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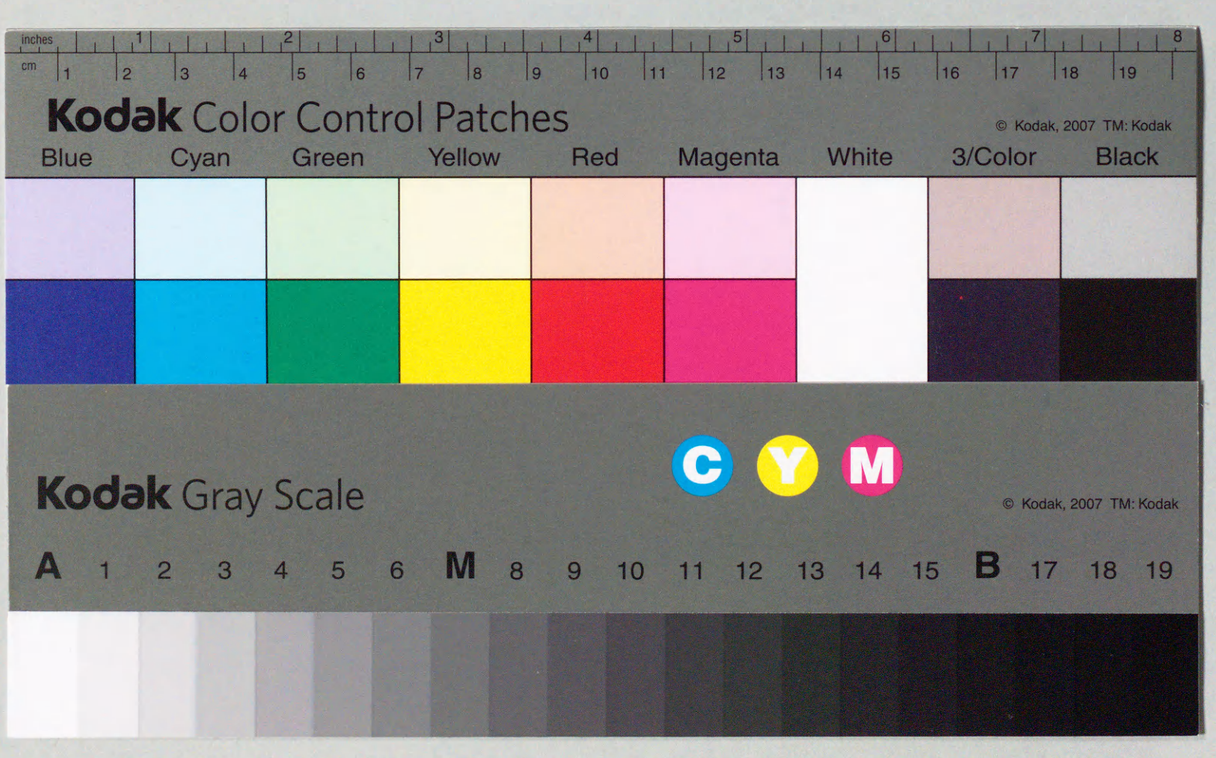

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Tonic 5-HT modulation of spinal dorsal horn neuron activity evoked by both noxious and non-noxious stimuli: a source of neuronal plasticity

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Summary The influence of tonic serotonergic modulation on the responses of spinal dorsal horn neurons to natural peripheral stimulation was examined in physiologically intact, awake, drug-free cats. Systemically administered methysergide (maximum cumulative dose 2 mg/kg) caused significant changes in responses of some dorsal horn neurons to both mildly noxious and non-noxious stimulation. Individual changes provide evidence, in this model, for tonic 5-HT modulation of many aspects of sensory transmission at the level of the spinal cord. Taken together, the changes demonstrate the significant degree of plasticity that exists for some spinal dorsal horn neurons. It is clear that the plasticity of some spinal dorsal horn neurons allows for a much broader response profile than would be apparent under the restricted circumstances of a normal neurophysiologic study. Removal of tonic inhibition on responses to noxious stimuli may be an aspect of neuronal plasticity that functions to provide an immediate change in the way that the nervous system responds to a noxious stimulus.

Key words: Spinal dorsal horn; Tonic modulation; Plasticity; Serotonin

Introduction

As our understanding of the physiology and pharmacology of spinal sensory transmission has developed, classification schemes have been employed in an attempt to effectively categorize neuronal types within the spinal dorsal horn [e.g., 6,8,22,27,31,38,42,43,46,51–53]. These schemes have typically defined a limited response profile for a given cell type (e.g., low threshold, high threshold, wide dynamic range (WDR)), in effect producing the impression that each spinal dorsal horn neuron is likely to fit into a single category based upon a constant response profile. Changes in these response profiles have usually only been assumed to occur as a result of phasic modulation. In contrast to the image of well-defined response profiles, more attention is now being paid to evidence that some spinal dorsal horn neurons may have their responses readily adapted to circumstances at hand [e.g., 7,11,17–19,37,41,54].

Modulation of spinal dorsal horn neurons is a well-established event. Serotonin (5-HT) has been shown to have an important role in the modulation of spinal sensory transmission. Intrathecal administration of 5-HT produces a dose-dependent, reversible behavioral analgesia in several species [e.g., 3,49,55]. There is evidence of 5-HT inhibition of both noxious and non-noxious input to spinal dorsal horn neurons in the cat [2,28,31,33,49]. Following development of the

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technique to conduct extracellular single neuron recordings from the spinal dorsal horn in intact, awake, drug-free cats [13], we observed evidence of tonic modulation of dorsal horn neuronal responses [14,16]. We hypothesized that a component of the tonic modulation may employ 5-HT as a neurotransmitter.

