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Peri-MAC Depression of a Nociceptive Withdrawal Reflex Is Accompanied by Reduced Dorsal Horn Activity with Halothane but not Isoflurane

Steven L. Jinks, Ph.D.,* John T. Martin, M.D.,† Earl Carstens, Ph.D.,‡ Sung-Won Jung, M.D.,§ Joseph F. Antognini, M.D.‖

Background: Anesthetics act in the spinal cord to suppress movement evoked by a noxious stimulus, although the exact site is unknown.

Methods: This study investigated sensorimotor processing in hind limb withdrawal reflexes, and effects of two general anesthetics, halothane and isoflurane, on simultaneously recorded responses of single dorsal horn neurons and hind limb withdrawal force, elicited by graded noxious thermal hind paw stimulation in rats. Minimum alveolar anesthetic concentration (MAC) needed to block gross movement to a supra-maximal mechanical stimulus was determined for each animal.

Results: Between 0.9 and 1.1 MAC, halothane and isoflurane greatly reduced or abolished withdrawal force (79 and 89% reduction, respectively). Halothane (0.75–1.4 MAC) depressed heat-evoked neuronal responses in a concentration-related manner (41% reduction between 0.9 and 1.1 MAC averaged across all stimulus temperatures, $P < 0.05$) and decreased stimulus-response function slopes, with corresponding reductions in withdrawal force. In contrast, isoflurane did not reduce neuronal responses in the 0.75–1.4 MAC range and slightly facilitated responses (by 16%) when concentration increased from 0.9 to 1.1 MAC, despite a concurrent withdrawal force reduction. Anesthetic depression of heat-evoked withdrawal force correlated well with MAC determination using a supra-maximal mechanical stimulus. At sub-MAC anesthetic concentrations, some units exhibited firing rate changes that preceded and paralleled moment-to-moment changes in force during a given withdrawal.

Conclusions: Halothane reduces noxious-evoked movement at least partly *via* depression of dorsal horn neurons, whereas isoflurane suppresses movement by an action at more ventral sites in the spinal cord.

LITTLE is known about spinal nociceptive reflex circuitry and how anesthetics act on sensory *versus* motor components of this circuitry to cause immobility, a desired anesthetic endpoint. A better understanding of these processes would aid both clinical practice as well as basic neuroscience research, in which many invasive procedures require anesthesia.

The spinal cord is the major site of anesthetic action for abolishing movement in response to a noxious stimulus,[1–3] although it is unclear where in the spinal circuitry anesthetics act to cause immobility. Since volatile anesthetics do not block peripheral nerve conduction and may even facilitate responses of nociceptors,[4,5] they presumably depress sensorimotor processing within the spinal cord. A direct depressant action on motoneurons partly contributes to anesthetic-induced immobility.[6,7] Depression of premotor intraspinal circuits, however, is also likely to play an important role. The initiation of nocifensive motor responses is mediated by excitation of nociceptors, which terminate in superficial and intermediate laminae of the spinal dorsal.[8,9] Previously, it has been reported that responses of dorsal horn neurons to noxious stimuli are depressed by halothane[10–12] and isoflurane.[13–15] However, prior studies employed relatively large changes in anesthetic concentration, and did not correlate changes in sensory responses with changes in motor output, as we did presently.

This study aimed to determine how volatile anesthetic effects on dorsal horn nociceptive processing might contribute to suppression of motor reflexes, using small concentration changes related to the minimum alveolar concentration (MAC) of these agents. MAC is the ED_{50} anesthetic concentration needed to block gross and purposeful movement evoked by a supra-maximal noxious stimulus and is considered to be the standard method for determining the immobilizing potencies of anesthetics.[16] Current methods to determine MAC are based on subjective observation of movement and do not permit quantitative detailed analyses of graded anesthetic-induced changes in sensorimotor processing. We sought to accomplish this in the present study by assessing the effects of anesthetics on dorsal horn neuronal and limb withdrawal reflexive responses to graded noxious thermal stimulation. We also sought to determine how anesthetic-induced changes in sensorimotor responses relate to MAC values. We hypothesized that halothane and isoflurane would depress both neuronal responses as well as withdrawal force, especially in the peri-MAC range, where noxious stimulus-evoked movement ceases.

