The Journal of Physiological Sciences (2019) 69:387–397
https://doi.org/10.1007/s12576-018-00656-3

ORIGINAL PAPER

Distributions of different types of nociceptive neurons in thalamic mediodorsal nuclei of anesthetized rats

Pen-Li Lu[1] · Meng-Li Tsai[2] · Fu-Shan Jaw[1] · Chen-Tung Yen[3]

Received: 20 June 2018 / Accepted: 18 December 2018 / Published online: 3 January 2019

Abstract

Mediodorsal thalamic nucleus (MD) is a critical relay of nociception. This study recorded responses of MD neurons to noxious mechanical and thermal stimuli in isoflurane anesthetized rats. We found the threshold of noxious mechanical stimulation was 141 gw and that of noxious heat stimulation was 46 °C. A significantly higher percentage of noxious inhibitory neurons were found in the medial and central part of the MD, whereas a higher percentage of noxious excitatory neurons were found in the lateral part of the MD and adjacent intralaminar nuclei. The differential distribution of excitatory and inhibitory neurons implies functional differentiation between the medial and lateral part of the MD in nociception processing. Furthermore, by an analysis of the stimulus–response function (SRF), we found 80% of these excitatory neurons had a step-function or hat-shape-like SRF. This suggests that most of the MD neurons may serve as a system to distinguish innocuous versus noxious stimuli.

Keywords Mediodorsal thalamic nucleus · Intralaminar thalamic nuclei · Stimulus–response function · Multiple single-unit recording

Introduction

Mediodorsal thalamic nucleus (MD) contains numerous nociceptive neurons [1]. They are mostly noxious-specific, containing a large receptive field including visceral organs [2]. The neurons of MD directly project to the medial prefrontal cortex [3, 4] and insular cortex [5]. These target cortex are consistently activated by noxious stimulations in humans [6–8] and in rodents [9, 10]. Medial thalamic lesioned rats, which had major lesions in MD, showed temporary antinociception to inflammatory nociception [11] and to visceral nociception [12]. The study using activity-dependent tracing technique indicated that the projection from MD to medial prefrontal cortex was more activated by noxious than innocuous electrical stimulation, and the activation was inhibited by morphine administration [13]. These results support that MD is a critical relay of nociception information.

The MD of rats contains three subnuclei, which are medial (MDm), central (MDc), and lateral (MDl) subnucleus. The cortical projections of rats of each MD subnucleus have been investigated by anterograde tracers injected in MD [3, 14]. The MDm projected to prelimbic cortex and dorsal agranular insular cortex; the MDc projected to ventral agranular insular cortex and lateral orbital cortex; the MDl projected to anterior cingulate cortex and medial precentral cortex. Retrograde tracing from various cortex showed that the ventral part of MDm projected to medial orbital cortex and dorsal part of MDl projected to ventral orbital cortex [15, 16]. Although the cortical connections are different, it is unclear whether the nociceptive responses of each MD subnucleus are different.

✉ Chen-Tung Yen
ctyen@ntu.edu.tw

Pen-Li Lu
penlilu@gmail.com

Meng-Li Tsai
mltsai@niu.edu.tw

Fu-Shan Jaw
jaw@ntu.edu.tw

[1] Institute of Biomedical Engineering, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taibei 10617, Taiwan

[2] Department of Biomechatronic Engineering, National Ilan University, 1, Sec. 1, Shen-Lung Road, I-Lan 26047, Taiwan

[3] Department of Life Science, National Taiwan University, 1 Roosevelt Road, Section 4, Taibei 10617, Taiwan

Furthermore, a linear stimulus–response function (SRF) indicates the coding capability of stimulus intensity, and a sigmoidal SRF is interpreted as providing a detection signal of stimulus occurrence [17, 18]. The SRFs of entire MD to visceral noxious stimuli of anesthetized rats [2] and to laser-heat of conscious rats [19] were reported, however, there was no study to access the nociceptive responses in subnuclei of MD qualitatively and quantitatively.

The goal of the present study was to investigate the details of nociceptive responses in MD, including the types of nociceptive neurons and their distributions in subnuclei. By multichannel microelectrode recording and SRF analysis, we found that more noxious-inhibitory units were aggregated in the medial and central part of the MD, and the shape of SRFs suggested MD and adjacent intralaminar nuclei (IL) were better at distinguishing occurrences of noxious stimuli rather than coding intensity. A part of the results of the present study were previously published in abstract form [20].

Materials and methods

Ethical approval

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan University and adhered to guidelines established by codes for experimental use of animals from the Council of Agriculture, Executive Yuan, Taiwan. This guideline is based on the "Guide for the Care and Use of Laboratory Animals" (Guide 2011 Edition), the "Checklist of the Office of Laboratory Animal Welfare" in the National Institutes of Health in the United States, and further revised according to the requirements of the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC).

Animal preparation

Thirteen male Wistar rats (300–450 g) were used in this study. Rats were initially anesthetized with ketamine (90 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.). A tracheotomy was performed, and the left jugular vein was cannulated. The anesthetic was switched to 2% isoflurane when the rat showed signs of lightly and quickly breathing or whisker vibrating. Craniotomies were performed over an area that ranged 0.5–2 mm lateral to the midline and 2–4 mm posterior to the bregma, and the recording depth was 4.8–6.4 mm ventral to the cortical surface. Rats were paralyzed by gallamine (50 mg/kg, i.v., with the same supporting dose given every hour) and artificially ventilated. Rats were allowed occasional windows without paralysis so that their flexor reflex and anesthetic condition could be checked. The body temperature was maintained at 37.5 °C with a homeothermic blanket (Harvard Apparatus, Holliston, MA, USA).

Experimental protocol

After a craniotomy, the concentration of isoflurane was adjusted to 0.7%, which was maintained through the end of the experiment.

A 16-channel linear Michigan probe with a 100-µm interelectrode distance (A1 × 16–10 mm-100-413, NeuroNexus Technologies, Ann Arbor, MI, USA) was inserted into the target nuclei of the right thalamus. The reference electrode was a stainless-steel wire inserted in the neck muscle. The signals were filtered at 150–8000 Hz, amplified at 10,000–32,000, and sampled at 40 kHz. The Michigan probe was advanced slightly until a position to yield maximal number of single unit and held this position for 30 min to record neuronal activities evoked by stimulation protocol. Complex waveforms for all 16 channels were sorted and discriminated into single unit by an interface package and recorded to a computer (MEA Workstation, Plexon, Dallas, Texas, USA).

The 0.7% isoflurane was close to 1 minimal alveolar concentration which determined by a series of experiments. First, three rats underwent the same initial anesthesia as the aforementioned. Once the ketamine/xylazine anesthesia became too light, 2% isoflurane was given through a mask for 30 min, which mimicked the period required for a craniotomy and electrode insertion. Then, the concentration was adjusted to 1% for 15 min to test the pinch reflex with a vessel clip (with a jaw size of 1.5 × 10 mm; World Precision Instruments, Sarasota, FL, USA) that was applied to the hind paw digits for 15 s. Depending upon the response, the anesthetic concentration was increased or decreased by 0.2%. After an equilibration time of 15 min, the clip was reapplied. This process was continued until the anesthetic concentration was found that just prevented minimal withdrawal. At this concentration, 1 ml of blood from a tail vein was sampled to determine the plasma concentration of isoflurane by gas chromatography [21]. The results for the three rats were 0.72, 0.79, and 0.57% (mean, 0.7% ± 0.1%). Second, we monitored blood pressure of three rats by additional femoral arterial cannulation under the same anesthesia treatment. Noxious pinch stimulations (up to 1200 g, handheld pincher, RP-1, Bioseb, Chaville, France) were applied to the intact hind paw every hour to 5 h. Blood pressure and heart rate results showed a gradual increasing trend, however, no fluctuations caused by noxious mechanical stimuli was observed (one example in Fig. 1). Therefore, we used 0.7% isoflurane in the present study. All rats in the preliminary tests were the same gender and body weight as the experimental rats.