The purpose of this study was to test for tonic 5-HT modulation of spinal dorsal horn neurons. By comparing neuronal response properties before and after the systemic administration of the non-selective 5-HT antagonist methysergide, we wanted also to determine if the tonic modulation, should it exist, was selective for noxious or non-noxious input.

Methods

This research protocol was approved by the Yale Animal Care and Use Committee. Electrical activity of single spinal dorsal horn neurons was recorded extracellularly from physiologically intact, awake, drug-free cats. A detailed description of the recording technique has been reported previously [13]. Animals were trained to sit quietly in a restraint box. Under general anesthesia and using sterile technique, a recording chamber was surgically attached to the animal's vertebral column over a 6×12 mm opening in the bone (dura mater remains intact) that was made over the lumbar enlargement. The chamber provided a window to the bone opening through which recording microelectrodes could be positioned in the dorsal horn of the spinal cord. An external jugular vein catheter was implanted and externalized on the head. Following a minimum of 2 weeks of recovery after chamber implantation, electrophysiological studies were begun.

For each experiment, a tungsten microelectrode (impedance 10 MΩ) was inserted through the dura into the spinal cord. Dural penetration produced no obvious animal discomfort. While the electrode was advanced in micron steps, likely receptive field areas were lightly brushed. Following isolation of a single cell, the receptive field of the neuron was mapped on the surface of the skin, and the most sensitive area of the receptive field was stimulated by brushing, pinching, and heating to evaluate the neuron's response properties. The border of the receptive field was determined by observing neuronal response to light rubbing or brushing of the skin. Pinch stimuli were produced with forceps that were modified so that a constant area (3 mm in diameter) was stimulated each time. The forceps were also instrumented with strain gauges to monitor stimulus intensity. Pinch was increased in intensity until a reflex withdrawal was initiated by the animal. Withdrawal usually occurred to stimuli in the range considered mildly noxious by the experimenters. Pinch stimuli were separated by a 2 min interstimulus interval. Heat stimuli of 40°C, 43°C, 45°C, and 47°C were delivered for a maximum of 8 sec either by focusing a radiant heat source or putting a contact thermal stimulator on the most sensitive part of the receptive field. Thermal stimuli were separated by a 2 min interstimulus interval and terminated upon reflex withdrawal by the animal. Radiant heating involved feedback control from a thermocouple glued to the center of the receptive field.

Following baseline studies (spontaneous activity, receptive field mapping, brushing, pinching, heating), the effects of intravenously administered methysergide were observed. An initial dose (0.05–0.25 mg/kg) of methysergide was administered and additional doses were, at times, given to a maximum cumulative dose of 2 mg/kg. Neuronal activity was typically recorded 5 min after each administration of methysergide and dosing typically occurred every 30 min. In one neuron, recovery from methysergide was recorded for 150 min and re-administration of methysergide was performed. In 5 control studies, the time course of the neuronal activity with no administration of methysergide was observed for periods of up to 120 min.

Spontaneous firing rates were determined by averaging the activity over two 10–20 sec periods when there was no contact with the receptive field. The brush-evoked activity was determined by averaging events per brush stroke. For pinch-evoked activity, the slope of the evoked activity and the slope of the stimulus intensity were determined and the ratio of these two slopes was analyzed (see Fig. 6). The outlines of receptive fields that had been mapped on the skin surface with non-toxic paints were transferred to tracing paper, digitized and used to determine receptive field areas.

All data were recorded on magnetic tape during experiments and later stored and analyzed on a personal computer.

Statistical analysis of the data was carried out using Student's t test and chi-square analysis. P values of less than 0.05 were accepted as significant.

Results

Data for this study were obtained from 5 cats. This report includes data from 33 neurons with receptive fields on the feet, legs, and hips of the animals. Twenty-nine neurons were utilized for the methysergide study. One of those 29 prior to methysergide administration and 4 additional neurons were used for time course control studies with no administration of methysergide.

Spontaneous neuronal activity

The spontaneous discharge rate of the 20 neurons that had some aspect of their response profile influenced by methysergide varied from 0 to 4 events per 0.5 sec. Most neurons had spontaneous discharge rates of less than 0.5 events/0.5 sec before and after methysergide administration. Table I summarizes the effect of methysergide on spontaneous activity. The mean rate was 0.44 ± 0.62 ($\bar{X} \pm$ S.D.) events/0.5 sec prior to methysergide administration. Following methysergide administration (0.25 mg/kg) the mean rate was 0.54 ± 0.84 events/0.5 sec. There was no significant difference between two mean rates (paired Student t test). Chi-square analysis revealed no significant change in the number of neurons with rates greater or less than 0.25 events/0.5 sec following methysergide administration.

TABLE I

DISTRIBUTION OF SPONTANEOUS FIRING FREQUENCIES BEFORE AND AFTER METHYSERGIDE (0.25 mg/kg)

Events/0.5 sec	Control	Post methysergide
0 –0.25	10	9
0.26–0.5	6	7
0.51–0.75	1	1
0.76–1.0	1	1
1.1 –2.0	1	0
2.1 –3.0	1	1
3.1 –4.0	0	1
$\bar{X} \pm$ S.D.	0.44 ± 0.62	0.54 ± 0.84

Non-noxious stimulation

We were able to evaluate neuronal response to non-noxious stimuli in 3 different ways: (1) response to brushing; (2) response to initial forceps contacts or contact by the thermal stimulator; (3) change in receptive field size.

