of Control | | | | |
|---|---|---|---|---|---|---|
| | | 10 Min | 30 Min | 60 Min | 90 Min | 135 Min |
| 0.25 mg/kg, (n = 6) | 17.1 ± 5.1 | 63.9* ± 9.2 | 81.7 ± 17.4 | 95.5 ± 12.7 | | |
| 0.50 mg/kg (n = 18) | 19.2 ± 2.4 | 43.0* ± 5.4 | 47.8* ± 5.7 | 63.5* ± 5.9 | 88.4 ± 9.3 | |
| 1.00 mg/kg (n = 15) | 18.0 ± 2.9 | 26.5* ± 6.0 | 32.1* ±5.4 | 37.2* ± 6.6 | 48.0* ± 7.1 | 90.8 ± 13.8 |

* $P < 0.01$ *vs.* control, by t-test.

## Discussion

There are at least four major ascending pathways within the feline lumbar spinal cord. The first, the dorsal column system, is believed to convey information responsible for tactile and proprioceptive sensation[15]; the second, the spinocervical pathway, conveys information thought to be essential for tactile and pain sensation via relays in the lateral cervical nucleus and the contralateral thalamus[16–17]; the third, the spinoreticular pathway, transmits information regarding noxious stimuli via relays in the nucleus gigantocellularis in the medial brain-stem reticular formation and forms a part of the ascending input to the medial thalamus[18]; the fourth, the spinothalamic pathway,[17] transmits information thought to be

** Digital Equipment Corp. PDP-12.
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essential for tactile, nociceptive and thermal sensation to the contralateral thalamus, mainly to ventrobasal regions. The cells of origin of the spinocervical tract in the feline lumbar spinal cord are located in Rexed laminae IV and V according to Bryan *et al.*,[16] Eccles *et al.*,[19] and Hongo *et al.*,[20] as demonstrated with antidromic stimulation techniques. Recently, Levante *et al.*[13] and Fields *et al.*[14] demonstrated that cells of origin of the spinoreticular tract are located predominantly in laminae VII and VIII in the feline lumbosacral enlargement, utilizing an antidromic stimulation technique. As regards the cells of origin of the spinothalamic tract, Trevino *et al.*[12] and Albe-Fessard *et al.*,[21] using an antidromic stimulation technique, found that in the lumbosacral enlargement of the feline spinal cord most of the cells of origin of the spinothalamic tract were located in Rexed laminae VII and VIII, although they were located in Rexed laminae IV and V in monkeys.[22,23] Szentagothai[24] found that axons decussating in the ventral commissure of the spinal cord arise from cells of Rexed laminae VI–VIII in cats, and suggested that spinothalamic neurons originate in these regions.

The mode and site of action of morphine and its surrogates are not well clarified. It is generally agreed that they have no significant effect on the impulse transmission of peripheral nerves.[25] They might act at spinal[8–10] or supraspinal[26–32] levels or at both levels. Direct effects of morphine upon spinal neuronal activity have been reported by several investigators. Using decerebrated and spinalized cats, Kitahata *et al.*[8] found that morphine given intravenously suppressed, in a dose-related manner, spontaneous single-unit activity of cells in Rexed laminae I and V known to respond to noxious peripheral stimuli, but did not affect spontaneous single-unit activity of cells in laminae IV and VI, known to respond primarily to non-noxious stimuli. In lamina V of spinal cats, Le Bars *et al.*[9] observed the suppressive effect of morphine upon spontaneous activity and upon the evoked responses elicited by natural nociceptive stimulation and by supramaximal electrical stimulation. In their experiment, after morphine sulfate, 2 mg/kg, intravenously, spontaneous firing and responses to intense pinch were decreased 50 per cent, and the responses to electrical stimulation were decreased 33 per cent.

