Central Lateral Thalamic Neurons Receive Noxious Visceral Mechanical and Chemical Input in Rats

Yong Ren, Liping Zhang, Ying Lu, Hong Yang, and Karin N. Westlund

Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, Texas; and Department of Physiology, University of Kentucky College of Medicine, Lexington, Kentucky

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Ren Y, Zhang L, Lu Y, Yang H, Westlund KN. Central lateral thalamic neurons receive noxious visceral mechanical and chemical input in rats. J Neurophysiol 102: 244–258, 2009. First published April 15, 2009; doi:10.1152/jn.90985.2008. Thalamic intralaminar and medial nuclei are a major route of transmission for noxious visceral input from the spinal cord to the DC nucleus and then to thalamus. In preparation for pain processing, unique to the present study were identification and characterization of spontaneously active neurons in the central lateral nucleus (CL) of the intralaminar thalamus, which were found to respond only to viscerally evoked noxious stimuli in animals under pentobarbital anesthesia. Responses to noxious colorectal distention, intraperitoneal bradykinin, intraportal dilute acetic acid, and greater splanchnic nerve electrical stimulation were characterized. Electrophysiological recordings revealed activity in most CL neurons (93%) was excited (69%) or inhibited (31%) in response to noxious visceral stimulation of visceral nerves. Expression of c-Fos observed in CL nucleus after extensive visceral stimulation confirmed the activation. However, excited CL neurons did not have somatic fields, except in 3 of 43 (7%) CL neurons tested for responses to somatic stimulation (innocuous brush and noxious pinch). Intrathalamic administration of morphine significantly reduced the increased responses of CL neurons to colorectal and pancreatic stimuli and was naloxone reversible. High-level thoracic midline dorsal column (DC) myelotomy also dramatically reduced responses, identifying the DC as a major route of travel from the spinal cord to CL input, in addition to input traveling ventromedially in the spinalnthalamic tract identified anatomically in a previous study. Spinal cord and lower brain stem nuclei sent to specific regions of the granular layers of the insular cortex (Allen et al. 1991). Medial and intralaminar thalamic structures are reported to relay information about nociceptive transmission, such as ventromedial medulla, raphe, and periaqueductal gray. In addition, terminations of the midline pathway innervate the medial and intralaminar thalamic nuclei, but not the ventrolateral thalamus. Electrophysiological studies activated the pancreas with bradykinin and the responses were recorded in the DC nucleus and lateral thalamus (Houghton et al. 2001; Wang and Westlund 2001). The latter were significantly reduced by DC lesions. The current study looks at neuronal responses in the central lateral thalamic nucleus, the largest of the intralaminar thalamic nuclei, after Bradykinin stimulation of the pancreas.

Previous studies report that most medial and intralaminar thalamic neurons respond to convergent noxious somatic and visceral stimuli (Berkley et al. 1995; Peschanski et al. 1981), supplied by projecting axons arising from high-threshold or wide-dynamic range neurons with large and often bilateral receptive field (Dong et al. 1978; Jones 1985). This suggests a minor role in discriminative pain perception in contrast to the lateral thalamus (ventroposterolateral parvicellular [VPLpc] and ventroposteromedial parvicellular [VPMpc] visceral relay nuclei), which receives viscerotopically organized input from brain stem nuclei sent to specific regions of the granular layers of the insular cortex (Allen et al. 1991). Medial and intralaminar thalamic structures are reported to relay information about pain from a wide array of sources to widespread cortical areas, particularly to the frontal and limbic cortex, participating in executive decisions and both affective and motivational aspects of pain (Albe-Fessard et al. 1985; Bushnell et al. 1989; Guilbaud et al. 1994; Jones 1985). Despite apparent lack of viscerotopy, other studies using tracing methods have been able to clarify that the small central lateral nucleus (CL) subnuclei of the intralaminar group do have discrete collateralized projections to cortex (somatosensory, medial parietal, and cingulate cortex) and medial striatum, with specific functions necessary for attention and cortical awareness (Berendse and Groenewegen 1990; Deschénes et al. 1996; Kamishina et al. 2008; Reep et al. 1984; Smith et al. 2004; Van der Werf et al. 2002).

INTRODUCTION

There is still much to be discovered about visceral pain transmission. Clinical evidence shows pelvic cancer pain can be successfully alleviated or abolished in patients with a limited surgical midline dorsal column (DC) myelotomy (Becker et al. 1999; Gildenberg and Hirshberg 1984; Hirshberg et al. 1996; Hu and Li 2002; Hwang et al. 2004; Nauta et al. 2000). Follow-up anatomical, electrophysiological, and lesion studies in animals have confirmed the existence of a dorsal midline route for transmission of noxious visceral input from spinal cord to the DC nucleus and then to thalamus, which, at least in the rat and human, plays a greater role in visceral pain transmission than the ventrolateral spinothalamic tract pathway (Al-Chaer et al. 1996a,b; Hirshberg et al. 1996; Houghton et al. 2001; Palecek et al. 2002; Wang et al. 1999; Willis et al. 1999). The anatomical studies found direct neuronal projections arising from lamina X cells near the central canal that travel both in the midline and at the medial edge of the ventrolateral spinothalamic tract. The DC pathway from lamina X innervates the DC nuclei. The ventral midline axonal projections innervate mediolaterally located structures (Wang et al. 1999) that many others report are involved in descending modulation of nociceptive transmission, such as ventromedial medulla, raphe, and periaqueductal gray. In addition, terminations of the midline pathway innervate the medial and intralaminar thalamic nuclei, but not the ventrolateral thalamus. Electrophysiological studies activated the pancreas with bradykinin and the responses were recorded in the DC nucleus and lateral thalamus (Houghton et al. 2001; Wang and Westlund 2001). The latter were significantly reduced by DC lesions. The current study looks at neuronal responses in the central lateral thalamic nucleus, the largest of the intralaminar thalamic nuclei, after bradykinin stimulation of the pancreas.