Response to brushing. The effects of methysergide on neuronal responses to brushing were variable. Some neurons showed an increased response to brushing, some a decrease and some no change following methysergide administration. Table II summarizes the change in response to brushing for the 12 neurons with a methysergide-induced increase in receptive field area. We considered these neurons to be the ones most likely to have their response to brushing influenced by methysergide.

TABLE II

RESPONSE TO BRUSH FOR NEURONS WITH INCREASED RECEPTIVE FIELDS

+ or – indicates a greater than 20% change, numbers in parentheses indicate the % change.

Neuron	Methysergide (mg/kg)				
	0.05	0.15	0.25	0.5	1.0
1				+ (139)	
2			– (25)	– (67)	
3			– (71)		+ (127)
4			– (46)	0 (81)	
5			+ (154)	+ (126)	
6	0 (110)	+ (161)	– (69)	– (77)	
7			– (77)	+ (144)	
8	– (65)	– (36)			
9			+ (143)	+ (176)	
10	+ (121)	0	0	0	
11	0	– (78)			
12	+ (155)	+ (178)	+ (139)		

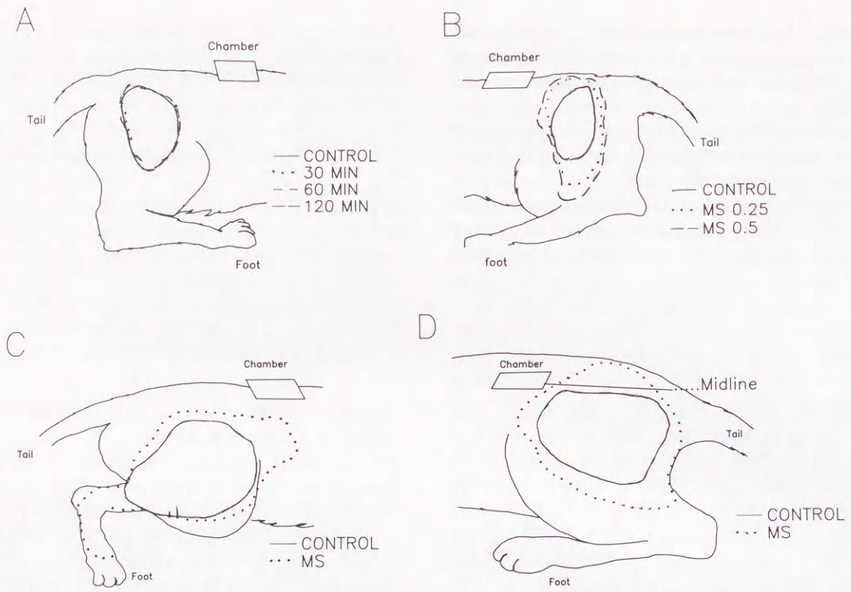

Fig. 1. Methysergide-induced change in receptive field area. A: limits of a spinal dorsal horn neuron's receptive field mapped at 3 different times during a 120 min observation period. The limits define the extent of the area from which spike activity could be elicited by gentle rubbing. B: dose-dependent increase in a neuron's receptive field following systemic methysergide administration. C: note the migration of this receptive field down the leg following the administration of methysergide (0.25 mg/kg). D: this is the only cell in this study in which migration across the midline was observed following methysergide administration (2.0 mg/kg).

Activity per brush stroke had to increase or decrease by 20% in order to be considered a change. According to that analysis, the changes were not constant. Five of 12 neurons had an increase in activity, 2 neurons had a decrease, 3 neurons showed both changes, depending upon the dose of methysergide, and 2 neurons had no change. The largest changes were 78% increase and a 75% decrease.

Response to initial forceps contact. As seen in Fig. 4, response to initial contact with the forceps was also enhanced for some neurons following methysergide administration. This change, however, was not consistently seen when initial contact of the forceps or contact thermal stimulator was observed. In some instances even though the neuronal response to higher intensities of stimulation may have been enhanced by methysergide the response to initial contact was unchanged.

Change of receptive field size. The change in receptive field area that was sensitive to light stroking was studied in 22 neurons with receptive fields that could be accurately mapped. Twelve neurons, including some (n = 3) that demonstrated increased sensitivity to noxious stimulation, had more than a 20% increase in receptive field area following methysergide administration. In contrast, 5 control neurons were observed for periods of up to 120 min with little change (0–16%) in receptive field area.

Fig. 1A demonstrates the small variation in receptive field area of a control neuron over a period of 120 min. In contrast, Fig. 1B–D demonstrate the types of change that were observed

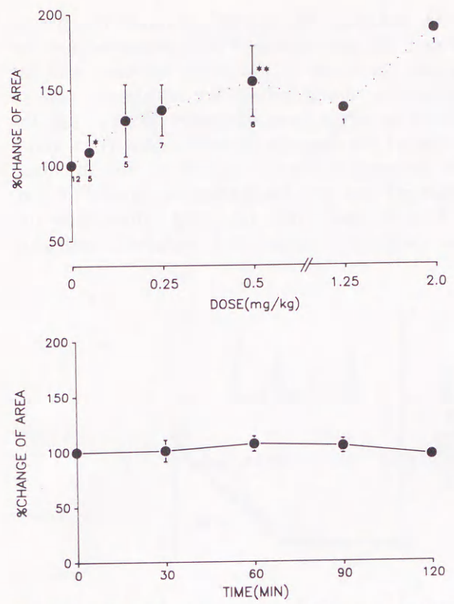

Fig. 2. Dose-dependent increase in receptive field area. Top: the mean % change ± S.E. for the 12 neurons that had at least a 20% increase in receptive field area is shown. Because of cumulative dosing the sample size is not the same at all points. The change in area at the lowest dose (0.15 mg/kg) was significantly different from control (*). The change in area at the 0.5 mg/kg dose was significantly different from the 0.15 mg/kg dose (**). Doses above 0.5 mg/kg caused the animals to become agitated. Bottom: control studies over 120 min (n = 5) revealed no significant change in receptive field area in the absence of methysergide administration.

