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Descending control of spinal nociceptive transmission. Actions produced on spinal multireceptive neurones from the nuclei locus coeruleus (LC) and raphe magnus (NRM)

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Summary. The effects of electrical stimulation in the nuclei locus coeruleus (LC) and raphe magnus (NRM) were examined on the background and/or evoked discharge of neurones in the spinal dorsal horn of anaesthetized cats. These were qualitatively, and in most cases quantitatively similar, in their action on multireceptive neurones. In these neurones an inhibitory action on the discharge evoked by noxious cutaneous stimuli or by activation of Aδ and C fibres was most prominent although in some neurones (22%) an initial excitation lasting up to 100 ms preceded the inhibition which could last up to 1 s. Excitation alone was observed in only 3% of multireceptive neurones. Electrical stimulation also produced an inhibitory action on the discharge of low threshold mechanoreceptive neurones (80%). In four of ten multireceptive neurones examined in detail, LC stimulation produced a selective inhibitory action on the discharge evoked by noxious cutaneous stimuli. In the remaining six multireceptive neurones it was partially selective against noxious as compared with non-noxious inputs. The inhibitory action was also more pronounced on the discharge evoked by activity in Aδ and C fibres than fast conducting afferents. The inhibitory action evoked by electrical stimulation in LC on nociceptive transmission in the spinal cord is suggested to play a part in mediating analgesia from LC.

Key words: Nucleus locus coeruleus – Nucleus raphe magnus – Spinal cord – Pain – Descending inhibition – Analgesia

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Introduction

It has been known for a long time that many areas of the brain can influence somatosensory and reflex transmission in the spinal cord. The recent surge of interest in the descending control of spinal nociceptive transmission, however, arose with the findings that excitation of several areas of the brain and in particular the medial brain-stem causes profound behavioral analgesia. Effective areas include the periaqueductal gray (PAG) (Lewis and Gebhart 1977; Mayer et al. 1971; Oliveras et al. 1974; Prieto et al. 1983; Reynolds 1969; Yaksh et al. 1976), dorsal raphe (Oliveras et al. 1974), nucleus raphe magnus (NRM) (Dickenson et al. 1979; Oliveras et al. 1975), nuclei reticularis gigantocellularis and paragigantocellularis, substantia nigra, hypothalamus, certain thalamic nuclei and internal capsule. It has been suggested that monoamines contribute to the generation of analgesia from the brain-stem but the exact role played by catecholamines and catecholaminergic nuclei is still controversial (Akil and Liebeskind 1975; Bodner et al. 1978; Hammond and Proudfit 1980; Sasa et al. 1977). There is, nevertheless, strong evidence which favours the concept that descending noradrenergic and serotonergic transmission in the spinal cord plays an important role in the generation of analgesia (Tyce and Yaksh 1981; Vogt 1974; Yaksh 1979). The pontine nucleus, locus coeruleus (LC) – once considered to be entirely composed of noradrenaline containing neurones – is one of the nuclei that could contribute to these analgesic mechanisms. It has been demonstrated to generate stimulation produced analgesia (SPA) (Sanberg and Segal 1978; Margalit and Segal 1979) and morphine analgesia (Yaksh et al. 1976). However, its role in opiate analgesia produced by systemic morphine is controversial since ablation studies have provided evidence in favour (Sasa et al. 1977) and against (Hammond and Proudfit 1980) this concept.

One of the neuronal mechanisms generating analgesia is by activation of descending pathways to the spinal cord. This in turn suppresses directly or indirectly via interneurones the discharge of nociceptive neurones ascending in somatosensory pathways and/or interposed in reflex pathways from cutaneous, muscle and visceral nociceptors. The neuronal discharge of spinal dorsal horn nociceptive neurones is under a powerful segmental and descending inhibitory influence from many areas including PAG (Carstens et al. 1981; Duggan and Morton 1983; Oliveras et al.1974), medullary raphe nuclei (Belcher et al. 1978; Gerhart et al. 1981; Fields et al. 1977; McCreery et al. 1979; Willis et al. 1977) hypothalamus (Carstens et al. 1983) and cortex (Yezierski et al. 1983). Some of these areas (medial part of the caudal brain-stem, NRM-Rmc) have previously been shown by Lundberg and collaborators (reviewed in Lundberg 1982) to be involved in the tonic and phasic control of transmission in flexor reflex pathways. However, the recent evidence, instead, suggests that the ventrolateral caudal medulla might play a role in the tonic descending control of nociceptive transmission in the spinal cord (Hall et al. 1982).

Neurones in the nucleus locus coeruleus project to the spinal cord in the cat (Hancock and Fougerousse 1976; Stevens et al. 1982; Kuypers and Maisky 1977; Tohyama et al. 1979a, b) and in several other species including the rat (Commissiong et al. 1978; Nygren and Olson 1977; Pickel et al. 1974; Westlund et al. 1983), monkey (Hancock and Fougerousse 1976; Westlund et al. 1984) and man (Papez 1925). There is some evidence that indicates the existence of afferent-efferent connections between LC and the raphe nuclei (Amaral and Sinnamon 1977; Chu and Bloom 1974; Sakai et al. 1977, 1979; Segal 1979) which in turn project to the spinal cord (Basbaum et al. 1978; Bobillier et al. 1976; Tohyama et al. 1979a, b).

The neuronal mechanisms generating analgesia from LC and the significance of the coeruleospinal projection are not well understood. Suppression of nociceptive transmission ascending in somatosensory pathways could be an important neuronal mechanism that contributes to the generation of analgesia. Multireceptive neurones which are abundant in the deeper laminae of the dorsal horn receive input from smaller diameter (Aδ and C) nociceptive afferents and large diameter (Aα) sensitive mechanoreceptive afferents. These neurones form a component of the spinothalamic tract (McCreery et al. 1979) and other ascending pathways relaying information to higher centres. The aim of the present study therefore was to examine the actions that LC may exert on the activity of multireceptive neurones in the dorsal horn of the cat spinal cord. A detailed report by Hodge et al. (1983) has recently appeared on this subject since the completion of our studies. Our findings confirm and extend their observations by addressing the following questions: a) Does the nucleus locus coeruleus exert a selective inhibitory control on primary afferent input relayed from cutaneous nociceptors? b) What are the temporal characteristics of its action? c) Are the actions evoked from LC comparable to those produced by NRM? Some of the results reported here have been published in brief (Iggo et al. 1981; Mokha and Iggo 1980; Mokha et al. 1983).

Material and methods

Surgical procedures

Experiments were performed on cats (2.5–3.5 kg) under chloralose anaesthesia (60–70 mg/kg i.v.) after induction with 4% halothane in a mixture of O_2/N_2O. The animals were paralysed with gallamine triethodide (Flaxedil) and artificially respired. Anaesthesia was monitored by allowing the animal to recover from paralysis at regular intervals, by observing the pupillary diameter and by detecting any sudden increases in the arterial blood pressure. Additional anaesthetic doses were given as required. Arterial blood pressure was continuously monitored and results reported here are from preparations with a minimum mean pressure of 80 mm Hg. The end tital CO_2 was continuously monitored and maintained between 3.5–4.5%. The body temperature was maintained constant at 37° C with an electric blanket thermostatically controlled by a rectal probe. A laminectomy was performed from L_2–L_7 and the exposed surface of the cord was covered with a warm paraffin pool and was maintained at 37° C by means of a feedback heating unit. Craniotomy was performed and the exposed surface of the brain was covered with fine cotton wool soaked in normal saline. Parts of the tentorium were carefully removed. The midline and the dorsal surface of the brain-stem were exposed in some experiments by removal of the cerebellum.