Address for reprint requests and other correspondence: K. Westlund High, Department of Physiology, MS-609 Chandler Medical Center, 800 Rose St., University of Kentucky, Lexington, KY 40536-0298 (E-mail: kwhigh2@uky.edu).
The visceral nociceptive information sent to the CL of the intralaminar thalamus has received almost no attention. The CL nucleus was chosen as an area of interest for the current study of visceral nociceptive responses since we have shown it receives direct spinal input from lamina X cells near the spinal cord midline (Wang et al. 1999). Our previous study in rats found increased functional magnetic resonance imaging signal, indicating increased activation in both the medial and lateral thalamus in response to pancreatic inflammation of 1-wk duration that was diminished by naloxone-reversible morphine treatment (Westlund et al. 2009). The present study characterizes electrophysiological responses of CL neurons to noxious visceral input in anesthetized rats. Colorectal balloon distention (CRD), shown by others to be a noxious visceral stimulus in awake animals (Ness et al. 1991), was used as the search stimulus after identifying spontaneously active cells through stereotaxic placement of carbon-fiber glass electrodes. Further characterization was done testing responses to visceral and somatic stimulation after intrapancreatic duct injection of bradykinin (BK). The acute mechanical visceral stimulation provided by CRD and the acute chemical visceral stimulation produced by BK application evoke reproducible physiologic responses and have been used in previous studies (Ness and Gebhardt 1988; Olivar et al. 2000; Wang and Westlund 2001). In some animals, responses of a few CL cells to intraperitoneal (ip) injection of acetic acid or splanchnic nerve electrical stimulation were also recorded. In addition, evidence of c-Fos expression in neurons of the thalamic CL nucleus was sought in some of the animals after intensive visceral stimulation with pancreatic duct injection of BK. Stereotaxic injections of retrograde tracer into the CL region allowed confirmation of spinal projections. Portions of this study were previously published in abstract form (Ren et al. 2006; Zhang et al. 2004).

METHODS

Animal preparation and anesthesia

Experiments were performed on adult male Sprague–Dawley rats (250–380 g). All experimental protocols were approved by the Institutional Animal Care and Use Committee and were consistent with the guidelines of the National Institutes of Health and the International Association for the Study of Pain.

Animals were initially anesthetized with sodium pentobarbital (50 mg/kg, ip). The trachea and jugular vein were catheterized for artificial respiration and anesthetic delivery, respectively. Anesthesia was maintained by continuous infusion of sodium pentobarbital (3–14 mg·kg⁻¹·h⁻¹ in lactated Ringer solution). Procedures for noxious visceral stimulation listed in the following text were initiated prior to preparing the animals for CL recordings, although animals remained anesthetized throughout all procedures. The carotid artery was catheterized for blood pressure monitoring. The level of anesthesia was monitored by frequent examination of pupillary size and responses to stimulation and confirmation of the absence of a flexion reflex. Once a stable level of anesthesia was reached, the animals were paralyzed with pancuronium (0.3–0.6 mg/h, administered intravenously [iv]) and artificially ventilated. The level of end-tidal CO₂ was kept between 3.5 and 4.5%. Arterial blood pressure was kept at 120–130 mmHg. Core body temperature was monitored by a rectal probe and maintained near 37°C with a servo-controlled heating blanket (Homoethermic Blanket Control Unit, Harvard Apparatus). At the end of the experiments, rats were killed with an overdose of pentobarbital (150 mg/kg) and the thorax was opened. The brain was removed and aldehydes fixed for histological confirmation of recording sites.

Noxious stimulation

COLORECTAL DISTENTION (CRD). To produce a colorectal (mechanical) stimulus, a 4- to 5-cm-long balloon from the finger of a latex glove was connected to a sphygmomanometer by polyethylene tubing and inserted through the anus into the rectum and descending colon of anesthetized animals prior to placement of the CL electrode (Fig. 1). The balloon was held in place by tapping the tubing to the tail. Prior to use the balloon was blown up and left overnight. CRD was induced by rapidly inflating the balloon with air. As an innocuous stimulus, a constant distention pressure of 30 mmHg was administered for 20 s and, as a noxious stimulus, distention with 80 mmHg pressure was applied for 20 s (Ness and Gebhardt 1987, 1988).