following methysergide administration. No reduction in receptive field area was observed following methysergide, if a change occurred it was always an increase in area. Fig. 1D is especially interesting because the increase extended across the midline.

Fig. 2 (top) demonstrates an apparent dose-dependent increase in receptive field area following methysergide administration for the 12 neurons that had their receptive fields increased by at least 20%. The small sample size at the 2 highest doses was due to those doses causing agitation in the animals that made it difficult to carry out the studies. The dose-dependent increase in receptive field area contrasts sharply with the constant area over time for the 5 control neurons as shown in the bottom of Fig. 2.

We attempted to determine if there was a directional selectivity associated with the change of receptive field area produced by methysergide. In Fig. 3, the directional change is indicated as a percent change of the distance from the most sensitive area (hot spot) of the receptive field to the outer limit of the receptive field in the 4 indicated directions for the 12 neurons shown in Fig. 2. The change in area was observed in all directions but the maximum extension indicated that the receptive fields tended to move the furthest in the distal direction.

Noxious stimulation

Response to pinch stimulation. As shown in Table III, prior to methysergide administration, 28 of the neurons in this study were classified as low threshold (LT) neurons. This classification was based upon the fact that the maximum firing frequency was elicited by light touch with no increase in activity when pinch or heat caused animal withdrawal. For 6 of the 28 LT neurons, a response to pinch (P) stimulation was remarkably increased within 5 min of systemic methysergide administration resulting in those 6 neurons being reclassified as WDR neurons. For 4 of the 6 neurons, a response to heat (H) was also present after methysergide. An example of a methysergide-induced change in response to pinch is shown

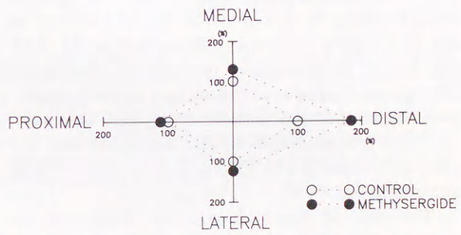

Fig. 3. Directional component of the maximum change in receptive field area. Although receptive field areas were observed to change in all directions, movement away from the 'hot spot' tended to be the greatest in the distal direction.

TABLE III

NUMBER OF LT AND WDR NEURONS BEFORE AND AFTER METHYSERGIDE

P = pinch stimulus; H = heat stimulus.

Control	Post methysergide	
LT 28	LT	21
	LT (?)	1
	WDR (P,H)	4
	WDR (P)	2
WDR 1	WDR	1

in Fig. 4. Prior to methysergide administration the neuron demonstrated an on- and off-response associated with initial contact by the forceps and removal of the forceps from skin as well as two transient responses to the rate of change of pinch that occurred at approximately 11 and 15 sec. Those responses were much greater than any other evoked activity during pinch. Following methysergide administration (0.15 mg/kg cumulative dose), the neuron demonstrated increased activity that was much greater than that seen during control and that increased gradually with increasing pinch intensity. This increased activity was seen in spite of the fact that the maximum intensity of pinch was slightly less than that during control. In both instances the pinch was terminated when the animal began to withdraw from the stimulus.

We were able to study recovery in one neuron that had its pinch response enhanced by methysergide. As shown in Fig. 5, this case demonstrated that the increase in the pinch-evoked activity following methysergide administration spontaneously returned to control levels 150 min after the cumulative systemic administration of 0.15 mg/kg of methysergide. In addition, the same change was reproduced following a second administration of methysergide (0.15 mg/kg). This neuron also demonstrated similar reversible and reproducible changes in its response to contact heat stimulation at 47°C.

The use of pinch stimulation produced by forceps that are instrumented with strain gauges has allowed us to observe an additional effect of methysergide on spinal dorsal horn neurons. It is apparent in Figs. 4 and 5 that methysergide not only enhanced the overall neuronal response to pinch but also increased the correspondence between the slope of the pinch intensity and the slope of neuronal activity. We calculated a ratio of the slope of the firing frequency (slope E) and the slope of the intensity of pinch (slope I) as shown in the insert in Fig. 6. Fig. 6 shows how the ratios changed over time for the neuron shown in Fig. 5. The ratio significantly increased following the initial dose and remained near a value of 1 until after

