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Ascending Pathways in the Spinal Cord Involved in Triggering of Diffuse Noxious Inhibitory Controls in the Rat

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SUMMARY AND CONCLUSIONS

1. Recordings were made from convergent neurons in trigeminal nucleus caudalis of the rat. These neurons were activated by both innocuous and noxious mechanical stimuli applied to their excitatory receptive fields located on the ipsilateral part of the muzzle. Transcutaneous application of suprathreshold 2-ms square-wave electrical stimuli to the center of the excitatory field resulted in responses to C-fiber activation being observed (mean latencies 63.6 ± 5.5 ms). This type of response was inhibited by applying noxious conditioning stimuli to heterotopic body areas, namely immersing either the left or right hindpaw in a 52°C water bath. A virtually total block of the response was observed during the application of the noxious conditioning stimulus, and this was followed by long-lasting poststimulus effects. Such inhibitory processes have been termed diffuse noxious inhibitory controls (DNIC) (39, 40).

2. The effects on these inhibitions of various transverse lesions of the cervical spinal cord were investigated in acute experiments; tests were performed before and at least 30 min after the spinal section. While the unconditioned C-fiber responses were unaltered, the inhibitory processes could be impaired by the cervical lesions, although these effects depended on the part of cervical cord destroyed and the side of application of the conditioning stimulus.

Lesioning dorsal, dorsolateral, and ventromedial parts of the cervical cord was found not to affect inhibitory processes triggered from either hindpaw. The overlapping of the regions of these ineffective lesions revealed that two remaining regions were not destroyed, that is, the left and right ventrolateral quadrants.

In experiments where the left anterolateral quadrant was affected by the surgical procedure the inhibition triggered from the right hindpaw was strongly reduced, whereas that elicited by left hindpaw stimulation was not diminished. The loss of inhibitory effects was characterized by a complete disappearance of poststimulus effects, whereas inhibition observed during the application of the noxious thermal conditioning stimulus was only partially, albeit very significantly, blocked.

3. To ascertain further the mainly crossed nature of the pathways responsible for the heterotopic inhibitory processes, the effects of lumbar commissurotomy were investigated. Again the unconditioned C-fiber responses were unaltered by this procedure, whereas the inhibitory processes, whether triggered from the left or right hindpaw, were strongly depressed in all the experiments. Once more, this depression was not complete, further suggesting that an uncrossed component plays a role in inhibitory processes of heterotopic origin.

4. In an additional series of experiments, lesions of the ventrolateral quadrant were performed before recordings. Unilateral lesions of the left ventrolateral quadrant resulted in a strong reduction of inhibitory processes triggered from the right hindpaw, whereas inhibition triggered from the left hindpaw was slightly, albeit significantly, decreased. Bilateral lesions of the ventrolateral quadrant resulted in a complete disappearance of inhibi-

tions, whether triggered from the right or left hindpaw.

5. In the last series of experiments, neurons in the right ventrobasal thalamic complex, which are the main targets for lateral spinothalamic tract axons, were destroyed by prior microinjections of kainic acid. In these animals, inhibitory processes were symmetrically triggered from either hindpaw and were essentially identical to those obtained in control animals.

6. We conclude that the ascending pathways involved in the triggering of DNIC are mainly crossed, but have a significant uncrossed component, and are confined to the ventrolateral quadrant. The postsynaptic fibers of the dorsal columns, the spinocervical tract, and the spinothalamic tract did not appear to play a role in these processes. Signals responsible for triggering DNIC seem to travel essentially in the spinoreticular tract.

INTRODUCTION

We have previously described the general characteristics of diffuse noxious inhibitory controls (DNIC) that act specifically, at least in the rat, on dorsal horn (39, 40) and trigeminal nucleus caudalis (17) convergent neurons, including those projecting toward the thalamus (16). These cells, also designated as class 2, wide dynamic range, or multireceptive neurons, are activated by both innocuous and nociceptive inputs through A- and C-afferent fibers. DNIC are triggered when heterotopic nociceptive stimuli are applied to the body; they are characterized by their strong efficacy, the existence of long-lasting poststimulus effects, and their ability to affect almost all convergent neurons but not other types of dorsal horn and trigeminal nucleus caudalis neurons (17, 40). DNIC affect all activities of convergent neurons, whether induced from the periphery (17, 39) or by microelectrophoretic application of an excitatory amino acid (63, 65), and a close relationship exists between the strength of the heterotopic nociceptive stimulus and the resultant inhibitory effect (38, 66).

Previous studies (12, 40) have demonstrated that DNIC acting on convergent lumbar neurons are abolished by a total section of the cervical spinal cord. It appears, therefore, that the involvement of a supraspinal loop is an essential characteristic of the triggering of DNIC. The aim of the present work was to determine the anatomical profile of the ascending limb of the loop subserving DNIC.

To approach this problem we made use of the fact that DNIC act on nucleus caudalis convergent neurons. By triggering DNIC from a posterior part of the body, we were able to undertake the study of a model in which the circuitry of the involved loop is successively organized in long ascending (Fig. 1*A*) and short descending (Fig. 1*B*) pathways. In such a situation, a blockade of DNIC by subtotal spinal section would have to be due to the impairment of nociceptive transmission in ascending spinal pathways rather than of transmission in descending pathways to the trigeminal system. Use of a thermal nociceptive conditioning stimulus applied to either the left or the right hindpaw allowed us to compare the strength of DNIC before and after lumbar commissurotomy (Fig. 1*a*) and cervical transverse sections (Fig. 1*b*).

Such an experimental design was chosen to answer the question of whether crossed or uncrossed ascending pathways are involved in the triggering of DNIC. In addition, it provided a situation wherein the distance between the sites of application of conditioned (the muzzle) and conditioning (the hindpaws) stimuli, minimized or ruled out the participation of propriospinal mechanisms in triggering inhibitions of activity of the recorded convergent neurons (12, 19, 24).

We will present analyses of the effects of *1*) restricted transverse lesions of the cervical spinal cord (Fig. 1*b*) corresponding to various sensory ascending pathways, *2*) lumbar commissurotomy, *3*) unilateral and bilateral sections of the ventrolateral quadrant at the cervical level, and *4*) thalamic lesions (Fig. 1*c*); this last experimental series was undertaken because the first three led to the conclusion that crossed pathways were to a large extent accountable for the triggering of DNIC. Preliminary reports of this work have already appeared in abstract form (64).

METHODS

Animal preparation

Experiments were performed on 27 male rats weighing 220–300 g. Following an intraperitoneal injection of 100 μg atropine sulfate, the animals

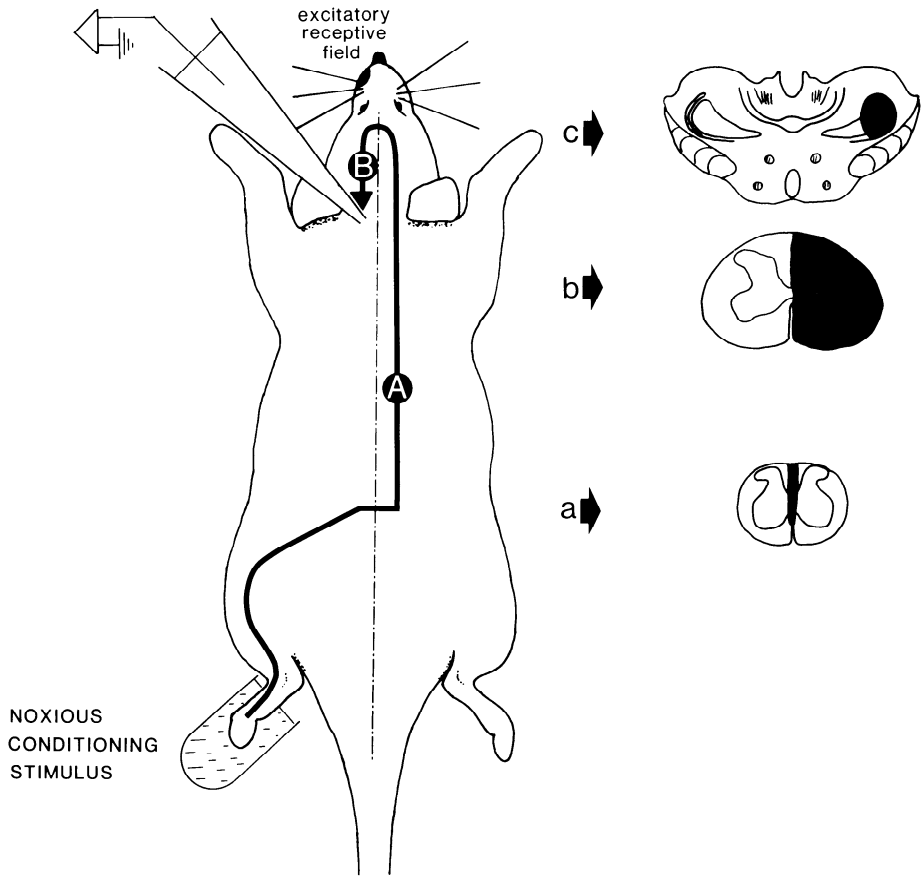

FIG. 1. Schematic representation of the experimental design. Convergent neurons with receptive fields located ipsilaterally on the muzzle were recorded in the left trigeminal nucleus caudalis. C-fiber responses were conditioned by immersion of either hindpaw in a 52°C water bath. With this experimental arrangement the supraspinal loop sustaining DNIC comprised a long ascending (*A*) and a short descending (*B*) pathway. The effects of 3 types of CNS lesions were studied: (*a*) lumbar commissurotomy, (*b*) cervical sections, and (*c*) destruction of the right ventrobasal thalamic complex.

were deeply anesthetized with 2% halothane in a nitrous oxide/oxygen mixture (⅔:⅓). A tracheal cannula was inserted, the jugular vein cannulated, and the animals were paralyzed by intravenous injection of gallamine triethiodide (Flaxedil) and were artificially ventilated; the rate (70–80 strokes/min) and volume of ventilation were adjusted to maintain a normal acid-base equilibrium (21). Heart rate was continuously monitored and core temperature maintained at 37 ± 0.5°C by means of an homeothermic blanket system. Animals were mounted in a stereotaxic frame, and laminectomies were performed either on vertebrae C_3 and C_5 to expose the cervical cord (1st and 3rd series of experiments) or on vertebrae T_{11} to L_1 to expose the lumbar cord (2nd series of experiments); no laminectomy was made in the 4th series of experiments (see below). The head was then mounted in a ventroflexed position and fixed by means of a metallic bar cemented to the skull. The caudal medulla was exposed by removing the overlying musculature, atlantooccipital membrane, and dura mater. After surgery the level of halothane was lowered to 0.5% to achieve an adequate level of anesthesia for ethical considerations while not excessively depressing neuronal responses to noxious stimuli (5, 41).