PANCREATIC DUCT INJECTION OF BRADYKININ. Pancreatic duct injection of bradykinin was used in this study as a chemical visceral stimulus (BK, dissolved in lactated Ringer solution; Sigma). Following a small (1-cm) midabdominal laparotomy, the pancreatic duct of anesthetized rats was cannulated through the duodenum and the papilla (Fig. 1) using polyethylene 10 tubing connected with a 0.5 ml U-100 insulin syringe. Then, the abdominal muscle and the skin were sutured to close the wound. Animals were then prepared for CL recordings as detailed in the following text. After characterization of the visceral responsiveness of a CL neuron to CRD, a 100-μl BK (10⁻⁵–10⁻⁶ M) injection was made into the pancreas through the duct by the syringe. The neuronal activity of a viscerally responsive CL neuron was recorded for 1 min. After an interval of ≥30 min, responses of the same CL neurons to CRD and BK stimuli were tested again.

INTRAPERITONEAL INJECTION OF ACETIC ACID. In animals (n = 13), 0.6% acetic acid in saline (pH 4.0, 4 ml/kg) was ip-injected as a second chemical visceral nerve activator. Blood pressure and neuron spike rate were monitored simultaneously as reactive indices for stimuli.

ELECTROSTIMULATION OF THE GREATER SPLANCHNIC NERVE. In a few anesthetized rats (n = 8), the left greater splanchnic nerve was accessed through a dorsolateral approach at the segment distal to the suprarenal ganglion. The nerve was dissected and cut and the proximal portion suspended with bipolar silver electrodes. The electrode was embedded in silicone sealant except for the bare tips of the two Teflon-coated silver wires (bare 250 μm; homemade). A train of electric pulses was generated from an Accupulser (WPI) and passed through a stimulus isolator (WPI, 10–70 V, 10 Hz, 0.1 ms, 20 s) onto the proximal splanchnic nerve fibers.

SOMATIC STIMULATION. Somatic stimulation consisted of innocuous and noxious cutaneous stimuli applied after the CL unit responding to noxious CRD was isolated. The innocuous stimuli were applied by repeated brushing (BR) of the skin with a camel hair brush. The pinch (PI) stimuli were delivered by sustained applications of small arterial clips to a fold of skin, producing pain in human subjects without causing overt damage to the skin. Each cutaneous stimulus (brush and pinch) was applied for 10 s followed by a 2-min pause at the end of the recording session. Multiple body regions were tested.

Electrophysiological studies

NEURONAL RECORDINGS. Recordings in the CL nucleus of the intralaminar thalamus were made after electrode placement at the appropriate brain site using the following method. Rats were mounted in a stereotaxic frame and then a unilateral craniotomy was performed at the coordinates 1.0–4.5 mm posterior to bregma and 0–3.0 mm lateral to midline for the recording of CL neurons (Fig. 1). The dura mater was opened; the arachnoid membrane and pia mater were removed over the recording area to allow the insertion of a recording
A warm mineral oil pool was formed over the exposed tissue. Extracellular single-unit recordings were made from neurons in the CL with low-impedance (3–5 MΩ) carbon-filament glass electrodes. The electrode was attached to an electronic micromanipulator that advanced in 5-μm steps and placed at depths of 4–6 mm below the cortical surface for recordings. Single-unit neuronal activity was isolated from the neighboring units on the basis of spike amplitude and waveform and its baseline activity was recorded. The units’ responses to innocuous CRD as the search stimuli were used to identify them as visceral-sensitive neurons. Single-unit neuronal activity to noxious-intensity graded CRD and pancreatic duct injection of BK was fully characterized. Then, the animal was searched for

**FIG. 1.** Schematic drawing depicting components of the experimental setup. The recording site is shown on the right side of thalamus in the central lateral (CL) nucleus in the top panel. The dorsal column (DC) lesion in the thoracic spinal cord is depicted in the panel below. The visceral stimulation of the common biliary pancreatic duct by injection of bradykinin and colorectal distention with a latex balloon is depicted in the bottom 2 drawings.
cutaneous receptive fields by consecutive applications of cutaneous stimuli (BR and PI) and the area mapped, if found. Deep blunt probe pressure was also applied in some cases. Recorded compound action potentials and neuronal responses to visceral (mechanical and chemical) stimuli before and after DC lesion or intrathecal morphine application were recorded. Single-unit neuronal activity was amplified and displayed on a digital oscilloscope (TDS-210) that allowed us to ensure that the same unit was being recorded throughout the experiment and that the relationship of the recording electrode to the neuron remained constant. The original signals recorded were also led to a data collection interface system (CED 1401+; Cambridge Electronic Design [CED]) and a computer for data compilation as wavermark or histogram files available to be quantified by Spike2 software (CED).

Some units had significantly increased activity after noxious visceral stimuli, referred to as excitatory responses; others had decreased activities, referred to as inhibitory responses; some units had no response to visceral stimuli. In this study, those units with excitatory responses were further studied pharmacologically. Typically, only one cell was recorded in an animal, except in seven animals.

Histology. At the end of each experiment, the recording site in the CL was marked with a lesion produced by passing DC current (250 μA for 3 min) through the tip of the carbon-filament glass electrode (Fig. 2, A and B). The brain was removed and put into 10% buffered formalin solution for 1 wk. Tissues were stored in 30% sucrose/0.1 M phosphate buffer (PB) overnight before embedding. Frozen coronal sections (15 μm) were cut through the thalamus and stained with cresyl violet. Recording sites were identified histologically and plotted on standard diagrams (from Paxinos and Watson 1986) of coronal brain sections (Fig. 2C).