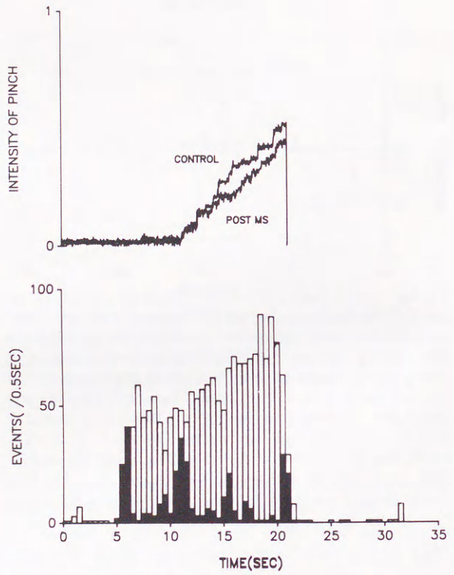

Fig. 4. Methysergide (0.15 mg/kg)-induced pinch response. Top: traces of the voltage output from strain gauges mounted on the forceps. A pinch intensity of 1 is equivalent to 750 g. (Note: the maximum pinch intensity during control was slightly greater than that achieved following methysergide (MS) administration.) Bottom: histogram of neuronal response to the pinch stimulus displayed in 0.5 sec bins. Filled bars, control; open bars, post methysergide. 0–5 sec: spontaneous activity in the absence of contact with the receptive field. 5–12 sec: initial contact of the forceps with the skin. Note that during control a rapid adaptation to contact was present. Following methysergide that adaptation was not seen. 12–21 sec: pinch intensity increased until the animal withdrew and the stimulus was terminated. Note the methysergide-induced response to pinch (open bars), that was not seen during control.

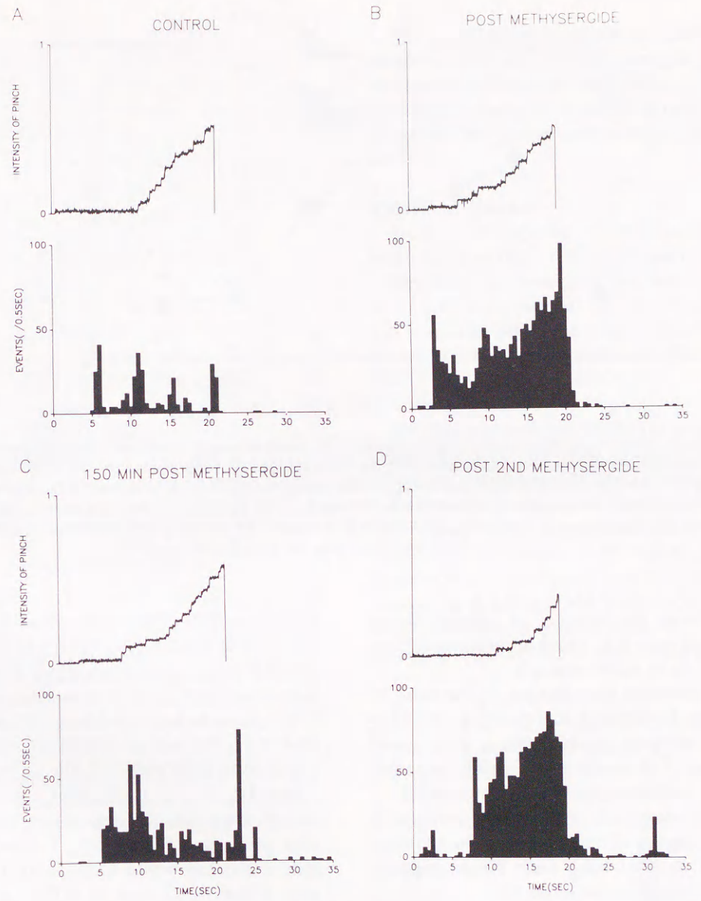

Fig. 5. Time course of methysergide-induced pinch response. A: control response to pinch. 0–5 sec: spontaneous activity with no contact with the receptive field. During control this neuron responded well to transient initial contact with the receptive field at 5 sec, initiation of pinch at 12 sec, and removal of the stimulus at 21 sec. B: response of the neuron shown in A to a similar stimulus but after a cumulative dose of 0.15 mg/kg of methysergide. Initial contact occurred at approximately 3 sec and pinch began at approximately 7 sec. C: neuronal response to pinch returning toward control values 150 min after methysergide. D: 30 min after C. An additional dose of 0.15 mg/kg of methysergide again induced a response to pinch stimulation (initial contact at 7.5 sec) but a stimulus that was of smaller intensity than that used in control.

the 90 min time point. The second administration produced a similar increase in the ratio of the 2 slopes. A ratio of 1 suggests a close correspondence between stimulus intensity and neuronal activity. The data were analyzed in this fashion because the rate of pinch varied from trial to trial, making more rigourous mathematical analysis invalid. Values in Figs. 6 and 7 and between 0 and

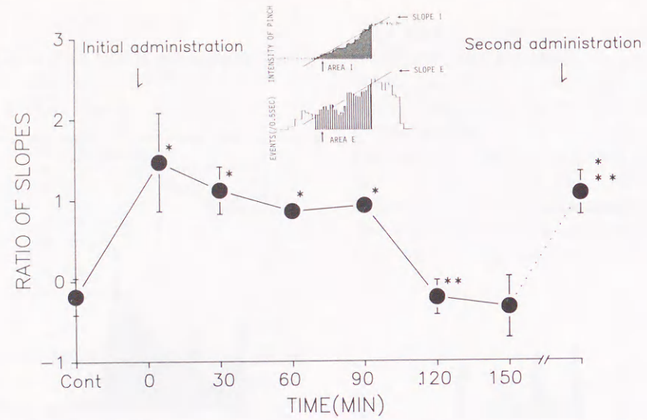

Fig. 6. Methysergide effect on correspondence between stimulus and response intensity functions. Inset: schematic indicating how slopes I and E were determined. Slope I was calculated from the start until the stop of the pinch stimulus. Slope E was determined for the neuronal activity during the same period of time as slope I. The ratio of the slopes (E/I) is plotted for the neuron shown in Fig. 5. During control there was no correspondence between slopes, 0.15 mg/kg of methysergide resulted in a close correspondence between the two slopes (i.e., a value close to 1). * Significantly different from control. ** Significantly different from the 90 min time point. *** Significantly different from the 150 min time point.

