Effects of Halothane and Isoflurane on Hyperexcitability of Spinal Dorsal Horn Neurons after Incision in the Rat

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Background: The aim of this study was to determine whether halothane and isoflurane used during and after surgical injury attenuate subsequent hyperexcitability of spinal dorsal horn (SDH) neurons by preventing development of central sensitization.

Methods: Activity of a wide-dynamic-range neuron of the SDH was isolated in decerebrate-spinal Sprague-Dawley rats, and neuronal activity (receptive field size and responses to nonnoxious and noxious stimuli) was recorded. A 1-cm-long incision was made through the skin, fascia, and muscle under anesthesia with halothane (1.1% or 2.2%) and isoflurane (1.4% or 2.8%). Anesthesia was discontinued just after the incision had been made or was continued until 30 min after the incision, and activity of the SDH neurons was measured for up to 2 h after the incision. In a control group, the incision was made without anesthesia.

Results: In the control group, the incision resulted in maximum excitation in the SDH neurons during surgery; spontaneous activity significantly increased for up to 30 min after the incision ($P < 0.05$) but did not significantly increase thereafter, returning to the preincision value. Halothane and isoflurane suppressed excitation of the neurons during the incision in a concentration-related manner. Administration of 2.2% halothane and 2.8% isoflurane during the incision and for up to 30 min after the incision almost abolished activity of the neurons for 30 min after the incision. The magnitude of neuronal activity 2 h after the incision was not significantly different among all groups, including the control group.

Conclusions: The results demonstrate that administration of halothane and isoflurane does not attenuate development of hyperexcitability of SDH neurons despite the fact that excitation and spontaneous activity during and after the incision were greatly suppressed by administration of halothane and isoflurane.

IN addition to sensitization of primary afferent nociceptors (peripheral sensitization), an enhanced sensitivity of spinal dorsal horn (SDH) neurons to sensory stimulation (central sensitization) is involved in hyperalgesia and allodynia after tissue injury. The central sensitization is triggered by excessive impulses in nociceptive C-fibers and Aδ-fibers. Similarly, repetitive electrical conditioning stimulation in fine primary afferents induces long-term enhancement of synaptic transmission in the SDH, which is comparable to long-term potentiation associated with learning and memory in the brain.[1] The barrage of action potentials in SDH neurons caused by surgery has thus been believed to be analogous to the conditioning stimulus for long-term potentiation and produce central sensitization.[1,2] Drugs administered before the conditioning stimulus have been shown to be more effective for inhibiting long-term potentiation than the same drugs administered after the conditioning stimulus,[3] suggesting that neural suppression in the SDH during surgery may block central sensitization and reduce the magnitude of postoperative pain. This is the basis for the assumption that preemptive analgesia should be of clinical value,[2,4] although the clinical evidence is less convincing.[5]

Volatile anesthetics are commonly used during surgery, and the effects of pretraumatic and posttraumatic analgesic treatments combined with volatile anesthetics have been investigated to determine the usefulness of preemptive analgesia for management of postoperative pain in a clinical setting.[5] Although volatile anesthetics have generally been believed not to have suppressive effects on central sensitization after tissue injury,[6] volatile anesthetics have been shown to be capable of blocking sensory transmission, including pain sensation, at the level of the SDH,[7] possibly preventing or attenuating development of central sensitization. Indeed, some previous studies showed volatile anesthetics suppressed central sensitization in the spinal cord after tissue injury.[8,9] If volatile anesthetics prevent or attenuate the occurrence of central sensitization and hence decrease the magnitude of postoperative pain, the different effects of pretraumatic and posttraumatic treatments with additional analgesics on postoperative pain may be minimum.

Because volatile anesthetics exert their actions *via* the central nervous system, including the spinal cord, with the minimum effects on the periphery and because the effects of volatile anesthetics can be completely abolished within a short period of time after administration, it appears that volatile anesthetics are good tools to investigate whether postoperative hyperexcitability is triggered by injury-induced excitation in SDH neurons during surgery. The aim of the current study was to determine the effects of two volatile anesthetics, halothane and isoflurane, on activity of SDH neurons during and after incision.

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Materials and Methods

All protocols of this study were approved by the Animal Care and Use Committee of our institution. Efforts were made to minimize the number of animals used, and the experiments followed the ethical guidelines of the International Association for the Study of Pain.[10] A total of 78 adult male Sprague-Dawley rats weighing 250–330 g was used in the experiments and were housed in groups of 4 in $40 \times 60 \times 30$-cm plastic cages with soft bedding under a 12:12 h day:night cycle until the day of the experiment. All of the experiments were carried out using acute preparations from decerebrate-spinal rats as described below.

Animal Preparation

Anesthesia was induced by placing each animal in an environment that contained 4% halothane in 100% oxygen. After loss of righting reflex, anesthesia was maintained by administering 2–3% halothane in 100% oxygen through a tightly fitting mask. The left carotid artery and external jugular vein were cannulated to allow for blood pressure monitoring and for drug and fluid administration, respectively. After a tracheotomy, the trachea was intubated and the animal was mechanically ventilated. To minimize spinal shock, lidocaine (0.05 ml of 1% lidocaine; Astra Zeneca, Tokyo, Japan) was injected into the spinal cord at the level of transection just before transection. The animal was then spinally transected at the interspace of T2–T3 *via* a small laminectomy and was decerebrated by aspiration of cranial contents rostral to the mesencephalon, after which administration of halothane was discontinued. The lumbar spinal cord (L2–L4) was exposed by a separate laminectomy of the T12 and T13 vertebrae, and the animal was then placed in a stereotaxic apparatus. Under a binocular microscope with $8\times$ to $40\times$ magnification, the dura over the lumbar spinal cord was carefully removed. The superficial dorsal gray matter lateral to the dorsal root entry zone was discernible as a relatively translucent band under Lissauer's tract. The pia-arachnoid membrane was cut to make a window large enough to allow insertion of an electrode into the spinal cord. According to a previously described method,[11] the surface of the exposed spinal cord was irrigated with Krebs solution (composition in mM: NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, glucose 11, $NaHCO_3$ 25) equilibrated with 95% oxygen + 5% carbon dioxide at $37° \pm 0.5°C$. The animals were paralyzed with intravenous pancuronium bromide (0.2 mg·kg^{-1}·h^{-1}). Body temperature was recorded with a rectal probe and maintained at 37°C by an infrared heat lamp and heat pad. The experiment was started no earlier than 1 h after surgery to allow for recovery from halothane and stabilization of the excitability of the preparation. Throughout the subsequent experiments, lactated Ringer's solution was intrave-nously administered (4–6 ml·kg^{-1}·h^{-1}), and arterial blood pressure (70–120 mmHg) and end-tidal P_{CO_2} (3–4%) were monitored; if these parameters could not be maintained within physiologic ranges, the experiment was discontinued and the data were excluded. At the end of each experiment, laminar locations of the recording sites were estimated from measurements of the depths of the electrodes from the surface of the cord. The animals were then killed with an overdose of potassium chloride. Only one dorsal horn neuron was examined in each rat.