Recordings

Unitary extracellular recordings of convergent neurons in trigeminal nucleus caudalis were made with glass micropipettes (10–15 MΩ) filled with a mixture of 5% NaCl and pontamine sky blue dye. All penetrations were made on the left side, 1.5–2.0 mm posterior to the obex and 1.5–2.5 mm lateral to the midline. Stability for recordings was achieved by placing over the surface of the medulla

a glass frame which was held in position with a micromanipulator and 2% Ringer-agar gel.

Nonnoxious facial stimuli were used to help isolate unitary activity, and neurons were identified as being convergent on the basis of their characteristic responses to both mechanical and electrical stimuli applied to their peripheral receptive fields; they all responded to innocuous (light touch, gentle stroking) and noxious (strong pinch) mechanical stimuli and gave responses with latencies corresponding to A- and C-fiber inputs during transcutaneous electrical stimulation through two needles inserted in the center of their excitatory receptive fields. Once a cell had been identified, the extent of its receptive field was determined. Other neuronal types, e.g., "noxious only" or "nonnoxious only," were not considered in the present study because they have been shown not to be influenced by DNIC (17, 40, 63, 65).

Only cells presenting no serious alterations in spike amplitude or waveform during the complete experimental procedure were considered.

Experimental design

The experimental procedure consisted of sequences of 100 suprathreshold electrical stimuli (single square-wave pulses of 2 ms duration) applied repetitively (0.66 Hz) to the excitatory receptive field; during these sequences, from the 45th to the 70th stimulus presentations (i.e., for 37 s), one hindpaw was immersed in a 52°C water bath. Two sequences involving left and right hindpaw conditioning were carried out at 5-min intervals.

During the experiments, a multichannel analyzer (Tracor TN 1710) was used to build poststimulus histograms (PSHs). The first 30 responses were not considered because, in some cases, the cell's responses showed habituation and, in most cases, "wind-up" (47, 57, 67) phenomena. The PSH built from the 31st to the 45th responses was used as a control for the sequence. The PSH built from the 56th to the 70th responses was taken as the conditioned response. The PSH built from the 71st–85th responses and 86th–100th responses allowed the posteffects to be observed during the 22 s and 22s to 44 s after the cessation of the conditioning period.

The PSH were analyzed to distinguish responses due to A- and C-fiber inputs, according to their latencies and using the classification of Gasser and Erlanger (23) and Burgess and Perl (11).

Inhibitions were expressed as percentage decreases in the number of spikes of both A- and C-fiber evoked responses with reference to the control PSH. The temporal evolution of individual responses was also visualized on a two-channel chart recorder on which the bins were set to give the cumulative individual responses due to either A- or C-fiber inputs. However, only the C-fiber component will be considered in the present study because it was not always easy to differentiate between A-α and A-ϑ responses; in addition DNIC, as previously described (17, 39), were found to be less potent upon these latter responses because of the supramaximal nature of the electrical shock applied to the peripheral field.

Statistical analyses were made using the paired or unpaired Student's t test.

Cervical sections

In the first experimental group (11 rats) the dura was slit over the cervical spinal cord at the end of the second control sequence, and a transversal lesion was made under a dissecting microscope by cutting the cord with a sharp blade.

Postlesion sequences were carried out 30 min after the lesions to minimize early possible changes induced by the surgery. Postlesion sequences identical to controls (see Figs. 5–6) indicated that this half-hour period was a sufficient time in this respect.

In some cases (see Fig. 9*A*, *a*, *b*, and *c*), two successive lesions (the first on C_3–C_4 segment, the second on C_5–C_6 segment) were made during the same experiment.

Figure 2 provides examples of cervical sections in which the lesions included, respectively, the dorsal columns, the dorsolateral part, and the dorso- and ventrolateral parts of the spinal cord.

Lumbar commissurotomy

In the second experimental group (6 rats) the total rostrocaudal extension of the lumbar cord giving dorsum potentials to electrical stimulation of the hindpaws was determined before recordings. This was taken as an index of the main rostrocaudal extension of the hindpaw representation. This whole region was included in the subsequent commissurotomy (Fig. 3) performed using a lancet diamond knife (A. Meyer Co).

Ventrolateral quadrant lesions

In the third experimental group a laminectomy was performed at the C_3–C_4 segment. Then the left (4 rats) or both ventrolateral quadrants (2 rats) were lesioned by using the lancet diamond knife. In these cases recordings began at least 3 h after the end of surgical procedure.

Thalamic lesions

In the fourth experimental group (4 rats) the right ventrobasal thalamic complex (VB) was lesioned (Fig. 4) using the following procedure. Operations were first performed on the rats 2 wk before the recordings. The rats were anesthetized with chloral hydrate (400 mg/kg), and a slow pressure injection of kainic acid (5 nM in 0.15 μl of water) was performed using a 1-μl Hamilton syringe at the level of the rostrolateral border of the VB, according to a technique described elsewhere (31).

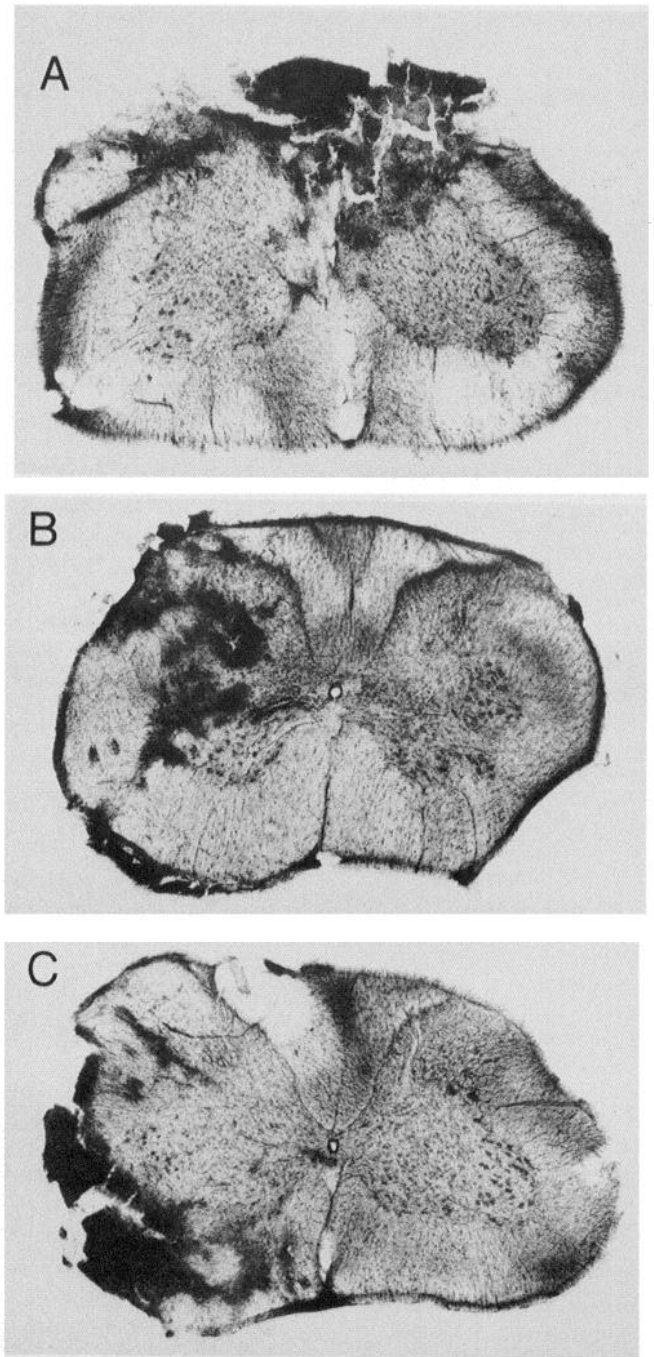

FIG. 2. Examples of 100-μm-thick cervical sections Nissl stained with cresyl violet, which allowed the reconstruction of the total extent of the lesions on camera lucida drawings. *A*: lesion of the dorsal columns. *B*: lesion of the dorsolateral funiculus. *C*: lesion of the dorso- and ventrolateral parts of the cord. See, respectively, Figs. 7*Aa*, 7*Ae*, and 9*Ad* for complete reconstruction of these 3 lesions.

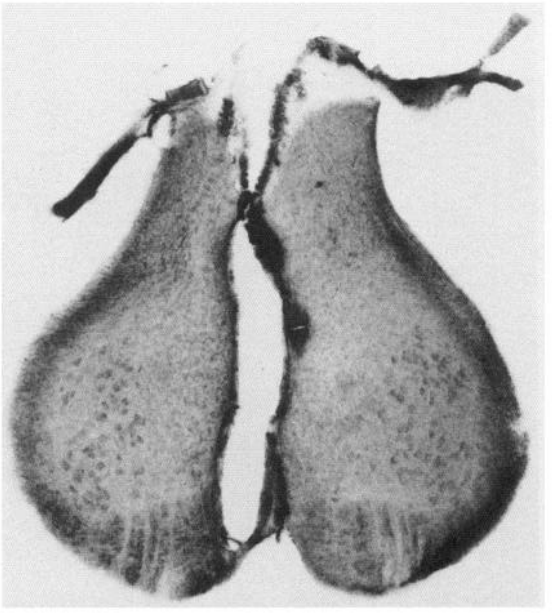

FIG. 3. Example of a commisurotomy in a 100-μm-thick Nissl-stained section in the lumbar cord. Note that the loss of medial structures is only apparent, being due to tissue displacement during histological procedures. There was no destruction of dorsal horns and intermediate zone.