Intrathecal administration of morphine. Animals were implanted with indwelling intrathecal catheters using the procedure described by Lu et al. (2003). Briefly, after rats were anesthetized and placed in a stereotactic frame, a 4.5-cm intrathecal catheter (32-gauge; ReCathCo, Allicon Park, PA) was implanted through an opening in the overlying musculature, and drilling the bone to expose the dura. Stereotaxic fluorescent dye was injected in six sites (total volume >2 μl) in the intermediate thalamus using the same coordinates as cited earlier for the central lateral thalamic nucleus (2.8–3.8 posterior; 1–1.5 mm lateral; 4–6 mm down). A 10% dextran amine Texas red (3,000 MW; Molecular Probes) solution in 0.1 M citrate-NaOH (pH 3.0) was injected with a glass microprobe with a tip diameter of 30 mm. The injection current was a 7-nA positive pulse, with 5 s on and off for 30 min. After injection of the dye, bone wax was applied to the bone. The surgical wound was closed with 3–0 polydioxanone PDS*II absorbable monofilament for closure of the musculature followed by subcuticular closure. The survival time was >4 wk. Ten tissue sections from each selected level were cut (50-μm thickness). Labeled neurons from five alternate sections (each section was ≥50 μm apart) at each level were counted. Dextran amine Texas red labeled neurons in the lower brain stem and spinal cord were mapped using the brain atlas of Paxinos and Watson (1986).

Immunohistochemistry studies

The c-Fos expression in the CL nucleus of the thalamus in rats with pancreatic duct injections of BK was investigated for comparisons to naïve animals. Rats were deeply anesthetized with isoflurane and a laparotomy was performed. Injections of 100 μl of 10−5 M BK solution were made into the pancreatic duct to evoke nerve activation and the wound was closed. One hour after the injection, rats were anesthetized with sodium pentobarbital (50 mg/kg, ip) and transcardially perfused with 50 ml of heparinized saline at 37°C, followed by 500 ml of 4% paraformaldehyde solution in 0.1 M PB (pH 7.4, 4°C). The brains were postfixed using the same fixative at room temperature for 4 h before cryoprotection in 30% sucrose/0.1 M PB overnight. Tissues were frozen embedded and sectioned. The thalamus was cut on a sliding microtome (30-μm thickness). Every second section was collected (n = 5) and stained free-floating. After rinsing (six times) in 0.1 M phosphate-buffered saline (PBS), sections were first incubated in 5% normal goat blocking serum for 40 min, followed by incubation in primary antibodies diluted in 1% NGSTB containing Triton X-100 at room temperature. The primary antibody was polyclonal rabbit anti-c-Fos (1:10,000; Calbiochem, San Diego, CA). The following day, the sections were washed in 0.1 M PBS and incubated with the secondary antibody, goat anti-rabbit IgG, conjugated with Alexa Fluor 568 (1:1,000, Molecular Probes, Eugene, OR) diluted in 1% NGSTB for 1 h at room temperature. After rinsing in 0.1 M PBS, sections were mounted onto gelatin-coated slides, air-dried, and covered with DAPI mounting medium hard set (Vector Laboratories, Burlingame, CA) and Microscope Cover Glass (Fisher, St. Louis, MO). The slides were visualized using the Metavue Program on a Nikon E1000 microscope (Nikon Instruments, Melville, NY). All sections from the different animal groups were processed at the same time with the same solutions.

c-Fos–labeled cells in the CL nucleus of thalamus were identified using the brain atlas of Paxinos and Watson (1986). Cell count estimates of c-Fos–labeled CL thalamic neurons were performed using a MetaMorph off-line program (Molecular Imaging Systems, Downingtown, PA). The average number of c-Fos–labeled cells counted in the CL thalamic nucleus from both sides of five tissue sections became the final value for each rat.

Iontophoretic injection of retrograde tracer

Animals (n = 8) were anesthetized with sodium pentobarbital (50–60 mg/kg, ip). Sterile surgery proceeded with skin incision, removal of overlying musculature, and drilling the bone to expose the dura. Stereotaxic fluorescent dye was injected in six sites (total volume >2 μl) in the intermediate thalamus using the same coordinates as cited earlier for the central lateral thalamic nucleus (2.8–3.8 posterior; 1–1.5 mm lateral; 4–6 mm down). A 10% dextran amine Texas red (3,000 MW; Molecular Probes) solution in 0.1 M citrate-NaOH (pH 3.0) was injected with a glass microprobe with a tip diameter of 30 mm. The injection current was a 7-nA positive pulse, with 5 s on and off for 30 min. After injection of the dye, bone wax was applied to the bone. The surgical wound was closed with 3–0 polydioxanone PDSII absorbable monofilament for closure of the musculature followed by subcuticular closure. The survival time was >4 wk. Ten tissue sections from each selected level were cut (50-μm thickness). Labeled neurons from five alternate sections (each section was ≥50 μm apart) at each level were counted. Dextran amine Texas red labeled neurons in the lower brain stem and spinal cord were mapped using the brain atlas of Paxinos and Watson (1986).