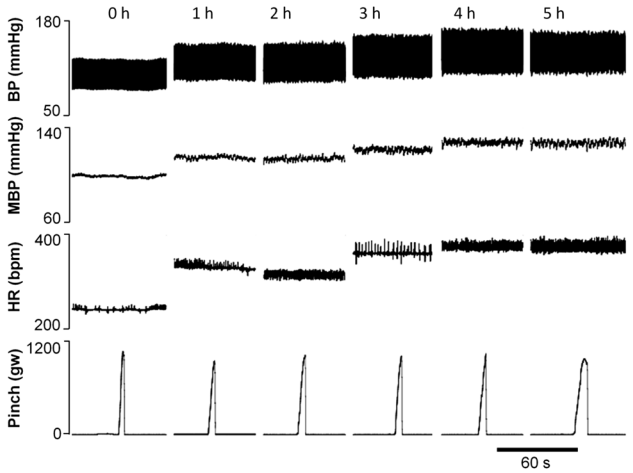

Fig. 1 No cardiovascular responses to noxious pinch stimuli (lowest traces reflecting the stimulus strength in gw). Blood pressure (BP), mean blood pressure (MBP), and heart rate (HR) did not obviously change before and after noxious pinch stimuli (> 1000 gw) over 5 h among three rats. One example showed

Noxious mechanical stimulation

The receptive fields of units were defined by pinching the four extremities, bilateral ears, and tail. A vessel clip (with a jaw size of 1.5 × 10 mm. World Precision Instruments) was applied for 10 s. The inter-stimulus interval was at least 1 min. Following the receptive field mechanical stimulation, the SRF was assessed by a calibrated hand-held pincher (RP-1, Bioseb, Chaville, France) to produce a ramp-increased force onto the receptive fields. The pinching force level was processed with a PC-based system (sampled at 1 kHz) that signal was recorded to synchronize with the neuronal signals.

Contact heat stimulation

Contact heat stimulation was applied after the noxious mechanical stimulation. A customized thermoelectric cooling chip coupled with a copper probe was used as the stimulation probe. The tip area for heat stimulation was 25 mm^2, and a thermistor was attached to the lateral surface of this area as a feedback control for the stimulation. The stimulation probe was held by a manipulator so that it lightly contacted the skin of the rat. The contacting surfaces of the stimulator and skin were moistened with a drop of saline for better thermal conduction. The baseline temperature was held at 35 °C for 3 min then gradually heated to 40, 45, or 50 °C. The heating process lasted 30 s, and the cooling process took 100 s back to the baseline temperature (see an example trace in Fig. 6c). Each stimulus was separated by a 3-min interval of the baseline temperature to avoid the skin being overstimulated. After the 50 °C stimulation, the skin turned red in all cases, but no permanent tissue damage was observed; the experiment was then terminated.

Histology

Each animal received only one microelectrode penetration. At the end of each experiment, a small electrolytic lesion (20 μA for 30 s, anodal DC) was made at the deepest channel of the recording electrode, and then after withdrawing the electrode by 1 mm, a second lesion was made. After the electrical lesions were made, the rats received one lethal bolus injection of sodium pentobarbital (80 mg/kg, i.v.) and were perfused with saline followed by 4% formalin. The brains were sectioned at 50 μm thick with a freezing microtome. Sections containing the recording track were examined fresh unstained using a ZEISS Axioplan 2 system equipped with a 1.25X Plan-NEOF objective to delineate the fiber-rich MDm [22], and stained for either acetylcholinesterase histochemistry [23] or cresyl violet. MD subnuclei were identified (Fig. 2) and each recording site was reconstructed to the nearest coronal atlas plate [24].

Data analysis

A commercial offline spike-sorting program was used (OfflineSorter, Plexon). Spikes were sorted by their waveform characteristics (e.g., principle components, peak-volley amplitudes et al.). Each spike can be plotted in a space which was defined by two or three waveform characteristics (2-D or 3-D feature space). Spikes with similar waveforms will cluster in the feature space. The single unit was defined as a distinguishable cluster in feature-space. Rate histograms and stimulus–response function analysis were carried out using NeuroExplorer (Nex Technologies, Littleton, MA, USA) and MatLab (MathWorks, Natick, MA, USA). Neuronal firings evoked by pinching and contact heat stimuli were determined from a histogram with a 1-s bin. Frequencies of neuronal firings were normalized to their baseline activities (60 s preceding the stimuli) and were represented by *Z* scores [25]. During the stimulus period, firings that exceeded a score of 2.33 (at the 99% confidence level) and were sustained for 3 s were considered to be excitatory responses. Since inhibitory responses were either entirely or partially silenced, they could not fit the definition as the excitatory responses (that is, the firings that exceeded a score of −2.33 during the stimulus period), so we defined the inhibitory response as bin values during noxious stimuli were significantly lower than baseline activities (according to a two-tailed Student's *t* test). The bin numbers of baseline activities were the same as that of noxious stimuli.

Units with repeated responses to pinching were regarded as pinch-responsive units; units that responded to 45/50 or 50 °C stimulation alone were regarded as contact

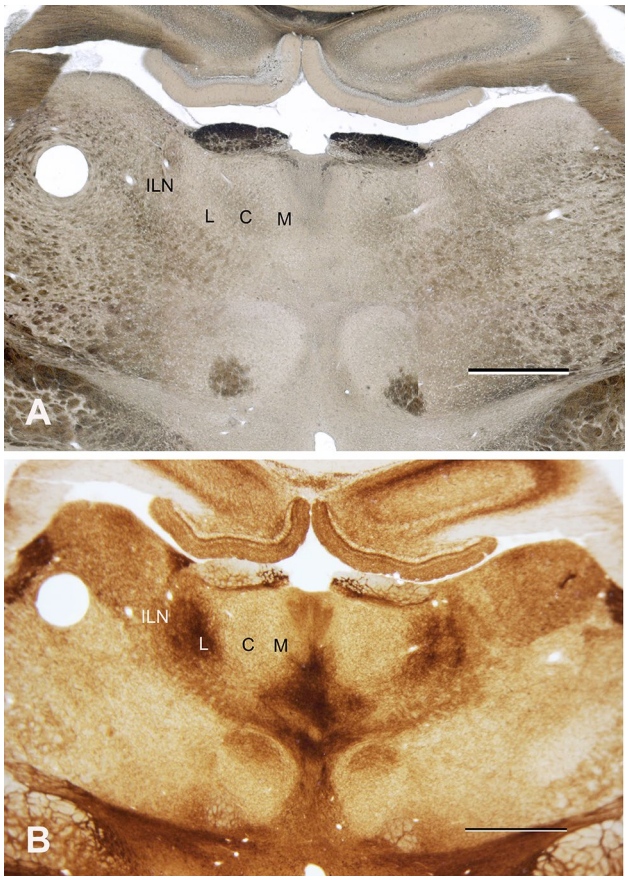

Fig. 2 Histological method for identifying MD subnuclei. **a** Photomicrograph of the medial thalamus in a wet unstained frozen section. **b** The same section after AChE histochemical staining. Note MDc is fiber-rich and can be clearly distinguished in the wet freshly prepared section in **a** and the MDl is AChE-rich and clearly distinguishable in the AChE stained section in **b**. *C* MDc, *ILN* intralaminar nuclei, *M* MDm, *L* MDl. Calibration bar is 1 mm

heat-responsive units. Units were catalogued as mechano-nociceptive alone (pinch, P), thermo-nociceptive alone (heat, H), both mechano- and thermo- nociceptive (PH), and non-responsive (NR) units. PH and P units that were activated during ramp pinching were used to calculate the mechanical SRFs, whereas PH and H units that were activated during heating to 50 °C were used to calculate the thermal SRFs. Coding units were defined as firing rate increased with stronger stimulation.