Methods

The University of California Davis animal care and use committee approved the present study. Acute terminal experiments were conducted on 19 adult male Sprague-Dawley rats (350–500 g) maintained under halothane (n = 8) or isoflurane (n = 11) anesthesia. Only one anesthetic was administered to each animal. Prior to the

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experiment, animals had continuous access to food and water, were housed individually in soft bedding, and were maintained on a 12-h light-dark cycle with lights on at 7:00AM.

Surgery and Monitoring

Rats were placed in an acrylic box, and anesthesia was induced with either halothane (Halocarbon Laboratories, River Edge, NJ) or isoflurane (Minrad, Buffalo, NY). The rat was then placed on mask anesthesia (1.4% halothane; 2.0% isoflurane), during which time a tracheostomy was performed (14-gauge catheter was inserted into the trachea), and the animal was mechanically ventilated with anesthetic mixed in 100% O_2 for the remainder of the experiment. Jugular vein and carotid artery cannulations were performed for saline administration and blood pressure monitoring, respectively. We continuously monitored rectal body temperature and blood pressure (model PB-240, Puritan-Bennett, Hazelwood, MO). End-tidal CO_2 and anesthetic concentration were monitored with a calibrated Ohmeda Rascal II analyzer (Helsinki, Finland). Inspired and expired anesthetic concentrations were always within 0.1% of one another during the time of testing. Mean arterial pressure was always maintained above 75 mmHg with saline administration, if necessary. Rectal temperature ($36.9 \pm 0.3°C$) was maintained with a lamp connected to a variable power supply.

A dorsal midline incision was made, and a laminectomy was performed to expose the lumbar enlargement of the spinal cord. The superficial muscle and tendons surrounding the laminectomized portion of the spine were severed to permit vertebral clamp placement immediately rostral and caudal to the laminectomy, and to provide for optimal mechanical stability of the preparation during hind limb withdrawal/electrophysiological recording. To support the hindquarters of the animal during testing, another incision was made more caudally to permit placement of another vertebral clamp on the S2-3 spinous processes. Following the animal's individual MAC (iMAC) determination (see MAC Measurement section), the rat was fixed to a stereotaxic frame (Trent H. Wells, South Gate, CA) *via* the vertebral clamps and earbars.

MAC Measurement

Each animal's iMAC was determined following all surgical procedures, before placing the animal in the stereotaxic frame and conducting electrophysiological recording and withdrawal force measurements. Prior to iMAC determination, we allowed the animal to equilibrate to an anesthetic concentration that corresponded to approximate MAC values for isoflurane (1.2–1.3%) or halothane (0.8–0.9%). The iMAC was determined by applying a supramaximal mechanical stimulus (30 cm hemostat that delivered 168 g [1.7 N]/mm²) midway down the length of the tail. The clamp was applied and

oscillated at approximately 2 Hz for 1 min, or until gross purposeful movement was observed during the 1 min of clamping. Head turning toward the stimulus and/or multilimb movement was interpreted as a positive response, whereas single limb withdrawals and tonic limb or neck extensions were interpreted as a negative response.[16] Depending on the response, the anesthetic concentration was increased or decreased by 0.2% for isoflurane or by 0.1% for halothane. After an equilibration time of 15–20 min, the clamp was reapplied. This process was continued until two anesthetic concentrations were found that just permitted and just prevented movement. The average of these values was iMAC.[14] The iMAC values were averaged to determine the group MAC.