−1 resulted from the presence of negative slopes for some event data (i.e., rapid adaptation following an initial burst upon contact).

Fig. 7 summarizes the changes of the ratio of slope E/slope I following methysergide administration in 18 neurons in which the analysis could be carried out. The neurons with pinch responses enhanced by methysergide had high values (1–5) which reached maximum points at the cumulative dose of 0.25 mg/kg of methysergide. In contrast, neurons that did not have their pinch response changed maintained ratios close to 0.

As shown in the insert in Fig. 6, we also compared the areas under the stimulus and response functions as a way of qualitatively examining drug effect. Quantitative analysis was not possible because the rate of change of stimulus intensity was not constant. In the absence of methysergide, the ratio of the areas for control neurons did not change for a period up to 120 min as shown in the insert in Fig. 8. The ratio of the areas also remained constant for those neurons that did not demonstrate a methysergide-induced increased pinch response (open bars). In contrast to that constancy, the ratio of the areas for the neurons that had enhanced responses was significantly increased at most test doses (filled bars). This effect was observed even at the low dose of 0.05 mg/kg.

Response to heat stimulation. Four of 6 neurons that demonstrated an increased response to pinch stimulation after methysergide administration also responded to heat stimulation following drug, although they did not respond to thermal stimulation prior to methysergide. Following methysergide 3 neurons began to respond to heat at 45°C and a fourth neuron at 47°C, although those neurons did not respond at any temperature from 40°C to 47°C during baseline observations. An example of this response is shown in Fig. 9. During baseline studies a 45°C stimulus elicited reflex withdrawal but no significant activation of the neuron. Following methysergide, 45°C elicited withdrawal in a shorter period of time and also produced significant activation of the neuron.

Unmasking effect of methysergide

Table III summarizes the change in neuronal classification as a result of methysergide administration. Twenty-eight neurons were characterized as LT neurons and one neuron was considered to be a WDR neuron in the physiologically intact, awake, drug-free animal (baseline observations). After methysergide, 6 of the LT neurons (21%) were reclassified as WDR because of their ability to respond to stimuli within the noxious range. For one LT neuron after methysergide administration, activity evoked by pinch stimulation at times was increased. However, the changes were not consistent so the neuron was classified as an LT (?) in Table III.

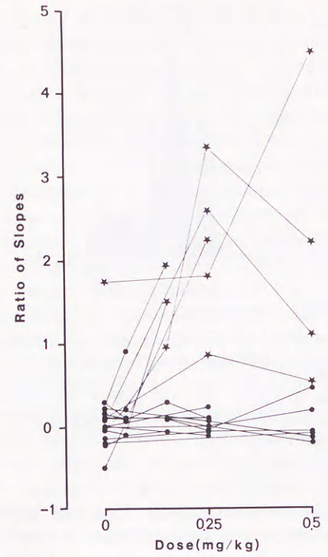

Fig. 7. Methysergide-induced changes in the ratio of the slopes of the stimulus and response functions. (Note: before the administration of methysergide all but one of the neurons had ratios clustered around 0 (filled circles). The neuron with a value of 1.7 in the absence of methysergide was one of the two WDR neurons observed in this study. Stars indicate a response to pinch that would be considered appropriate for a WDR neuron. This figure demonstrates that, as would be expected, the change in the ratio of the slopes was seen in all the neurons that had their classification changed from LT to WDR. Also, methysergide had an effect on the one neuron that was classified as WDR during baseline studies.

The unmasking by methysergide typically occurred within the 0.15–0.25 mg/kg dose range. Additional administration of methysergide did not enhance the response to pinch or heat even though receptive field areas were enlarged by higher doses.

Behavioral changes

Behavioral responses to the stimuli are difficult to quantify in this protocol. As seen in Fig. 9, the presence of methysergide was sometimes associated with a shorter latency to withdrawal. This was seen with both pinch and thermal stimulation. It was not, however, a consistent finding.

Blood pressure changes

We did not monitor blood pressure changes in awake animals. We did, however, examine the effect of methysergide on blood pressure in anesthetized (n = 1) or decerebrate (n = 5) cats. For example, at cumulative doses of 0.1–0.15 mg/kg, we saw small (< 10 mm Hg), short duration (< 5 min) changes in systolic pressure in the anesthetized animal. No profound changes were seen until doses greater than 0.5 mg/kg were achieved. Similar results were observed in the de-

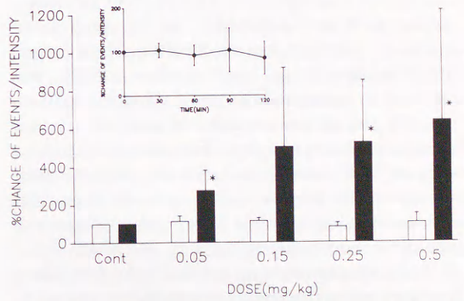

Fig. 8. Methysergide effects on total pinch response. Figs. 6 and 7 demonstrated methysergide effects on the slope of the pinch response. This figure demonstrates the drug effect on the total neuronal response during pinch. Open bars, % change of event area/intensity area for those neurons whose pinch response was unaffected by methysergide. Filled bars, neurons whose response to pinch was enhanced by methysergide. * Significantly different from unchanged cells represented by open bars. Inset: plot of % change of event area/intensity area for the 5 control neurons studied without methysergide.