Electrophysiological Recording

A tungsten microelectrode (impedance, 10–12 MΩ; FHC Inc, Brunswick, ME) was advanced by a hydraulic micromanipulator into the dorsal horn of the spinal cord up to 1000 μm until activity from a single neuron that had a receptive field (RF) on the shaved skin of the hindquarter (the lumbar and gluteal regions of the rat) could be isolated (fig. 1A). The activity of a single neuron was considered to be isolated when the spike was clearly distinguishable from background neuronal noise and had uniform spike amplitude with a signal-to-noise ratio of at least 4:1. Neurons that had spontaneous firings of 3 impulses/s or less were used in this study. When extracellular activity of a single dorsal horn neuron with minimal spontaneous firing was identified, the response profile of the neuron was determined by a series of stimuli, including application of hand-held calibrated nylon filaments (von Frey hairs), brushing, heating, and pinching with forceps. A neuron was classified as a wide-dynamic-range (WDR) neuron if responses were elicited by both low-intensity stimuli (light touch and brushing) and high-intensity stimulus (pinching) and also if it responded to a radiant heat stimulation for 20 s that was shown in a preliminary experiment to induce pain sensation in examiners and to induce an increase in rat skin temperature to 50°C. It was confirmed that the firing frequency of the WDR neuron increased as the stimulus intensity increased, with maximum activation occurring only with presentation of the most intense stimuli. In the current study, only WDR neurons were examined because WDR neurons, but not high-threshold neurons, are responsible for hyperexcitability of the spinal dorsal horn after incisional injury.[12]

After completion of classification of the neurons as WDR neurons and obtaining stable baseline values over a period of 20–30 min, the low-threshold and high-threshold RF areas of WDR neurons were carefully mapped. The edges of the RF areas were defined as follows. A 4-g von Frey hair was applied along 18 to 36 radial linear paths beginning outside and moving to the center of the RF at a rate of 2 mm per second until light touch stimulation with the tip of the probe elicited a response. If a response was observed at a point, stimulation was applied to that point 6–10 times at 5-s inter-
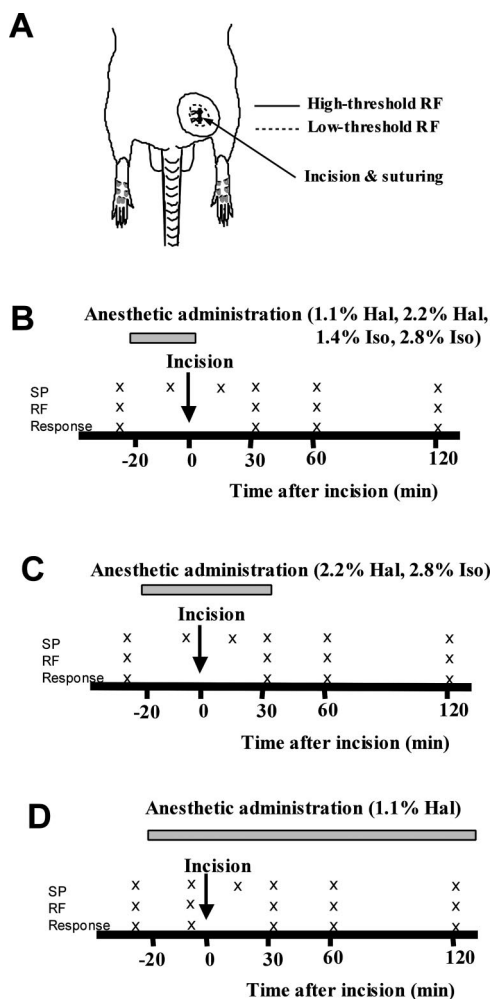
Fig. 1. (*A*) Schematic drawing of location of a typical receptive field (RF) responding to nonnoxious (low-threshold, *dotted line*) and noxious stimuli (high-threshold, *solid line*). A 1-cm-long incision was made in the center of the RF through the skin, fascia, and muscle, and the skin was apposed with two sutures. Nonnoxious and noxious mechanical stimuli were applied on the injured site (1–2 mm from the incision site). (*B*, ,*C*, *D*) Schematic illustration of the experimental protocols. In the 1.1% halothane (Hal), 2.2% halothane (Hal), 1.4% isoflurane (Iso), and 2.8% isoflurane (Iso) groups, administration of the anesthetics was started and continued for 20 min, and the incision was then made; administration of the anesthetics was discontinued just after suturing (*B*). In the 2.2% halothane (Hal) (30 min) and 2.8% isoflurane (Iso) (30 min) groups, administration of the anesthetics was started and continued for 20 min, and the incision was then made; administration of the anesthetics was continued for the next 30 min and then discontinued (*C*). In some neurons (n = 5), administration of 1.1% halothane was started at 20 min before the incision and continued during the experiment. The timings of assessment of neuronal activity are shown in (*B, C, D*). SP, spontaneous activity; RF, receptive field; Response, evoked responses to nonnoxious and noxious stimuli.

vals. The first point of the edge on which the stimulation elicited a response 50% of the time was marked on the skin with a felt pen. The dots that enclosed the RF area were later connected to form a continuous border. Then, the edge of the high-threshold RF was determined as the area in which a response to high-intensity mechan-

ical stimulation with a tungsten tip with a diameter of 100 μm attached to a nylon filament (calibrated force of 16 g) evoked a response 50% of the time in the same manner as used for mapping the low-threshold RF. This filament, which produced pricking pain in examiners when stimulated, was made according to a previously described method.[13]

After determination of the RF areas, a camel hairbrush was used to stimulate each neuron's RF with a dynamic low-intensity stimulus in the neuron. A 1-cm-long line, to be incised, was drawn in the most sensitive region of the RF, and the brush was slowly moved in a stereotyped manner 1–2 mm next to the line. The responses to brush stimulation were recorded at 30-s intervals. A punctate stimulation was then applied 1–2 mm from the line to be incised for 5 s at 1-min intervals using the von Frey filament (4 g) and a tungsten tip attached to a nylon filament (calibrated force of 16 g). Sustained application of a small arterial clip (#19–8080, Codman and Shurtleff Inc., Randolph, MA), which exerted a force of 250 g/mm^2, was performed for 3 s to a skin fold 1–2 mm next to the line to be incised two or three times at 3-min intervals.

