

## Fentanyl Suppression of Nociceptive Neurons in the Superficial Dorsal Horn of the Cat

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This study was designed to examine the influence of spinally administered fentanyl on the spontaneous and noxiously evoked activity of high threshold (HT) and wide dynamic range (WDR) neurons in the superficial layers (lamina I and II) of the dorsal horn of cats made decerebrate and in which the spinal cord had been transected. Single unit activity was recorded using extracellular microelectrode recording techniques. Neuronal activity was evoked by the presentation of noxious radiant heat (51° C) to the cells' receptive fields on the hind paws. Evoked activity of WDR neurons was monitored, both before and after the spinal administration of either 10 µg (n = 9) or 25 µg (n = 10) of fentanyl. HT neurons were examined before and after either 25 µg (n = 7) or 50 µg (n = 7) of spinally administered fentanyl. In all cases 31 min after fentanyl administration naloxone (0.1 mg) was administered intravenously (iv), and its antagonistic effect on the fentanyl suppression was determined. All doses of fentanyl tested suppressed both spontaneous and evoked activity of both types of neurons. Within 30 minutes 10 and 25 µg of fentanyl reduced the mean evoked activity of WDR neurons to 61% and 19% of control values, respectively, and 25 and 50 µg of fentanyl reduced the mean evoked activity of HT neurons to 70% and 47% of control values, respectively. Naloxone reversed the suppression seen in all cells studied. The results of the present study demonstrate that HT neurons are significantly less suppressed by the spinal administration of fentanyl than WDR neurons located in the same superficial layers of the dorsal horn. This difference in the sensitivity of HT neurons and WDR neurons to fentanyl suppression may reflect an important difference in the mechanisms of spinal opiate analgesia. (Key words: Analgesics; fentanyl. Spinal cord: high threshold neuron; wide dynamic range neuron.)

THE DORSAL HORN of the spinal cord is the site of the first synaptic relay in afferent pathways and has been recognized as an important site for modifying the transmission of noxious input. Neurons in the dorsal horn thought to be responsible for relaying afferent pain information to higher centers are wide dynamic range (WDR) neurons and high threshold (HT) neurons.<sup>1</sup> WDR neurons have been reported in Rexed laminae I, II, IV, V, and VI, and HT neurons in laminae I and II.<sup>1-7</sup> The superficial layers of the dorsal horn (laminae I and II) also have specific opiate receptors.<sup>8,9</sup>

Recent interest has centered on the importance of spinal pharmacology in relation to the blocking of pain transmission. It is generally agreed that opiates suppress the evoked activity of WDR neurons in the deep layers (laminae IV, V, and VI) of the dorsal horn,<sup>10-15</sup> but the effect of opiates on the activity of WDR neurons and HT neurons in the superficial layers is controversial.<sup>10,16,17</sup>

The present study was designed to examine the effects of spinally administered opioids on the activity of both HT and WDR neurons in the superficial layers of the dorsal horn. In contrast to our earlier study, we have examined the effects of the more lipid-soluble fentanyl administered intrathecally rather than intravenously (iv) to better understand the mechanism of action of intrathecally administered opioids. The administration of a lipid-soluble drug decreased concerns about the ability of the drug to quickly gain access to the dorsal horn. Results from this study will also enable us to compare the opiate effect on superficial WDR neurons with previously reported effects on the WDR neurons located deeper in the lamina propria.<sup>13</sup>

### Materials and Methods

This study was approved by the Yale Animal Care and Use Committee, and appropriate guidelines for the humane care and use of animals were observed during all aspects of this study. Thirty-three cats of both sexes (weighing 3.2-4.7 kg) were used. During general anesthesia (halothane-nitrous oxide-oxygen) a jugular vein and a carotid artery were catheterized to provide routes for (iv) fluid and drug administration and monitoring of arterial blood pressure. A tracheostomy was performed, and the animals were paralyzed with pancuronium bromide (0.1-0.15 mg · kg<sup>-1</sup> · h<sup>-1</sup>) and mechanically ventilated. End-tidal CO<sub>2</sub> was monitored and maintained between 3.0% and 4.0%. The animals were then placed in a stereotaxic apparatus and rendered decerebrate by placing bilateral electrolytic lesions in the midbrain reticular formation. The spinal cord was transected at T-11 or T-12 (spinal cord transection allowed us to monitor fentanyl effects on the dorsal horn of the spinal cord in the absence of descending supraspinal influences). A laminectomy was then performed at L4-6. The dura was cut and reflected, and the arachnoid was removed at the site of microelectrode placement. The