Recording and stimulation procedures

Single unit activity in neurones of the lumbosacral spinal cord was recorded extracellularly using glass microelectrodes filled with a solution of 4% pontamine sky blue in 0.5 M sodium acetate. The recordings were made from multireceptive neurones that were excited by an input from Aδ and C nociceptive afferents. However, in some instances recordings have also been made from neurones receiving input from sensitive mechanoreceptors only (mechanoreceptive). The hind limbs were shaved and peripheral receptive fields were mapped mechanically using a camel hair brush, sharp and blunt probes, toothed forceps and serrated clips. A radiant heat source was used for applying a controlled noxious heat stimulation. The effect of LC and NRM stimulation was also tested on the discharge evoked by electrical stimulation through bipolar electrodes, 20 mm apart on the ipsilateral tibial nerve. The thresholds for the activation of Aα, Aδ and C fibres were determined by monitoring the compound action potential recorded from the tibial nerve.

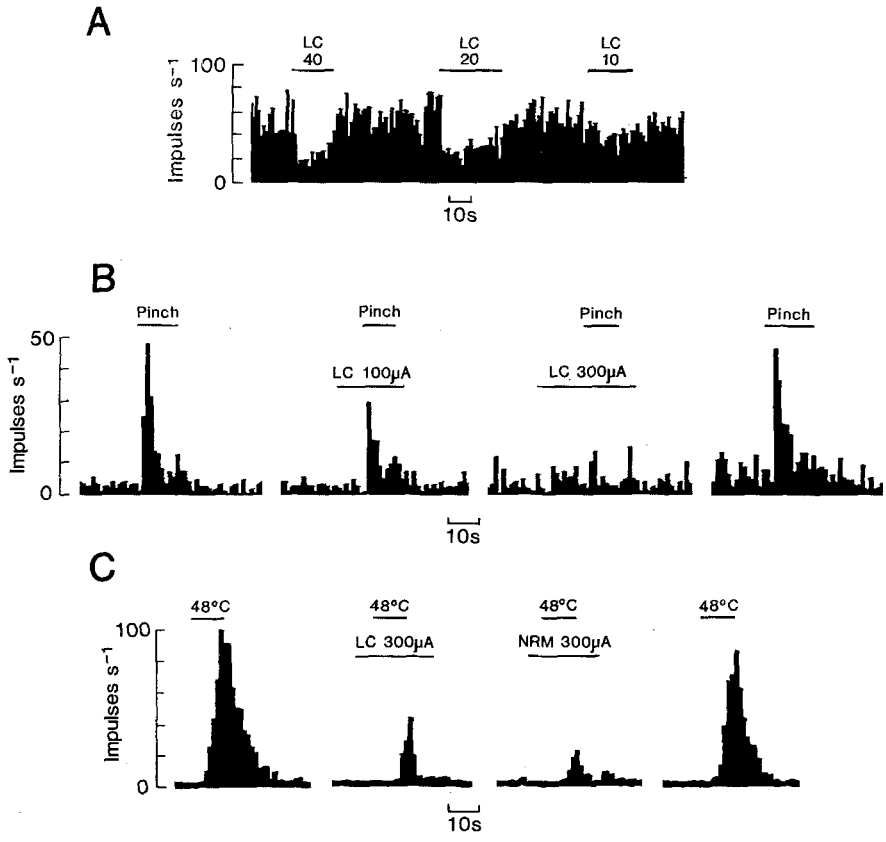

Fig. 1A–C. The histograms show the effect of stimulation in LC or NRM on the background or evoked discharge of three multireceptive neurones. **A** Frequency histograms illustrate the inhibitory effect on the background discharge. Stimulation parameters were 80 ms bursts of pulses (0.2 ms square pulses at 300 μA current intensity) repeated at intervals of 0.5 s for the durations indicated by the bars above the histograms. The number of pulses in each stimulus burst was changed systematically from 40 to 10 as indicated by the numbers above the bars. **B and C** The frequency histograms illustrate the inhibitory effect on the discharge evoked by noxious pinch (**B**) or noxious thermal stimulation (48° C for 10 s). Noxious stimuli were repeated at intervals of 3 min. Stimulation parameters for LC, except where indicated, were 16 pulses at 200 Hz duration 0.2 ms and current intensity 300 μA whereas parameters for NRM stimulation were 6 pulses at 200 Hz. Trains of stimuli were repeated 1/s. Threshold for producing inhibition from NRM was 100 μA whereas from LC it was 150 μA when using a train of 6 pulses at 200 Hz

Activity elicited by the natural cutaneous and by electrical stimulation was amplified and displayed using conventional means and were recorded on a FM data tape. These amplified action potentials were also led to a spike processor (Digitimer D130) and a chart recorder (Devices DC5H) which enabled construction of instantaneous frequency histograms of the activity. The 'dot raster display' technique was also used for data display.

An array of 6 (3 pairs, 1 mm grid) insulated concentric bipolar stainless steel electrodes (SNE-100, Rhodes Medical Instruments; 200 μm tip diameter, 500 μm tip separation) was placed in LC at an angle of 45° C to the horizontal plane and aimed at the stereotaxic coordinates $P_{2\text{-}4}$, $L_{2.0\text{-}3.5}$, $H_{-2\leftrightarrow-2.5}$ according to the atlas of Berman (1968). In a few experiments the most rostral parts of the nucleus were also stimulated using a single electrode. Stimulation in NRM was applied using an electrode similar to those used for LC stimulation and was directed at the stereotaxic coordinates P8, H-8, LO in the majority of experiments. However, the more rostral levels of NRM were also stimulated. The inhibitory action from LC and NRM was evoked by using stimulus intensities ranging from 50 μA–300 μA of constant current (HI-MED constant current stimulator). Square pulses (200 μs) in 60–100 ms trains at 200–500 Hz were repeated at intervals ranging from 330 ms to 1 s. Stimulation parameters used in the present study are comparable to those used in other studies (Carstens et al. 1981; Hodge et al. 1983; Fields et al. 1977; Willis et al. 1977). Gross stimuli can also produce activation of nearby structures and axons of passage. However, the concentric bipolar electrode configuration gives better current localisation compared to monopolar electrode configuration. There was a current spread of 1 mm at the maximum stimulus intensities used in the investigation since effects induced at one electrode in the array were not produced at adjacent electrode in the array (Fig. 10).

Histological procedures

Recording sites in the spinal cord were marked by the electrophoretic deposition of the pontamine sky-blue dye contained in the recording microelectrode and the spot was reconstructed from 40 μm thick transverse frozen sections of the spinal cord.