Calvillo *et al.*[10] described a depressant effect of morphine applied by microiontophoresis upon dorsal horn neurons that respond to noxious radiant heat, and almost no effect upon neurons responding to non-noxious stimuli, but, in their report, histologic correlates are not available. Satoh *et al.*,[27] using macroelectrode techniques, recorded responses from the ventrolateral funiculus following electrical stimulation of the splanchnic nerves. They found that these responses were suppressed by morphine (2–4 mg/kg, iv) in intact cats, but were only slightly suppressed in cats with high spinal-cord transection, suggesting that the suppressive effect of morphine is exerted through its descending inhibitory influences on the spinal cord. This concept has been supported by several investigations.[28–30] However, in our experiment described here, the possible effects of descending supraspinal mechanisms interacting with or mediating the effect of administered morphine were eliminated by spinal-cord transection. As a consequence of the present study and the results shown previously,[8–10] any theory of the action of morphine that neglects the direct effects of morphine on spinal-cord mechanisms is incomplete.

Compared with findings in our previous study,[8] the extent of suppression of the activity of neurons in lamina VII at each dose of morphine was significantly greater than that in lamina I or lamina V. In laminae I and V, morphine, 1 mg/kg, iv, for example, suppressed spontaneous activity to 82.4 ± 7.3 per cent (mean ± 1 SE) and 62.5 ± 5.9 per cent of control values, respectively, whereas in lamina VII the same dose of morphine suppressed activity to 26.5 ± 6.0 per cent ($P < 0.01$ *vs.* laminae I and V). The time required for the recovery of firing in the neurons of lamina VII was 120–150 min, which is also longer than corresponding times for the neurons of lamina I and lamina V (10–40 min). As we reported previously,[11] the intraspinal-cord latencies of cells in laminae V and VII are 2.0 ± 0.8 (mean ± 1 SE) and 3.9 ± 0.3 msec, respectively. Thus, the intraspinal-cord latency of the cells in lamina VII is longer than that of cells in lamina V by 1.9 msec, indicating the greater number of intraspinal synapses to lamina VII. The greater and longer effects of morphine on the neurons in lamina VII may be explained either by the sequential suppressive effect on the intervening synaptic junctions or by a vigorous direct suppressive effect on the synaptic junctions at lamina VII.

In summary, using an extracellular microelectrode recording technique, we found that the spontaneous activity of neurons in Rexed lamina VII was significantly suppressed by intravenously administered morphine sulfate. At each incremental dose of morphine, the degree of suppression of the activity of cells in lamina VII was greater and the duration of suppression was longer than those of dorsal horn interneurons in laminae I and V, also known to respond preferentially to noxious stimuli.