During the recording session, trigeminal convergent neuronal responses to electrical stimulation of the excitatory receptive fields were conditioned by immersion of the hindpaws in 52°C water baths in sequences identical to those previously described.

Histological controls

At the conclusion of the experiments, the recording sites were marked by electrophoretic deposition of pontamine sky blue, the cervical or lumbar spinal cord and medulla were removed and fixed by immersion in a 10% formalin solution for 72 h, and these were then soaked in a 30% buffered sucrose solution for 48 h. When thalamic lesions were performed the animals were perfused with a 10% formalin solution. Samples were frozen, cut in serial 100-μm-thick sections, and Nissl stained with cresyl violet or carmin. Cord lesions and thalamic sites of neuronal loss were reconstructed from camera lucida drawings of serial sections.

RESULTS

We will successively show results concerning "acute" lesions in which individual neurons were recorded before and after cervical lesion (experiment *1*) or commissurotomy (experiment *2*) and then, results concerning neurons in rats lesioned before recordings (ventrolateral quadrant: experiment *3*; ventrobasal thalamic complex: experiment *4*). General properties of recorded units will first be presented; in this section quantitative data were taken from control sequences recorded during experiments *1* and *2*.

General properties of recorded units

The convergent neurons recorded in the present study were located within the magnocellular layer of the left nucleus caudalis and in the adjacent reticular formation, as depicted by electrophoresis of dye at the end of the experiments. Their excitatory receptive fields were found on the ipsilateral part of the muzzle. The cells could be activated by both innocuous (hair movements, stroking, light pressure) and noxious (pinch) mechanical stimuli applied to their excitatory receptive fields.

By applying 2-ms transcutaneous electrical square-wave stimuli to the centers of their excitatory receptive fields, responses due to peripheral activation of A- (mean latency: 2.73 ± 0.26 ms) and C- (mean latency: 63.6 ± 5.5 ms) fibers could be observed. C-fiber responses were obtained with a mean threshold of 8.1 ± 0.9 mA. The current was systematically increased to a suprathreshold value (mean 2.9 times threshold) giving reproducible and regular C-fiber responses (mean: 13.0 ± 1.9 spikes per stimulus). The latency of the maximal firing of such responses occurred at a mean latency of 89.8 ± 5.0 ms, which corresponds to peripheral fibers with conduction velocities ~0.6 m/s.

All the units were under the influence of DNIC. When one of the hindpaws was immersed in a 52°C water bath, a strong inhibition of the responses due to the activation of A- and C-fibers occurred; in most cases, the responses due to C-fiber inputs were completely abolished during the conditioning period; this blockade was always followed by long-lasting poststimulus effects (see control sequences in upper parts of Figs. 5, 6, and 10).

There was no difference between the inhibitory effects triggered from the left and right hindpaws (mean inhibitions: $96.3 \pm 1.9\%$ and $97.7 \pm 1.3\%$ respectively).

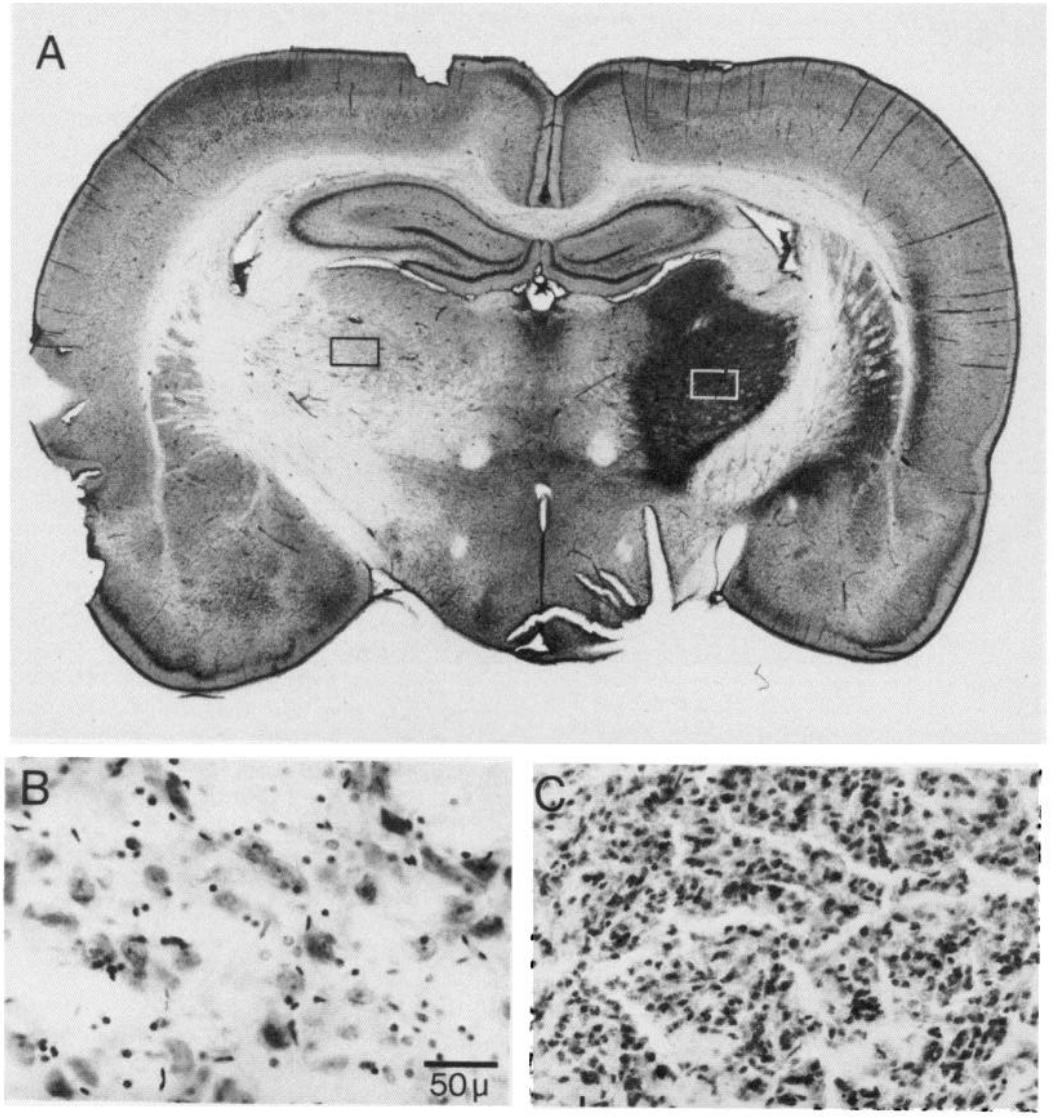

FIG. 4. Example of thalamic lesion induced by microinjection of kainic acid. *A*: photomicrograph (40×) of a diencephalic section showing, on *right*, a lesion induced by a kainic acid injection performed 2 wk earlier (*arrow*); thalamic neurons were eliminated in this area, and a glial reaction is visible. *B*: higher magnification (400×) photomicrograph of the lesioned area of the thalamus. Note that the structure is filled with small (<10 μm diam) dark structures corresponding to glial cells staining with cresyl violet. *C*: photomicrograph, with same magnification as in *B*, of a corresponding area in the opposite thalamus. Note the presence of larger (~20 μm diam) structures, which are stained neurons.

The A-fiber responses were also inhibited, but to a lesser extent (mean 67.0 ± 9.5%), probably because of the supramaximal nature of the electrical shock applied on the muzzle; they were not considered in the studies devoted to lesions, which will be presented in the following sections.

Effects of cervical transection upon DNIC (experiment 1)

For convenience and clarity of presentation, two representative individual experiments will first be presented. They have been chosen because they comprise two successive lesions,

with the first not affecting DNIC and the second reducing DNIC. Then the results will be analyzed by considering ineffective and effective lesions successively.

Before considering these experiments, it is important to specify that the unconditioned C-fiber trigeminal responses were almost identical before and after lesioning the cervical spinal cord (mean postlesion responses: 92.8 ± 9.7% of prelesion responses). One can