Electrophysiological Recording

Once the animal was fixed in the stereotaxic frame, the dura was removed, and a layer of transparent agar was poured over and surrounding the exposed spinal cord. After the agar solidified, a 12–15 MΩ tungsten microelectrode (FHC, Bowdoinham, ME) was advanced into the dorsal horn in increments of 5 μm with a hydraulic microdrive (D. Kopf Instruments, Tujunga, CA). All units were searched at an anesthetic depth of 0.9 MAC. Only one unit was recorded in each animal. Single units were identified by tactile stimulation and occasional pinching with forceps, and then tested for their response to noxious heat (50°C, 5 sec) delivered by a feedback-regulated Peltier thermode (Thermal Devices, Golden Valley, MN) with a surface area of 0.42 cm². The units that clearly responded to noxious heat were selected for further analysis. Thermally responsive units that responded to von Frey filaments with less than 1.5 g bending force were classified as wide-dynamic-range (WDR), and the two units classified as nociceptive-specific (NS) only responded to mechanical stimulation with von Frey filaments with more than 4 g bending force. Single-unit activity was amplified and displayed on an oscilloscope as well as digitized using two PC systems. On one computer, single-unit activity was recorded and used for off-line analysis with spike recording and template-matching software.[17] On another computer, single-unit activity was digitized and displayed simultaneously with hind limb force and thermode temperature, using a Powerlab interface and Chart software (AD Instruments, Grand Junction, CO).

Hind Limb Withdrawal Force Measurement

The method for hind limb withdrawal force measurement was nearly identical to a previously described method used for awake rats,[18] except the apparatus was adapted to the stereotaxic frame. A schematic of the setup is shown in figure 1. After single-unit activity was isolated, the hind paw was strapped with tape to the Peltier thermode such that the midportion of the plantar hind paw surface was in contact with the thermode. The
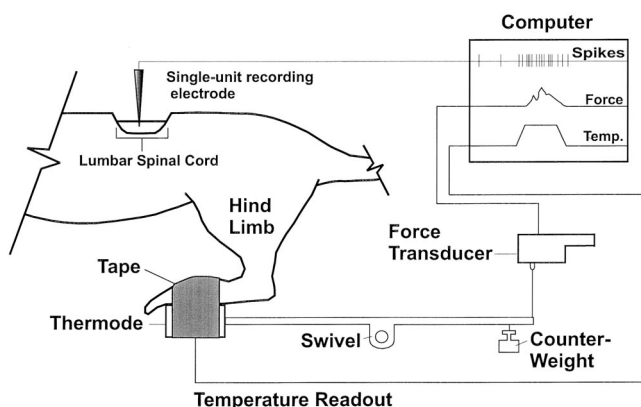
Fig. 1. Schematic diagram showing the experimental setup for simultaneous recording of single unit and hind limb withdrawal responses. After a single dorsal horn unit was isolated, the rat's hind paw was strapped to a Peltier thermode fixed to one end of a pivoting lever, of which the other end was attached to a force transducer *via* a suture. Graded noxious thermal stimuli were delivered to the receptive field of the dorsal horn unit. When the heat stimulus was of sufficient intensity to elicit a motor reflex, the rat's hind limb pulled one end of the lever up, which caused the other end to pull down on the force transducer. The thermode temperature, single-unit response, and hind limb withdrawal force were simultaneously recorded on a computer.

Peltier device was fixed to one end of a horizontal and counterbalanced lever, the midpoint of which was fixed to a free-moving swivel. The other end of the lever was attached to a force transducer (model FT03, Grass Instruments, West Warwick, RI) *via* a 1-0 silk suture, such that the suture and the center of the hind paw were attached to the lever at equal distances from the lever's pivoting point, and the suture was in vertical alignment with the force transducer. The transducer output was zeroed, and 50–60 g of passive tension was imposed on the hind limb by slightly raising or lowering the lever apparatus, after which the force tracing was zeroed. The thermode was then left in place for the remainder of the experiment. Force transducer output (calibrated in grams) was digitized and recorded on a PC, along with thermode temperature and single-unit activity, using a Powerlab interface and Chart software (AD Instruments).

Experimental Design

At least 15 min were allowed to lapse between changes in anesthetic concentration before retesting unit or withdrawal responses. Using each animal's iMAC, the unit responses and withdrawal responses were obtained at 0.75, 0.9, 1.1, and 1.4 MAC. The order in which different anesthetic concentrations were tested was randomized, except that testing at 0.9 and 1.1 MAC concentrations was usually performed in succession, with the order counterbalanced across experiments. This was because we were more interested in changes that occurred over the 0.9–1.1 MAC range, where noxious-evoked movement is greatly reduced or abolished. For some animals, we retested responses at 0.9 and 1.1 MAC, toward the end of the experiment.