Distribution of excitatory and inhibitory units over thalamic nuclei was analyzed by a Chi-square test or by Fisher's exact test if any observation was <5. Percentage of excitatory and inhibitory units over thalamic nuclei was analyzed by a *Z* test. Neuronal activities between groups were analyzed with the nonparametric Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks, and Dunn's test was used as a post hoc analysis to detect sources of significant difference between groups. The statistical significance threshold was set to 0.05. All statistics were calculated using SigmaStat 3.5 for Windows (Systat Software, Chicago, IL, USA).

Results

In total, 179 units had been isolated (2.2 ± 0.1 units/channel of microelectrode) and histological confirmed within the MD/IL from the thalamus of 13 rats (Fig. 3). The units were recorded from MDm ($n=25$), MDc ($n=40$), MDl ($n=50$), and IL ($n=64$, Table 1). No unit had responses when the mechanical or thermal stimulator gently touched on the rat's skin. One hundred and twelve units responded to either noxious pinching or heating in form of excitatory ($n=73$), inhibitory ($n=29$), or mix ($n=10$). Mix units had excitatory responses on some receptive fields and had inhibitory responses on other receptive fields. We further distinguished units into PH, P, and H (Table 2); these functionally classified units were intermingled each other (Fig. 3). Since we pinched four limbs of every rat, we can describe the pinch receptive fields (pinch-RFs) over four limbs (Table 3). Most neurons had bilateral pinch-RFs. Minor portion of neurons had pinch-RFs contained only contralateral limbs. It was worth noting that only noxious-excitatory neurons had ipsilateral pinch-RFs.

Differential distribution of noxious-excitatory and noxious-inhibitory units

Inhibitory units were all recorded from the MDm and MDc thalamic nuclei except one from the central medial (CM) nucleus. A 2-by-4 Chi-square test indicated that there was a significant correlation (Table 1, $p<0.001$) between response types (excitatory/inhibitory) and MDm/MDc/MDl/IL thalamic nuclei. Two-by-two Chi-square tests further showed no differences between the MDm and MDc thalamic nuclei ($p=0.47$), or between the IL and MDl thalamic nuclei ($p=1$, Fisher's exact test). However, different distributions were found for MDm/MDc and IL/MDl thalamic nuclei ($p<0.001$, * in Table 1). The percentage of inhibitory units over responsive units in the MDm/MDc thalamic nuclei was 60% (28/47), which was higher than that in the MDl/IL thalamic nuclei ($1/65=1.5\%$, *Z* test, $p<0.001$). The percentage of excitatory units over responsive units in the MDm/MDc thalamic nuclei was 23% (11/47), which was lower than that in the MDl/IL thalamic nuclei ($62/65=95\%$, *Z* test, $p<0.001$). Thus, our data demonstrated a large number of noxious-inhibitory units aggregated in the MDm/MDc thalamic nuclei, whereas a larger number of noxious-excitatory units aggregated in the MDl/IL.

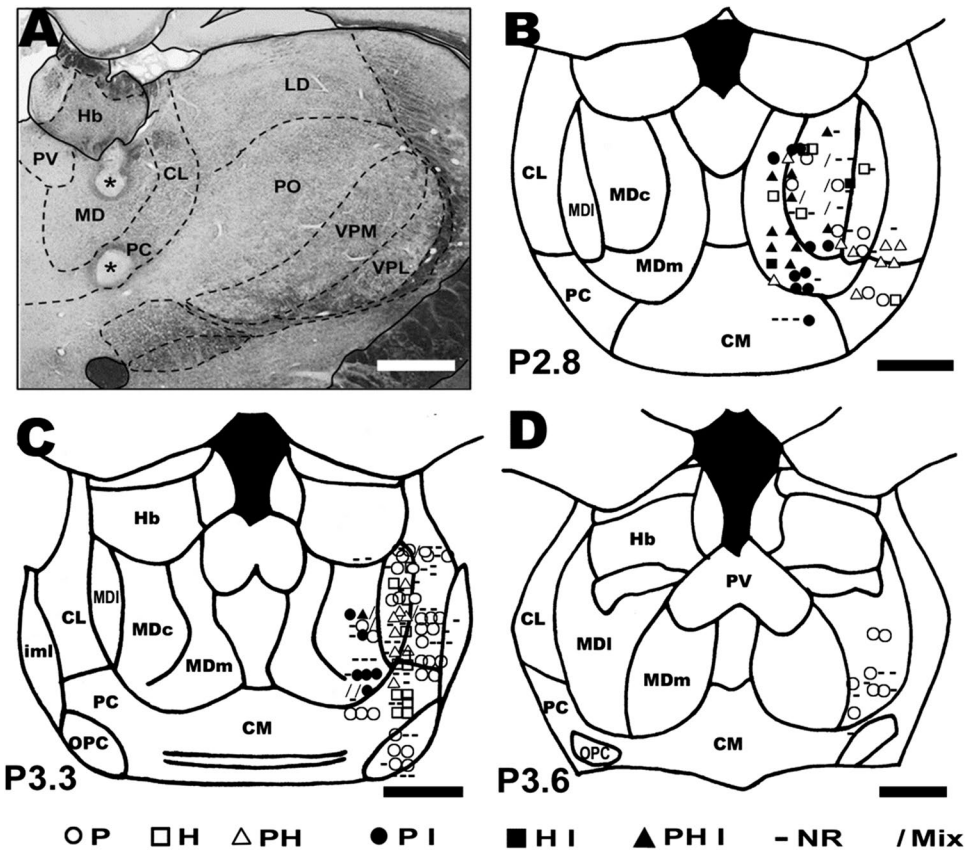

Fig. 3 Recording sites. **a** An exemplary photomicrograph of a wet unstained thalamic section showing the method for localization. Two electrolytic lesions (asterisk), separated by 1 mm, were made in the recording track. Sixteen equally spaced recording sites were identified by interpolation. The subnuclei were confirmed with acetylcholinesterase staining. Scale bar: 1 mm. **b**–**d** Distribution of the recording sites in all rats is shown on coronal diagrams of the medial thalamus in a standard atlas [24], 2.8 (**b**), 3.3 (**c**), and 3.6 (**d**) mm caudal to the bregma. Eight categories of units were identified: open circles, pinch excitatory; open squares, heat excitatory; open triangles, pinch and heat excitatory; solid circles, pinch inhibitory; solid squares, heat inhibitory; solid triangles, pinch and heat inhibitory; dashes, units responding to neither stimuli; and slash, mix responsive. Scale bar of (**b**–**d**): 0.5 mm