Protocols of Administration of Anesthetics

After obtaining the basal data of activity in the neurons, the animals were randomly divided into five groups: a control group, a 1.1% halothane group, a 2.2% halothane group, a 1.4% isoflurane group, and a 2.8% isoflurane group (fig. 1B). In the control group, anesthetics were not given, and 1.1% of halothane, 2.2% of halothane, 1.4% of isoflurane, and 2.8% of isoflurane were given in the 1.1% halothane group, 2.2% halothane group, 1.4% isoflurane group, and 2.8% isoflurane group, respectively. In each anesthesia group, halothane and isoflurane was adjusted to each concentration and allowed to equilibrate for 20 min. A 1-cm-long incision was then made on the marked line with a number 11 blade through the skin, fascia, and muscle of the hindquarter, with care taken to prevent damage to superficial veins and nerves in the muscle. The skin was apposed with two sutures of 4–0 nylon using an FS-2 needle, and administration of halothane and isoflurane was discontinued. Spontaneous activity in the neurons was recorded before administration of halothane and isoflurane, before the incision, and at 15 min, 30 min, 1 h, and 2 h after the incision (fig. 1B). The RF mapping and responses to nonnoxious and noxious stimuli (brush, punctate stimulation using a 4-g von Frey filament, pinprick stimulation using a 16-g tungsten tip, and pinch stimulation by an arterial clip) were recorded as described in "Electrophysiological Recording" before administration of halothane and isoflurane and at 30 min, 1 h, and 2 h after the incision.

To determine the effects of high concentrations of halothane and isoflurane administered for a long period on postsurgical hyperexcitability in SDH neurons, two

groups, in addition to the above-described five groups, were established: one group in which 2.2% of halothane was continuously administered until 30 min after the incision had been made (2.2% halothane [30 min] group) and one group in which 2.8% isoflurane was continuously administered until 30 min after the incision had been made (2.8% isoflurane [30 min] group) (fig. 1C). Spontaneous activity in the neurons was recorded before administration of halothane and isoflurane, before the incision, and at 15 min, 30 min, 1 h, and 2 h after incision. The RF mapping and recordings of responses to nonnoxious and noxious stimuli were as described in "Electrophysiological Recording" before the anesthetics were administered and at 30 min, 1 h, and 2 h after the incision had been made (fig. 1C).

To know whether hyperexcitability of spinal neurons is observed under anesthesia with the low concentration of halothane, which has been commonly used in animal preparations, activity was recorded from some neurons (n = 5) in which anesthesia with 1.1% of halothane was continued during the experiment (a 1.1 halothane [C] group, fig. 1D). After obtaining the basal activity, 1.1% of halothane was administered and activity was repeatedly recorded in the same manner as described above.

Data Analysis

Data obtained from animals in which arterial blood pressure decreased to less than 60 mmHg during administration of anesthetics were excluded because activity of spinal neurons (RF size and responses to stimuli) is shown to decrease in association with such a decrease in blood pressure.[14] The outline of the RF mapped on the skin was transferred to tracing paper, digitized, and used to determine and analyze RF areas. Activities of isolated single neurons and blood pressure were digitized (CED 1401; Cambridge Electronic Design Ltd., Cambridge, UK) and stored in computer format (IBM-AT personal computer, ThinkPad; IBM Japan, Tokyo, Japan). Collected data were analyzed off-line using the computer program Spike 2 (Cambridge Electronic Design Ltd.).

Spontaneous firing rates were determined by averaging the activity over 2-min to 3-min periods when there was no contact with the RF. To evaluate evoked activities of dorsal horn neurons, firing rates in response to various stimuli were quantified as the mean firing rates during stimuli after subtracting spontaneous firing rates; the subtracted firing rates before and after the incision were compared among the seven groups by one-way and two-way analyses of variance for repeated-measures analysis of variance with Fisher exact test for intragroup comparison and paired Student *t* tests for differences from the control values.

The variables are expressed as percentages of control values in the absence of anesthetic administration (preincision values). Evaluation of evoked responses to incision and suturing during surgery *versus* expansions in

RF sizes was done by multiple regression analysis using the data from all of the groups together. Evaluation of the changes in spontaneous activities *versus* expansions in RF sizes was also done by multiple regression analysis. For the analysis, the changes in spontaneous activities were calculated from the mean rates of spontaneous activity in each neuron before anesthetic administration and at 2 h after the incision had been made. The expansions in RF sizes were calculated from the basal sizes before anesthetic administration and 2 h after the incision had been made. Data are shown as means ± SD. *P* values of < 0.05 were considered statistically significant.

Results

Because blood pressures decreased to 60 mmHg or less during administration of the anesthetics in one neuron in the 2.2% halothane group, in three neurons in the 2.2% halothane (30 min) group, and in two neurons in the 2.8% isoflurane (30 min) group, data from these neurons are not presented in this article. The number of neurons, depths of cell location, basal RF sizes of, spontaneous firing rates of and responses of these neurons to various stimuli in each group are shown in table 1. From the findings based on the depths of the electrodes from the surface of the spinal cord, locations of the neurons are thought to be located in the superficial (~400 μm) or deep (400–1000 μm) laminae.[15] Most of the neurons examined in the current study were thus deeper neurons of the spinal dorsal horn neurons.

There were no significant differences in the spontaneous activities or responses to various stimuli among the groups examined. The duration of surgical incision and suturing and blood pressures before, during, and after surgery in each group are shown in table 2. There were no significant differences between durations of surgical incision and suturing in these groups of neurons (*P* > 0.2). Although administration of 1.1% and 2.2% halothane and of 1.4% isoflurane and 2.8% isoflurane significantly decreased blood pressures (*P* < 0.05), the blood pressures returned to control values within 30 min after administration of anesthetics had been completed.