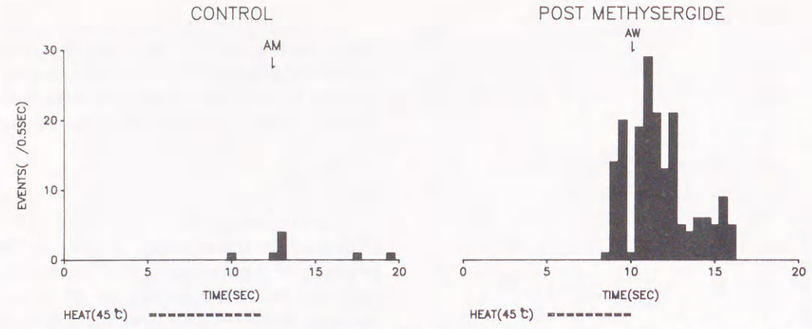

Fig. 9. Methysergide-induced response to thermal stimulation. An 8 sec, 45°C control thermal stimulus caused no activation of the neuron. Animal movement (AM) was detected following the end of the stimulus. Following the systemic administration of a cumulative dose of 0.25 mg/kg of methysergide, the neuron was activated by the 45°C stimulus even though the animal withdrew from it after approximately 6 sec (AW). The brief reduction in activity at the time of animal withdrawal may be explained by slight animal movement. Resumption of activity after withdrawal was not due to contact with the receptive field and was judged part of the response to the thermal stimulus.

cerebrate animals. The absence of significant blood pressure changes suggests that the observed changes in neuronal responsivity were not due to methysergide effects on the cardiovascular system.

Discussion

As stated in the Introduction, the purpose of this study was to determine if in the awake, intact, drug-free model that was employed, 5-HT was involved in tonic modulation of spinal dorsal horn neurons and if the modulation was selective for noxiously evoked activity. The data presented here support 5-HT involvement in tonic modulation of spinal dorsal horn neurons, a modulation that influences activity evoked by both noxious and non-noxious stimulation.

Tonic inhibition of spinal dorsal horn neurons has previously been demonstrated in acute preparations [5,31,52]. Wall [52] using a reversible cold block of the spinal cord demonstrated that descending systems are capable of changing neuronal response properties of some spinal dorsal horn neurons. He reported that when descending inhibition was presumably disrupted by reversible cold block at the thoraco-lumbar junction, some neurons below the level of the block began to respond to noxious stimuli and had expanded receptive fields. This present study, in conjunction with other work from our laboratory [12,14,16], provides evidence of tonic inhibition of some spinal dorsal horn neurons in physiologically intact, awake, drug-free cats.

The tonic nature of the modulation contrasts with the phasic modulation of medullary dorsal horn neurons that has been shown in awake monkeys [e.g., 7,18,21,32]. In the present study, the absence of a behavioral paradigm that allows the animal control over the environment may have been a source of stress that activated the observed inhibition. Alternatively, involvement in a behavioral paradigm may alter the level of tonic modulation. As will be emphasized below, it is important that we recognize the plasticity that may result from modulation of the type observed in this and other studies.

The involvement of 5-HT in descending modulation of spinal dorsal horn neurons is well established, although there has been controversy about how selective 5-HT modulation may be for noxious input. Electrical stimulation of a major source of descending 5-HT input to the spinal dorsal horn, the nucleus raphe magnus, can cause inhibition of dorsal horn neuronal responses to noxious and non-noxious stimuli [1,4,9,20,23–26, 29,30,35,48,56]. The results of the present study provide evidence for significant 5-HT modulation of non-noxiously evoked activity in the physiologically intact animal. In addition, in this study, animals reflexly withdrew from mechanical and thermal stimuli that were considered by humans to be mildly noxious. The unmasking of some neuronal responses to those stimuli provide evidence for tonic 5-HT modulation of noxiously evoked activity as well. Because methysergide was administered systemically, we do not know the site of action at which tonic inhibition was altered.

The effect of methysergide on non-noxiously evoked activity is of particular interest. Three separate measures of low intensity stimulation were available for analysis. The different effect of methysergide on each of those measures suggests a complex effect of 5-HT on low threshold activity of some spinal dorsal horn neurons. We see in Table II that some neurons with enhanced receptive field area evidenced no change or a reduced response to brushing of the receptive field. It is possible that a reduced response to brushing was due to a shift in the levels of sensitivity across the receptive field associated with changes in the receptive field margins. It appears that for some neurons, although more synaptic input had been made available (i.e., a larger receptive field area), the security of the initial synaptic connections had been reduced or altered (i.e., decreased sensitivity within the control area).

As shown in Fig. 3, the greatest percent change in the receptive field areas was observed to occur in the distal direction. If the reduction in tonic modulation acts to enhance the nervous system 'sensitivity' to external stimuli, then the increased distal extension of receptive field areas may be of value in increasing the animal's ability to detect stimuli that impinge on more distal regions of the skin. While such a change could be viewed as an aid in alerting the animal to external events, it will also be expected to decrease stimulus localization.

If we examine the ability of methysergide to change the neuronal response to pinch, we may be attracted by the obvious change in total activity elicited during the pinch stimulus. However, the change in response to initial contact is also of interest. Usually, the neurons rapidly adapted to the initial contact. However, as we see in Figs. 4 and 5, methysergide at times inhibited the rapid adaptation to light touch. Clearly, 5-HT has a profound and variable tonic effect on low-threshold input to some spinal dorsal horn neurons.