Table 1 Distribution of units in subnuclei of the MD and adjacent IL

Subnuclei	MDm	MDc	MDl	IL	Total
Total	25	40	50	64	179
NR	4	14	19	30	67
Responsive	21	26	31	34	112
E	4 (19%)	7 (27%)	29 (94%)[a]	33 (97%)[a]	73 (65%)
I	15 (71%)[b]	13 (50%)[b]	0 (0%)[a]	1 (3%)	29 (26%)
Mix	2 (10%)	6 (23%)	2 (7%)	0 (0%)	10 (9%)

E excitatory, *I* inhibitory, *Mix* inhibitory in one limb and excitatory in other limbs, *NR* non-responsive to pinch nor contact heat, *MDm, MDc, MDl* medial, central, and lateral part of the mediodorsal thalamic nucleus, *IL* intralaminar thalamic nuclei including centrolateral, paracentral, oval paracentral, and central medial thalamic nuclei

[a]Excitatory units of MDl/IL (95%) was higher than that of MDm/MDc (23%), *Z* test, $p < 0.001$

[b]Inhibitory units of MDm/MDc (60%) was higher than that of MDl/IL (1.5%), *Z* test, $p < 0.001$

Stimulus–response functions of noxious-excitatory and noxious-inhibitory neurons

Sixty of 65 excitatory and mixed PH and P units were successfully tested by ramp pinching. Eight units with after-discharge responses were excluded from the mechanical SRFs since they were not activated during ramp pinching. Figure 4a shows an example of one unit with coding property for which firing increased with an increasing pinch force (upper raster); while the other (lower raster) showed a trend as a hat-function like for which firing was strongest at 424 gw but diminished at higher intensity. Eighty percent (42/52) of units were classified as non-coding units which fired extremely at certain intensity and sustained or declined responses at higher intensity. We further separated these non-coding units into two types based on the features of SRFs, one is the hat-function like and another is step-function like. The averaged SRF of neurons with hat-function like feature (25/52, 48%) had significant firing at 141 gw, reached peak at 247 gw and diminished with increasing force (Fig. 4b(a)). For the step-function like group (17/52, 33%), the averaged SRF was stepped up a plateau of 565-1062 gw (Fig. 4b(b)). The averaged SRF of coding group (10/52, 19%) was proportional to the pinching force (Fig. 4b(c)). Half (13/25, 52%) of the hat-function- like units were recorded from IL and the others were from MDc (6/25, 24%), MDl (4/25, 16%), and MDm (2/25, 8%). Equal amounts of step-function-like units were from IL (7/17, 41%) and MDl (7/17, 41%) and the others were from MDc (2/17, 12%), and MDm (1/17, 6%). Equal amounts of units of coding units were from IL (4/10, 40%), MDc (3/10, 30%), and MDl (3/10, 30%).

Table 2 Distribution of mechanonociceptive-alone (pinch, P) and thermonociceptive-alone (heat, H) units in subnuclei of the MD and adjacent IL

Subnuclei	MDm				MDc				MDl				IL			
Responsive	E	I	Mix	Total	E	I	Mix	Total	E	I	Mix	Total	E	I	Mix	Total
PH	1	7	0	8	1	1	3	5	7	0	1	8	6	0	0	6
P	2	6	2	10	4	10	3	17	15	0	1	16	19	1	0	20
H	1	2	0	3	2	2	0	4	7	0	0	7	8	0	0	8

Other abbreviations refer to Table 1

PH mechano- and thermonociceptive (pinch-heat, PH)

Table 3 Pinch-receptive fields (pinch-RFs) of noxious-excitatory (E), noxious-inhibitory (I), and mix units (Mix)

Pinch-RFs	E	I	Mix
Bilateral	44 (80%)	17 (68%)	6 (60%)
Contralateral	7 (13%)	8 (32%)	4 (40%)
Ipsilateral	4 (7%)	0	0

Thirty-two of 36 PH and H units were excited by 50 °C ramp stimulation and the remaining units were exclusively excited by 40 ($n=2$) and 45 °C ($n=2$). Three units had delayed responses to 50 °C and thus were excluded from the SRF analysis. One example unit showed a robust response to 45 °C and 50 °C, but not to 40 °C (Fig. 5a). In Fig. 5b, color-coded responses of 36 PH and H units revealed that more neurons responded to 50 °C (30/36, 83%) than to 45 °C (18/36, 50%), and more units showed after-discharge phenomena emerged after 50 °C. In addition, the cyan color bands (arrows in Fig. 5b) indicated that most units had similar response thresholds. Figure 5c illustrated one example unit with coding property (upper unit) and one example unit with non-coding property (lower unit) to 50 °C. Seventy-six percent (22/29) of units

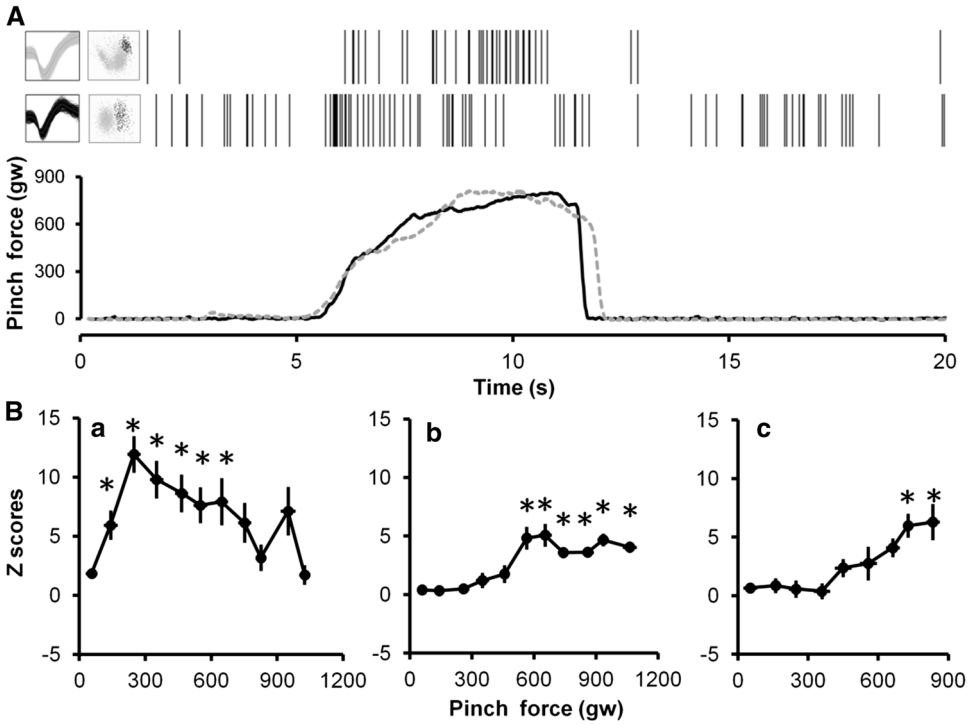

Fig. 4 The stimulus–response functions (SRFs) of noxious-excitatory units to graded pinching. **a** Raster plots of the responses of two units (upper two panels) and the corresponding applied forces (lower panel) by a hand-held pincher. The light-gray unit in the upper panel had coding property, i.e., its firing frequency increased with an increasing pinching force. In contrast, the black unit in the lower panel showed that its firing frequency was the strongest at a certain threshold of the pinching force, but firing frequency did not increase any further with an increasing force. The two examples are from two different rats. **b** (a) Averaged SRF (mean ± SE) of 25 units with hat-function-like features reached threshold at 141 gw, peaked at 247 gw, and diminished with an increasing pinching force. (b) Averaged SRF of 17 units with step-function-like features reached a peak at 565 gw. (c) Averaged SRF of 10 units with coding property significantly increased at 727 gw. *$p<0.05$, compared to the baseline data