Effects of Halothane and Isoflurane on Evoked Responses to Incision and Suturing and Spontaneous Activity after Surgery

Examples of evoked responses to surgical incision and suturing of WDR neurons under various anesthetic depths of halothane and isoflurane are shown in figure 2. The surgical incision resulted in maximum excitation in the neurons in the control group (fig. 2A). Administration of halothane and isoflurane significantly suppressed the incision-induced firing rates of the neurons in a dose-related manner (*P* < 0.01, fig. 2, B through E and fig. 3A). Although the mean rates of spontaneous activity

Table 1. Physiology of cells in groups

Groups	No. of cells	Depth (μm)	Receptive field size (cm^2) von Frey	Receptive field size (cm^2) Pinprick	Spont. (/sec)	Brush (/event)	von Frey (/sec)	Pinprick (/sec)	Pinch (/sec)
Control	19	551 ± 129	5.3 ± 3.0	17.0 ± 6.8	0.6 ± 1.4	17 ± 16	6.6 ± 6.1	37 ± 27	116 ± 67
1.1% Hal	6	601 ± 182	4.9 ± 2.7	19.4 ± 5.8	0.8 ± 0.5	11 ± 7	6.1 ± 4.5	23 ± 21	134 ± 65
2.2% Hal	7	589 ± 155	5.3 ± 3.4	18.9 ± 11.7	0.4 ± 0.5	21 ± 11	5.7 ± 3.7	35 ± 31	114 ± 62
2.2% Hal (30 min)	8	534 ± 211	4.6 ± 2.9	21.6 ± 9.0	0.4 ± 0.6	14 ± 9	5.6 ± 1.9	21 ± 26	118 ± 68
1.4% Iso	9	568 ± 191	5.7 ± 3.2	18.4 ± 15.2	0.2 ± 0.5	10 ± 12	6.3 ± 3.1	27 ± 19	129 ± 77
2.8% Iso	6	556 ± 125	5.4 ± 2.7	16.8 ± 11.5	0.5 ± 1.2	16 ± 13	6.9 ± 3.9	34 ± 24	119 ± 63
2.8% Iso (30 min)	6	611 ± 203	4.5 ± 3.1	20.1 ± 7.3	0.2 ± 0.7	19 ± 8	5.5 ± 4.1	22 ± 20	108 ± 51
1.1% Hal (C)	5	703 ± 338	7.9 ± 3.0	16.4 ± 8.9	1.1 ± 0.9	11 ± 5	5.1 ± 3.6	24 ± 7	138 ± 70

The numbers of cells, receptive fields (RFs) sizes, spontaneous activities (Spont.), and responses to various stimuli before incision in the groups. Hal = halothane; Iso = Isoflurane. In the control group, anesthetics were not given, and 1.1% of halothane, 2.2% of halothane, 1.4% of isoflurane and 2.8% of isoflurane were given during the incision and suturing. In the 2.2% Hal (30 min) group, 2.2% of halothane was continuously administered until 30 min after the incision had been made and 2.8% isoflurane was continuously administered in the 2.8% Iso (30 min) group until 30 min after the incision had been made. In the 1.1% Hal (C) group, 1.1% of halothane was continued during the experiment. Cell location was determined as the distance of the electrode tip from the surface of the spinal cord. RF was determined by a von Frey filament (4 g) and a pinprick with a tungsten tip (100 μm-diameter, 16 g). Data are shown as means ± SD. There are no significant differences in the spontaneous activities, RF sizes, or responses to various stimuli among the groups.

were significantly higher at 15 and 30 min after the incision than those before the incision in the control group ($P < 0.01$), the mean spontaneous activity of the neurons had almost returned to the preincision values at 1 h and 2 h after the incision (fig. 3B). The mean rates of spontaneous activity were abolished after 1.1% and 2.2% halothane and 1.4% and 2.8% isoflurane had been administered before the incision. In the 1.1% halothane group, 1.4% isoflurane group, and 2.8% isoflurane group, spontaneous activity significantly increased only at 15 and 30 min after the incision had been made ($P < 0.05$). In the 2.2% halothane group, spontaneous activity significantly increased only at 30 min after the incision had been made ($P < 0.05$). In the 2.2% halothane (30 min) group and the 2.8% isoflurane (30 min) group, spontaneous activity was abolished until 30 min after the incision had been made, at which time the anesthetics were discontinued, and spontaneous activity did not significantly increase as compared to basal values before surgery (fig. 3B).

Effects of Halothane on Responses to Nonnoxious and Noxious Stimuli and Receptive Fields after Surgery

Examples of RF areas and responses to various stimuli in a WDR neuron after halothane administration and incision are shown in figure 4. Responses to nonnoxious and noxious stimuli had significantly increased 30 min after the incision and continued to increase thereafter in the control group, the 1.1% halothane group, and the 2.2% halothane group ($P < 0.05$) (fig. 4C). In the 2.2% halothane (30 min) group, administration of 2.2% halothane completely suppressed responses to nonnoxious and noxious stimuli at 30 min after the incision had been made (fig. 4A). Expansions in low-threshold and high-threshold receptive fields were seen in all of the groups at 1 and 2 h after the incision had been made even though RF was abolished until 30 min after the incision had been made in the 2.2% halothane (30 min) group. The time courses of expansions in RFs were similar in all the groups, although RF size and responses to nonnox-

Table 2. Duration of surgery and changes in blood pressure in each group

Groups	Duration of surgery	Systolic blood pressure (mmHg) Pre	Anesthetic administration	During incision	30 min after incision	2 hr after incision
Control	157 ± 41	110 ± 18	109 ± 16	114 ± 19	112 ± 12	108 ± 11
1.1% Hal	169 ± 22	108 ± 10	92 ± 8*	88 ± 4*	103 ± 11	101 ± 19
2.2% Hal	170 ± 23	104 ± 19	81 ± 8*	78 ± 7*	92 ± 13	108 ± 15
2.2% Hal (30 min)	172 ± 20	112 ± 16	77 ± 16*	74 ± 5*	72 ± 4*	105 ± 14
1.4% Iso	163 ± 31	107 ± 14	99 ± 11	103 ± 13	102 ± 8	106 ± 18
2.8% Iso	141 ± 38	111 ± 9	92 ± 6*	83 ± 6*	104 ± 11	102 ± 10
2.8% Iso (30 min)	186 ± 53	101 ± 14	84 ± 5*	82 ± 8*	80 ± 9*	99 ± 11
1.1% Hal (C)	153 ± 42	113 ± 12	88 ± 7*	92 ± 10*	84 ± 8*	80 ± 14*