Statistical analysis

Recorded neuron activity was analyzed off-line from peristimulus time histograms (bin width = 1) using Spike2 software (CED 1401+) to obtain the average rate of evoked discharges. Background discharges were first recorded for 20 s before application of a mechanical or chemical visceral stimulus. The responses of CL neurons to mechanical and chemical visceral stimuli, as well as mechanical cutaneous stimuli, were measured and expressed as a percentage of baseline, with baseline set at 100%. All responses evoked by stimulation were calculated by subtracting the background discharges from the total number of action potentials that occurred during each stimulus to produce net response values of discharge rate. Statistical significance was tested using paired t-test for the pairwise comparisons of responses before and after treatment. The Mann–Whitney U test was used to compare the differences in responses between groups having different treatments. All averaged values were given as the means ± SE. Statistical significance was accepted at the level P < 0.05.
FIG. 2. A: diagram depicting the rostrocaudal distribution of 37 neurons recorded in the CL thalamic nucleus plotted on representative serial sections through the CL nucleus at 6 stereotaxic levels posterior to bregma. Numbers adjacent to coronal sections are coordinates (in millimeters) relative to bregma. Diagrams are adapted from Paxinos and Watson (1986). Filled circles (●) show the locations of visceral responsive neurons. The CL neurons recorded from the morphine treatment group and the DC lesion group are shown as ○ and ▲, respectively. A coronal section of medial thalamus stained with cresyl violet illustrates placement of one of the glass electrodes in the CL nucleus (bottom). The thalamic midline is located directly below the third ventricle (3V). The bar in the bottom left represents 500 μm.
RESULTS

General response characteristics of the CL nucleus neuronal subgroup responding to noxious visceral stimulation

In all, 62 spontaneously active neurons within the central lateral (CL) thalamic nucleus that responded to visceral stimulation were recorded from 55 rats. Noxious visceral stimuli produced an excitatory response in 43 of the CL neurons (69%). Two of the 43 neurons (5%) were excited only by intrapancreatic duct injection of BK and were not excited by noxious CRD. Three units (7%) responded to CRD only. The remaining 38 neurons (88%) were excited by both noxious CRD and pancreatic duct injection of BK. In the neurons with excitatory responses, only 3 units (7%) responded to both noxious visceral and somatic (cutaneous) stimulation, despite a thorough search. For these 3 neurons, their receptive fields were found only on the face, the left side of the thigh, or the scrotum, respectively. Among the 62 neurons responding to graded colorectal stimulation, there were 19 neurons (31%) with inhibitory responses only, with reduced activity to applied noxious mechanical or chemical visceral stimulation. An additional 9 neurons were responsive to noxious cutaneous stimulation but not to noxious visceral stimulation. For a summary of cellular responses see Table 1.

Histological study revealed that recording sites for cells in the central lateral thalamus with excitatory responses to visceral stimuli were most often located at 2–3.8 mm posterior from bregma, at a depth of 5–6 mm and lateral 1–1.2 mm. Figure 2 shows the rostrocaudal distribution of 37 of the 43 neurons recorded in the CL nucleus that were responsive to noxious visceral stimulation. All of the 37 recovered recording sites were found to be in the CL and it was assumed that the remaining 6 were also in CL. Furthermore, neurons recorded in the immediately surrounding nuclei did not respond to visceral stimuli.

<table>
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<tr>
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<th>Inhibitory Response (n = 19)</th>
<th>Excitatory Response (n = 43)</th>
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<tbody>
<tr>
<td>BK</td>
<td>0</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>CRD</td>
<td>0</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>BK + CRD</td>
<td>19 (100%)</td>
<td>38 (88%)</td>
</tr>
<tr>
<td>Visceral only</td>
<td>—</td>
<td>40 (93%)</td>
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<tr>
<td>Visceral + cutaneous</td>
<td>—</td>
<td>3 (7%)</td>
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<td>Cutaneous only</td>
<td>—</td>
<td>9</td>
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BK, bradykinin; CRD, colorectal distention.

**TABLE 1. Neurons responding to visceral stimuli and/or vigorous cutaneous stimuli**

**Responses of CL neurons to CRD and pancreatic duct injection of BK**

The majority of the CL neurons (38/43, 88%) with excitatory responses to noxious visceral stimuli responded to both noxious...