Stimulation sites in LC and NRM were marked by passing 300 μA DC current for 30–100 s through the stimulating electrode at the end of each experiment. The animals were perfused with formal-saline and the brain-stem was removed and left in formal-saline for five days. The lesions made in LC and NRM were reconstructed from 50 μm thick serial, transverse sections stained with cresyl-violet. Sections were examined under a light microscope (Zeiss or Nikon) and reconstructed using a camera lucida attachment (Zeiss) or a similar drawing tube (Sankei). The position of each site of stimulation in LC and NRM was studied, determined and reconstructed from the serial transverse sections with the help of the atlas of Berman (1968).

Results

The effects of stimulation in LC were studied on the background and/or evoked discharge of 150 neurones in 45 cats. This sample of 150 dorsal horn neurones included multireceptive (145/150) and low threshold mechanoreceptive (5/150) neurones. The effect of stimulation in LC was studied on the background discharge (spontaneous discharge) and on the dis-

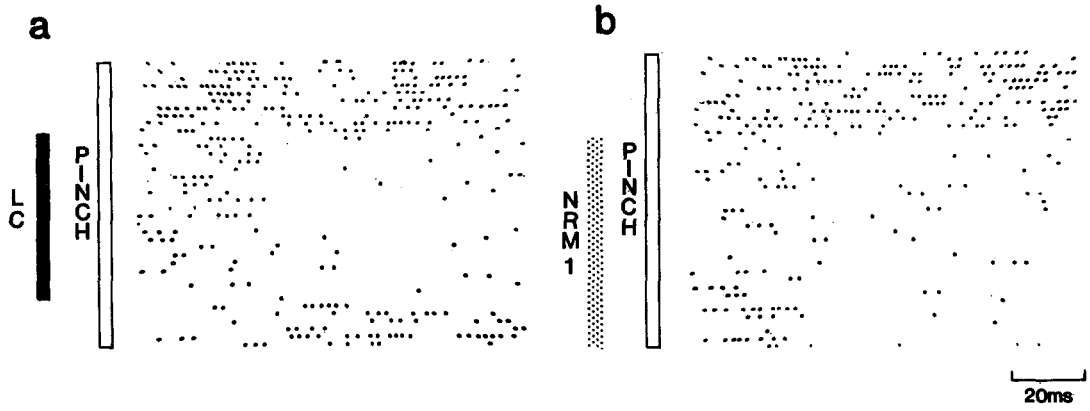

Fig. 2a and b. The inhibitory effect of LC and NRM stimulation on the pinch evoked discharge of a multireceptive neurone is illustrated using the dot raster display technique in **a** and **b.** Each dot in **a, b** and in subsequent figures using this display represents one action potential. Each horizontal line (sweep) was triggered by the stimulator (triggering pulse) and each successive sweep triggered at the end of every cycle (1 s in this example) was displayed below the preceding one. The time of application of LC and NRM stimulation is represented respectively by the black and stippled bars whereas the duration of peripheral stimulation is represented by the open vertical bar. A train of 2 ms pulses at current intensity 300 μA applied at the start of each sweep in LC or NRM was repeated once every second. **a** Show the effect on the pinch-evoked discharge of stimulation in LC with trains of 3 pulses at 200 Hz. **b** The effect of stimulation in NRM with a single pulse is shown in the dot-raster display

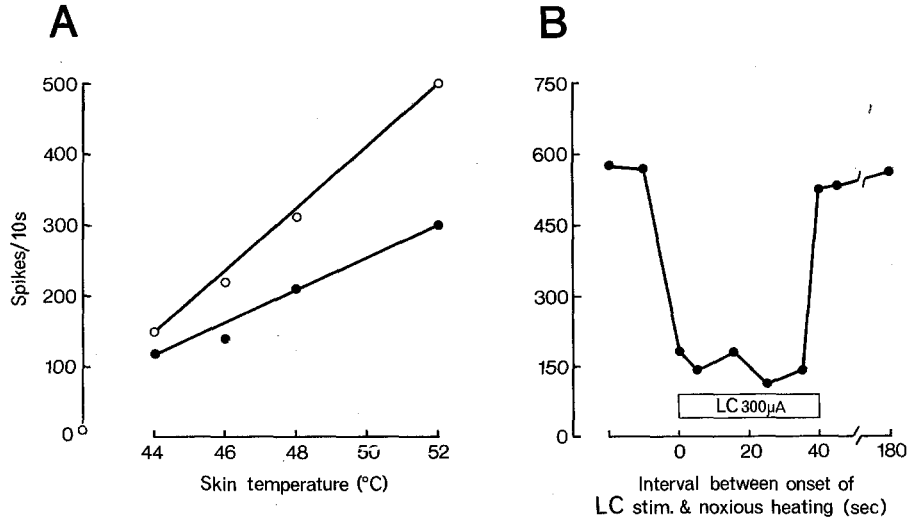

Fig. 3. A The graph shows the control responses (open circles) of a multireceptive neurone evoked by noxious heat stimuli (abscissa) applied for 10 s and the reduction in these responses when LC was stimulated electrically with a train of stimuli repeated once every second. Stimulation parameters were 16 pulses at 200 Hz, 0.2 ms duration and current intensity 300 μA. **B** The graph illustrates the time course of the inhibitory effect produced by stimulation in LC on the response of a multireceptive neurone evoked by noxious heat (48° C for 10 s) which was applied at different intervals after the start of stimulation in LC that lasted for 40 s. The discharges were counted for 10 s, starting 5 s after the onset of each heat stimulus, and are plotted (●) against the time at the start of each count

charge evoked by natural cutaneous stimulation and/or electrical stimulation of the tibial nerve (TN).

Effects of LC stimulation on the discharge of multireceptive neurones

LC stimulation inhibited both background (Fig. 1A) and stimulation evoked discharge of 114 multireceptive neurones. In 20 neurones there was an initial excitation followed by pronounced inhibition. Excitation alone was also observed on 8 multireceptive neurones. Two of the latter neurones also received input from joint and muscle afferents in addition to cutaneous input. Threshold for the excitatory action was less than 50 μA whereas it varied from 50–150 μA for the inhibitory action. Only three neurones were unaffected by stimulation of LC.