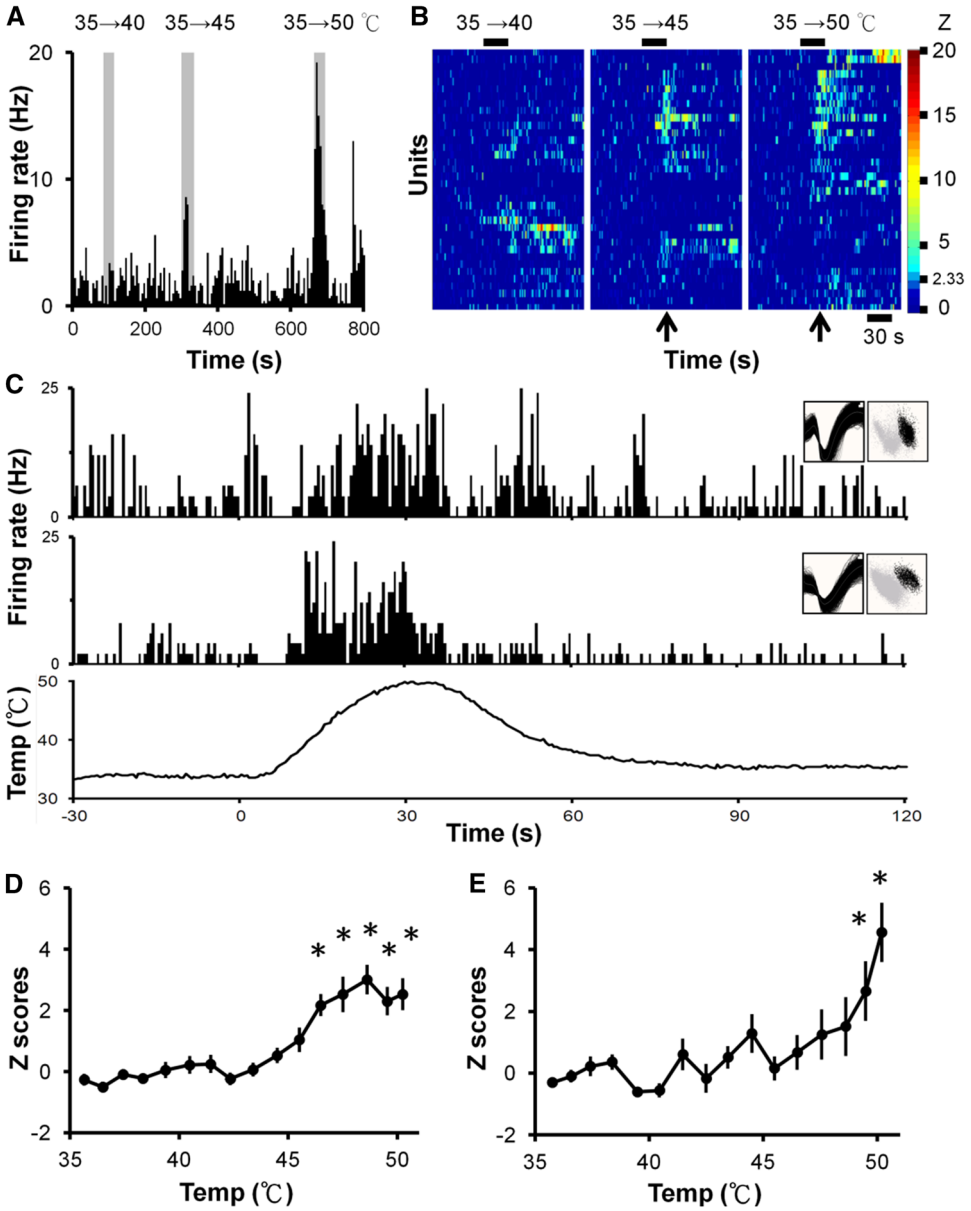

Fig. 5 The stimulus–response functions (SRFs) of noxious-excitatory units to contact heat stimuli. **a** An example response of a MD thalamic nuclear unit to 40, 45, and 50 °C contact heat applied to the fore paw. The baseline temperature of the probe was held at 35 °C. Bin = 6 s. **b** The responses of all MD/IL thalamic nuclear units ($n = 36$) plotted against time. Each row represents data from a single unit, and units' activities are represented by the color-coded *Z* score. Note that there was a sudden increase in activity for most heat-responsive units (arrows). **c** The firing histograms of two example units responding to 50 °C contact heat applied to the fore paw. The upper and lower units, respectively, show coding and non-coding properties. The two units were recorded at the same time in the same rat. The baseline temperature was held at 35 °C. At 10 s, the Peltier thermal probe was turned on, and it had reached 50 °C by the end of the 30-s heating period. The 50 °C stimulation-response data were used to construct the SRFs. **d** Averaged SRF (mean ± SE) of 22 units with step-function-like features showed the responses significantly increased from 46 to 50 °C. **e** Averaged SRF (mean ± SE) of 7 units with coding property showed the responses significantly increased from 49 to 50 °C. $*p < 0.05$, compared to the baseline activity at 35 °C (Kruskal–Wallis one-way ANOVA on ranks, post hoc analysis: Dunn's test)

demonstrated step-function-like features characterized by the strongest firing at a certain threshold temperature, and the averaged SRF exhibited a plateau from 46 to 50 °C (Fig. 5d). Twenty-four percent (7/29) of units showed coding property with the averaged SRF significantly higher at 49–50 °C (Fig. 5e). Most units of the step-function-like group were from MDl (11/22, 50%), IL (8/22, 36%), and the remaining were from MDc (3/22, 14%). Most units of the coding group were from IL (5/7, 71%) and the others were from MDm (2/7, 29%).

Twenty-five units were inhibited by noxious pinch stimuli. Twenty-one units were further tested by pincher (one example unit in Fig. 6a). The averaged SRF showed the threshold of inhibitory response was 455 gw. The units did not respond differently when the pinch intensity was larger than the threshold, and the shape of the averaged SRF was similar to step-down function (Fig. 6b). Twelve units were inhibited by contact heat stimuli (one example unit in Fig. 6c). The averaged SRF showed the responses significantly attenuated when the temperature higher than 48 °C (Fig. 6d).

Discussion

By multi-channel recording, we found a higher percentage of noxious-inhibitory units aggregated in MDm and MDc and noxious-excitatory units aggregated in MDl and IL thalamic nuclei. Through the quantitative analysis, we found that most noxious-excitatory and noxious-inhibitory units had SRFs that peaked at a lower but noxious intensity followed by sustained or declined responses when stimulus increased. The results implied potential difference between MDm/MDc and MDl/IL and these units may serve as the role of distinguishing noxious/innocuous stimuli.