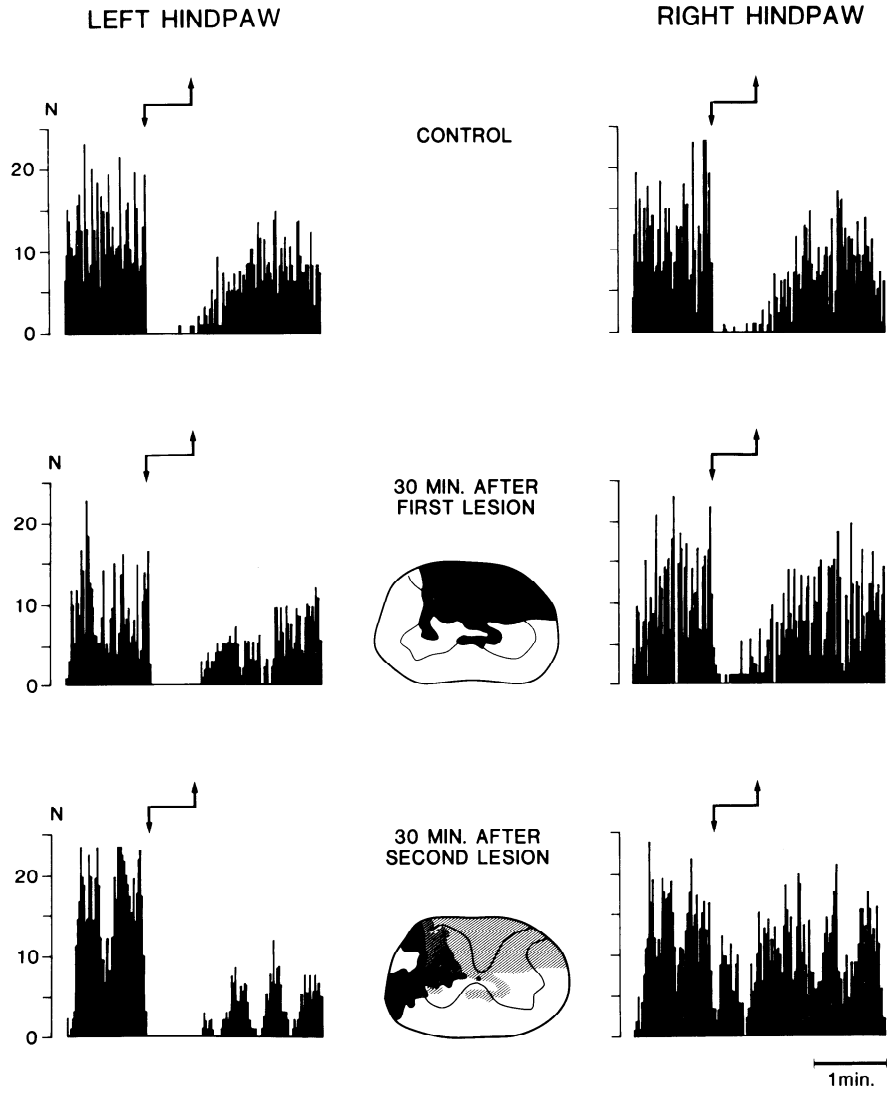

FIG. 5. First example of the effects of cervical lesions on DNIC. The histograms represent the temporal evolution (*abscissa*: time) of C-fiber responses (*ordinate*: number of spikes computed within the 60–100 ms following the stimulus) of a nucleus caudalis convergent neuron to transcutaneous electrical stimulation (2 ms; 22 mA; 0.66 Hz). During the period between the 46th and 70th stimulus presentations (*arrowed*), the extremity of the left (*left* histograms) or right (*right* histograms) hindpaw was immersed in a 52°C water bath. In the control situation (*upper* histograms) either of the conditioning noxious stimuli induced a blockade of activity followed by posteffects. The first lesion, which involved both dorsal horns, the dorsal column, and the right DLF (as indicated in *black* on the *central drawing*), did not modify the general profile of the histograms. The second lesion, which involved in addition the left DLF and VLQ (as indicated in *black* on the lower drawing with the *hatched area* recalling the extention of the first lesion), did not block the inhibitory processes triggered from the left hindpaw. By contrast inhibitions triggered from the right hindpaw were markedly reduced.

therefore consider that a minimal bias, if any, was introduced in our study by the variability of the neuronal responses to C-fiber activation.

TWO TYPICAL EXAMPLES. Figures 5 and 6 show typical examples of inhibition of C-fiber responses induced by immersion of either the right or left hindpaw in a 52°C water bath (upper traces). Following a lesion that involved the dorsal columns and the right dorsolateral funiculus almost totally (Fig. 5, middle traces), these inhibitory effects persisted with essentially the characteristics of those observed in the control sequences. Following a second lesion, which included a well-delineated region of the left ventrolateral quadrant (Fig. 5, lower traces), inhibition triggered from the right hindpaw was strongly reduced with the almost complete disappearance of poststimulus effects, whereas the inhibition triggered from the left hindpaw was not significantly affected.

In a second example (Fig. 6) a first lesion

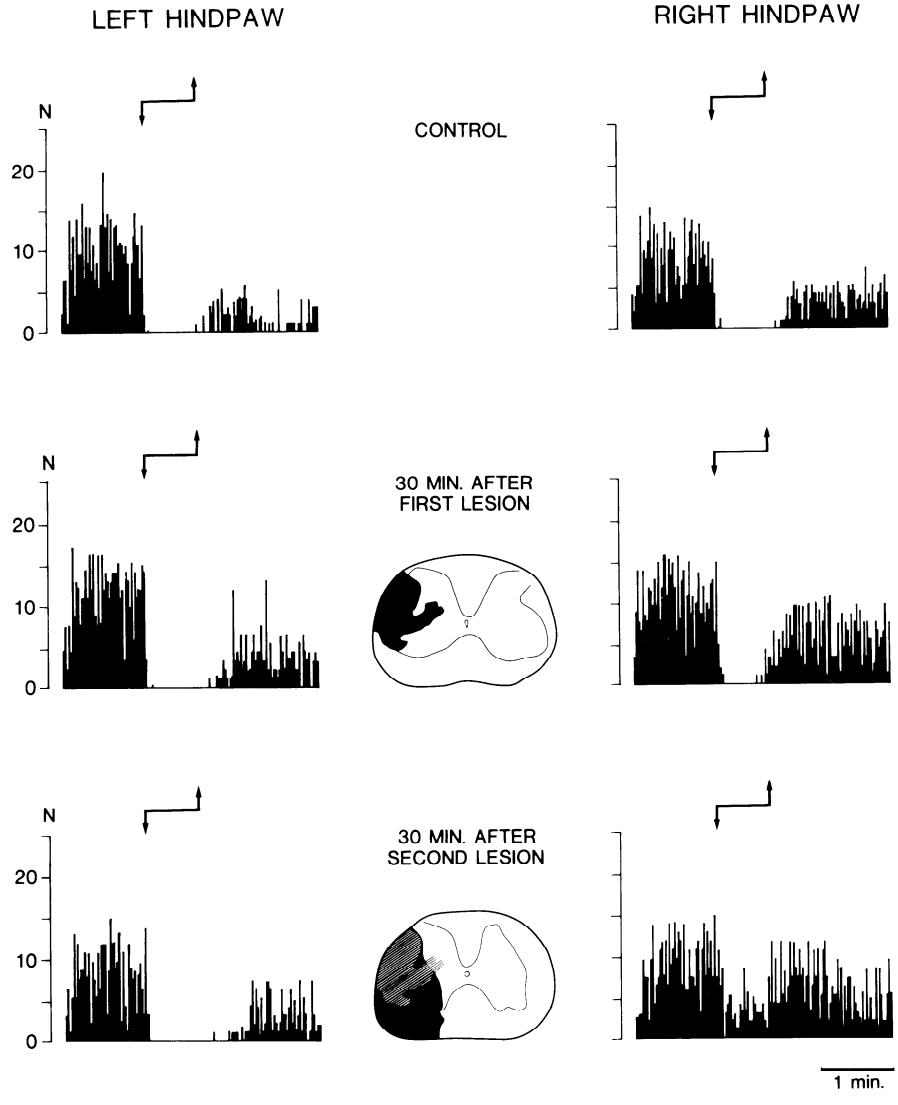

FIG. 6. Second example of the effect of cervical lesions upon DNIC (experimental design, representation, and symbols as in Fig. 5). Note the strength of inhibitory processes in the control situation, especially in terms of the posteffects. The 1st lesion, which involved the left DLF, did not modify the general profile of the histograms. Only inhibitions triggered from the right hindpaw were reduced by the 2nd lesion, which in addition involved the left VLQ.

including the left dorsolateral funiculus did not affect the inhibition triggered from either hindpaw. A further lesion including the whole left ventrolateral quadrant induced a great diminution of the inhibition triggered from the right hindpaw with inhibition triggered from the left hindpaw being essentially unchanged.

Lesions that did not affect DNIC. Figure 7*A* regroups the drawings of camera lucida reconstruction of lesions that did not reduce the inhibitions triggered by immersion of either hindpaw in a 52°C water bath. On some occasions, lesions had a narrow extent including mainly the dorsal columns (Fig. 7*Aa*) or the left dorsolateral funiculus (Fig. 7*A*, *c*, *d*, and *e*) without significantly affecting other structures. The remaining widest lesions included to various degrees the dorsal (Fig. 7*A*, *b* and *g*) or the ventromedial (Fig. 7A, *f* and *g*) part of the spinal cord. In all eight cases, inhibitions triggered by thermal noxious conditioning stimuli applied to either hindpaw, presented features similar to those observed during control sequences.

Figure 7*B* summarizes the mean results obtained in these experiments: Note that inhibitions observed during the conditioning period were unaffected by the lesions depicted in the upper part of the figure; this lack of