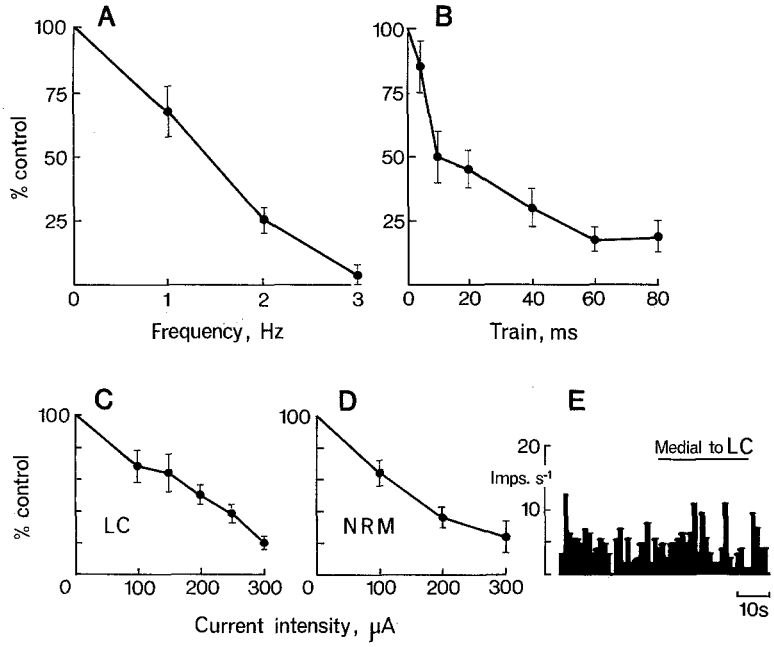

Fig. 4. A–E Show the relationship of the inhibitory action produced from LC or NRM. This is illustrated on the background discharge of a multireceptive neurone. Stimulation parameters were 16 pulses at 200 Hz, 0.2 ms duration and current intensity of 300 μA. Trains of stimuli were repeated once every second. Changes in parameters of stimulation for individual records are indicated as appropriate. **A** Shows the quantitative data on the effect produced by increasing the repetition rate of the train of stimuli. **B** Shows the recruitment of inhibition produced by increasing the length of a train from 5 ms to 80 ms. Trains of stimuli were repeated at intervals of 0.5 s. **C and D** Recruitment of inhibition from LC or NRM on increasing the intensity of stimulation in LC (**C**) or NRM (**D**). The trains of stimuli were repeated twice a second. **E** Shows that using identical stimulation parameters as above through an electrode lying approximately 1 mm medial to the electrode that produced the inhibitory effect (**A–C**), did not produce any action on this neurone. Such sites as these are represented by open triangles in Fig. 10. Each point in the graphs (**A–D**) represents a mean of 4 trials and the standard errors of the mean are marked as shown at each point. 100% in **A–D** equals the averaged number of impulses over several trials, counted prior to each LC or NRM stimulation trial for a period that equalled the duration of stimulation (20 s)

a) Inhibition of excitatory responses to noxious input

The effect of LC stimulation was studied on the discharge evoked by noxious cutaneous stimulation such as pinch and/or noxious radiant heat (43–55° C) applied for 10 s on the cutaneous receptive field of the unit. There was a partial or complete inhibition of the response (Figs. 1B and C, 2).

The noxious cutaneous stimuli were applied at regular intervals of 3–5 min. Multireceptive neurones show a progressive rise in their discharge on increasing the level of noxious heat applied on the cutaneous receptive field. Stimulation in LC when tested on such a neurone reduced the slope of the curve (Fig. 3A).

Relation of inhibition to stimulation parameters. The standard parameters chosen for electrical stimulation in LC were 0.2 ms square pulses in 80 ms trains at 200 Hz that were repeated at intervals ranging from 330 ms to 1 s. Results for a typical multireceptive neurone are illustrated in Fig. 4. Increasing the repetition rate of trains of stimuli from 1.0 to 3.0/s (Fig. 4A) or train length (Fig. 4B) or current intensity (Fig. 4C) enhanced the inhibition of the background discharge and at a repetition rate of 3/s there was complete inhibition (Fig. 4A). Increasing the current intensity or the repetition rate of trains of stimuli also caused a progressive increase in inhibition of the heat evoked discharge. There was a 20–80% reduction in discharge evoked by heat or pinch depending on the LC stimulation parameters but occasionally complete inhibition of the heat evoked discharge was observed.

Time course of inhibition evoked by stimulation of LC. The inhibitory action of a single 80 ms train of stimuli to LC on the background discharge lasted for as long as a second. The time course of the inhibitory action was measured by examining the degree of inhibition of the heat evoked discharge tested at various intervals during stimulation in LC as indicated in Fig. 3B. The heat stimulus, of 10 s duration, was applied at given delays after the start of stimula-

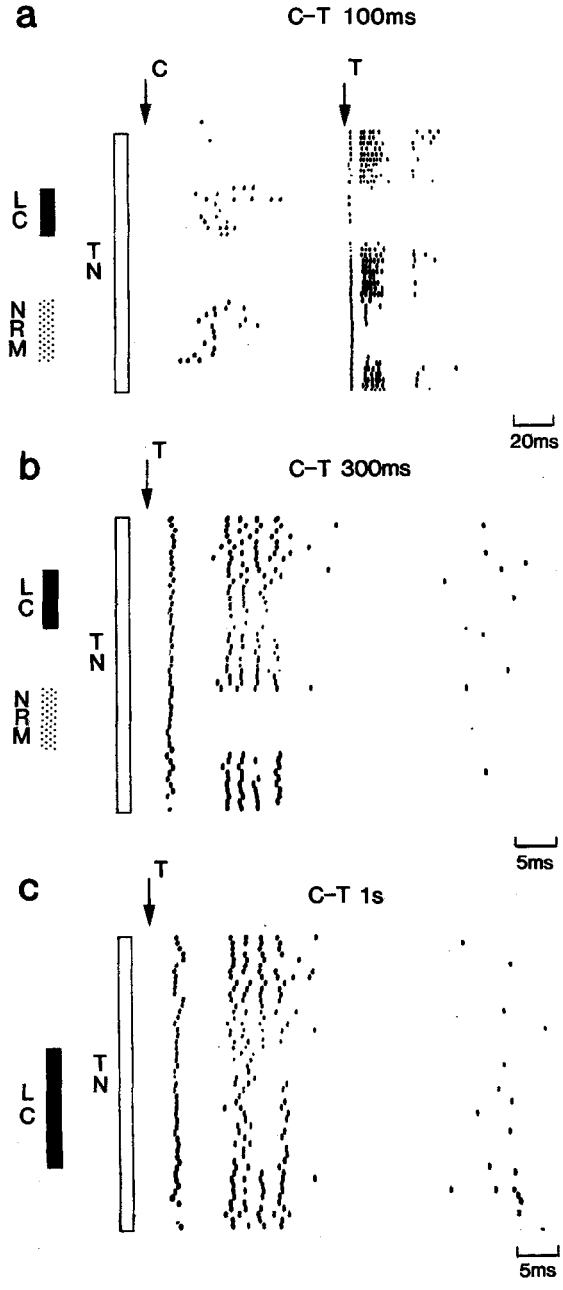

Fig. 5a–c. Effects of LC or NRM stimulation on the discharge of a multireceptive neurone evoked by supramaximal tibial nerve stimulation (TN) at various conditioning-test intervals are shown in a dot-raster display. **a** Shows the effect of conditioning trains of stimuli applied in LC or NRM at the start of each cycle (arrow C) on the discharge evoked by a test stimulus applied to the tibial nerve (0.5 ms pulse at 10 V) at a conditioning-test interval of 100 ms (arrow T). Stimulation parameters of the conditioning stimuli were 12 pulses at 200 Hz, 0.2 ms duration and current intensity 300 μA, repeated once every second. Stimuli in either LC or NRM produced an initial excitation followed by an almost complete inhibition of the tibial nerve evoked discharge. **b** The inhibition was less at longer conditioning test intervals of 300 ms. Increasing the pulse duration of LC stimulus pulses from 0.2 ms to 2 ms restored the inhibition at C–T intervals up to at least 1 s **c.** In **b** and **c** the oscilloscope was triggered by the test pulse and the C–T stimuli were repeated 1/330 ms

tion in LC. The response was strongly inhibited when the heat stimulus was applied at the onset or during the course of LC stimulation. There was no inhibition if the heat stimulus preceded by 5 s the end of LC stimulation (Fig. 3B). The temporal characteristics of the inhibitory action on the discharge evoked by electrical stimulation of peripheral afferents were investigated by varying the conditioning-test interval. If the test stimulus was applied to the tibial nerve or dorsal root, the inhibition was present when the conditioning test interval was at least 30 ms. The inhibition became progressively weaker as the conditioning test interval was increased. It was still powerful at an interval of 100 ms (Fig. 5a) weak at 300 ms (Fig. 5b) and absent at 500 ms. Increasing the stimulus pulse duration from 0.2 to 2 ms led to a reappearance of the inhibition, and it was still present at conditioning-test interval of at least 1 s (Fig. 5c).