Distribution of nociceptive neurons in subnuclei of MD and in adjacent IL nuclei

A possible mechanism of the aggregated noxious-inhibitory neurons may be the nociceptive, GABAergic neurons that preferential projected to the MDm and MDc thalamic nuclei. The ventral pallidum, the vertical limb of the diagonal band, and the reticular thalamic nucleus are the three major GABAergic inputs to the MD thalamic nucleus [26]. No study indicated that the vertical limb of the diagonal band had nociceptive responses. The reticular thalamic nucleus was reported to respond to noxious pinch stimuli [27], however, the MDm, MDc, and MDl thalamic nuclei all receive topographically reticular projections [28]. Neurons in the ventral pallidum of rats respond to noxious mechanical [29] and noxious thermal stimuli [30]. Locally, electrical stimulation in the ventral pallidum induced inhibitory responses or excitatory followed by prolonged inhibitory responses in the MD thalamic nucleus [31, 32]. In addition, neurons in the ventral pallidum had restricted projections to the MDm [28]. Thus, we suggest that the ventral pallidum may have contributed to the aggregated noxious-inhibitory responses.

The function of the aggregated noxious-inhibitory neurons in MDm and MDc is poorly understood. However, we speculated that it was involved in modulation of pain

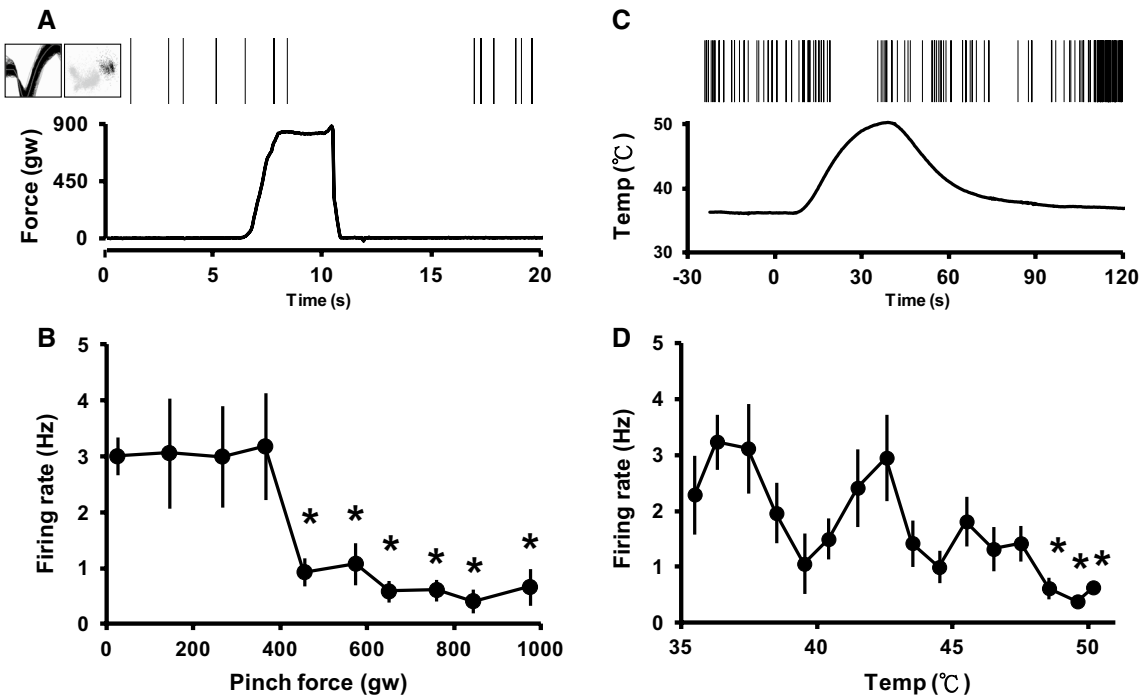

Fig. 6 The stimulus–response functions (SRFs) of noxious-inhibitory units to graded pinching and to contact heat. **a** Raster plots of a MDm unit responded to applied forces (lower panel) by a hand-held pincher. **b** Averaged (mean ± SE) SRF of 21 noxious-inhibitory units showed the response significantly reduced at 455 gw. **c** Raster plots of the same MDm unit responded to 50 °C contact heat (lower panel). **d** Averaged SRF of 12 noxious-inhibitory units showed the response significantly reduced at 48 °C. $*p < 0.05$, compared to the baseline data (Kruskal–Wallis one-way ANOVA on ranks, post hoc analysis: Dunn's test)

threshold through rostral agranular insular cortex (RAIC) circuit. Unlike MDl thalamic nucleus predominately projects to medial prefrontal cortex [3, 4, 33], MDm and MDc thalamic nuclei have a distinct project to RAIC [5]. Increasing local GABA concentration in RAIC produced analgesia in freely moving rats. Selectively activation of $GABA_B$ receptor containing neurons in RAIC which projects to amygdalate produced hyperalgesia [34]. The excitatory and inhibitory neurons in MDm and MDc might modulate this circuit from thalamic level.

Step-function-like and linear function of SRFs in MD and adjacent IL nuclei

A lot of the SRFs of the MD and adjacent IL neurons were step-function-like to either noxious mechanical or heat stimuli. This type of SRF is supposed better in distinguishing the presence of noxious stimuli than coding the stimuli intensity. Indeed some units in IL could encode faithfully to pinching intensity [35], however, the ensemble response in MD cannot follow the intensity of visceral expansion [2]. The ensemble response of MD neurons increased when the intensity was increased from 20 to 80 mmHg, but declined at 100 mmHg. The decline response was also found in inhibitory ensemble responses of MD [2]. Ascending modulation by dorsal raphe nucleus may account for the reduced responses of medial thalamus when receiving extremely hight intensity stimuli. Many medial thalamic nuclei, including CL, CM, OPC, PC, and MD thalamic nuclei received moderate-to-dense projections from dorsal raphe [36]. Electrical stimulation at dorsal raphe nucleus reduced nociceptive responses of parafascicularis (Pf, one of the IL nuclei) thalamic neurons [37–39].

The coding ability of MD and adjacent IL neurons was 0.01 spikes/g (see the slope of c in Fig. 4b). In contrast, the coding ability of rat wide-dynamic range neurons in the ventrobasal (VB) thalamic complex is 0.8–0.9 spikes/g [40, 41], and that of noxious-specific neurons is 0.125 spikes/g [40]. Thus, the coding ability of medial thalamic neurons is much weaker than that of the VB complex. This situation is the same as that with contact heat for which slopes were 0.5–1.3 spikes/ °C in the MD and adjacent IL (see the slope in Fig. 5e) and 1–4 spikes/°C in the VB complex [42], however, some units also had sigmoid-like SRFs in the VB complex of the raccoon [43]. Studies from awake animals demonstrated that IL [44] and MD [19] thalamic neurons can encode stimulus intensity. Bushnell et al. [44] demonstrated one CM neuron of awake monkey had excellent coding ability, which was 6.6 spikes/°C from 46 to 49 °C. However, the coding ability of ensemble response was shown at limited temperature of 37, 45, and 47 °C. It was unknown that the ensemble responses to temperature higher than 47 °C. Zhang et al. [19] had demonstrated coding ability of MD neurons to noxious laser heat. Either the number of responded neurons or the ensemble activities showed sigmoidal SRFs with plateau from 150 to 180 mJ. By linear regression of SRFs, coding ability of MD neurons to laser-heat stimuli was 0.018 *z*-score/mJ.