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TABLE 1. Modalities of WDR and HT Neurons

Type of Stimuli	WDR Neuron	HT Neuron
Air puff	+	-
Brushing	+ ~ ++	-
Pinch	++ ~ +++	+ ~ ++
Radiant heat (51° C)	++ ~ +++	+ ~ ++
Cooling (ethyl-chloride)	++ ~ +++	-

+ = response; - = no response.

spinal cord was bathed with 37° C physiologic saline. Esophageal temperature was maintained between 37° C and 38° C by a servocontrolled heating lamp. After surgical preparation and decerebration anesthesia was discontinued. This sequence allowed us to ensure that the animals were pain-free during both surgical preparation and neurophysiologic recordings, and yet permitted us to study the effects of fentanyl in a preparation that was drug-free (except for the neuromuscular blocking agents). After cessation of general anesthesia we allowed 2 h to pass before recording the neuronal activity.

Tungsten microelectrodes were advanced into the dorsal horn of the spinal cord, while receptive fields on the ipsilateral hind paw were stimulated with air puffs, brushing, and pressure. Maximum penetration of the microelectrodes was no deeper than 1500  $\mu$ m from the surface of the spinal cord. When the activity of a single cell was encountered, amplitude discrimination was used to record single unit activity above the baseline electrical activity.

Upon isolation of a single WDR or HT neuron, radiant heat (51° C), cooling of the skin (ethyl-chloride spray), and pinching with nonserrated forceps were ap-

plied to the cell's receptive field. If the response profile indicated a WDR or HT neuron, as determined by the criteria shown in table 1, control studies were performed in which both spontaneous and noxiously evoked activity were recorded. Noxiously evoked activity was produced by the presentation of a 51° C radiant heat stimulus for 8 s to the receptive field on the hind paw. In all cases an attempt was made to position the stimulator to maximize receptive field stimulation (*i.e.*, the center of the radiant heat stimulus was focused on the center of the most excitable area of the receptive field). Following control studies the saline over the spinal cord was carefully removed, and the fentanyl-saline solution (0.5 ml, 37° C) was gently applied onto the spinal cord (10 or 25  $\mu$ g for WDR neurons, 25 or 50  $\mu$ g for HT neurons). The 10- and 25- $\mu$ g doses for WDR neurons were chosen because they had been shown to be capable of suppressing WDR neurons in deeper laminae.<sup>13</sup> Higher doses for HT neurons were employed based on preliminary results of this study. The solution placed on the spinal cord contained no preservatives. After application of fentanyl spontaneous and noxiously evoked activity was measured every 3 min for 30 min. At 31 min 0.1 mg iv of naloxone was given to 28 cats. In two cats HT neuron activity was observed for 60 min and 120 min, after which naloxone was given. Only one neuron with one dose of drug was studied in each animal to avoid cumulative effects of the drugs. At the end of the experiment, before the electrode was removed from the recording site, electrolytic lesions were made in the dorsal horn by passing 20–30  $\mu$ A for 30 s through the recording microelectrode. The position of the tip of the microelectrode was later examined histologically. At the end of each experiment animals were killed by anesthetic overdose.