Graded noxious thermal stimuli were delivered to the hind paw using a feedback-regulated Peltier thermode that was calibrated before each experiment. Graded noxious thermal stimuli (43, 45, 48, 51, and 55°C) were delivered in ascending order at 4-min interstimulus intervals, from an adapting temperature of 35°C. The duration of each heat stimulus was 10 sec. In a pilot experiment, the intradermal temperature was measured using a fine-gauge thermocouple (Physitemp, Clifton, NJ) inserted into the epidermis with a 25-gauge needle. Thermode temperatures of 43, 45, 48, 51, and 55°C corresponded to peak intradermal temperatures of 42.1, 44.3, 47.3, 49.2, and 52.0°C, respectively. Although in pilot studies we used graded mechanical stimuli, in the present study, we used a thermal stimulus because of its reproducible application to the receptive field and because we wanted to measure simultaneous withdrawal.[18]

Data and Statistical Analysis

Spontaneous activity was recorded 1 min prior to each heat stimulus. Dorsal horn neuronal responses were quantified by summing the number of impulses during the 10-s thermal stimulus (10-s response), or during the 60 sec following the onset of heat (60-s response). A hind limb withdrawal was defined as a change in tension of 0.8 g or more above baseline (tension at the onset of the thermal stimulus). Withdrawal threshold was defined as the lowest temperature at which 50% or more of the animals had a detectable withdrawal response. Peak force and force integrated over time following the onset of heat (10 and 30 s) were also calculated. Peak force was the maximum force attained during the withdrawal, regardless of when the peak occurred, although it always occurred within 13 sec following the heat onset. Total withdrawal force was calculated by summing 30-s integrated forces for all heat stimuli in each animal. Statistical comparisons were made by normalizing values of each of these withdrawal parameters as percent of maximum response. Neuronal or withdrawal responses at different stimulus temperatures and different MAC concentrations were analyzed by a three-factor ANOVA (MAC × temperature × unit, or animal) with *post hoc* Tukey multicomparisons, using statistical software (SPSS, Chicago, IL). Single pairwise comparisons were made using a paired *t* test. Neuronal and withdrawal responses were correlated using a multivariate regression analysis (SPSS). In all cases, a *P* value of less than 0.05 was considered statistically significant.

Results

MAC Determination and Unit Samples

The MAC for halothane was 0.8% ± 0.1 SD (n = 8). The MAC for isoflurane was 1.2% ± 0.1 SD (n = 11). The

A   Halothane

B   Isoflurane

Fig. 2. Bar graph plots of mean spontaneous activity under different minimum alveolar concentrations (MAC) of halothane (*A*) or isoflurane (*B*). Mean spontaneous activity was higher under halothane anesthesia (n = 8) compared to isoflurane anesthesia (n = 11) at each concentration (*P* < 0.05), except for 1.4 MAC. Error bars = SEM. *Significantly different from 0.75 MAC (*P* < 0.05).
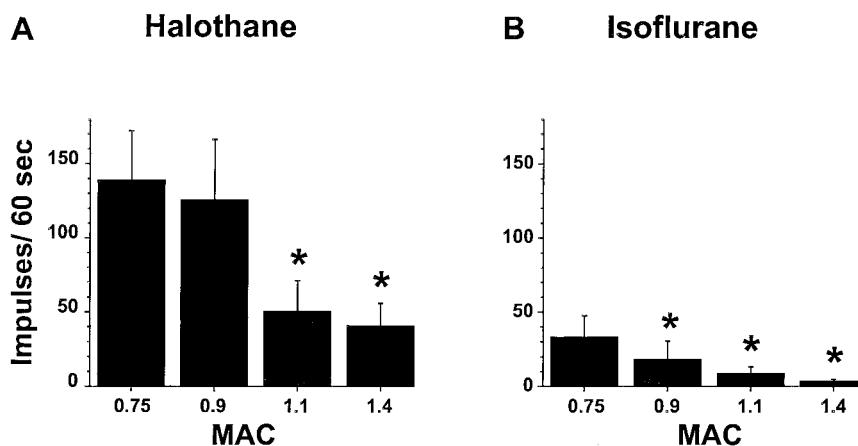
unit sample consisted of 19 dorsal horn units (17 WDR and 2 NS). Eight dorsal horn units (7 WDR and 1 NS) were tested under halothane anesthesia and 11 units (10 WDR and 1 NS) were tested under isoflurane anesthesia. The mean recording depths were 353 ± 136 μm for units recorded under halothane anesthesia (range, 270–670), and 360 ± 219 μm for units recorded under isoflurane anesthesia (range, 50–810). Both anesthetics had uniform effects on their respective sample, and thus effects of either anesthetic were not significantly different for superficial neurons (<350 μm, corresponding to laminae I-III) *versus* deep neurons (>350 μm, corresponding to laminae IV-VI), assessed by their spontaneous or evoked (10 or 60-s response) activity (three-factor ANOVA).