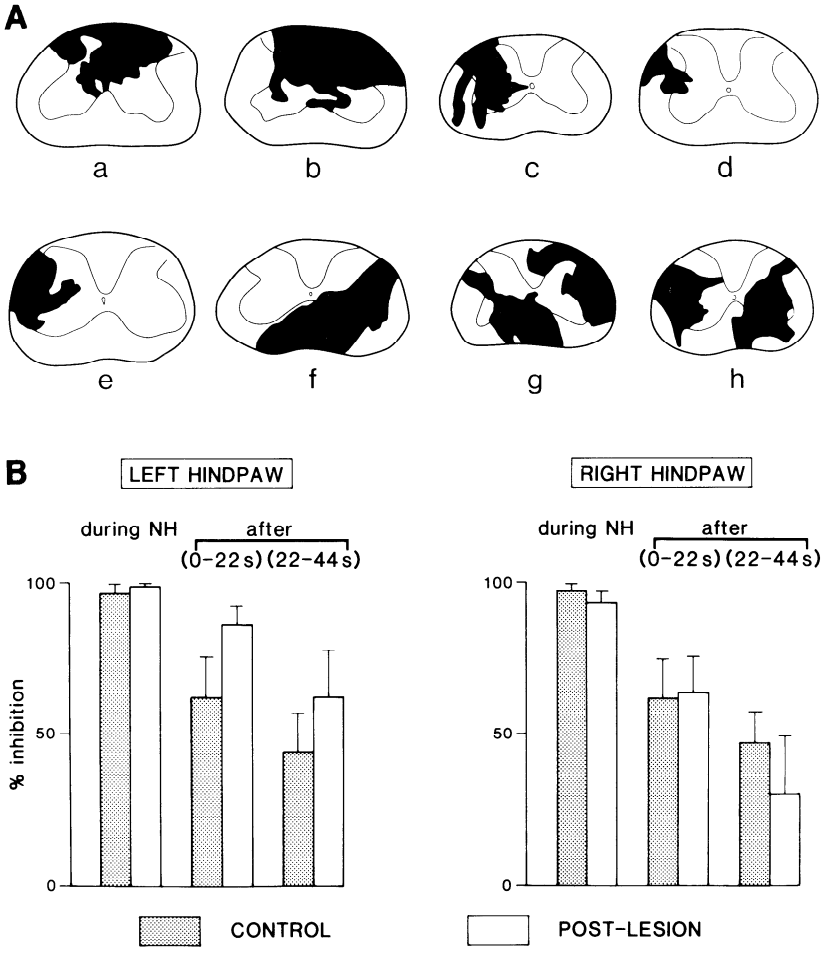

FIG. 7. Summary of experiments during which DNIC were unaffected by cervical lesions. *A*: extent of individual lesions. *B*: mean results obtained during these 8 experiments. Histograms represent the percentage inhibitions observed during ("during NH") and within the 44 s after ("after 0–22 s"; "after 22–44 s") the immersion of the left (*left* histograms) and the right (*right* histograms) hindpaws. Note that inhibitory processes were not essentially different before (*hatched columns*) and after (*open columns*) the lesions.

effect was found for the inhibitions triggered from both the left and the right hindpaws. With regard to the posteffects calculated during the 44 s after the end of the conditioning period, no reduction in the inhibitory effects, whether triggered from the left or right hindpaw, was observed following these lesions.

With the aim of summarizing the experimental data set out in the present section, an overlapping of ineffective lesions is presented in Fig. 8. Note that the cumulated lesions include the dorsal, dorsolateral, and ventromedial regions of the cervical spinal cord; the two remaining regions correspond to portions of the left and right ventrolateral quadrants.

Lesions that affected DNIC. Figure 9*A* shows camera lucida drawings corresponding to the lesions that were able to reduce the inhibition triggered by noxious heterotopic stimuli. Note henceforth that the inhibition triggered from the right hindpaw was affected, whereas those emanating from the left hindpaw were not reduced. Since a dorsal approach was used to perform the lesions, a dorsal part of the spinal cord was always affected. However, as shown in the previous section, lesions including the dorsal or dorsolateral part of the spinal cord did not reduce the inhibitions triggered from either hindpaw. The main observation in the present experiments was therefore that the left ventrolateral quadrant was affected in each individual case in which the inhibition was reduced. These data and those summarized in Fig. 8 suggest that the integrity of this region is required for the triggering at the trigeminal level of inhibitions originating from the right hindpaw. More precisely, detailed analysis of the effective (Fig. 9) and ineffective (Fig. 7) lesions revealed that the destruction of a well-delineated area, corresponding to the lateral part of the left ventrolateral quadrant, should be sufficient to obtain a significant reduction of the inhibitory processes triggered from the right hindpaw.

Figure 9*B* illustrates the quantitative data obtained in these experiments. It can be seen that inhibition triggered from the right hindpaw was very significantly diminished after lesioning. Note, however, that poststimulus effects were completely abolished in all cases while inhibition observed during the application of the noxious thermal conditioning stimulus was only partially blocked. With regard to the inhibitory processes triggered from the left hindpaw, they always persisted after the lesions with the poststimulus effects being slightly facilitated; note that this slight facilitation only concerned inhibitory processes triggered from the limb ipsilateral to the lesion (see also Fig. 7*B*).

Effects of lumbar commissurotomy upon DNIC (experiment 2)

The results presented above demonstrate that the ascending pathways that mediate the triggering of DNIC involve the anterolateral

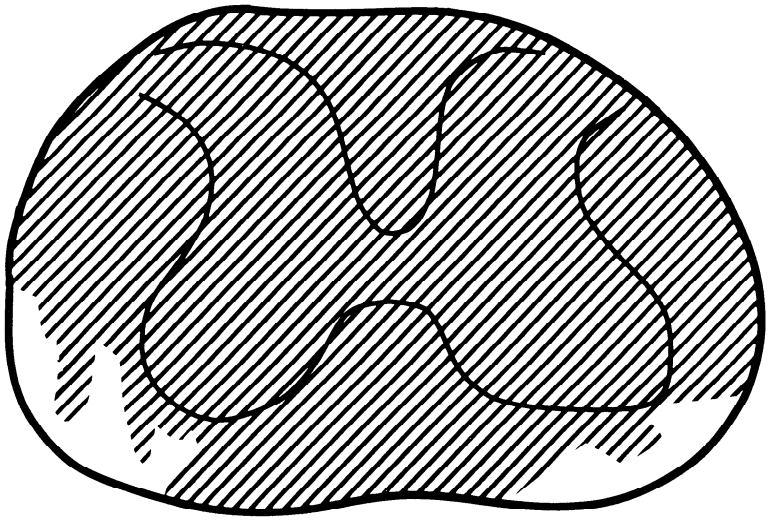

FIG. 8. Schematic representation of the overlapping of lesions that did not affect DNIC.

quadrant and, to a large extent, are crossed. To confirm this latter proposition the effects of lumbar commissurotomy were investigated with an experimental design identical to that described in the previous section. The rostrocaudal extent of the commissurotomies was large enough for sectioning all the crossed pathways involved in the transmission of messages emanating from the hindpaw extremities (see METHODS).

A typical example of these experiments is illustrated in Fig. 10: After a complete commissurotomy, inhibitions triggered from left and right hindpaws were similarly affected with a large reduction of the effects observed during the conditioning period and an almost complete abolition of poststimulus effects. This was a consistent finding in all six experiments. Figure 11 summarizes these results with camera lucida reconstructions of lesions shown in the upper part, and the mean data illustrated in the lower part. Note the symmetry between the results obtained with the left and right hindpaw conditioning respectively and their rough similarity with results illustrated in Fig. 9*B* (right).

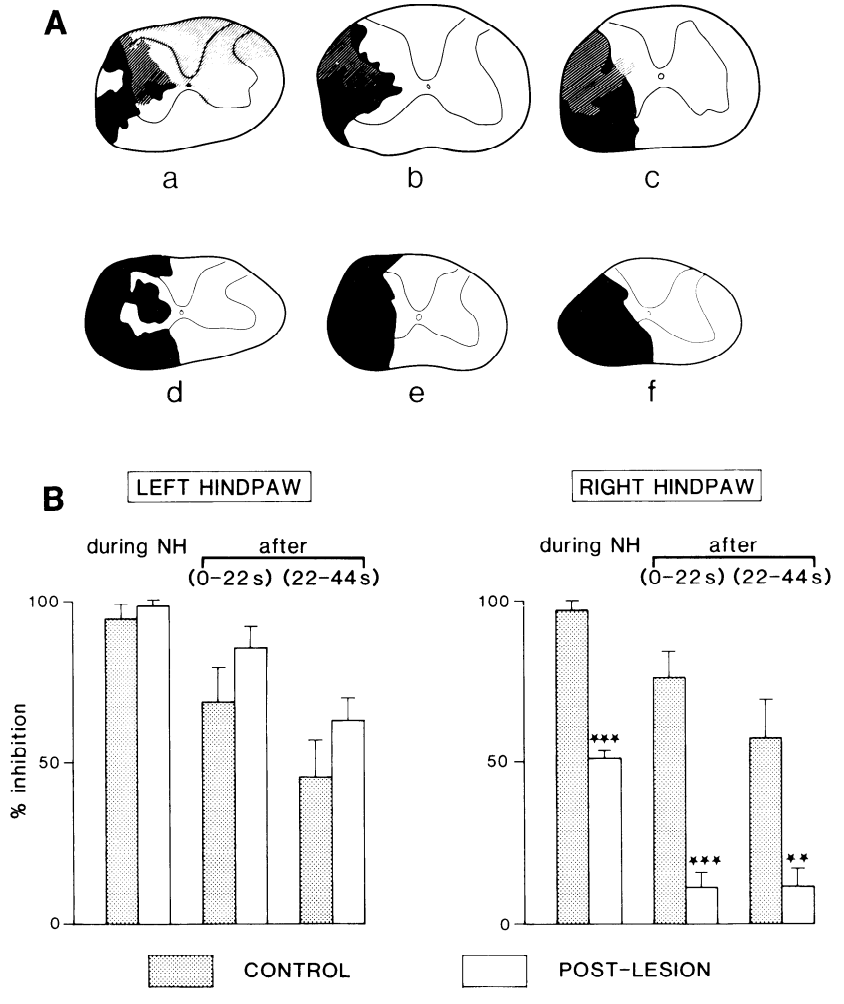

FIG. 9. Summary of experiments during which DNIC was depressed by cervical lesions. *A*: extent of individual lesions; *black area*. In the three 1st cases (*a*, *b*, *c*), this was a 2nd lesion, and the 1st one is indicated by the *hatched area*. *B*: mean results obtained during these 6 experiments (experimental design, representation and symbols as in Fig. 7). Note that inhibitory processes triggered from the right hindpaw were strongly reduced, with the posteffects disappearing almost completely. **, $P < 0.01$; ***, $P < 0.001$; paired t test.