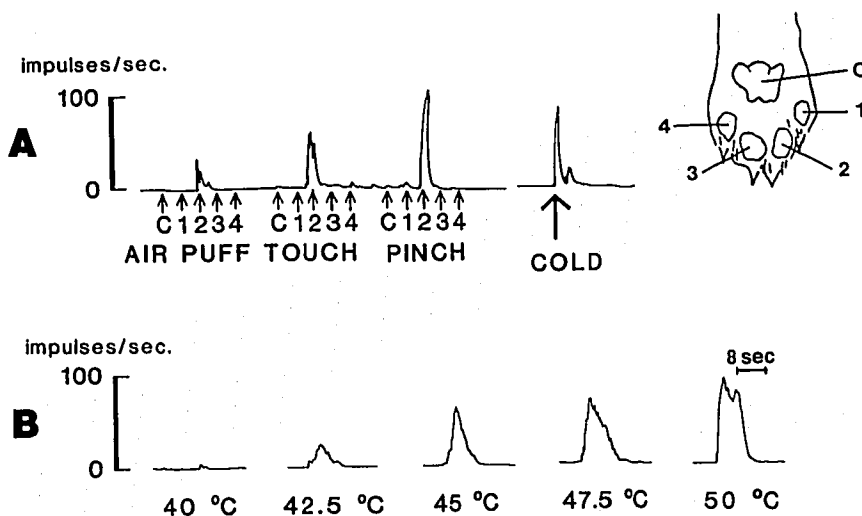
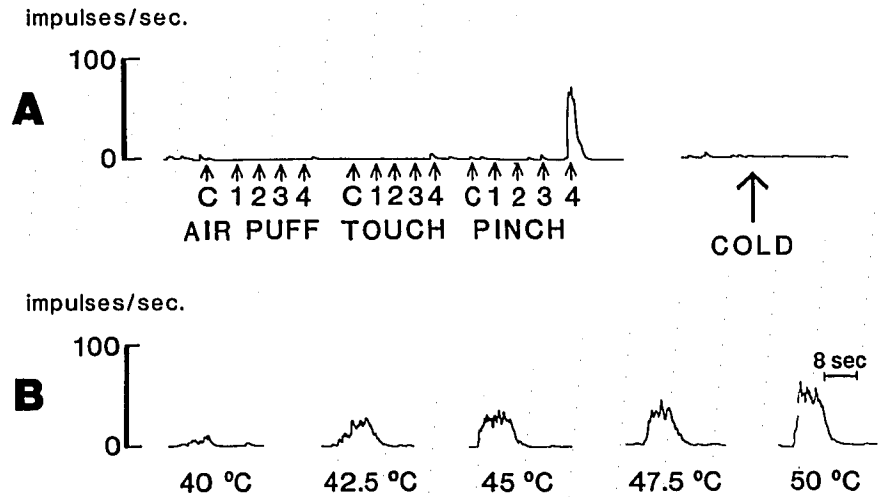


FIG. 1. An example of a response profile of a WDR neuron. A. WDR neuronal responses to various stimuli are shown (numbers and C represent the site of stimulation on the foot). The response increases with increasing intensity of stimuli. Note that the spontaneous discharge rate is low and the receptive field is small (only pad 2 stimulation evoked the activity). B. WDR neuronal responses to increasing intensity of radiant heat. The response became greater as the temperature was increased. (For data in this study only one thermal stimulus was used—51° C.)

FIG. 2. An example of a response profile of an HT neuron. A. HT neuronal responses to various stimuli. The HT neuron only responded to noxious pinch on pad 4 (see fig. 1 for description of stimulation sites). Note that the spontaneous discharge rate is low. B. HT neuronal responses to radiant heat on pad 4. The response became greater as the temperature was increased.



All data collected were analyzed on-line as well as off-line by a PDP® 11/40 computer. Spontaneous activity was averaged for 30 s prior to stimulus presentation. The evoked discharge frequency was observed during the time that the radiant heat stimulus caused the firing rate to rise above the average spontaneous rate. Student's paired and unpaired t-tests were used for statistical analysis. Differences were considered to be significant if *P* values were less than 0.05.

**Results**

Data were obtained from 33 neurons. Fourteen were classified as HT neurons, and the remaining 19 were classified as WDR neurons, based upon response properties shown in table 1. Examples of response properties of WDR neurons and HT neurons are shown in figures 1 and 2. The location of the neurons was established by the depth of the microelectrode tips. The depth of the neurons recorded was between 682 and 1,390 μm from the dorsal surface of the cord. Although spontaneous discharge rates of all neurons were low (average impulses/s ≈ 1.1), they were increased after 51° C radi-

ant heat stimulation. Successful markings of about one-half of the recording locations were verified to be in the superficial layers (laminae I and II) of the dorsal horn.