The hat- and step-function-like non-coding neurons may be comparable with the thresholds of noxious-tap and the nociceptive–specific neurons of IL neurons of cats [35]. The threshold of noxious-tap neurons was 50–200 gw and the threshold of nociceptive-specific neurons was 500–700 gw [35]. Medial thalamic neurons of rats were classified as nociceptive-specific and noxious-tapping [1, 45, 46] but the responsive thresholds were not quantified. Our data indicated that there were also low (141 gw for hat-function-like) and high (565 gw for step-function-like units and 726 gw for coding units) mechanical thresholds of MD and adjacent IL in rats. Furthermore, our data showed the neuronal activities sustained or declined even the stimulus intensity increased.

Functional interpretation of step-like non-coding neurons in MD and adjacent IL

Different patterns of SRF may show different functional characteristics of a neuron. The step-function-like feature of SRF would show a neuron could discriminate two different types of stimuli. In our case, such step-function-like features of these non-coding neurons imply they could discriminate noxious and non-noxious stimuli. This may indicate that they serve as an "alarm" function, not only did these units have step-function-like responses, but also most of them had a fairly high threshold.

The medial thalamus receives strong inputs from mesencephalic reticular formation and thus is regarded as an anterior part of ascending reticular arousal system [47]. Infarctions of medial thalamus caused conscious deficiencies in patients [48]. The cerebral blood flow of the IL thalamic nucleus increased when the subjects became attentive in human [49]. The firing rate and firing patterns of the IL thalamic nucleus changed correlatively with the sleep–wake cycles in cats [50]. Moreover, conscious level of rats decreased by abolishing neuronal activities [51] and increased by activating the neuronal activities of IL thalamic nucleus [52, 53]. Electrical stimulation of the IL thalamic nucleus can desynchronize EEG in cats [54], and this technique was adapted to improve the arousal of patients in a minimally conscious state [55]. By combining the above-mentioned knowledge, the MD and adjacent IL neurons may provide a robust activation to cortex, and this activation may elevate arousal and allow the organism to be more aware of further noxious stimuli.

Conclusions

The medial aggregated of inhibitory and lateral aggregated of excitatory neurons in the MD and adjacent IL highlights the differences of the related pain-pathways. The step-function-like SRFs implied that many of the MD neurons may serve the function of distinguishing innocuous versus noxious stimuli.

Acknowledgements This research was supported by grants from the National Science Council (NSC100-2311-B-002-002-MY3, NSC100-2221-E-002-064, and NSC 100-2923-E-002-007-MY3) and National Health Research Institute (NHRI-EX101-10104NI), Taiwan.

Author contributions PLL, MLT, FSJ, and CTY contributed to the conception and design of the experiments. PLL conducted the experiments. PLL and MLT analyzed the data. PLL and CTY drafted the manuscript. All authors participated in interpreting the data, revising the article, and approving the final version for publication. Experiments were conducted at the Department of Life Science, National Taiwan University, Taipei, Taiwan.

Funding This study was funded by grants from the National Science Council (NSC 100-2311-B-002-002-MY3, NSC 100-2221-E-002-064, and NSC 100-2923-E-002-007-MY3) and National Health Research Institute (NHRI-EX101-10104NI), Taiwan.

Compliance with ethical standards

Conflict of interest Author Pen-Li Lu declares that she has no conflicts of interest. Author Meng-Li Tsai declares that he has no conflicts of interest. Author Fu-Shan Jaw declares that he has no conflicts of interest. Author Chen-Tung Yen declares that he has no conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Dostrovsky JO, Guilbaud G (1990) Nociceptive responses in medial thalamus of the normal and arthritic rat. Pain 40:93–104
2. Yang SW, Follett KA, Piper JG, Ness TJ (1998) The effect of morphine on responses of mediodorsal thalamic nuclei and nucleus submedius neurons to colorectal distension in the rat. Brain Res 779:41–52
3. Krettek JE, Price JL (1977) The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J Comp Neurol 171:157–191
4. Wang CC, Shyu BC (2004) Differential projections from the mediodorsal and centrolateral thalamic nuclei to the frontal cortex in rats. Brain Res 995:226–235
5. Jasmin L, Burkey AR, Granato A, Ohara PT (2004) Rostral agranular insular cortex and pain areas of the central nervous system: a tract-tracing study in the rat. J Comp Neurol 468:425–440
6. Casey KL, Minoshima S, Berger KL, Koeppe RA, Morrow TJ, Frey KA (1994) Positron emission tomographic analysis of cerebral structures activated specifically by repetitive noxious heat stimuli. J Neurophysiol 71:802–807
7. Coghill RC, Talbot JD, Evans AC, Meyer E, Gjedde A, Bushnell MC, Duncan GH (1994) Distributed processing of pain and vibration by the human brain. J Neurosci 14:4095–4108
8. Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MC (1997) Pain affect encoded in human anterior cingulate but not somatosensory cortex. Science 277:968–971
9. Luo Z, Yu M, Smith SD, Kritzer M, Du C, Ma Y, Volkow ND, Glass PS, Benveniste H (2009) The effect of intravenous lidocaine on brain activation during non-noxious and acute noxious stimulation of the forepaw: a functional magnetic resonance imaging study in the rat. Anesth Analg 108:334–344
10. Chang C, Shyu BC (2001) A fMRI study of brain activations during non-noxious and noxious electrical stimulation of the sciatic nerve of rats. Brain Res 897:71–81
11. Wilson HD, Uhelski ML, Fuchs PN (2008) Examining the role of the medial thalamus in modulating the affective dimension of pain. Brain Res 1229:90–99
12. Wang HC, Chai SC, Wu YS, Wang CC (2007) Does the medial thalamus play a role in the negative affective component of visceral pain in rats? Neurosci Lett 420:80–84
13. Yang PF, Chen DY, Hu JW, Chen JH, Yen CT (2011) Functional tracing of medial nociceptive pathways using activity-dependent manganese-enhanced MRI. Pain 152:194–203
14. Groenewegen HJ (1988) Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography. Neuroscience 24:379–431
15. Ray JP, Price JL (1992) The organization of the thalamocortical connections of the mediodorsal thalamic nucleus in the rat, related to the ventral forebrain-prefrontal cortex topography. J Comp Neurol 323:167–197
16. Reep RL, Corwin JV, King V (1996) Neuronal connections of orbital cortex in rats: topography of cortical and thalamic afferents. Exp Brain Res 111:215–232
17. Buchel C, Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C (2002) Dissociable neural responses related to pain intensity, stimulus intensity, and stimulus awareness within the anterior cingulate cortex: a parametric single-trial laser functional magnetic resonance imaging study. J Neurosci 22:970–976
18. Timmermann L, Ploner M, Haucke K, Schmitz F, Baltissen R, Schnitzler A (2001) Differential coding of pain intensity in the human primary and secondary somatosensory cortex. J Neurophysiol 86:1499–1503
19. Zhang Y, Wang N, Wang JY, Chang JY, Woodward DJ, Luo F (2011) Ensemble encoding of nociceptive stimulus intensity in the rat medial and lateral pain systems. Mol Pain 7:64
20. Lu PL, Jaw FS and Yen CT (2009) Response pattern of medial thalamic neuron to thermal and mechanic nociceptive stimulation in anesthetized rat. In: XXXVI international congress of physiological sciences, Kyoto, Japan, p 424
21. Lu CC, Lin TC, Yu MH, Chen TL, Lin CY, Chiang JS, Ho ST (2009) Effects of changes in alveolar ventilation on isoflurane arterial blood concentration and its uptake into the human body. Pharmacology 83:150–156
22. Ray JP, Price JL (1992) The organization of the thalamocortical connections of the mediodorsal thalamic nucleus in the rat, related to the ventral forebrain-prefrontal cortex topography. J Comp Neurol 323:167–197
23. Woolf NJ, Butcher LL (1981) Cholinergic neurons in the caudate-putamen complex proper are intrinsically organized: a combined Evans blue and acetylcholinesterase analysis. Brain Res Bull 7:487–507