While measuring simultaneous hind limb withdrawal force, we intentionally searched units that had mechanical receptive fields that included, or were restricted to, the midportion of the plantar surface of the hind paw (*i.e.*, the area between the pads and heel). We chose units with such receptive fields so that we could consistently place the thermode on the same approximate area of the hind paw for all animals, and because hind limb withdrawals were more consistently elicited by stimulation of this area of hind paw skin. Four of the 19 total units had receptive fields located mainly on the toes and/or pads, and only neuronal response data were used for these four units.

Effects of Halothane and Isoflurane on Spontaneous Activity

Average spontaneous activity was significantly greater under halothane compared to isoflurane anesthesia (fig. 2). Under halothane anesthesia, mean spontaneous activity decreased from 2.32 ± 0.56 Hz at 0.75 MAC to 0.68 ± 0.25 Hz at 1.4 MAC (*P* < 0.0001). Under isoflurane anesthesia, mean spontaneous activity was significantly lower than that with halothane (*P* < 0.0001) and progressively decreased from 0.55 ± 0.24 Hz at 0.75 MAC to 0.06 ± 0.02 Hz at 1.4 MAC. For halothane, a significant 60% decrease in mean spontaneous activity

occurred between 0.9 and 1.1 MAC (*P* < 0.001; fig. 2A), whereas with isoflurane, significant decreases in mean spontaneous activity of 46% and 51% occurred from 0.75 to 0.9 MAC, and 0.9 to 1.1 MAC, respectively (*P* < 0.02; fig. 2B). At a given anesthetic concentration, spontaneous activity did not change significantly during the series of graded noxious thermal stimuli (fig. 3A, B), nor did it change significantly if responses were tested again later at the same anesthetic concentration.

Responses to Graded Noxious Heat

For both isoflurane and halothane, responses to noxious thermal stimuli increased with increases in stimulus intensity (43–55°C) at every anesthetic level tested (*P* < 0.0001). Responses usually consisted of a sharp increase in firing rate followed by a poststimulus after-discharge, which usually returned to baseline levels within 60 s. Figure 3 shows individual examples of graded heat-evoked responses under halothane (fig. 3A) and isoflurane (fig. 3B) anesthesia at 0.9 and 1.1 MAC. Mean graded heat-evoked responses under different concentrations of halothane or isoflurane are shown in figure 4.

From 0.75 to 1.4 MAC, halothane significantly (*P* < 0.0001) and consistently (in 7 of 7 WDR neurons and 1 NS neuron) caused a concentration-dependent depression of noxious heat-evoked responses (fig. 4A, C). There were overall significant differences between responses at 0.75 and 0.9 MAC, and between 0.9 and 1.1 MAC (*P* < 0.05 for both comparisons). Most of the depression occurred in the 0.9–1.1 MAC range, in which areas under the curve were reduced by 30 and 41% for the 10 and 60-s responses, respectively. However, responses to 48°C had a slightly greater reduction of 39 and 47% for the 10 and 60-s responses, respectively. Individual examples are shown in figure 3, mean stimulus-response curves in figures 4A and 4C.