Comparison of inhibitory action on response to noxious vs non-noxious stimuli. A selective reduction in the discharge evoked by noxious stimuli was observed in 4 of 10 multireceptive neurones (Fig. 6a). Discharges evoked by non-noxious stimuli were unaffected (Fig. 6b). In the other multireceptive neurones tested the response to noxious stimuli was more powerfully affected although the non-noxious responses were also reduced but to a lesser extent. A typical example is illustrated in Fig. 7a.

Effects on the discharge evoked by electrical stimulation of the tibial nerve. The effects of stimulation of LC were tested on the discharge evoked by electrical stimulation of the tibial nerve at various intensities. The inhibitory action of stimulation in LC was more effective against the discharge evoked by activity in Aδ and C fibres than the more rapidly conducting afferent fibres (Figs. 5 and 8a).

b) Neurones both excited and inhibited from LC

LC stimulation produced mixed excitation and inhibition on 20 multireceptive neurones.The excitation lasting up to 100 ms usually appeared first (Figs. 5a, 8a, b) and was followed by a longer lasting inhibition (Fig. 8b). Rarely, a late period of excitation was present after the inhibition.

Comparison of LC and NRM actions on different neuronal classes

a) Multireceptive neurones. Effects of LC stimulation tested on both the background discharge and on

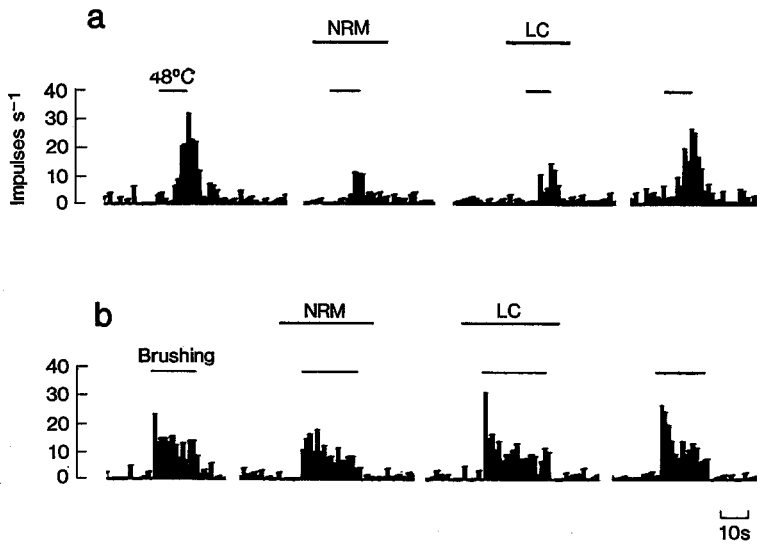

Fig. 6a and b. The frequency histograms illustrate the selective inhibitory action of both LC and NRM on the discharge evoked in a multireceptive neurone by noxious stimuli. Stimulation parameters were 12 pulses at 200 Hz, 0.2 ms duration and current intensity 300 μA. These trains of pulses when repeated once every second, produced a selective reduction in the heat evoked discharge as shown in **a** but did not affect that evoked by brushing **b**. The labelled bars on top of histograms represent the duration of various stimuli

responses evoked by peripheral stimulation were compared with effects produced by NRM stimulation on the same 92 neurones. Short trains (60–100 ms) consisting of 0.2 ms square wave pulses at 200–500 Hz (repetition rate = 0.5–2 Hz) were used for stimulation in LC and NRM. An inhibitory action was produced on 67 neurones from both LC and NRM (Figs. 1C, 4CD, 6, 7a, b). In two other neurones only NRM was effective. The threshold for the inhibitory action from LC varied from 50–150 μA whereas it was 50–100 μA in case of NRM. In 20 neurones LC and NRM stimulation both caused an initial period of excitation followed by a period of inhibition (Figs. 5a, 8a, b) which lasted longer than the excitation. The initial excitation lasted up to 100 ms (Figs. 5a, 8a, b) whereas the subsequent inhibition lasted up to 1 s or more (Fig. 8b). Four of these 20 neurones were excited and then inhibited by NRM but were only inhibited by LC. Three were excited and then inhibited by LC but were only inhibited by NRM (Fig. 8a). Stimulation also produced only excitation on some neurones (3/92). LC produced excitation on all of these whereas NRM was found effective in only one of them.

In a majority of neurones the LC and NRM evoked inhibition was similar in magnitude and duration (Fig. 8b). Occasionally, inhibition was greater from LC or from NRM (Figs. 1C, 7b, 8a). The inhibitory action of LC and NRM was more effective against the discharge evoked by noxious cutaneous stimuli than that evoked by non-noxious cutaneous stimulation. NRM inhibited the latter discharge more potently than did LC. NRM inhibition like the inhibitory effect from LC was more marked on the later part of the discharge evoked by activity in Aδ and C fibres than on that evoked by activity in faster conducting fibres (Fig. 8a). Postinhibitory rebound was observed on some multireceptive neurones and was not as frequent from LC as it was from NRM.

b) Low threshold mechanoreceptive neurones. Stimulation of both LC and NRM was tested in 5 low threshold mechanoreceptive neurones, four were inhibited from both whereas there was no effect on the remaining one neurone.

Locations of recording and stimulation sites

a) Spinal cord locations of multireceptive neurones. The location of recording sites of the majority of multireceptive neurones as marked by the electrophoretic deposition of pontamine from the recording microelectrode is shown in Fig. 9a, b. Multireceptive neurones irrespective of their location in the dorsal horn (in laminae I or V) were inhibited or excited-inhibited by stimulation in LC or NRM (Fig. 9a, b). Stimulation in LC and NRM produced inhibition (3 neurones) or mixed excitation-inhibition (2 neurones) in a small number of neurones recorded within the boundaries of the substantia gelatinosa. These neurones also displayed similar characteristics to the other dorsal horn neurones examined.

b) LC stimulation sites. The rostrocaudal extent of the nucleus from P_2–P_4 was investigated in the majority of the experiments. The composite pictures (Fig. 10 P_2–P_4) show that the majority of the sites stimulated in the present investigation were located either within or directly adjacent to the nucleus locus