**FIG. 3. Traces A and B show recordings of spontaneous and evoked activity of a single nociceptive CL neuron from the CL nucleus of the medial thalamus. In the top rows are recorded action potentials and the responses to colorectal balloon distention (CRD) and bradykinin (BK) injection. The middle row shows the recorded action potential converted into analog signals (wavemarks) by Spike2 software that filtered the baseline and other unidentified neuron activities. The 3rd row shows the recorded action potentials converted into histograms so that discharge rates could be counted. In C–E are responses of a CL neuron that only responded to noxious 80 mmHg CRD, as well as to the 1st and 2nd BK injections. Horizontal lines above the histograms indicate timing of application of the different stimulations. F: the mean firing rates of CL neurons (n = 43) in response to visceral (CRD, BK) and cutaneous (repeated brushing [BR], pinch [PI]) stimulations. ***P < 0.001 compared with baseline, paired t-test.**
CRD and intrapancreatic duct injection of BK. None of the neurons in the CL nucleus responded only to innocuous mechanical visceral stimulation (30 mmHg CRD). Figure 3 shows the response of a CL neuron excited by both noxious mechanical CRD (Fig. 3A) and chemical BK stimulation of the pancreas (Fig. 3B). The rate histograms shown in Fig. 3, C–E are from an example of a CL neuron that responded only to noxious 80 mmHg grade CRD, as well as first and second BK injections in the pancreas. There was no significant increase in discharge rate after 30 mmHg grade CRD, innocuous cutaneous stimuli (BR), or noxious cutaneous stimuli (PI). Grouped data in Fig. 3F show that the average increases in response to noxious mechanical CRD and first BK injection in spinal cord intact rats were 174.2 ± 7.54% (P < 0.001) compared with baseline level for CRD and 242.6 ± 16.4% (P < 0.001) for BK injection. The average increase in discharge rate in response to innocuous and noxious cutaneous stimuli was to 104.9 ± 1.78% (P > 0.05, compared with baseline) and 106.2 ± 1.68% (P > 0.05), respectively. Intraductal bradykinin stimulation produced the maximal responses recorded from cells in these studies; however, there was no significant increase in the discharge rate of CL neurons between the first and second BK injections.

Effects of intrathecal administration of morphine

Five CL visceral nociceptive neurons were further tested after administration of morphine. Figure 4 shows the rate histograms of one representative CL visceral nociceptive neuron that responded to 80 mmHg CRD and BK injection before (Fig. 4A), 45 min after morphine (Fig. 4B), and 10 min after naloxone (Fig. 4C) with intrathecal administrations of morphine. Morphine dramatically inhibited the responses of all five CL neurons to noxious CRD. Intravenous naloxone reversed the effects of morphine on the visceral responses. Administration of morphine or naloxone alone did not produce a significant change in the spontaneous firing rate of these visceral nociceptive neurons as in our previous report (Houghton et al. 2001).

Effects of DC lesion at T3 segment of thoracic spinal cord

Responses of another five CL visceral nociceptive neurons to mechanical and chemical visceral stimulation were investigated before and after a high thoracic level DC lesion. The DC lesion dramatically decreased the responses of these neurons to 80 mmHg CRD and intrapancreatic duct injection of BK. Figure 5 shows an example of responses of a CL neuron to noxious visceral stimulations (CRD and BK) before (Fig. 5A) and 20 min after a DC lesion (Fig. 5B). Group data (Fig. 5C) show that the average firing rate of five CL neurons after a high thoracic level DC lesion were significantly reduced following 80 mmHg CRD from 198.3 ± 29.02% (P < 0.05) responses before DC lesion to 124.3 ± 5.023%. In animals with DC lesions, responses to BK injection were significantly reduced from 329 ± 33.20 to 170.6 ± 18.22% (P < 0.05), respectively. The DC lesion did not significantly alter the spontaneous firing rate.

**Fig. 4.** Rate histograms of CL visceral nociceptive neuron responses to 80 mmHg CRD and BK injections before (top row), after morphine (middle row), and after morphine + naloxone (bottom row) administration.
Responses of CL neurons to greater splanchnic nerve stimulation

In a small group of additional animals (n = 8), responses of CL neurons to stimulation of the greater splanchnic nerve were tested (Fig. 6). In all, 11 cells were recorded. The cells in CL had variable low-baseline spontaneous activities (11.74 ± 1.6 spikes/s) and did not have a clear somatic receptive field. The cells could be divided into three different subgroups according to their response to electrical stimuli of the greater splanchnic nerve (GSPN), used as another form of stimulation of nociceptive visceral input into CL. Cells with increases in their firing rate immediately after electric stimuli of GSPN were defined as the excitatory response cells (n = 3). A robust increase in neuronal activity lasted for 5–6 min and then returned back to normal baseline levels (Fig. 6A). Cells whose firing rate was reduced corresponding to the input of nociceptive input from GSPN were defined as the inhibitory response cell subgroup (n = 3). Five minutes after GSPN stimulation these cells showed a decreased firing rate from baseline, which lasted for hours (Fig. 6B); in some cells the low-baseline spontaneous activity was maintained for hours, regardless of any stimulation, and these neurons were defined as a no response subgroup (n = 5) (Fig. 6C).

Responses of CL neurons to intraperitoneal acetic acid

In a small group of additional animals (n = 13), responses to dilute intraperitoneal acetic acid (0.6%) were recorded (Fig. 7).
None of the cells tested in the CL thalamic nuclei had clear somatic receptive fields. Cells were divided into three categories according to cell responses to intraperitoneal acetic acid stimulation: 1) excitatory responses \((n = 7)\), with a firing rate increase two- to tenfold over baseline after simulation (Fig. 7A); 2) inhibitory responses \((n = 3)\), with a firing rate decrease to 20–50% of baseline after simulation (Fig. 7B); and 3) nonresponsive cells \((n = 3)\), with no change from baseline (Fig. 7C).

**c-Fos expression in CL nucleus evoked by noxious visceral stimulation**

Cells with expression of Fos protein were mapped bilaterally in the CL nuclei of thalamus in rats, after intensive visceral stimulation with pancreatic duct injection of BK, and compared with naïve rats. Figure 8 shows examples of c-Fos staining in histological sections of the CL nucleus.
from naïve control rats for comparison to rats with inflamed pancreatic ducts. Although Fos-labeled cells were observed in the medial thalamus (not shown), few cells were seen in nuclei adjacent to CL.