With changes in halothane concentration, an overall change in the slope of the stimulus-response functions of dorsal horn unit responses was supported by a significant MAC × temperature interaction (*P* < 0.001; fig. 4A, C). At 0.75 MAC, only the highest stimulus intensity
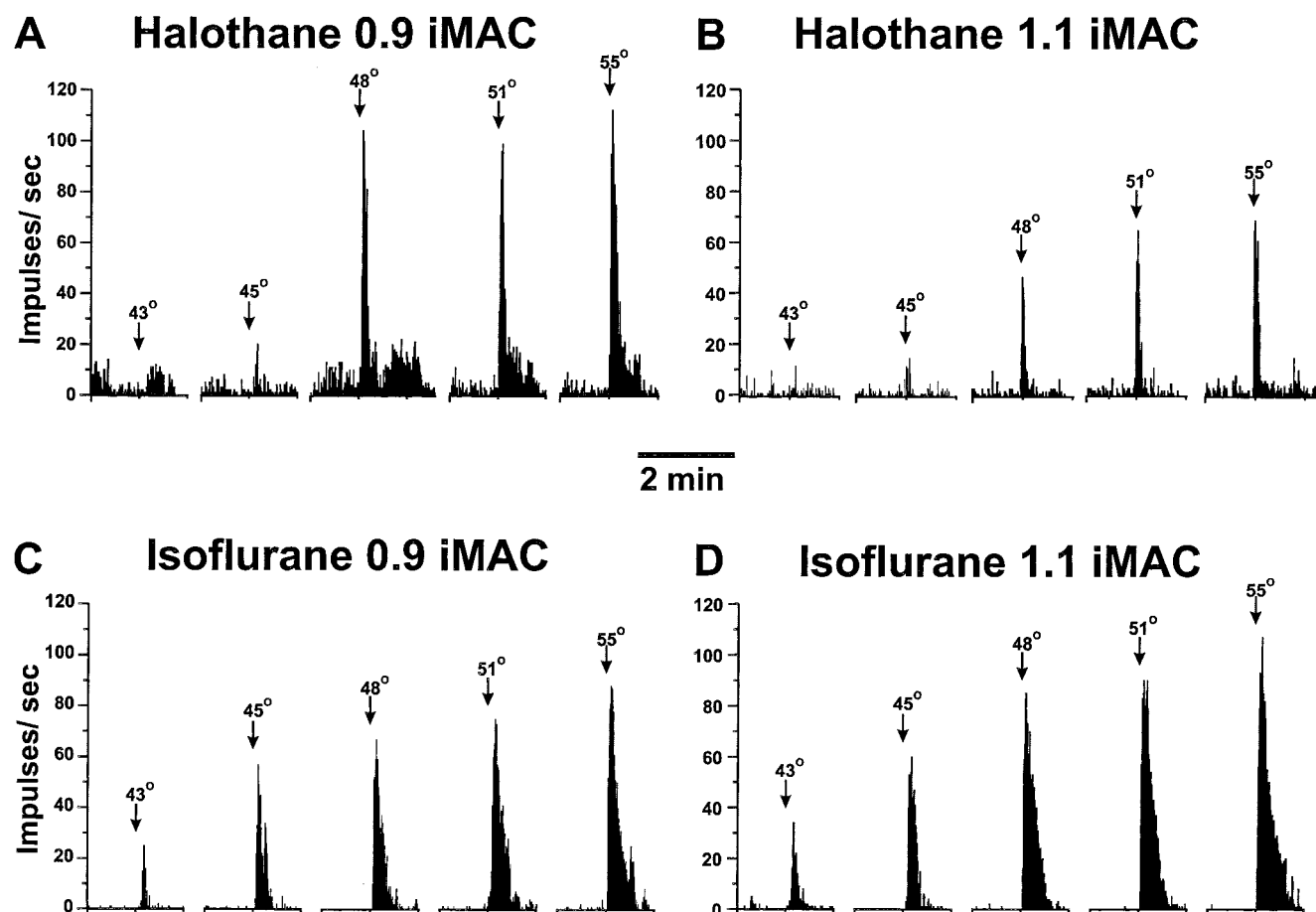
Fig. 3. Peristimulus time histograms (bin width = 1 sec) of individual single-unit responses to graded noxious heat. One unit was recorded under halothane anesthesia (*A*, *B*), and another unit was recorded under isoflurane anesthesia (*C*, *D*), at 0.9 of the animal's individual minimum alveolar concentration (iMAC) (*A*, *C*), and at 1.1 iMAC (*B*, *D*). Halothane depressed the unit's response to heat when halothane concentration was increased from 0.9 to 1.1 iMAC (*A*, *B*), whereas isoflurane slightly enhanced another unit's responses at equipotent concentrations (*C*, *D*).