Durations of an incision made through the skin, fascia and muscle and suturing the incision (sec) and systolic blood pressures before, during and after surgery (mmHg) in the groups examined in the present study. Hal = halothane; Iso = Isoflurane; Pre = basal values before administration of anesthetics. Data are shown as means ± SD.
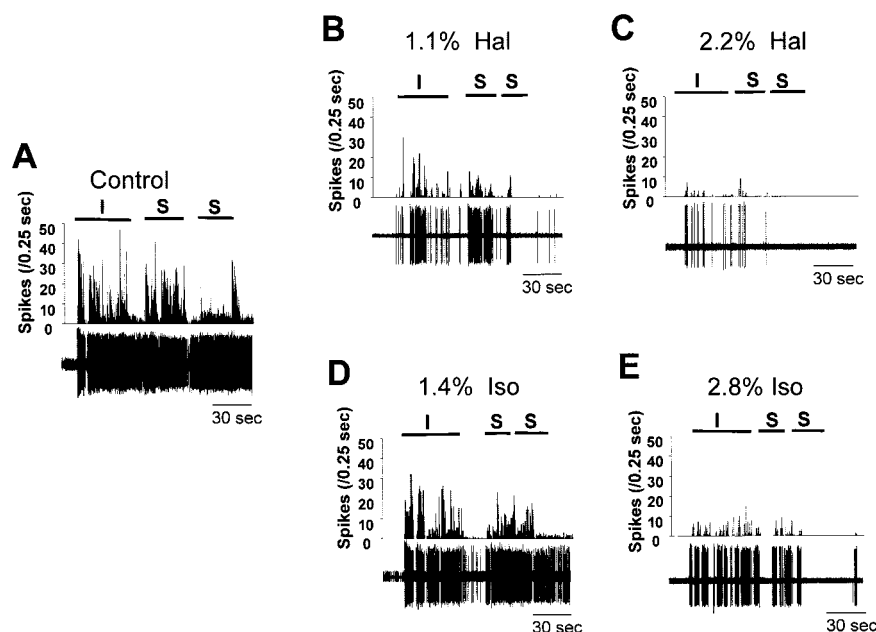
P < 0.05 versus Pre.

Fig. 2. Examples of responses of spinal dorsal horn neurons to the incision and suturing during surgery in the control group (*A*), the 1.1% halothane (Hal) group (*B*), the 2.2% halothane (Hal) group (*C*), the 1.4% isoflurane (Iso) group (*D*), and the 2.8% isoflurane (Iso) group. Firing rates and raw spike data are shown in the upper and lower panels, respectively. I, incision; S, suturing.
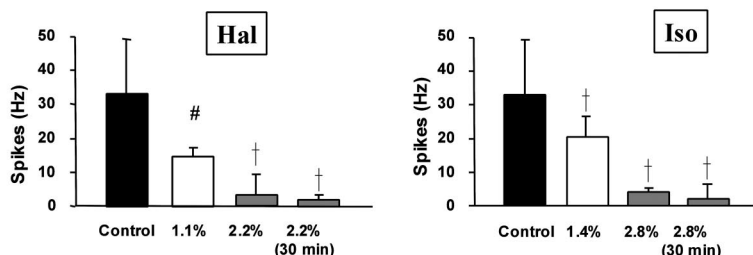
ious and noxious stimuli were almost completely abolished in the 2.2% halothane (30 min) group at 30 min after the incision until the time when 2.2% halothane was administered (fig. 4, B and C).

Effects of Isoflurane on Responses to Nonnoxious and Noxious Stimuli and Receptive Fields after Surgery

Examples of RF areas and responses to various stimuli in a WDR neuron after isoflurane administration and incision are shown in figure 5. Similar to the results of the halothane experiments, administration of 2.8%

isoflurane almost completely suppressed responses to nonnoxious and noxious stimuli in the 2.8% isoflurane (30 min) group at 30 min after the incision had been made (fig. 5A). Expansions in low-threshold and high-threshold RFs were seen in all of the groups at 1 and 2 h after the incision had been made. The time courses of expansions in RFs and increases in responses to various stimuli were similar in all of the groups, although RF size and responses to nonnoxious and noxious stimuli were almost completely abolished in the 2.8% isoflurane (30 min) group at 30 min after the incision until the time when 2.8% isoflurane had been administered (fig. 5, B and C).
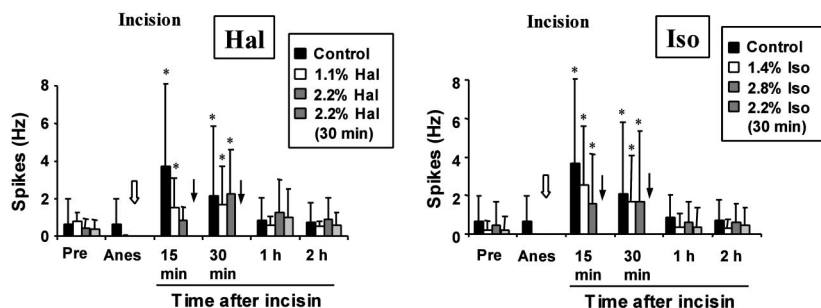

Fig. 3. Mean change in evoked responses during incision and suturing (*A*) and spontaneous activity after incision (*B*) in each group. †*P* < 0.05 *versus* control group. #*P* < 0.05 *versus* the 1.4% isoflurane group. *P* < 0.05 *versus* prevalues. Pre, preadministration of anesthetics; Anes, during administration of anesthetics. Administration of 1.1% and 2.2% of halothane and 1.4% and 2.8% of isoflurane abolished spontaneous activity (white block arrow). In the 2.2% halothane (30 min) group and 2.8% isoflurane (30 min) group, spontaneous activity was abolished (*arrow*) until 30 min after the incision had been made, at which time at administration of the anesthetics was discontinued. There are no significant differences in spontaneous activity among the groups before administration of anesthetics and at 1 and 2 h after the incision had been made.