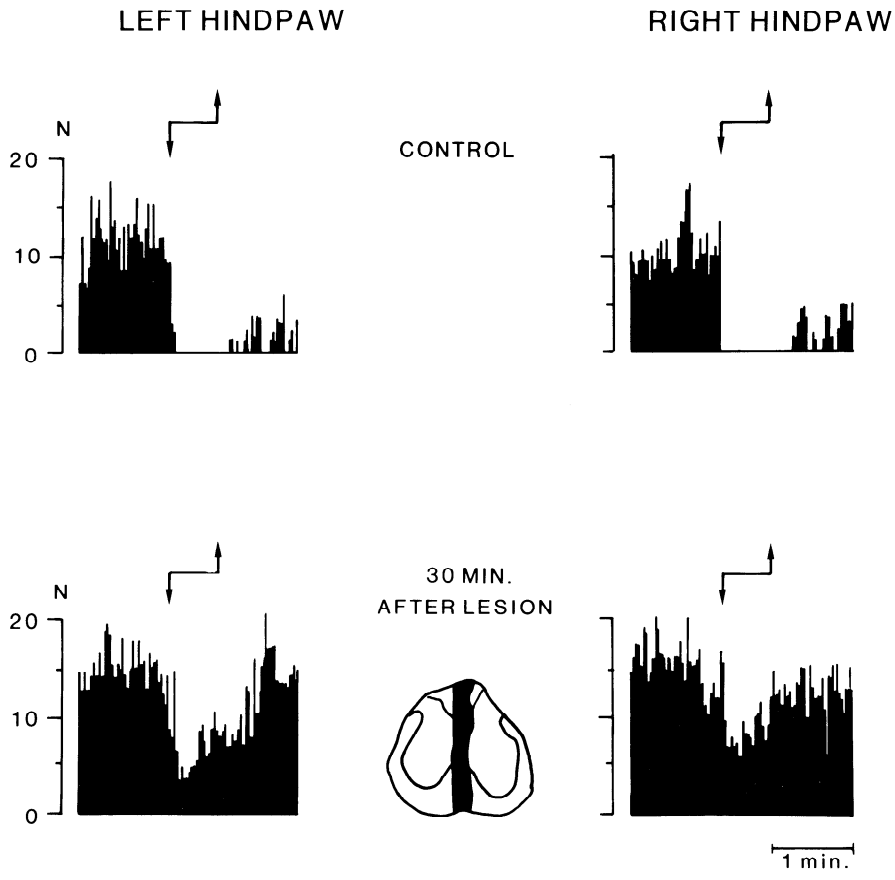

FIG. 10. Example of the effect of a lumbar commissurotomy upon DNIC (experimental design, presentation, and symbols as in Fig. 5). Note the strong reduction of the inhibitory processes triggered from either the left or right hindpaw, which was induced by the commissurotomy (*insert drawing*).

In every case, the postlesion efficacy of DNIC was ascertained by triggering strong inhibitory effects from a forepaw and/or the contralateral muzzle thus ruling out the possibility of a nonspecific degradation of the inhibitory processes induced by the commissurotomy.

Since in every case DNIC triggered from either hindpaw were strongly reduced but never abolished, these experiments indicate that both crossed and uncrossed pathways are involved in these processes. This assertion is confirmed in the following section.

Effects of ventrolateral quadrant transection upon DNIC (experiment 3)

The results presented above strongly suggested the involvement of the ventrolateral quadrant in the triggering of DNIC. However, since a dorsal approach was necessarily used to perform the lesions acutely while recording the neurons, each ventrolateral quadrant section was combined with a more dorsal lesion. One could therefore argue that the decrease of DNIC was the result of the combination of dorsal and ventral cord lesions.

To test this hypothesis, additional experiments were performed in which unilateral or bilateral sections of the ventrolateral quadrant were made before recordings.

In such a preparation, the mean unconditioned C-fiber responses contained 13.6 ± 1.8 spikes/stimulus (n = 14), which was very close to those described in control situation in experiments *1* and *2*, indicating that minimal bias was introduced by the lesions. Inhibitions of C-fiber responses induced by immersion of either the right or left hindpaw in a 52°C water

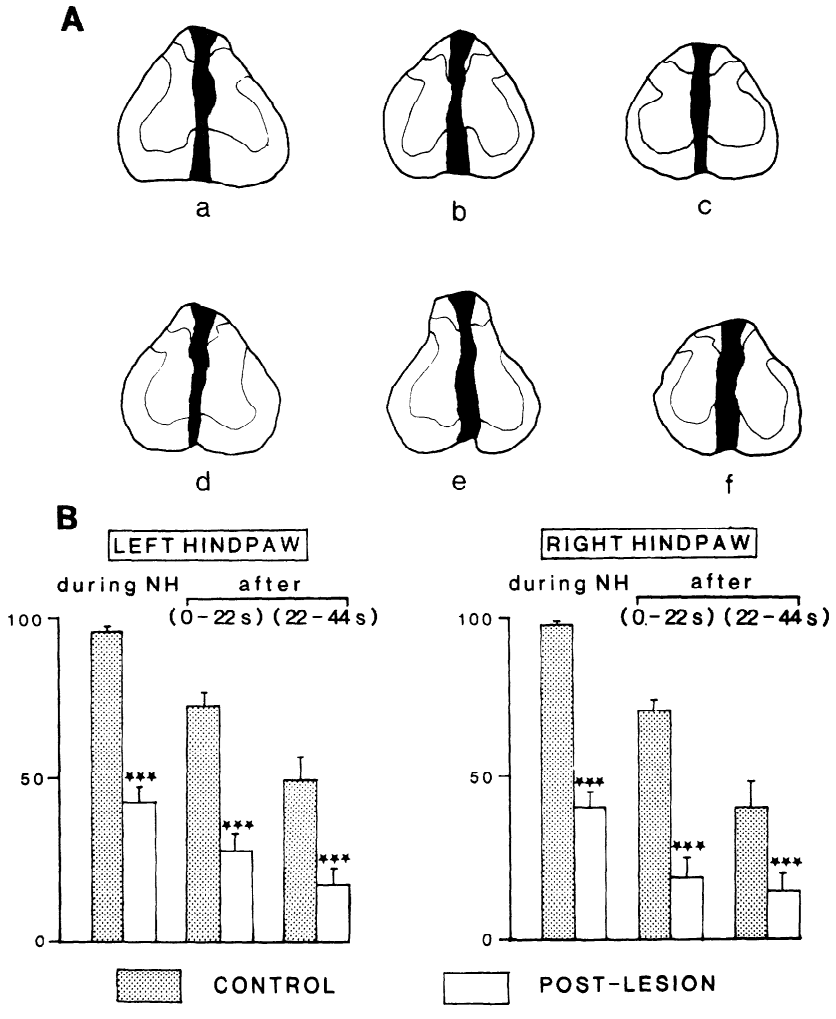

FIG. 11. Summary of experiments involving lumbar commissurotomy. *A*: extent of individual lesions. Note that the lateral spread of lesions was due to tissue displacement during histological procedures and was therefore overestimated (see Fig. 3). *B*: mean results obtained during these 6 experiments (experimental design, representation, and symbols as in Fig. 7*B*). Note the symmetrical nature of the reductions of the inhibitory processes triggered from either hindpaw, which were induced by the commissurotomy.

bath were compared in three experimental situations (Fig. 12): untransected animals (controls of experiments *1* and *2*: $n = 17$ cells), four rats with lesion of the left ventrolateral quadrant ($n = 9$ cells), and two rats with a bilateral lesion of the ventrolateral quadrant ($n = 5$ cells). Histological controls of the lesions are depicted on Fig. 12*A*. Figure 12*B* illustrates the data obtained in this experimental series.

In rats with previous lesion of the left ventrolateral quadrant, the inhibitory processes triggered from the right hindpaw were strongly reduced with a disappearance of poststimulus effects; this result is essentially similar to that described after an "acute" section of the ventrolateral quadrant (right part of Fig. 9*B*).

A decrease of inhibition was also observed during the immersion of the left hindpaw and within the following 22 s. Although of a small amplitude, this effect was statistically significant.

When a chronic bilateral section of the ventrolateral quadrant was performed, an almost complete suppression of inhibitions occurred, whether triggered from the left or right hindpaw. In all these cases, the efficacy of DNIC triggered by a pinch applied to the controlateral muzzle was ascertained, indicating that