(55°C) evoked responses that were significantly larger than those evoked by 55°C at 0.9 MAC ($P < 0.02$), and this is indicated by a reduction in the stimulus-response slope in the 51–55°C range (fig. 4A, C). As halothane concentration was increased to 1.1 and 1.4 MAC, there were further reductions in the slope of the stimulus–response functions.

Halothane effects and the magnitude of the heat-evoked responses were reproducible when we repeated the series of stimuli toward the end of the experiment, for 0.9 and 1.1 MAC (data not shown), indicating that repeated application of the ascending stimulus series did not produce any significant sensitization or desensitization of responses.

The effects of isoflurane on heat-evoked responses in dorsal horn neurons were markedly different from those of halothane. Whereas halothane significantly depressed responses when the concentration was increased from 0.9 to 1.1 MAC, isoflurane modestly but significantly enhanced responses, by 29 and 16% for the 10 and 60-s responses (areas under the curve), respectively ($P <$

0.02; fig. 4B, D). All neurons (10 of 10 WDR neurons and 1 NS neuron) had enhanced 10-s responses at 1.1 MAC isoflurane, and 8 of 11 neurons had enhanced 60-s responses, compared to those at 0.9 MAC. In the remaining 3 of 11 neurons, 60-s responses were reduced by a mean of only 15%. This enhancement at 1.1 MAC was not dose-dependent in that responses at 1.4 MAC were similar to those at 0.75 MAC and 0.9 MAC.

Hind Limb Withdrawal Force and Correlation with MAC

Hind limb withdrawals evoked by noxious heat were usually characterized by an abrupt phasic increase in tension followed by a more prolonged component of lesser force. Mean values for withdrawal force parameters are shown in figure 5, and individual examples of raw withdrawal force traces are shown in figure 6. Most of the response occurred within 30 sec of the onset of the heat stimulus, although it appeared that low levels of tension (≥ 3 g) sometimes persisted for several minutes. For both anesthetics at 0.75 and 0.9 MAC, the mean