Fig. 4. Effect of halothane on activity of spinal dorsal horn neurons. (*A*) Examples of responses of a spinal dorsal horn neuron to nonnoxious and noxious stimuli before and 30 min and 2 h after the incision. (*B*) Examples of low-threshold and high-threshold receptive fields (RFs) of a spinal neuron. The data in (*A*) and (*B*) were obtained from the same neuron in which administration of 2.2% halothane was started 20 min before the incision and continued for up to 30 min after the incision and then discontinued (fig. 1C). BR, brush stimulation; VF, 4-g von Frey hair stimulation; PP, pinprick stimulation; PI, pinch stimulation. (*C*) Mean changes in responses to nonnoxious and noxious stimuli and RF sizes in the control group, the 1.1% halothane (Hal) group (n = 6), the 2.2% halothane (Hal) group (n = 7), and the 2.2% halothane (Hal) (30 min) group (n = 8). When administration of 2.2% halothane was continued for up to 30 min after the incision in the 2.2% Hal (30 min) group, low-threshold RF and responses to brush and pinch stimuli at 30 min after the incision were abolished (*arrow*). There are no significant differences in the increased responses to brushing and pinching stimuli and increased RF sizes among the groups before administration of anesthetics and at 1 and 2 h after the incision had been made.
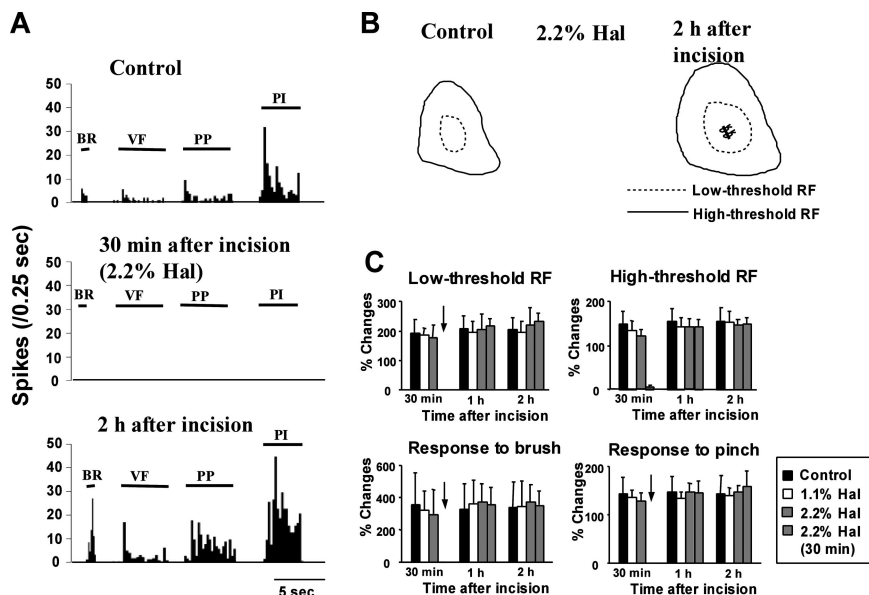
Evoked Responses to Incision and Suturing and Hyperexcitability of Spinal Neurons after Surgery under Anesthesia with 1.1% Halothane

Although administration of 1.1% halothane significantly decreased the responses to nonnoxious stimuli, the incision significantly increased the responses particularly to noxious stimuli (fig. 6A). The administration of 1.1% halothane abolished the low-threshold RF areas, and the low-threshold RF areas were not observed in 40% of the neurons examined (two of five) even after the

incision had been made (fig. 6, B and C). The mean low-threshold RF sizes did not significantly increase after the incision had been made (fig. 6C). The mean response to brush stimulation was suppressed by administration of 1.1% halothane (6 ± 5% of preanesthetic values), but the mean rates significantly increased 1 and 2 h after the incision had been made (*P* = 0.046). The administration of halothane decreased the mean responses to noxious stimuli (pinching) to 22 ± 11% of the preanesthetic values and the high-threshold RF sizes to 28 ± 14% of the