24. Paxinos G, Watson C (1998) The rat brain. Academic Press, San Diego
25. Tsai ML, Kuo CC, Sun WZ, Yen CT (2004) Differential morphine effects on short- and long-latency laser-evoked cortical responses in the rat. Pain 110:665–674
26. Churchill L, Zahm DS, Kalivas PW (1996) The mediodorsal nucleus of the thalamus in rats—I. Forebrain GABAergic innervation. Neuroscience 70:93–102
27. Yen CT, Shaw FZ (2003) Reticular thalamic responses to nociceptive inputs in anesthetized rats. Brain Res 968:179–191
28. Cornwall J, Phillipson OT (1988) Afferent projections to the dorsal thalamus of the rat as shown by retrograde lectin transport—I. The mediodorsal nucleus. Neuroscience 24:1035–1049
29. Lamour Y, Dutar P, Rascol O, Jobert A (1986) Basal forebrain neurons projecting to the rat frontoparietal cortex: electrophysiological and pharmacological properties. Brain Res 362:122–131
30. Chudler EH (1998) Response properties of neurons in the caudate-putamen and globus pallidus to noxious and non-noxious thermal stimulation in anesthetized rats. Brain Res 812:283–288
31. Mogenson GJ, Ciriello J, Garland J, Wu M (1987) Ventral pallidum projections to mediodorsal nucleus of the thalamus: an anatomical and electrophysiological investigation in the rat. Brain Res 404:221–230
32. Vives F, Mogenson GJ (1985) Electrophysiological evidence that the mediodorsal nucleus of the thalamus is a relay between the ventral pallidum and the medial prefrontal cortex in the rat. Brain Res 344:329–337
33. Conde F, Audinat E, Maire-Lepoivre E, Crepel F (1990) Afferent connections of the medial frontal cortex of the rat. A study using retrograde transport of fluorescent dyes. I. Thalamic afferents. Brain Res Bull 24:341–354
34. Jasmin L, Rabkin SD, Granato A, Boudah A, Ohara PT (2003) Analgesia and hyperalgesia from GABA-mediated modulation of the cerebral cortex. Nature 424:316–320
35. Dong WK, Ryu H, Wagman IH (1978) Nociceptive responses of neurons in medial thalamus and their relationship to spinothalamic pathways. J Neurophysiol 41:1592–1613
36. Krout KE, Belzer RE, Loewy AD (2002) Brainstem projections to midline and intralaminar thalamic nuclei of the rat. J Comp Neurol 448:53–101
37. Andersen E, Dafny N (1983) An ascending serotonergic pain modulation pathway from the dorsal raphe nucleus to the parafascicularis nucleus of the thalamus. Brain Res 269:57–67
38. Andersen E, Dafny N (1983) Dorsal raphe stimulation reduces responses of parafascicular neurons to noxious stimulation. Pain 15:323–331
39. Prieto-Gomez B, Dafny N, Reyes-Vazquez C (1989) Dorsal raphe stimulation, 5-HT and morphine microiontophoresis effects on noxious and nonnoxious identified neurons in the medial thalamus of the rat. Brain Res Bull 22:937–943
40. Chiang CY, Zhang S, Park SJ, Hu JW, Dostrovsky JO, Sessle BJ (2005) Mechanoreceptive field and response properties of nociceptive neurons in ventral posteromedial thalamic nucleus of the rat. Thalamus Relat Syst 3:41–51
41. Martin WJ, Hohmann AG, Walker JM (1996) Suppression of noxious stimulus-evoked activity in the ventral posterolateral nucleus of the thalamus by a cannabinoid agonist: correlation between electrophysiological and antinociceptive effects. J Neurosci 16:6601–6611
42. Peschanski M, Guilbaud G, Gautron M, Besson JM (1980) Encoding of noxious heat messages in neurons of the ventrobasal thalamic complex of the rat. Brain Res 197:401–413
43. Simone DA, Hanson ME, Bernau NA, Pubols BH Jr (1993) Nociceptive neurons of the raccoon lateral thalamus. J Neurophysiol 69:318–328
44. Bushnell MC, Duncan GH (1989) Sensory and affective aspects of pain perception: is medial thalamus restricted to emotional issues? Exp Brain Res 78:415–418
45. Peschanski M, Guilbaud G, Gautron M (1981) Posterior intralaminar region in rat: neuronal responses to noxious and nonnoxious cutaneous stimuli. Exp Neurol 72:226–238
46. Berkley KJ, Benoist JM, Gautron M, Guilbaud G (1995) Responses of neurons in the caudal intralaminar thalamic complex of the rat to stimulation of the uterus, vagina, cervix, colon and skin. Brain Res 695:92–95
47. Van der Werf YD, Witter MP, Groenewegen HJ (2002) The intralaminar and midline nuclei of the thalamus. Anatomical and functional evidence for participation in processes of arousal and awareness. Brain Res Brain Res Rev 39:107–140
48. Schmahmann JD (2003) Vascular syndromes of the thalamus. Stroke 34:2264–2278
49. Kinomura S, Larsson J, Gulyas B, Roland PE (1996) Activation by attention of the human reticular formation and thalamic intralaminar nuclei. Science 271:512–515
50. Glenn LL, Steriade M (1982) Discharge rate and excitability of cortically projecting intralaminar thalamic neurons during waking and sleep states. J Neurosci 2:1387–1404
51. Miller JW, Ferrendelli JA (1990) Characterization of GABAergic seizure regulation in the midline thalamus. Neuropharmacology 29:649–655
52. Alkire MT, McReynolds JR, Hahn EL, Trivedi AN (2007) Thalamic microinjection of nicotine reverses sevoflurane-induced loss of righting reflex in the rat. Anesthesiology 107:264–272
53. Alkire MT, Asher CD, Franciscus AM, Hahn EL (2009) Thalamic microinfusion of antibody to a voltage-gated potassium channel restores consciousness during anesthesia. Anesthesiology 110:766–773
54. Dempsey EW, Morison RS (1941) The production of rhythmically recurrent cortical potentials after localized thalamic stimulation. Am J Physiol 135:293–300
55. Schiff ND, Giacino JT, Kalmar K, Victor JD, Baker K, Gerber M, Fritz B, Eisenberg B, Biondi T, O'Connor J, Kobylarz EJ, Farris S, Machado A, McCagg C, Plum F, Fins JJ, Rezai AR (2007) Behavioural improvements with thalamic stimulation after severe traumatic brain injury. Nature 448:600–603