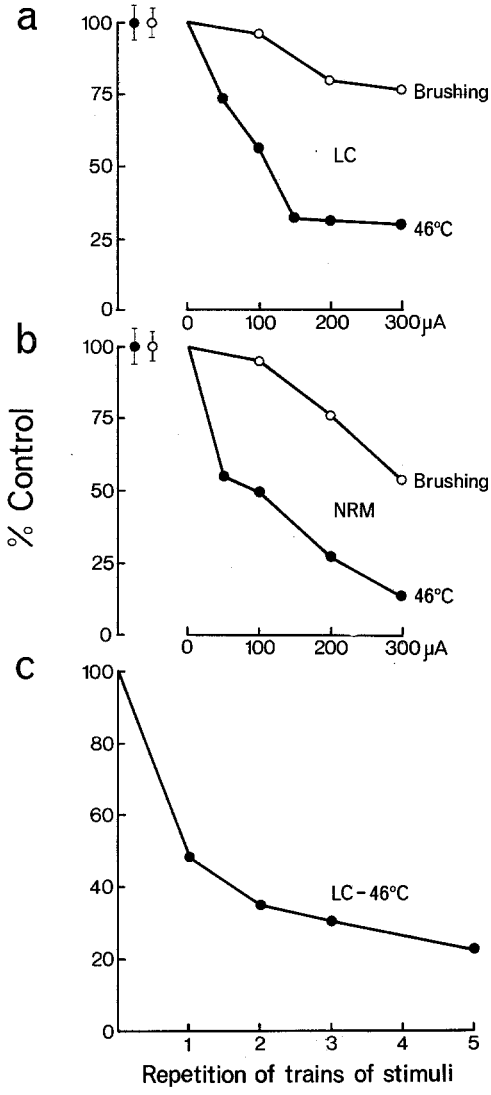

Fig. 7a–c. The graphs illustrate a typical example showing the relationship between the intensity of electrical stimulation of LC and of NRM and the degree of inhibition of the discharge evoked by noxious heating (46° C) (filled black circles) and brushing of the receptive field (open circles). Stimulation parameters were 6 pulses at 200 Hz, 0.2 ms and current intensity of 300 μA. Trains of stimuli were repeated at intervals of 0.5 s. The variability of the control responses to heat and brushing is shown at the top left hand corner of each graph, as the SE of mean. **c** Increasing the repetition rate of the train of stimuli in LC produced progressively increased inhibition of the discharge evoked by noxious heat. 100% equals the number of impulses counted over a period of 10 s – generated by brushing or noxious heat applied for 10 s in the absence of LC or NRM stimulation. Absolute size of the control response was different for each modality

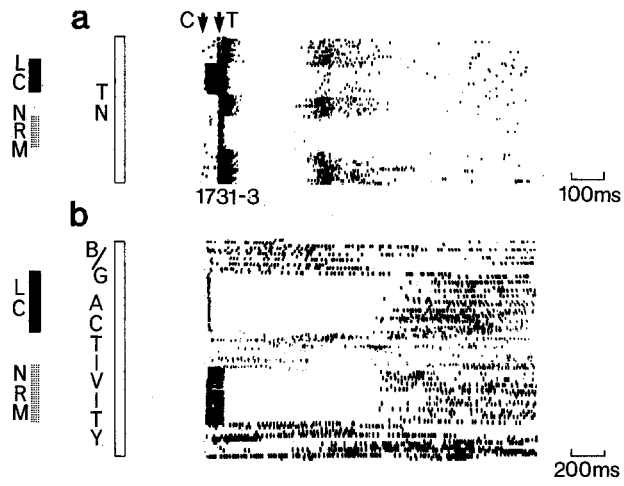

Fig. 8a and b. Comparison of the effects evoked from LC and NRM. The figures show the effects of stimulation in LC and NRM on the responses of two multireceptive neurones. **a** Stimulation in LC with trains of stimuli repeated at 1.2 s intervals produced an initial excitation that was followed by inhibition of the discharge evoked by tibial nerve stimulation (0.5 ms pulse at 2 V) applied 40 ms after the onset of LC stimulation. Stimulation parameters were 12 pulses at 200 Hz, 0.2 ms duration and current intensity of 300 μA. Stimulation in NRM with identical stimulation parameters produced only an inhibitory action. **b** Stimulation in LC or NRM with trains of stimuli repeated at 2.2 s intervals inhibited the background discharge (B/G Activity) for longer than a second. NRM also caused a much stronger initial excitation. Stimulation parameters were 20 pulses at 200 Hz, 0.2 ms duration and current intensity 300 μA

coeruleus. The filled circles represent stimulation sites that produced an inhibitory action whereas the open circles represent sites that produced an excitatory action. Electrical stimulation of the rostral part of the nucleus at P0.9 also produced effects which were similar to the actions produced from other parts of the nucleus. However, stimulation at this most rostral site was not used routinely since it is embedded in the ventrolateral part of the periacqueductal gray. In order to obtain an indication of current spread, the medial electrode of the pair was placed so that it was approximately 1 mm outside the nucleus and the lateral electrode was within the nucleus. In four experiments the maximum current intensities of 300 μA used did not produce any effect (Fig. 4E) from the medial electrode in the pair (Fig. 10P2, P3) represented by open triangles whereas the lateral electrode (represented by filled triangles) did. This observation was repeated in several experiments that indicated a current spread of less than 1 mm at the maximum current intensities used in this study. Locus coeruleus stimulation at any place along the rostrocaudal extent (P_2–P_4) produced consistent effects on the discharge of neurones.

c) NRM stimulation sites. The effective NRM stimulation sites are shown as filled triangles, located within or adjacent to the nucleus raphe magnus (Fig. 10 P6–P8). Stimulated at various sites along the rostrocaudal extent of the nucleus, from P_6–P_9, produced consistent effects. Sites of stimulation

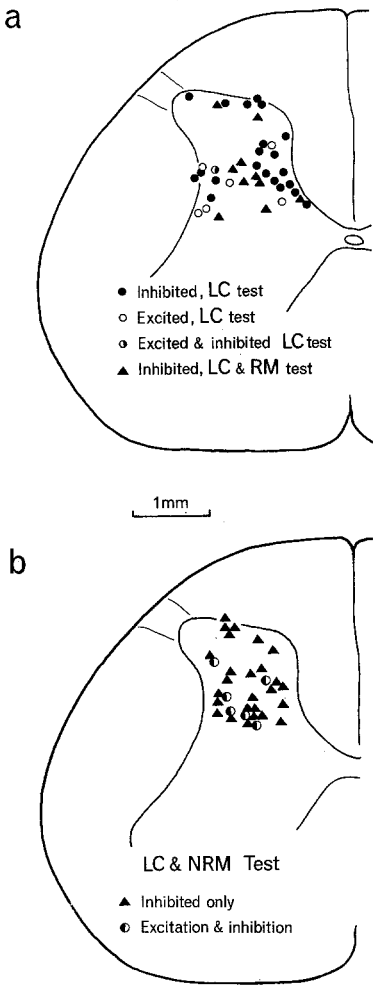

Fig. 9a and b. Location of marked recording sites of multireceptive neurones in the spinal cord. The keys for the figures are shown at the bottom of each figure. "LC test" **a** means that only the effect of LC stimulation was studied whereas "LC and NRM test" **a and b** means that the effects of stimulation in both LC and NRM were examined on the activity of multireceptive neurones (represented by filled triangles)

located outside the nucleus required higher stimulus intensities to produce effects similar to those produced from the sites within the nucleus. The NRM effects could be evoked at current intensities of less than 50 μA at the centre of the nucleus, even when the stimulating electrode was placed 2 mm dorsal to the pyramidal tract. This finding ruled out current spread to the pyramidal tract.