**FENTANYL EFFECTS ON SPONTANEOUS ACTIVITY OF WDR AND HT NEURONS**

Table 2 shows the effects of fentanyl on spontaneous activity of neurons studied and subsequent naloxone reversal of those effects. All doses produced significant suppression of mean spontaneous activity. After 30 min mean spontaneous activity of WDR neurons was reduced to 58.3% and 21.0% of control values by 10 and 25 μg of fentanyl, respectively, and that of HT neurons was reduced to 42.9% and 22.9% of control values by 25 and 50 μg of fentanyl, respectively. Spontaneous rates ranged from 0 to 19.2 impulses/s for WDR neurons and from 0 to 14.4 impulses/s for HT neurons. The 25-μg dose produced significantly greater suppression of the spontaneous activity of the WDR neurons than of the HT neurons. Naloxone, 0.1 mg iv, produced reversal of fentanyl suppression, although the

TABLE 2. The Effects of Fentanyl on Spontaneous Activity of WDR and HT Neurons

	Control	15 min	30 min	Naloxone‡
WDR neuron				
10 μg (n = 9)	100	69.7 ± 11.1	58.3 ± 8.7*	114.1 ± 22.9
25 μg (n = 9)	100	23.4 ± 8.8	21.0 ± 10.2†‡	102.1 ± 37.1
HT neuron				
25 μg (n = 7)	100	51.6 ± 11.3	42.9 ± 12.4*†‡	91.6 ± 12.7
50 μg (n = 5)	100	44.9 ± 19.9	22.9 ± 10.4†‡	67.5 ± 40.2

Values are given as mean percent of control ± SE.  
\* *P* < 0.05, significantly different from control.  
† *P* < 0.01, significantly different from control.

‡ *P* < 0.05, significantly different, WDR versus HT.  
§ Administered 31 minutes after fentanyl.

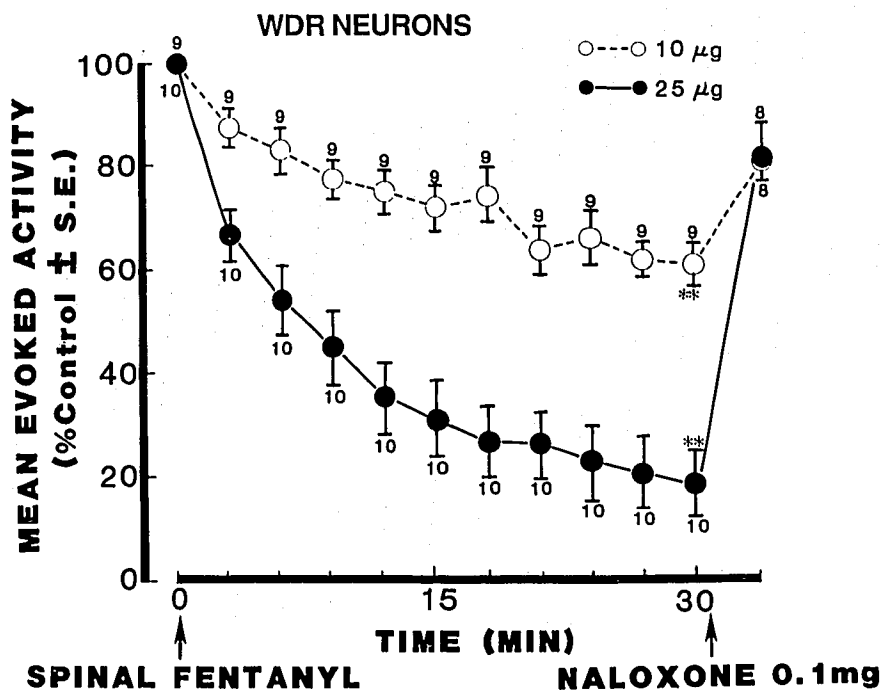


FIG. 3. Effects of two doses of fentanyl on the mean evoked activity of WDR neurons elicited by an 8-s application of a 51° C radiant heat stimulus to their receptive fields. Both 10 µg (dashed line, n = 9) and 25 µg (solid line, n = 10) of fentanyl produced a significant reduction of evoked activity of WDR neurons at 30 minutes. The suppression was reversed by naloxone, 0.1 mg iv. \*\* $P < 0.01$ , compared with control values.

effect on the 50-µg dose was not as great as that produced on the lower doses.