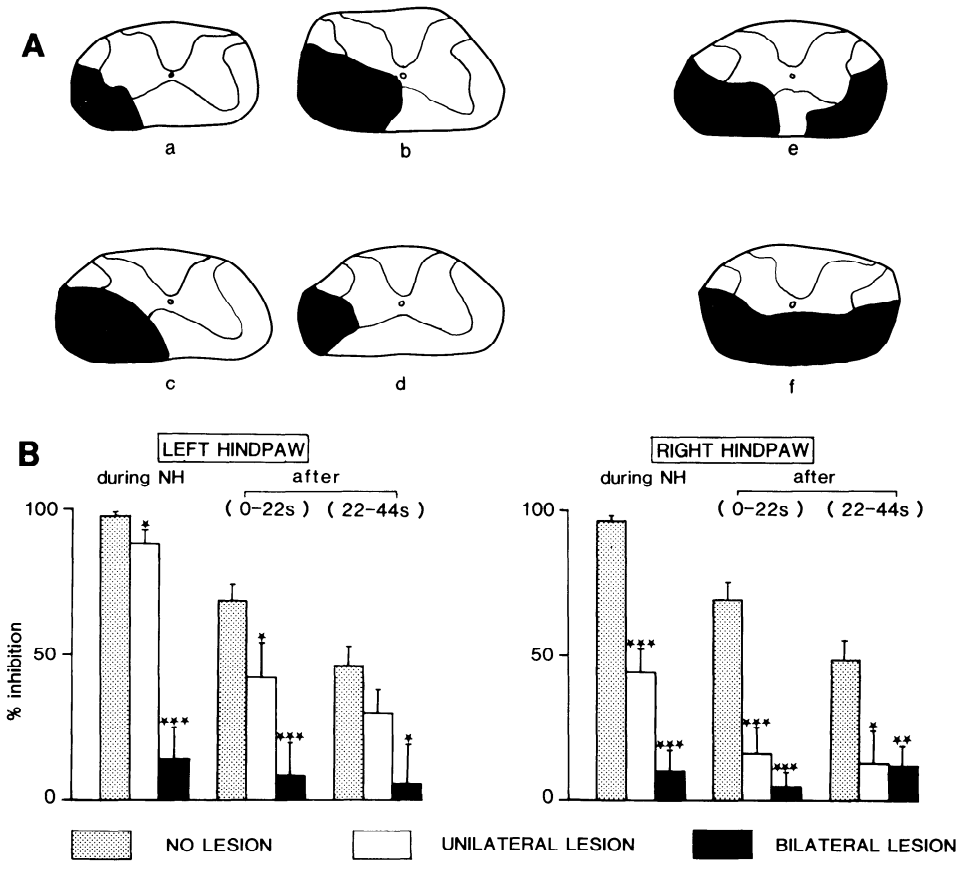

FIG. 12. Summary of experiments involving ventrolateral lesions. *A*: extent of individual unilateral (*a*, *b*, *c*, *d*) and bilateral (*e*, *f*) lesions. *B*: mean results: inhibitions observed in rats with unilateral (*open columns*) or bilateral lesions (*dark columns*) are compared with those observed in untransected animals (*hatched columns*). *, $P < 0.05$; **, $P > 0.01$; ***, $P > 0.001$; *t* test. Note that *1*) in unilateral lesions, inhibitory processes triggered from the right hindpaw were strongly reduced, with the poststimulus effects disappearing almost completely; inhibitory processes triggered from the left hindpaw were slightly decreased, and *2*) in bilateral lesions, the inhibitions, whether triggered from the left or the right hindpaw, disappeared almost completely.

the central mechanisms subserving DNIC were not impaired by the bilateral lesion.

In conclusion, this third experimental series confirmed that the ascending pathways involved in the triggering of DNIC are confined to the ventrolateral quadrant. The involvement of dorsal fasciculi appears very unlikely. The restriction of the lesion to the ventral part of the cord clearly revealed the participation of an uncrossed component in the circuitry involved in the triggering of DNIC in addition to the main crossed component.

Effects of thalamic lesions upon DNIC (experiment 4)

According to the results described above (i.e., involvement of both crossed and uncrossed pathways) and the data in the literature (see DISCUSSION), we wish to propose that spinoreticular pathways play a role in the triggering of DNIC; however the predominance of crossed pathways could suggest an additional involvement of the lateral spinothalamic tract in these processes (see DISCUSSION).

To test this hypothesis, experiments were designed to determine whether lesions of the right ventrobasal complex of the thalamus (VB), which is the main target for the lateral spinothalamic tract (25), could interfere with DNIC triggered from either hindpaw. Recordings were made 15 days after kainic acid injections in the VB (see METHODS), which systematically induced a total loss of neurons as confirmed by histological controls (see Fig. 4).

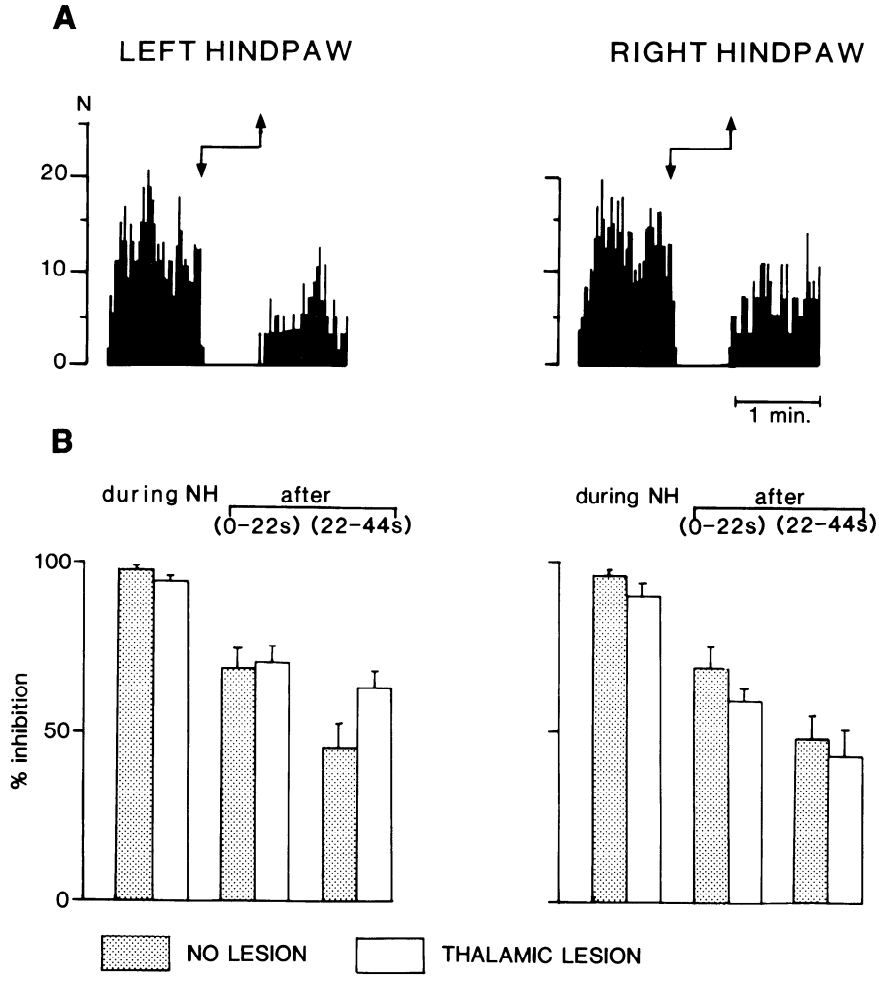

FIG. 13. Effects of the lesion of the right ventrobasal thalamic complex upon DNIC. *A*: individual example. Note the strength of inhibitory processes triggered from either hindpaw. *B*: mean results obtained during 4 experiments (*open columns*). Note that the inhibitions could be triggered equally from either hindpaw; they did not differ significantly from those observed in control situations in intact rats (*hatched columns*).

Figure 13 illustrates a typical example (upper part) and the mean data obtained for eight convergent neurons (lower part). The mean unconditioned C-fiber responses were close to those described in the previous sections (10.6 ± 3.7 spikes/stimulus), and when the general characteristics of the inhibitory effects triggered from either hindpaw were considered it was found that DNIC were similarly triggered from either side with resultant inhibitions, including poststimulus effects, almost identical to those obtained in the control sequences for the intact rats.

These experiments therefore exclude a significant involvement of the VB, and therefore of the lateral spinothalamic fibers that reach it, in the supraspinal loop subserving DNIC.

DISCUSSION

The results obtained in these experiments demonstrated that diffuse noxious inhibitory controls (DNIC) triggered by noxious stimulation of the hindpaw and acting on trigeminal convergent neurons depend on ascending pathways located in the ventrolateral quadrant of the spinal cord. In addition, such pathways were found to include a crossed and an uncrossed component. After briefly discussing some technical aspects of this study, we will

analyze the present results in relation to those ascending pathways that may be involved in nociception.

Technical consideration

The present study, which confirms earlier reports of DNIC acting on convergent neurons of the rat trigeminal system (17), was made possible by the powerful nature of these inhibitions and by their constancy and reproducibility: DNIC were again found to affect all convergent neurons, and the inhibitions produced were almost identical in magnitude and temporal pattern when comparing the effects induced by nociceptive thermal conditioning stimulation of one hindpaw compared with the other.

In a first step, we chose "acute" lesions of the spinal cord in preference to "chronic" lesions performed several days before the recordings. The great advantage of this lies in the possibility of comparing, in a standardized fashion, the behaviors of neurons before and after a lesion. A possible drawback could have been the introduction of bias in results by the acute surgical procedure. In a pilot study we observed that such perturbations were obvious in the early minutes that followed the lesion but progressively disappeared within 20 min. A 30-min latency was therefore considered a sufficient safety margin in these experiments. Indeed, the consistency of the results and, more particularly, the facts that *1*) inhibitions were found to be unchanged by large sections (e.g., see Fig. 7*A*, *g* and *h*) and *2*) there was a clear asymmetry of effects observed in the first series of experiments with inhibitions being blocked when triggered from one hindpaw and essentially unchanged when triggered from the other hindpaw (see Fig. 9*B*) lead us to believe that only a minimal bias was introduced by the surgical procedure. In addition, in those cases where inhibitions triggered from either hindpaw were impaired (2nd series of experiments), we systematically checked the capacity of the neuron to be inhibited by heterotopic stimuli applied to the anterior part of the body: In all cases DNIC could be triggered from a forepaw or the muzzle after lumbar commissurotomy. Finally, the conclusions reached with these two first series of experiments were confirmed by the third series in which lesions of the ventrolateral quadrant were performed several hours before the recordings.

Involvement of the ventrolateral quadrants

In an experimental situation comparable with that described in the present paper, the threshold for triggering DNIC was found in the range of 40 to 44°C, and a very significant correlation was observed between conditioning temperatures of 44–52°C and the degree of inhibition (38, 66). These data were interpreted as proof that DNIC are triggered specifically by the activation of peripheral nociceptors whose signals are carried by A-ϑ- and C-fibers. The immersion of a paw in a 52°C water bath is obviously a nociceptive stimulus. The application of such a temperature to the skin has been demonstrated in many experiments to excite A-ϑ and C nociceptors (4, 7, 11, 15, 30, 36) and both "noxious only" and convergent neurons of the dorsal horn (29, 32, 37, 48, 56). The ascending tracts involved in the triggering of DNIC in our experimental conditions would a priori concern pathways implicated in nociception. As discussed below, this was indeed the case.