Significantly increased numbers of c-Fos–labeled cells were observed in CL nucleus in rat after pancreatic duct injection of BK compared with the naïve rat as shown in the group summary data for c-Fos–labeled cells in Fig. 8 (bar graph). In rats with intensive visceral stimulation using pancreatic duct injection of BK the average number of c-Fos–labeled cells was 54.2 ± 13.49% ($P < 0.05$, compared with 3.75 ± 0.92% in naïve rats).

**Spinal and medullary localization of neurons with CL input**

Following injection of dextran amine Texas red, using the same stereotaxic coordinates used in the recordings, neurons were identified in the spinal cord containing fluorescent dye retrogradely transported from the injection sites in the CL nucleus. Retrogradely labeled neurons with CL terminations are shown mapped in one animal for comparison (Fig. 9). Labeled neurons were localized at all spinal cord levels in laminae III–V, VII, X. Many more were found at the level of the spinomedullary junction, including neurons in the reticular formation and the trigeminal caudalis and DC nuclei. Neuron counts from eight rats in

**FIG. 7.** Examples of the firing patterns of CL neurons after intraperitoneal injection of dilute acetic acid. A: excitatory responses. These cells had variable baseline firing rates. After injection of acetic acid, indicated by the arrow, the firing rate gradually increased and remains increased over baseline for hours. B: inhibitory responses. These cells had stable baseline spike activity. The spike rate was soon reduced after acetic acid injection and remained decreased for hours. C: no responses. This cell type had very low but stable baseline activity. The spike activity did not change due to the acetic acid injection (arrow) nor did the baseline firing rate deviate.
selected lower brain stem and spinal cord segmental levels are summarized in Table 2.

DISCUSSION

CL intrathalamic nuclei neurons respond to visceral input

In this study, a subpopulation of spontaneously active neurons in the CL intralaminar nucleus of the thalamus in rats was identified as being responsive to noxious visceral stimulation. In fact, most CL neurons (93%) with increased responsivity to noxious visceral stimuli did not respond to somatic stimuli, although others have reported responses of CL neurons to somatosensory input when using cutaneous search stimuli (Albe-Fessard et al. 1985; Dong et al. 1978; Dostrovsky and Guilbaud 1990; Peschanski et al. 1981). We and others have also reported responses to both visceral and cutaneous stimuli from neurons in the ventral posterolateral nucleus in previous studies (Houghton et al. 2001). Noxious colorectal distention, as well as chemical pancreatic stimuli, produced 1) an excitatory response in 69% of the visceral responsive cells, 2) an inhibitory response in 31%, or 3) no response. Expression of c-Fos in the CL nucleus after intense noxious visceral stimulation confirmed activation of neurons in this intralaminar thalamic subnucleus. This is the first report of c-Fos in the CL thalamus. Intrathecal administration of morphine significantly reduced the increased responses of CL neurons to colorectal and pancreatic stimuli. This reduction was reversed by an iv injection of naloxone. A midline dorsal column (DC) my-
FIG. 9. Diagram depicting placement of the retrograde tracer (arrow) used in this study shown with a rectangle in the left side of the CL nucleus of the thalamus. Cells filled with retrogradely transported dye were found contralaterally in the spinomedullary junction of the brainstem in the DC nuclei and trigeminal caudalis nucleus, as well as in spinal cord laminae III–V, VII, and X at all spinal levels examined. Abbreviations according to Paxinos and Watson (1986).
elotomy at a high thoracic level also dramatically reduced the excitatory responses of CL neurons to visceral stimuli, identifying this as a route of travel of these axons. We also previously described (Wang et al. 1999) a small direct ventromedial white matter axonal projection to CL from spinal cord lamina X. Spinal cord cells providing CL input, mapped after stereotaxic injections of a retrograde dye into CL, were identified in laminae III, IV, V, VII, and X. Cells with axonal projections to CL were also found in the parvocellular DC nuclei, dorsal medullary reticular nucleus, and deep layers of the spinal trigeminal caudalis.

A multitude of pain studies have used cutaneous stimulation and concluded that nociceptive input to the medial and intralaminar thalamus is limited and nondiscriminative. However, only a rare study related to visceral pain is available. A few reports are available demonstrating electrophysiological recordings of somatosensory input to this nucleus, including input to the medial and intralaminar thalamic nuclei due to collaterals of VPL innervation (Albe-Fessard et al. 1985). Responses of the cells in the medial thalamus are frequently high-threshold cells and have large cutaneous receptive fields. On occasion, cells \( n = 9 \) were recorded in the present study using cutaneous search stimuli. In this case, the cells in CL responded to whole body, bilateral cutaneous input, as previously reported by others (Albe-Fessard et al. 1985; Carstens et al. 1990; Dong et al. 1978; Dostrovsky and Guilbaud 1990; Peschanski et al. 1981). Dostrovsky and Guilbaud (1990) reported that 34% of the cells in the medial thalamus respond to bilateral cutaneous stimulation. Of these cells, they found that 26% were localized in the central lateral thalamic nucleus. These cells responding to cutaneous stimuli were not the subject of the present study. Cutaneous stimulation with nocuous mechanical stimuli has also been shown to evoke c-Fos expression at the border between centromedian (CM) and CL (Bullitt 1990), but the nocuous visceral stimuli applied in the present study provided abundant staining for Fos protein throughout the CL as shown and in the CM (data not shown). Thus although the CL and other medial thalamic nuclei such as the CM nuclei are reportedly responsive to nocuous visceral input, no previous study has identified thalamic neurons responsive only to visceral input. Identification of this visceroresponsive cell group in CL is attributed to the two unique search strategies used in the current study not previously used to investigate thalamic activation in response to noxious input.