The very low level of spontaneous activity recorded in this preparation was observed by others in the awake cat spinal cord [50]. In acute studies, reversible cold block of the spinal cord enhances spontaneous activity [e.g., 52] suggesting that tonic descending inhibition normally keeps spontaneous activity at a low level. Results from the present study suggest that in the intact awake cat tonic 5-HT modulation is not responsible for the low levels of spontaneous activity observed. We have previously hypothesized that tonic inhibition of increased afferent input in an acute preparation may be important in maintaining low levels of spontaneous activity [15].

As previously reported [14] the ability of some spinal dorsal horn neurons to respond to a noxious stimulus can be unmasked by pharmacologically altering tonic systems. This study demonstrates that 5-HT does play a role in that process. In this study 21% of the sample evidenced an unmasking of response to noxious stimulus. A summary of published and unpublished results of work from our laboratory indicates that unmasking of that type is likely to be seen in 20–30% of the neurons studied. These results suggest that the modality specificity of some spinal dorsal horn neurons is dependent upon tonic modulation.

A particularly interesting aspect of the effect of methysergide on noxious stimulation was the change in the slope of the response function that occurred during pinch stimulation. Removal of tonic inhibition resulted in the appearance of a positively accelerating response profile that reflected changing stimulus intensity. We have seen similar changes following pentobarbital administration [16]. Although the lack of a uniform rate of pinch in this study complicates quantification, the change in the response function represented by the ratios of the two slopes E over I demonstrates the effect of methysergide. As we see in Fig. 7, the activity of 6 neurons was changed so that after methysergide, the firing frequency carried infor-

mation about the intensity of the stimulus, information that was not present prior to drug administration. If firing frequency codes stimulus intensity, then for those 6 neurons interference with 5-HT modulation allowed them to better signal the change in the intensity of the pinch (i.e., tonic inhibition altered modality specificity). Such a change in the neuronal firing frequency, by increasing the dynamic sensitivity of the neurons, could enhance the warning capability of the nervous system by conveying more information about changing stimulus intensity.

This observed change is of particular interest if we consider the role that WDR neurons has been attributed in signaling information about pain. According to our classification, the neurons that had an enhanced response to pinch were LT before methysergide and WDR after. The firing frequency of the 6 during control pinch contained no obvious information about the changing intensity of the stimulus. After methysergide the firing frequency reflected changes in stimulus intensity. The likely role of WDR neurons in signaling stimulus intensity has been shown in a series of studies in awake monkeys [e.g., 7,34,36,39]. If removal of tonic 5-HT inhibition unmasks the ability of a neuron to convey information about stimulus intensities approaching the noxious range, such plasticity may be a means by which the spinal cord modifies its ability to signal tissue damaging stimuli.

As discussed by others [e.g., 32,45], it is possible that sensory processing is more dependent upon activation of a 'large group of unique central neurons' [45] than on activation of a few labeled lines. If the modality of these neurons has truly been changed from LT neurons to ones that respond best to noxious stimuli, then detection of a tissue damaging stimulus may be enhanced because more WDR neurons are now available for participation in the 'large group of unique central neurons' that subserve that purpose.

The role of the WDR neuron in the signaling of pain has been widely debated. The two extremes of the argument range from the assumption that the activation of WDR neurons is a sufficient condition to produce pain in man [40,47] to the belief that, at best, WDR responses to painful stimuli may, under pathological conditions, be a source of abnormal somatosensory experience [44]. If the neurons sampled in this study are involved in sending information centrally (as previously reported [12] we typically record from lamina III through VI but do not know about the central connectivity of the neurons under study), then there is limited evidence from this study that both points of view may be correct. WDR neurons are present in the intact animal. WDR response profiles may also be unmasked, perhaps by pathological states.

Although the above discussion deals with individual points of interest, perhaps the most important implications of these studies are apparent when all of these changes are observed together. Spinal dorsal horn neurons have been classified based upon their ability to respond to stimuli of increasing intensity [42]. An underlying assumption that allowed those classification schemes to be developed was that any one neuron had a unique response profile that was considered to be invariant. The results of this study provide one more piece of evidence to argue against invariant response profiles for all spinal dorsal horn neurons. They support an argument in favor of a level of plasticity that can significantly change a neuron's ability to respond to a given series of stimuli. When we first observed changes of the type reported here, we referred to them as an unmasking of WDR response profiles [14]. We continued, for the ease of data presentation in this paper, to suggest as we see in Table III that some of the neurons have been changed from LT to WDR neurons. Although this nomenclature provides an easy means of communicating the types of changes that we encountered, it also perpetuates an incorrect image that all neurons have fairly constant response profiles.

The problem of defining the response range of a neuron has been recognized by many individuals and most recently has been dealt with by an attempt to define, using cluster analysis, a larger number of groupings into which neurons could be placed based upon their response profiles [5,10]. All of these schemes, however, are problematic because they are still associated with an underlying assumption that there is a particular profile for any one neuron. It is much more likely that for at least some spinal dorsal horn neurons there is a broad-ranging response profile that is modulated by all of the factors that can control input to that neuron. In this study, we have only examined the effects of one of many possible transmitter systems. Only after evaluating several transmitter systems will we be able to appreciate the level of plasticity that may exist in the spinal dorsal horn of the physiologically intact, awake, behaviorally naive, drug-free animal.

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