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## References

1. Rexed B: The cytoarchitectonic organization of the spinal cord in the cat. J Comp Neurol 96:415–495, 1952
2. Wall PD: The laminar organization of dorsal horn and effects of descending impulses. J Physiol (Lond) 188:403–423, 1967
3. Pomeranz B, Wall PD, Weber WV: Cord cells responding to fine myelinated afferents from viscera, muscle and skin. J Physiol (Lond) 199:511–532, 1968
4. Christensen BN, Perl ER: Spinal neurons specifically excited by noxious or thermal stimuli: Marginal zone of the dorsal horn. J Neurophysiol 33:293–307, 1970
5. deJong RH, Wagman IH: Block of afferent impulses in the dorsal horn of monkey. A possible mechanism of anesthesia. Exp Neurol 20:352–358, 1968
6. Kitahata LM, Taub A, Sato I: Lamina-specific suppression of dorsal horn unit activity by nitrous oxide and by hyperventilation. J Pharmacol Exp Ther 176:101–108, 1971
7. Kitahata LM, Taub A, Kosaka Y: Lamina-specific suppression of dorsal-horn unit activity by ketamine hydrochloride. ANESTHESIOLOGY 38:4–11, 1973
8. Kitahata LM, Kosaka Y, Taub A, et al: Lamina-specific suppression of dorsal-horn unit activity by morphine sulfate. ANESTHESIOLOGY 41:39–48, 1974
9. Le Bars D, Menétrey D, Conseiller C, et al: Depressive effects of morphine upon lamina V cells activities in the dorsal horn of the spinal cat. Brain Res 98:261–277, 1975
10. Calvillo O, Henry JL, Neuman RS: Effects of morphine and naloxone on dorsal horn neurones in the cat. Can J Physiol Pharmacol 52:1207–1211, 1974
11. Kitahata LM, Yamashita M, Ghazi-Saidi K, et al: Effects of halothane on single-unit activity of cells in Rexed lamina VII, Advances in Pain Research and Therapy. Volume 1. Edited by Bonica JJ, et al. New York, Raven Press, 1976, pp 111–115
12. Trevino DL, Maunz RA, Bryan RN, et al: Location of cells of origin of the spinothalamic tract in the lumbar enlargement of cat. Exp Neurol 34:64–77, 1972
13. Levante A, Albe-Fessard D: Localisation dans les couches VII et VIII de Rexed des cellules d'origine d'un faisceau spino-réticulaire croisé. C R Acad Sci D (Paris) 274: 3007–3010, 1972
14. Fields HL, Wagner GM, Anderson SD: Some properties of spinal neurons projecting to the medial brain-stem reticular formation. Exp Neurol 47:118–134, 1975
15. Kruger L, Siminoff R, Witkovsky P: Single neuron analysis of dorsal column nuclei and spinal nucleus of trigeminal in cat. J. Neurophysiol 24:333–349, 1961
16. Bryan RN, Trevino DL, Coulter JD, et al: Location and somatotopic organization of the cells of origin of the spinocervical tract. Exp Brain Res 17:177–189, 1973
17. Morin F, Schwartz HG, O'Leary JL: Experimental study of the spinothalamic and related tracts. Acta Psychiat Neurol Scand 26:371–396, 1951
18. Casey KL: Somatic stimuli, spinal pathways, and size of cutaneous fibers influencing unit activity in the medial medullary reticular formation. Exp Neurol 25:35–56, 1969
19. Eccles JC, Eccles RM, Lundberg A: Types of neurone in and around the intermediate nucleus of the lumbosacral cord. J Physiol (Lond) 154:89–114, 1960
20. Hongo T, Jankowska E, Lundberg A: Post-synaptic excitation and inhibition from primary afferents in neurones of the spino-cervical tract. J Physiol (Lond) 199:569–592, 1968
21. Albe-Fessard D, Levante A, Lamour Y: Origin of spinothalamic and spinoreticular pathways in cats and monkeys, Advances in Neurology. Volume 4. Edited by Bonica JJ. New York, Raven Press, 1974, pp 157–166
22. Trevino DL, Coulter JD, Willis WD: Location of cells of origin of spinothalamic tract in lumbar enlargement of the monkey. J Neurophysiol 36:750–761, 1973
23. Albe-Fessard D, Levante A, Lamour Y: Origin of spinothalamic tract in monkeys. Brain Res 65:503–509, 1974
24. Szentagothai J: Neuronal and synaptic arrangement in the substantia gelatinosa Rolandi. J Comp Neurol 122:219–239, 1964
25. Kosterlitz HW, Wallis DI: The action of morphine-like drugs on impulse transmission in mammalian nerve fibers. Br J Pharmacol 22:499–510, 1964
26. Tsou K, Jang CS: Studies on the site of analgesic action of morphine by intracerebral micro-injection. Sci Sinica 13: 1099–1109, 1964
27. Satoh M, Takagi H: Enhancement by morphine of the central descending inhibitory influence on spinal sensory transmission. Eur J Pharmacol 14:60–65, 1971
28. Satoh M, Nakamura N, Takagi H: Effect of morphine on bradykinin-induced unitary discharges in the spinal cord of the rabbit. Eur J Pharmacol 16:245–247, 1971
29. Akil H, Mayer DJ, Liebeskind JC: Comparaison chez le Rat entre l'analgésie induite par stimulation de la substance grise périaqueducale et l'analgésie morphinique. C R Acad Sci D (Paris) 274:3603–3605, 1972
30. Liebeskind JC, Guilbaud G, Besson J, et al: Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: Behavioral observations and inhibitory effects on spinal cord interneurons. Brain Res 50:441–446, 1973
31. Pert CB, Snyder SH: Opiate receptor: Demonstration in nervous tissue. Science 179:1011–1014, 1973
32. Sun CL, Gatipon GB: Effects of morphine sulfate on medial bulboreticular response to peripherally applied noxious stimuli. Exp Neurol 52:1–12, 1976