First, this study relied on previous anatomical and physiological data that identified the CL as a site of interest receiving direct axonal projections from lamina X neurons, a spinal cord region known to receive and process visceral input. It is known that lamina X cells are visceroresponsive, responding to colonic distentions in a graded manner and to mustard oil in the colon with increases in background activity (Hirshberg et al. 1996; Honda 1985; Honda and Perl 1985). From minute anterograde tracer injection sites confined to lamina X, we followed axonal projections to nociceptive integration sites not previously considered as visceral pain integration sites including the parvo-cellular portions of the DC nuclei, medial, and intralaminar thalamic nuclei, brain stem nuclei (raphe nuclei, ventromedial medulla, noradrenergic cell groups, periaqueductal gray), and the parvocellular insular cortex (Wang et al. 1999). Studies reported in the literature indicate that the CL nucleus receives extensive projections from brain stem areas identified as having a relationship to pain and analgesia (Albe-Fessard 1975; Carstens et al. 1990; Moore et al. 1978). This includes the periaqueductal gray, raphe nuclei, and DC nuclei. Anatomical studies in rats (Gebhart 1982; Wang et al. 1999), cats (Craig and Burton 1985), monkeys (Ammons 1989; Apkarian and Hodge 1989), and humans (Mehler 1966) found that intralaminar nuclei receive input from the spinal cord, although it was noted by Albe-Fessard and colleagues (1975) that there are no direct projections into CL from lamina I, IV–V, the spinal laminae that are involved in discriminative cutaneous pain transmission. Thus it has been assumed that CL receives only multisynaptic nociceptive input. The present study used an excellent modern retrograde tracer that indicates a small number of cells in spinal laminae (IV, V, X) and the lower medulla do send projections to medial thalamus.

Second, in the present study we used colon distention in the noxious range (Ness et al. 1991) as the initial search stimulus rather than the usual cutaneous search stimuli. Cells responsive to visceral stimuli were identified by tracking through the CL nucleus and testing responses of spontaneously active cells to colon distention. The responses of cells to graded colon distention, noxious pancreatic stimulation, and other visceral stimuli were then characterized. Intensive search for cutaneous receptive fields followed the characterization of responses to visceral stimuli. However, no cutaneous receptive fields were identified for CL thalamus cells in most cases. Previous studies characterizing responses of thalamic neurons to visceral input have used somatic stimuli as the initial search stimulus by convention (Berkley et al. 1995). This practice would have eliminated the CL neurons under study here since they were not responsive to somatic input. Visceral stimuli may have produced a more intense arousal response than the vigorous noxious cutaneous stimuli we tested (strong forceps pinch). This could explain the much lower incidence of responses to cutaneous noxious stimuli observed in CL neurons identified by first searching using the CRD stimulus.

Neurons responsive only to visceral input have not been previously identified in the thalamus, although previous studies have noted convergence of cutaneous and visceral input onto...
neurons in other regions of the thalamus (Albe-Fessard et al. 1985). Spinothalamic tract cells in the spinal cord with projections to the ventral posterolateral nucleus have been characterized as responsive to both visceral and cutaneous stimulation (Al-Chaer et al. 1996a,b; Hancock et al. 1975; Milne et al. 1981). In the present study a second population is identified. In this population only 3 of the 43 CL neurons characterized with visceral stimuli were also responsive to cutaneous input. Thus thalamic visceroreceptive neurons identified using visceral search stimulus in CL (as the initial search stimulus) rather than cutaneous stimuli are unique among thalamic cells. Lack of CL cell response to distention after morphine implies elimination of noxious nociceptive input arising from spinal cord levels, although direct inhibition of response is possible. Sensitization at the spinal level and/or thalamic level could have modified the neuronal response properties of CL neurons, although noxious visceral and cutaneous stimuli were not applied repetitively.

Conclusion

In conclusion, visceral-specific nociceptive signaling neurons have been identified in the CL nucleus of the intralaminar thalamus responding to noxious mechanical and chemical visceral stimuli. The excitatory responses of CL neurons to noxious visceral stimuli could be reduced by spinal morphine or by a lesion of the dorsal column (DC). The midline DC myelotomy identified a route of travel to the CL thalamus, in addition to the direct ventromedial spinothalamic tract projection we have described anatomically in a previous study. Combined with evidence of c-Fos expression after noxious visceral stimulation in this nucleus, the data suggest that the CL nucleus is an integral component of the median visceral pain matrix that relays visceral nociceptive information from brain stem and spinal cord cells to higher centers important for affective, endocrine, and autonomic responses to visceral pain.

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References


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