#### FENTANYL EFFECTS ON EVOKED ACTIVITY OF WDR NEURONS

Both the 10 and 25 µg doses of spinally administered fentanyl affected the noxiously evoked activity of WDR neurons (fig. 3). Figure 3 contains plots indicating the effects of the two doses of fentanyl on the mean evoked activity of WDR neurons and subsequent naloxone reversal of those effects. Both the 10- and 25-µg doses produced a significant reduction of the mean evoked activity at 30 min, to 61% and 19%, respectively. Those effects were reversed by 0.1 mg of naloxone iv.

#### FENTANYL EFFECTS ON EVOKED ACTIVITY OF HT NEURONS

Spinally administered fentanyl, 25 and 50 µg, significantly suppressed the noxiously evoked activity of HT neurons (fig. 4). A comparison of the degree of suppression produced on the two neuron types indicates that the HT neurons were significantly less suppressed than were the WDR neurons. The 25-µg dose of fentanyl produced a statistically ( $P < 0.01$ ) greater suppression of noxiously evoked activity of the WDR neurons than of HT neurons. Fifty micrograms of fentanyl did not produce a significantly greater degree of suppression of the HT neurons than did 10 µg when employed with WDR neurons. The fentanyl suppres-

sion of HT neurons was reversed by 0.1 mg of naloxone iv.

The neuronal activity was observed for 60 min in one neuron and 120 min in another neuron after the application of 25 µg fentanyl, and no recovery was observed during that time period. In one of the 14 neurons excitation (by 12%) was briefly seen shortly after the drug application (6 min).

#### Discussion

There have been many anatomic and physiologic studies concerning the marginal layer of the dorsal horn since Christensen and Perl demonstrated that the marginal layer (rexed lamina I) represents a specialized sensory nucleus containing neurons important for nociception.<sup>2</sup> Several studies have demonstrated nociceptive HT neurons in laminae I and II,<sup>2,3,18</sup> and others have reported the presence of WDR neurons in the superficial layers of the dorsal horn in rats,<sup>4</sup> cats,<sup>5,6</sup> and primate,<sup>7</sup> in addition to the presence of WDR neurons in the deep layers of the dorsal horn.<sup>1</sup> Thus, WDR neurons exist both in superficial and deep layers of dorsal horn, whereas HT neurons are predominantly in laminae I and II.

WDR neurons respond to both innocuous and noxious mechanical stimuli, whereas HT neurons respond almost exclusively to noxious stimulation. The heat threshold of WDR neurons is between 35° C and 50°