Discussion

The findings of the present investigation show that stimulation in the nucleus locus coeruleus produces a predominantly inhibitory action on nociceptive input through multireceptive dorsal horn neurones. Our findings that LC exerts a predominantly inhibitory action on multireceptive and low threshold mechanoreceptive neurones is in general agreement with the observations of Hodge et al. (1983). Less frequently observed mixed effects of an initial excitation that was followed by inhibition were also reported by these authors. The inhibitory action produced by LC stimulation was most effective against the discharge evoked in multireceptive neurones by activity in Aδ and C fibres rather than that evoked by more rapidly conducting afferent fibres. LC stimulation reduced responses evoked by noxious cutaneous stimuli, such as heat and pinch, and in some neurones this was selectively inhibitory. In some of those neurones when inhibition was non-selective, there was a relatively stronger magnitude of inhibition on the discharge evoked by noxious stimuli.

Particular attention was paid to multireceptive neurones in the present study since they are considered to be important for the sensory discriminative aspects of pain and although not tested for projections in this study some may form a component of the spinothalamic tract (McCreery et al. 1979) or project into other ascending spinal hind limb pathways such as the spinocervical tract, spinoreticular tract and the dorsal column postsynaptic system. The inhibitory action on these neurones may therefore represent an important neuronal mechanism contributing to the generation of analgesia from LC.

A widespread inhibitory action of LC has also been reported including in the spinal trigeminal nucleus (Sasa et al. 1974) the cerebellum, hippocampus, diencephalon and cerebral cortex (Amaral and Sinnamon 1977). Interestingly its inhibitory action in the visual cortex also dominates the excitatory action as observed in the spinal cord. It is therefore conceivable that locus coeruleus produces antinociception by exerting its action at various levels of the neuraxis which are involved in the processing of nociceptive information and perception of pain.

It was suggested by Lundberg and collaborators (reviewed in Lundberg 1982) that a descending noradrenergic pathway participates in the control of reflex transmission in the spinal cord. It is interesting in this regard that locus coeruleus stimulation has recently been reported to reduce the inhibitory effect of high threshold cutaneous and muscle afferents on the extensor monosynaptic reflex (Yakhnitsa and Pilyavsky 1980). However, it did not produce any effect on the facilitatory action of high threshold afferents on the extensor and flexor monosynaptic reflexes (Yakhnitsa and Pilyavsky 1980) thus reflecting a selective modulatory influence on reflex transmission.

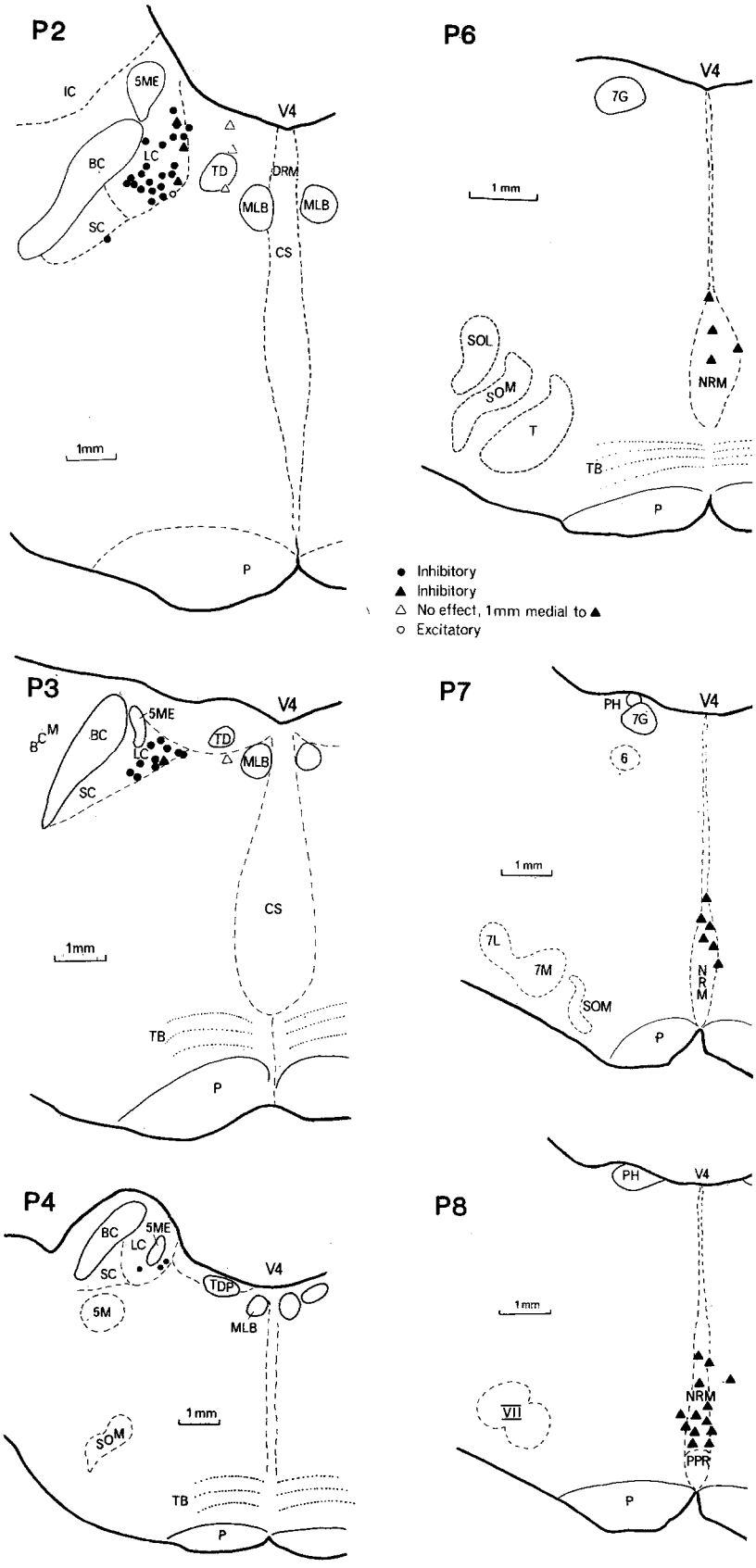

Fig. 10. Diagram of cross-section of the brainstem at P2 to P8 to illustrate stimulation sites. P2–P4: P2–P4 are composite pictures showing the sites of stimulation in LC at the posterior coordinates P2, P3 and P4. Filled circles (●) sites of stimulation that evoked inhibition; open circles (○) sites that produced excitation of dorsal horn neurones. The filled triangles at P2 and P3 correspond to the lateral electrode in the pair of stimulating electrodes and represent sites that were effective in producing inhibition, the unfilled triangles correspond to the position of the ineffective medial electrodes. P6–P8: P6–P8 are composite pictures showing the sites of stimulation in or adjacent to NRM at the posterior coordinates P6, P7 and P8. The filled triangles are sites of stimulation that evoked inhibition, or excitation-inhibition on the discharge of multireceptive neurones