Several sensory pathways for which a role in nociception has been suggested could have been involved: the postsynaptic fibers of the dorsal columns (2, 3, 61), the spinocervical tract which travels through the dorsolateral funiculus (14, 27, 43, 53), part of the spinothalamic tract, i.e., those fibers projecting to the medial and intralaminar thalamus and traveling through the ventromedial funiculus (25), and the lateral spinothalamic tract (9, 25, 26, 33, 46) and the part of the spinoreticular system which travels within the ventrolateral quadrant (33, 45, 46, 72).

It was not at all possible to reduce the inhibitions triggered by noxious conditioning stimulation of either hindpaw when the lesions included the dorsomedial, dorsolateral, and ventromedial regions of the cervical spinal cord. DNIC acting on trigeminal convergent neurons always persisted and presented essentially the same characteristics as those observed before performing such lesions. On the other hand, our results did underline an essential role for the ventrolateral quadrant in the triggering of DNIC, since it was this single region that remained undamaged when the overlap of the lesions that were unable to attenuate DNIC was considered (see Fig. 8). The conclusion, therefore, is that the lateral spinothalamic and/or the spinoreticular tracts are involved in these processes.

Conversely, all the lesions that reduced DNIC triggered from hindpaw included the ventrolateral region of the opposite side or at least the lateral part of that region. Interestingly, the effective lesions entirely blocked the poststimulus effects that followed the cessation of conditioning stimulation, whereas the effects observed during the conditioning period, although strongly reduced, were still present to some extent; this was observed even in the case of a virtually total hemisection. Such an observation was confirmed in the third series of experiments where the ventrolateral quadrant was unilaterally lesioned.

The possibility arose that the residual inhibitions were triggered by mechanisms different from DNIC, for instance propriospinal mechanisms triggered by heteretopic noxious stimuli. However, such a possibility appears very unlikely, not only because such a spinotrigeminal system has not yet been described, but also for the following reasons: Such propriospinal mechanisms were described in the spinal dorsal horn acting on convergent neurons of the "spinal" rat (12, 19) and monkey (24); however in the rat they were reported (12, 19) as affecting only about half of the convergent neurons when the conditioned and conditioning stimuli were applied onto neighboring regions (e.g., both hindpaws or a hindpaw and the tail) and only about 15% of the convergent neurons when such stimuli were applied to remote regions (e.g., a hindpaw and a forepaw). In the present experiments, a residual inhibition was observed in every case in which the posteffects disappeared (i.e., 6/6 experiments with cervical sections and 6/6 experiments with commissurotomies), which suggested that all trigeminal convergent neurons were concerned by such a phenomenon. In addition, inhibitions mediated by propriospinal mechanisms were reported to be weak, to adapt rapidly, i.e., within 30 s, whereas we have no evidence from the present work that the residual inhibitions adapted during the 37 s conditioning period.

We therefore conclude that ascending pathways involved in the triggering of DNIC are mainly crossed, but that an uncrossed component cannot be neglected. Experiments in which lumbar commissurotomies were performed confirmed such a conclusion unambiguously: For the reduction of DNIC, the lumbar commissurotomy appeared equivalent to a section of the contralateral ventrolateral quadrant (compare Fig. 9*B* with Fig. 11*B* right and 12*B* right).

It is important to note that although the uncrossed component could be revealed by the existence of residual inhibitory effects after contralateral cervical sections or commissurotomies, large ipsilateral cervical sections did not block the inhibitory effects triggered from the left hindpaw (e.g., see Fig. 9*A*, *c–f*). Two hypotheses could be advanced to explain such results: *1*) that the ipsilateral component plays a subordinate role that is largely transcended by signals conveyed by the crossed pathways and/or *2*) that the ipsilateral cervical lesions resulted in two opposing effects that may have masked each other, namely, a partial blockade of the uncrossed ascending pathways and a facilitation of nociceptive transmission in the crossed ascending pathways resulting from the lifting of tonic descending inhibitory controls from the brain stem.

These two hypotheses are consistent with findings obtained in our third experimental series in which the dorsal parts of the cord were not lesioned; in such a situation the dorsolateral funiculi were undamaged, and one could postulate that tonic inhibition from brain stem origin was preserved at the level of the lumbar transmission of nociceptive information; this could result in an unmasking of the decrease of DNIC triggered from the hindpaw ipsilateral to the cervical lesion (compare Fig. 12*B* left to 9*B* left). Note however that such a decrease, albeit significant was of low magnitude.

Involvement of spinoreticular pathways

The mainly crossed nature of the ascending pathways subserving DNIC could suggest the participation of spinothalamic tract neurons in these processes; with such an hypothesis, the effectively lesioned cervical regions, in these experiments in the rat, would contain fibers of the spinothalamic tract projecting to the lateral thalamus (25). Furthermore, it has been shown recently, in the rat, that ascending projections reaching the lateral thalamus are completely crossed (54), and the axons of spinothalamic neurons are classically described as crossing the midline at the level of the segment containing the cell body (20, 71).

All these considerations prompted us to undertake the last series of experiments in

which the neurons of the right lateral thalamus were destroyed by prior microinjection of kainic acid. The results were unambiguous: DNIC were triggered equally well from either hindpaw after large lesions involving the right ventrobasal thalamic complex. These experiments therefore eliminate a possible lateral thalamic link in the loop subserving DNIC.

The remaining candidate for playing a role in triggering DNIC was the spinoreticular tract, since it has been clearly established that its axons are located in the ventrolateral region of the spinal cord (33, 45, 46). Interestingly, in the rat these pathways have been shown to comprise a crossed and an uncrossed component (45, 46, 72).

In the rat, as in other mammalian species, the spinal projections to the reticular formation reach areas located all along the brain stem (45, 72), but it seems reasonable in this analysis of a pain-related system to put aside the projections to the lateral reticular nucleus and the medial pontine reticular formation that are involved in spinocerebellar loops (13, 51, 59). Because of the lack of a significant spinal projection to rostral mesencephalic reticular areas (58, 62), two major reticular sites of spinal projections remain as candidates for a role in the triggering of DNIC: the medial bulbar gigantocellular area (nucleus reticularis gigantocellularis, NGC) and the caudal mesencephalic reticular area (namely area cuneiformis, CUN), which have both long been recognized as parts of a "pain system" on the basis of electrophysiological and behavioral studies (6, 10). From the anatomical standpoint two quite different populations of spinal neurons project to NGC and CUN, respectively: NGC receives primarily projections from neurons located in laminae VII and VIII, with few from laminae V and VI (1, 13, 22, 34, 35, 55). Authors account for more than two-thirds of the neurons labeled after injections of horseradish peroxidase (HRP) in NGC to these sources in lamina VII and VIII. In sharp contrast, all authors agree that the CUN and adjacent areas receive dense projections from the dorsal horn, and in particular from laminae I and V (13, 42, 50, 68). Although electrophysiological studies of these two populations of spinoreticular neurons are still quite few in number and incomplete, it is clear that these populations exhibit large differences in their responses to noxious stimulation in the rat and in other mammalian species. Nociceptive neurons projecting to the CUN have relatively small ipsilateral receptive fields, respond to noxious thermal stimulation quite consistently, and have a threshold of response of 43–45°C (18, 28, 49). Their properties are essentially those of convergent neurons or noxious-only neurons. In contrast, neurons projecting to NGC and responding to noxious stimulation usually have large bilateral receptive fields, which in some cases do not seem to be clearly limited and change with time; their responses to a given stimulus are not very reproducible, and importantly, a strong noxious stimulus is necessary to activate these neurons (18, 28, 44, 52, 60).

We have, at present, no evidence of the particular involvement of one or the other of these structures in the triggering of DNIC. However, the above-mentioned anatomical and electrophysiological data indicate that the two spinoreticular systems are quite different, and some features of DNIC could help us to speculate on this matter: *1*) DNIC are very reproducible events that can be obtained repetitively; *2*) inhibitions are sustained throughout the period of noxious conditioning stimulation; *3*) the threshold for triggering DNIC by thermal stimulation lies between 40 and 44°C; and *4*) there is a significant correlation between the conditioning temperature and the degree of inhibition in the range of 44 to 52°C (38, 66). It therefore appears that the spinal mechanisms involved in the triggering of DNIC require a relatively high level of sensory-discriminative properties including reproducibility, a capability of being sustained throughout a period of stimulation, and the ability to encode thermal noxious stimuli.

In this respect the involvement of the spinomedullary (NGC) pathway seems unlikely since it has been precisely stated by several authors that "neurons of this system are unlikely to be involved in discriminative functions due to their complex receptive-field characteristics" (34) and "their habituation to repetitive stimuli" (44). In addition, the most prominent response of spino-NGC neurons to strong peripheral stimulation was a cessation of ongoing discharge and not an excitation (18, 44, 60). In the present study, it was also demonstrated that, although the triggering of DNIC was supported by bilateral ascending pathways in the anterolateral quadrants, the

crossed component seemed predominant. Such a unilateral feature does not fit well with the bilateral receptive fields of spino-NGC neurons (18, 28, 44, 60).

However, consideration of the characteristics of spino-CUN neurons with those of DNIC gives a different impression: Indeed, dorsal horn neurons responding to nociceptive stimuli seem to present response characteristics in agreement with those required for the triggering of DNIC, namely reproducibility of responses to a given stimulus, the encoding of graded noxious thermal stimuli (29, 32 37, 48, 56), and the unilaterality of the receptive fields that are associated with a predominantly, although not completely, crossed pathway to the mesencephalon (8, 34, 42, 45, 50, 68, 72), thus correlating well with the results of the present study. Note that many authors have emphasized the fact that this spinal projection to CUN overwhelmingly originates from lamina I (34, 42, 50, 68), and it is well known that many neurons in this lamina are responsive to noxious stimulation (see Refs. 6, 70). This latter feature is interesting because DNIC are also exclusively driven by noxious, not by nonnoxious, stimulation. Therefore, although speculative at present, the hypothesis can be proposed that the mesencephalic reticular formation, and more particularly the area cuneiformis, may play a major role in the spinoreticular systems that carry the ascending signals for triggering DNIC.

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