Fig. 5. Effect of isoflurane on activity of spinal dorsal horn neurons. (*A*) Examples of responses of a spinal dorsal horn neuron to nonnoxious and noxious stimuli before and at 30 min and 2 h after the incision. (*B*) Examples of low-threshold and high-threshold receptive fields (RFs) of a spinal neuron. The data in (*A*) and (*B*) were obtained from the same neuron in which administration of 2.8% isoflurane was started 20 min before the incision and continued for up to 30 min after the incision and then discontinued (fig. 1C). BR, brush stimulation; VF, 4-g von Frey hair stimulation; PP, pinprick stimulation; PI, pinch stimulation. (*C*) Mean changes in responses to nonnoxious and noxious stimuli and RF sizes in the control group, the 1.4% isoflurane (Iso) group (n = 9), the 2.8% of isoflurane (Iso) group (n = 6), and the 2.8% isoflurane (Iso) (30 min) group (n = 6). When administration of 2.8% isoflurane was continued for up to 30 min after the incision in the 2.8% Iso (30 min) group, low-threshold RF and responses to brush and pinch stimuli at 30 min after the incision were abolished (*arrow*). There are no significant differences in the increased responses to brushing and pinching stimuli and increased RF sizes among the groups before administration of anesthetics and at 1 and 2 h after the incision had been made.
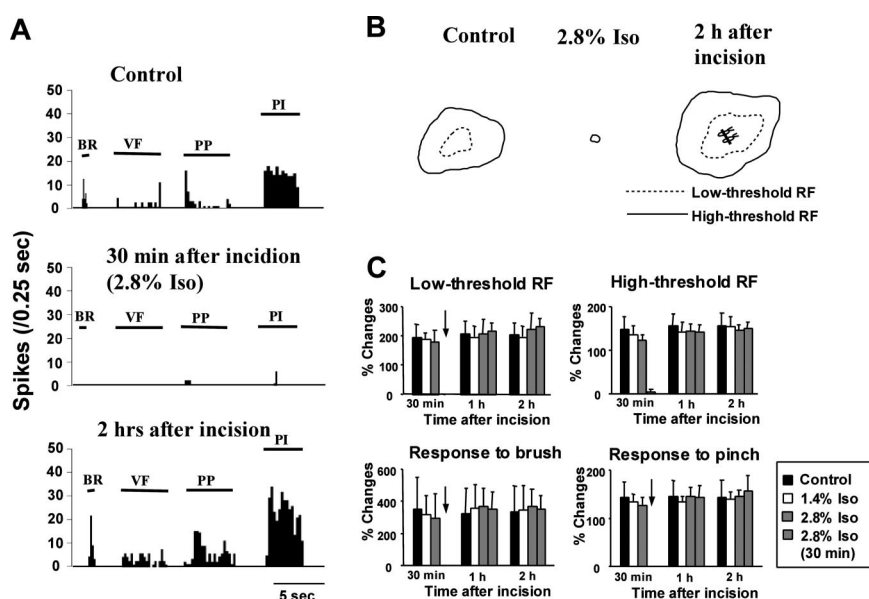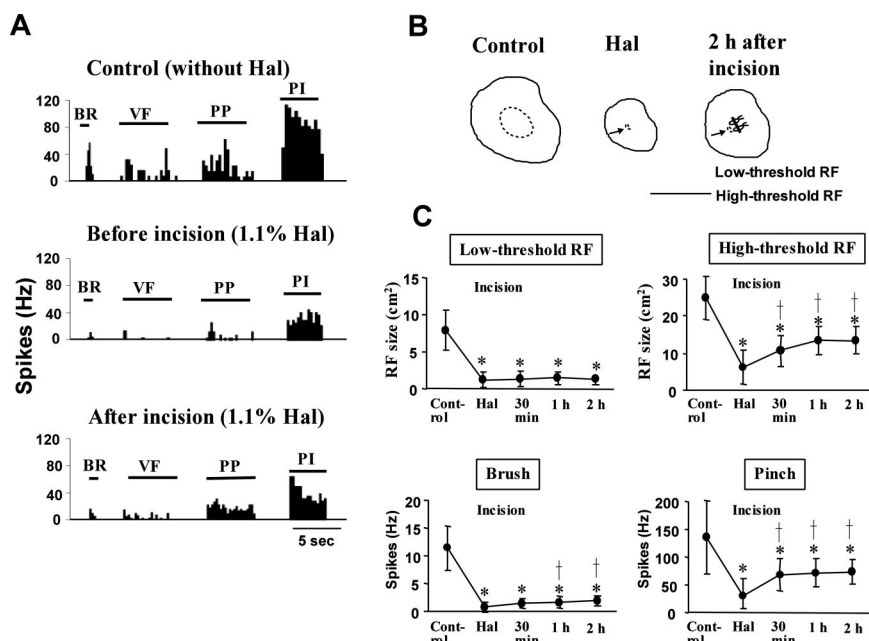
Fig. 6. Effect of halothane (1.1%) anesthesia on activity of a spinal dorsal horn neuron. (*A*) Examples of responses of a spinal dorsal horn neuron to nonnoxious and noxious stimuli before administration of 1.1% halothane and before and 2 h after the incision under anesthesia with 1.1% halothane. BR, brush stimulation; VF, 4-g von Frey hair stimulation; PP, pinprick stimulation; PI, pinch stimulation. (*B*) Examples of low-threshold and high-threshold receptive fields (RFs) of a spinal dorsal horn neuron before administration of 1.1% halothane and before and 2 h after the incision under anesthesia with 1.1% halothane. The data in A and B were obtained from the same neuron. Administration of 1.1% halothane decreased the low-threshold RF, and expansion of the low-threshold RF is minimum (*arrow*). (*C*) Mean changes in responses of spinal dorsal horn neurons (n = 5) to brushing and pinching stimuli and low-threshold and high-threshold RF sizes before ad-ministration of 1.1% halothane and before and after the incision under anesthesia with 1.1% halothane. *P < 0.01 *versus* pre (before administration of 1.1% halothane).†P < 0.05 *versus* before incision under anesthesia with 1.1% halothane.

preanesthetic values, but the incision significantly increased the high-threshold RF sizes and responses to the noxious stimuli (*P* < 0.01) (fig. 6, B and C).

Relationships of Evoked Responses to Incision and Suturing and Changes in Spontaneous Activities to Expansions in RF Sizes

No significant relationships were found between surgical injury-induced responses during surgery (incision and suturing) and increases in low-threshold RF sizes (correlation coefficient = 0.01, *P* = 0.99) or between surgical injury-induced responses during surgery and increases in high-threshold RF sizes (correlation coefficient = −0.18, *P* = 0.25). The results also did not show linear relations between increases in spontaneous activity and increases in low-threshold RF sizes 2 h after surgery (correlation coefficient = 0.19, *P* = 0.19) or between increases in spontaneous activity and increases in high-threshold RF sizes 2 h after surgery (correlation coefficient = 0.17, *P* = 0.13).

Discussion

Effects of Halothane and Isoflurane on Excitation during Surgery and Postoperative Spontaneous Activity in SDH Neurons

In the current study, 1.1% and 2.2% of halothane and 1.4% and 2.8% of isoflurane were used because previous studies showed that minimum alveolar concentration (MAC) for halothane and isoflurane in rats were approximately 1.1% and 1.4%, respectively.[16-18] The relevant site of action for MAC determination of volatile anesthetics has been shown to be within the spinal cord rather than at supraspinal sites of the central nervous system.[7] However, it has also been suggested that the ventral horn rather than the dorsal horn is involved in MAC determination of isoflurane.[19] Volatile anesthetics may be thus considered poor analgesics despite potent anesthetic actions on the spinal cord to produce surgical immobility. However, early studies[20,21] and recent studies[22,23] have demonstrated that halothane suppresses noxiously evoked activity of SDH neurons, including WDR neurons, that play a central role in spinal transmission of nociceptive information. Accordingly, various volatile anesthetics could differently affect the ventral and dorsal horn, possibly resulting in different effects on excitation of SDH neurons during surgery even though the volatile anesthetics are administered at the same MAC.

In the current study, both halothane and isoflurane administered at different concentrations and different durations suppressed evoked responses to incision and suturing in concentration-related manners. High concentrations of halothane (2.2%) and isoflurane (2.8%) greatly suppressed responses of SDH neurons caused by incision and suturing. Thus, use of volatile anesthetics may attenuate development of postoperative hyperexcitability in SDH neurons if central sensitization is triggered by excitation in SDH neurons during surgery. On the other hand, in the control animals, spontaneous activity in SDH neurons significantly increased after the incision, and the increased spontaneous activity was seen until 30 min after the incision had been made and then returned to the preincision values. When halothane and isoflurane were administered at high concentrations and for a long period (30 min) after the incision, spontaneous activity was abolished during administration and did not significantly

increase during the experimental period. Thus, clinically relevant concentrations of halothane and isoflurane could suppress the increase in spontaneous activity of SDH neurons, depending on the concentrations.