The nucleus locus coeruleus projects to the spinal cord and, in addition, is also linked with other brainstem nuclei such as NRM and other raphe nuclei (Amaral and Sinnamon 1977; Chu and Bloom 1974; Sakai et al. 1979; Westlund and Coulter 1980). However, the most recent evidence does not show any substantial projection to NRM in the cat (Abols and Basbaum 1981; Sakai et al. 1979). Electrical stimulation in NRM (Oliveras et al. 1975) and microinjection of morphine (Dickenson et al. 1979) produce analgesia. Lesion studies also suggest the involvement of NRM in mediating opiate analgesia (Proudfit and Anderson 1975) and stimulation produced analgesia (SPA) generated from PAG (Behbehani and Fields 1979; Fields and Anderson 1978). Descending raphe spinal projections (Basbaum et al. 1978; Fields and Anderson 1978) are thought to play an important part in the generation of these analgesic mechanisms. The question arises, therefore, whether the LC acts through the NRM. The effects of stimulation in LC were tested and compared with those produced by NRM stimulation on the activity of the same multireceptive neurone in order to determine the contribution of NRM in mediating LC actions in the spinal cord. The actions produced from both nuclei were qualitatively, and in most cases quantitatively, similar. The inhibitory action was more effective on the discharge evoked by activity in Aδ and C fibres than in more rapidly conducting afferents and was partially selective against the discharge evoked by nocuous stimuli. The predominantly inhibitory action of NRM is in agreement with previous studies (Belcher et al. 1978; Fields et al. 1977; Gerhart et al. 1981; Willis et al. 1977). The mixed excitation and inhibition or excitation alone observed in the present study was observed in some (Belcher et al. 1978; Fields et al. 1977b; McCreery et al. 1979) but not in other studies (Willis et al. 1977; Gerhart et al. 1981). The evidence provided in the present investigation may indicate the involvement of NRM in mediating the effects of LC stimulation but our subsequent experiments suggest that this is not the case (Mokha et al. 1983).

The evidence on current spread together with the histological localisation of the stimulation sites within LC and NRM suggests that the actions observed were produced by the activation of structures within the nuclei. The actions produced from LC are possibly mediated via direct coeruleospinal projections (Hancock and Fougerousse 1976; Stevens et al. 1982; Kuypers and Maisky 1977; Tohyama et al. 1979a, b) which at least in some species contain noradrenaline (Commissiong et al. 1978; Nygren and Olson 1977; Westlund et al. 1983, 1984; Westlund and Coulter 1980). Although noradrenaline (NA) is reportedly involved in mediating the actions of LC in the spinal cord (Fung and Barnes 1980; Mokha et al. 1983) and the trigeminal nucleus caudalis in the cat (Sasa et al. 1977a), recent evidence provided by Hodge and colleagues (Hodge et al. 1983; Stevens et al. 1982) does raise some doubts. The descending pathways from LC may, however, in addition to NA, also contain a variety of other known and unknown neurotransmitters. It is known for example that there are neurones in the nucleus that contain 5-HT (Wiklund et al. 1981) or enkephalin, neurotensin, substance P and avian pancreatic polypeptide. Electrical stimulation in LC may therefore cause the release of more than one neurotransmitter in the spinal cord. These neurotransmitters released from descending fibres can be expected to produce a variety of actions on neurones examined in the present investigation by acting directly or indirectly via interneurones in the superficial dorsal horn that contain peptides, γ-aminobutyric acid (GABA) (Hunt et al. 1981) or glycine etc. Locus coeruleus is important but not unique in providing noradrenergic innervation to the spinal cord. Noradrenaline applied microiontophoretically in the dorsal horn (Belcher et al. 1978; Headley et al. 1978) produces actions which are similar to those evoked by electrical stimulation in LC in the present study. Our own studies (Mokha et al. 1983) indicate the involvement of noradrenaline, GABA and opioid peptides in mediating descending inhibition from LC. Similarly, the nucleus raphe magnus may also produce its actions through a variety of neurotransmitters since medullary raphe neurones projecting to the spinal cord are known to contain 5-HT (Bowker et al. 1981b; Dahlström and Fuxe 1965), or 5-HT, Substance P and TRH (Johansson et al. 1981) or substance P, enkephalin and 5-HT (Bowker et al. 1981a) possibly co-existing in the same neurone.

Locus coeruleus has been implicated in stimulation produced analgesia that depends on noradrenergic, serotonergic and opioid transmission (Sandberg and Segal 1978; Margalit and Segal 1979). One should be cautious in trying to correlate the inhibition of nociceptive transmission in an animal under anaesthesia to the behavioral state of analgesia. It is not unreasonable to suggest that the inhibitory effect of LC on nociceptive transmission observed in the present investigation could be one of the neural mechanisms that generates analgesia from LC. Its actions could also play a part in mediating SPA from the dorsal raphe nucleus (Oliveras et al. 1974) since neurones in the dorsal raphe establish a link with LC (Sakai et al. 1979; Segal 1979) and do not project beyond the cervical spinal cord (Tohyama et al. 1979b). This possibility, however, does not seem very

likely since the recent findings of Prieto et al. (1983) show that NRM lesions disrupt SPA from dorsal raphe. Animals during analgesia are responsive to other sensory modalities such as tactile input (Oliveras et al. 1974, 1975). Selective or partially selective inhibition evoked from LC could be relevant for the generation of analgesia. Tactile information during analgesia can, however, be transmitted through tactile pathways which may not be under inhibitory control from LC. Its actions at other levels of the neuraxis such as thalamus can also contribute to analgesia. Neurones in LC are known to respond to noxious cutaneous stimuli which could therefore act as a trigger to activate the coeruleospinal system thus contributing to the generation of antinociception or analgesia.

The nucleus locus coeruleus innervates wide areas of the CNS such as the spinal cord, brain-stem, thalamus, hypothalamus, cerebellum, basal telencephalon and cortex (Amaral and Sinnamon 1977; Pickel et al. 1974) and has been suggested to play a role in such diverse functions as sleep, analgesia, motivation, reward and micturition (Amaral and Sinnamon 1977). The actions observed in the present investigation are suggested to play a part in anti-nociception and may also be relevant for other functions such as sleep. Although on the surface LC may appear to give rise to a diffuse system its actions could be state-dependant and therefore selective. It could, for example, contribute to anti-nociception in an awake state whereas in sleep it acts as a general inhibitor of all sensory inputs to the CNS. One has to assume the existence of different sub-sets of neurones in the nucleus subserving different functions.

Abbreviation for structures

Abducens nucleus (6); Brachium Conjunctivum (BC); Brachium Pontis (BP); Dorsal Nucleus of Raphe, Medial division (DRM); Dorsal Tegmental nucleus (TD); Facial Nucleus, lateral division (7L); Facial Nucleus, medial Division (7M); Fourth ventricle (V4); Genu of the Facial Nerve (7G); Inferior Colliculus (IC); Lateral, Medial Nucleus of Superior Olive (SOL, SOM); Marginal Nucleus of the Brachium conjunctivum (BCM); Mesencephalic trigeminal nucleus (5ME); Motor Trigeminal Nucleus (5M); Nucleus locus coeruleus (LC); Nucleus praepositus hypoglossi (PH); Nucleus Raphe Magnus (NRM); Pyramidal tract (P); Postpyramidal Nucleus of the Raphe (PPR); Subcoeruleus (SC); Superior Central Nucleus (CS); trapezoid body (TB).

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