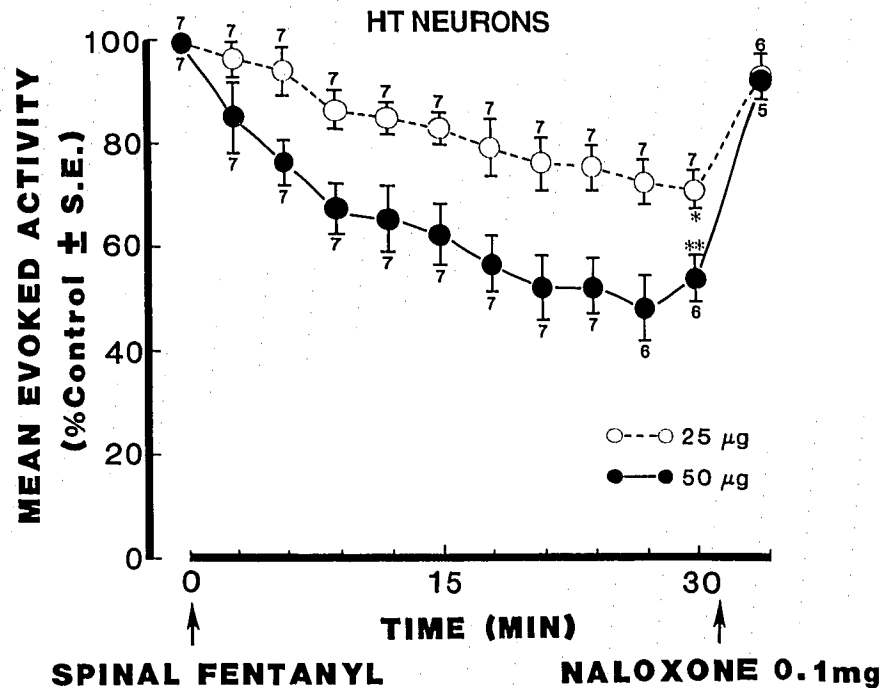


FIG. 4. Effects of two doses of fentanyl on the mean evoked activity of HT neurons. Both 25 µg (dashed line, n = 7) and 50 µg (solid line, n = 7) of fentanyl produced a significant suppression at 30 minutes. Drug effects on HT neurons were less suppressive and of slower onset when compared with WDR neurons. \* $P < 0.02$ , \*\* $P < 0.01$ , compared with control values.

C,<sup>19</sup> whereas that of HT neurons is greater than 40° C.<sup>2,19</sup> WDR neurons respond also to cold stimulation, whereas HT neurons do not.<sup>2,3</sup> Both types of neurons in the superficial layers have low rates of spontaneous activity, are excited by small afferent fibers of peripheral nerves (A delta and/or C fibers) and have projections to the thalamus.<sup>7,20</sup> For this reason both HT neurons and WDR neurons are considered essential elements of pain transmission systems.

Because we still do not know what neurons must be suppressed in order to produce spinal analgesia, it is of interest to know the effects of opiates on the superficially located nociceptive neurons. Kitahata *et al.* demonstrated that the spontaneous and mechanically evoked activity of HT neurons in lamina I are suppressed by iv administered morphine (0.5–2.0 mg/kg) in cats.<sup>10</sup> Woolf *et al.* reported that morphine (5 mg/kg, iv) produced variable changes in the C-evoked activity of units recorded within lamina II in rats, some units showing excitation, some inhibition, and some an alteration in the timing and pattern of the C-evoked activity.<sup>16</sup> Dickensen *et al.* also reported that higher concentrations of morphine (3–150 µg, intrathecally) produced suppression of C-fiber and pinch evoked activity of neurons in the superficial layers, although lower concentrations of morphine (1.5–2.5 µg, intrathecally) produced excitation.<sup>17</sup> In the present study all neurons were suppressed by fentanyl in a dose-dependent manner except for one neuron excited (12% of control) during the initial 3–6 min.

The importance of the findings of the present study is that the degree of suppression of HT neurons is significantly less than that of WDR neurons located in the superficial layers of the dorsal horn. This difference in sensitivity agrees with the findings of Cervero *et al.* who showed that descending inhibition of HT neurons was less than that of WDR neurons in the superficial layers.<sup>7</sup> Thus, HT neurons appear to be less sensitive to factors that are known to inhibit noxiously evoked activity.

As to the functional difference between these two types of neurons, Price has indicated that discriminative aspects of pain may be signaled by the combined output of WDR neurons and HT neurons, whereas the function of the latter group of neurons may be to provide additional information about the quality and spatial localization of noxious stimuli.<sup>1</sup> Thus, our results may explain a general clinical impression that opiate analgesia produces significant antinociception yet maintains appreciation of location and quality of nociception.

The effects of the same doses of fentanyl (10 µg and 25 µg) on WDR neurons in the deep layers (laminae IV, V, and VI) were previously reported from our laboratory.<sup>13</sup> Comparing results from both the superficial and deep layers of the dorsal horn, it is clear that the rate of onset and the degree of suppression by fentanyl are similar. Duggan *et al.*<sup>21</sup> demonstrated that morphine administered iontophoretically to the substantia gelatinosa suppresses nociceptive neurons in laminae IV and V, and they postulated that these effects might have occurred through dendritic processes.<sup>14</sup> They based

their argument on the fact that the superficial layers are high in opiate binding sites.<sup>8,9</sup> The results of the present study support this hypothesis.

In summary, the results of the present study demonstrate that intrathecally administered fentanyl suppresses the noxiously evoked activity of both HT neurons and WDR neurons located in the superficial layers of the dorsal horn. The degree of the suppression of the activity of HT neurons was less than that of WDR neurons.

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