Effects of Halothane and Isoflurane Used during Surgery on Postoperative Increased Responsiveness to Sensory Stimulation and RF Expansion after Incision

It has been postulated that light anesthesia using volatile anesthetics failed to prevent central sensitization after tissue injuries by application of chemical irritants.[6,24] However, it has also been shown that exposure of 1 MAC of halothane and isoflurane from 5 min before to 6 min after formalin suppressed pain-related behavior during the late phase (10–60 min) after subcutaneous formalin injection[8] and that isoflurane administration depressed neuronal activity in the spinal cord as determined by fos-like immunoreactivity after supramaximal mechanical stimulation.[25] These results suggest that volatile anesthetics can attenuate or prevent central sensitization in some animal models of tissue injury-induced pain and intense mechanical stimulation-induced pain. However, because physiologic and pharmacological characteristics and mechanisms of postoperative pain differ from those of tissue injury-induced pain in conventionally used animal models as a result of different processes of tissue injury and subsequent inflammation,[12,26] the effects of volatile anesthetics on central sensitization after incisional injury may be different.

In the current study, suppression of the activity of SDH neurons during surgery to various degrees by halothane and isoflurane did not affect the magnitude of increased responses of SDH neurons to nonnoxious or noxious stimuli on the injured site, which reflect peripheral sensitization much more than central sensitization.[27] More importantly, the suppression caused by these anesthetics did not affect the magnitude of expansion in RF sizes, which is though to reflect central sensitization much more than peripheral sensitization. Central sensitization, measured by expanded RFs, has been suggested to be triggered by excessive inputs from nociceptors during tissue injuries.[28–30] However, this was not the case in the current study. The results of the current study seem to be consistent with the results of a previous study in which bupivacaine was pretraumatically injected in a site to be incised.[31] Even if the excessive inputs during injuries are blocked, inputs from the periphery after the injury and hence an increase in spontaneous activity in SDH neurons may sensitize the SDH neurons, induce central sensitization, and expand the RFs. However, in the current study, when 2.2% of halothane and 2.8% of isoflurane were administered for up to 30 min after the incision had been made, spontaneous activity did not significantly increase, but RF expansion developed to a

degree similar to that of control animals and with a similar time course.

Furthermore, no significant relationships were found between evoked responses to incision and suturing and increases in RF sizes. The results of the current study also did not show significant relationships between increases in spontaneous activity and increases in RF sizes 2 h after incision. Therefore, RF expansion develops independently of excessive inputs from nociceptors during incision and changes in postincisional spontaneous activity, suggesting unique aspects of an incision.[31]

Injury-induced Excitation during Incision and Hyperexcitability after Incision in SDH Neurons under Anesthesia with 1.1% Halothane

Halothane has commonly been used at a concentration of approximately 1% for *in vivo* electrophysiological recordings in a variety of animal models for persistent pain, including the plantar incision model.[12,31] Because halothane affects spontaneous activity, responsiveness to various stimuli, and RF sizes, determination of the effects of halothane anesthesia on incision-induced excitation and on subsequent hyperexcitability of SDH neurons should be required. In the current study, 1.1% of halothane suppressed incision-induced excitation of SDH neurons to $43 \pm 8\%$ of control values that were obtained without halothane anesthesia in the control animals (fig. 3A). Administration of 1.1% halothane more greatly suppressed the sizes of the low-threshold RFs than the high-threshold RFs. There was no significant increase in the low-threshold RFs and a barely significant increase in response to brush stimulation ($P = 0.046$) after incision under anesthesia with 1.1% halothane. In contrast, the sizes of the high-threshold RFs and responses to noxious stimuli were less suppressed by administration of 1.1% halothane than were the sizes of the low-threshold RFs and responses to nonnoxious stimuli. These results suggest that injury-induced excitation of SDH neurons during incision and hyperexcitability of SDH neurons after incision can be seen under anesthesia with 1.1% halothane but that care should be taken for analysis of incision-induced effects on the low-threshold RF sizes and responses to nonnoxious stimuli. Furthermore, because spontaneous activity could be greatly affected by the type of anesthesia (pentobarbital *versus* halothane, for example) and depth of anesthesia, various effects of anesthetics and depth of anesthesia should be considered for analysis of changes in spontaneous activity in animal models of tissue injury-induced pain, including incision-induced pain.

Supraspinal Contribution to Hyperexcitability of SDH Neurons after Incision

It should be pointed out that the current experiments were carried out using acute preparations from decere-

brate-spinal rats in which descending inhibitory and facilitatory influences from the brain were disrupted. The site of action of halothane is well known to be within the SDH,[7,18] whereas isoflurane has been shown to have a supraspinal pronociceptive effect in addition to a supraspinal antinociceptive effect.[32] Thus, effects of isoflurane on activity of SDH neurons in the current study might not be the same as those in animals in which the brains and spinal cords are intact, although it has also been shown that isoflurane can suppress SDH neurons.[7,33] On the other hand, spinal transection results in increases in spontaneous activity and responses to nonnoxious and noxious stimuli,[34,35] in part by elimination of descending inhibition from the rostral medial medulla including the rostroventral medulla and its adjacent medullary sites.[36] In contrast, spinal transection may attenuate behavioral hyperalgesia, particularly secondary hyperalgesia, and central sensitization in animal models of persistent inflammatory pain, in part by elimination of descending facilitatory influences from the rostral medial medulla.[36,37] However, RF expansion, which would reflect secondary hyperalgesia, was seen after incision in the current study. It has also been shown that primary hyperalgesia and secondary hyperalgesia after an incision is not modulated by descending influence from the rostral medial medulla.[38] Taken together, our results suggest that incision-induced hyperexcitability of SDH neurons develops independently from influences from the brain and that unique peripheral and spinal mechanisms more contribute to behavioral hyperalgesia/allodynia after incisional injury.

In summary, although administration of halothane and isoflurane during surgery suppressed evoked responses of SDH neurons to incision in a concentration-related manner, it did not prevent or attenuate subsequent hyperexcitability of the SDH neurons, characterized by expansion in RF size and increases in response to nonnoxious and noxious stimuli. There were no significant relations between excitation during incision and RF expansion after incision or between spontaneous activity and RF expansion after incision.

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