Fig. 4. Line graphs showing stimulus-response functions for mean responses to graded noxious heat under halothane (*A*, *C*; n = 8) or isoflurane (*B*, *D*; n = 11) anesthesia. Mean impulses discharged during the 10-s heat stimuli are shown in *A* and *B*, and mean impulses discharged during 60-s following the onset of heat stimuli are shown in *C* and *D*. Increases in halothane concentration significantly reduced the stimulus–response slope ($P < 0.001$) and increased heat thresholds ($P < 0.01$), whereas isoflurane had no effect on heat-evoked responses, except for a slight enhancement at 1.1 of the minimum alveolar concentration (MAC), compared to responses at 0.9 MAC ($P < 0.02$). Error bars = SEM.
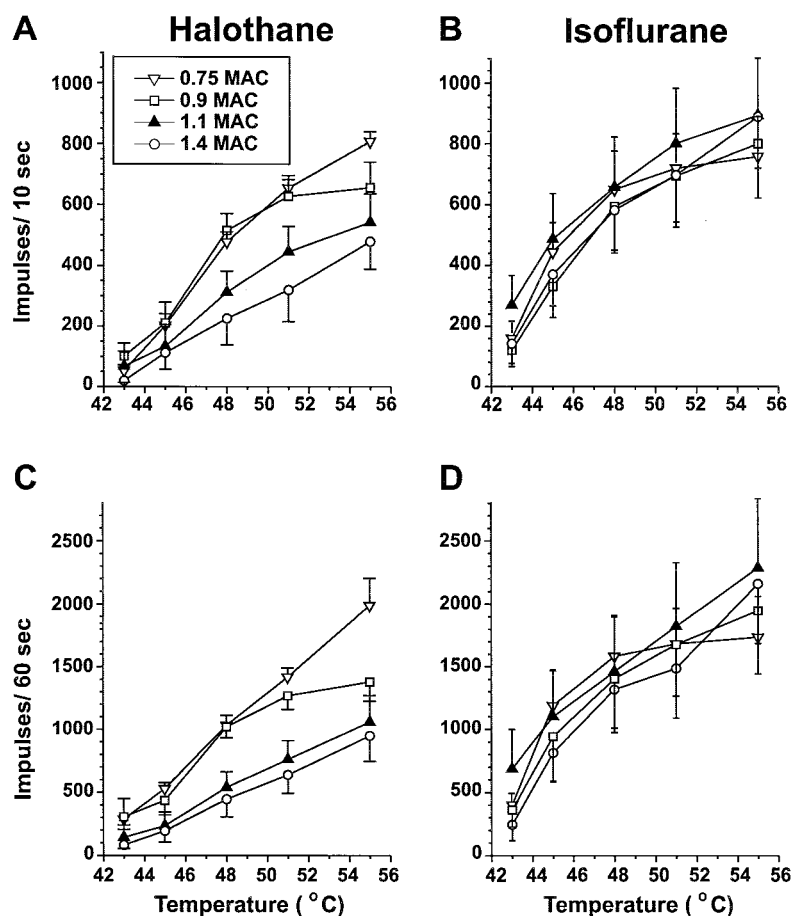
magnitude of withdrawals (peak and integrated forces) increased with increases in stimulus temperature ($P < 0.05$ in all cases; fig. 5). However, withdrawals at 0.75 MAC halothane tended to plateau at higher heat stimulus intensities (fig. 5A, C). At 1.1 MAC, there was no significant increase in withdrawal force with stimulus intensity for either anesthetic.

Withdrawal forces (peak, 10-s integral, and 30-s integral) under isoflurane anesthesia were all greater than withdrawal forces measured under halothane anesthesia ($P < 0.02$ in all cases). Increasing the concentration of both halothane and isoflurane from 0.75 to 1.1 MAC resulted in an increase in withdrawal threshold. For halothane, withdrawal thresholds increased from 48°C at 0.75 MAC to 51°C at 0.9 MAC, and at 1.1 MAC only 1 of 6 animals had a detectable withdrawal response. For isoflurane, withdrawal thresholds at 0.75, 0.9, and 1.1 MAC were 48, 51, and 55°C, respectively. Both anesthetics also caused a significant decrease in peak and integrated force when the concentration was changed from 0.9 to 1.1 MAC (figs. 5 and 7; $P < 0.015$ in all cases). For both anesthetics at 0.9 MAC, the series of thermal stimuli produced withdrawal responses in all animals tested, however, withdrawals at 1.1 MAC were either substantially reduced or absent (figs. 5–7). With halothane, the incidence of withdrawals was 100% (6 of

6 rats) at 0.9 MAC, and dropped to 17% (1 of 6 rats) at 1.1 MAC. With isoflurane at 0.9 MAC, the incidence of withdrawals was 100% (9 of 9 rats), but when isoflurane concentration was changed from 0.9 to 1.1 MAC, the incidence dropped to 56% (5 of 9 rats), with an 89% reduction in mean total withdrawal force (fig. 7). In the five rats alone that did show withdrawal responses at 1.1 MAC isoflurane, the mean reduction in total withdrawal force was still 85%. However, the weak withdrawal responses at 1.1 MAC would not have been considered gross and purposeful, as is normally sought in an MAC study.

We controlled for possible sensitization or habituation of motor responses by randomizing the order in which different anesthetic concentrations were delivered. However, in some animals, we also retested motor responses at 0.75 MAC (n = 3), 0.9 MAC (n = 7), or 1.1 MAC (n = 7) and did not find any significant changes in peak force, integrated force, or withdrawal incidence between the two trials (data not shown).

Correlation between Withdrawal Force and Responses of Dorsal Horn Neurons

For all dorsal horn units, heat-evoked responses preceded hind limb withdrawals (examples shown in fig. 6). Increases in halothane concentration caused progressive
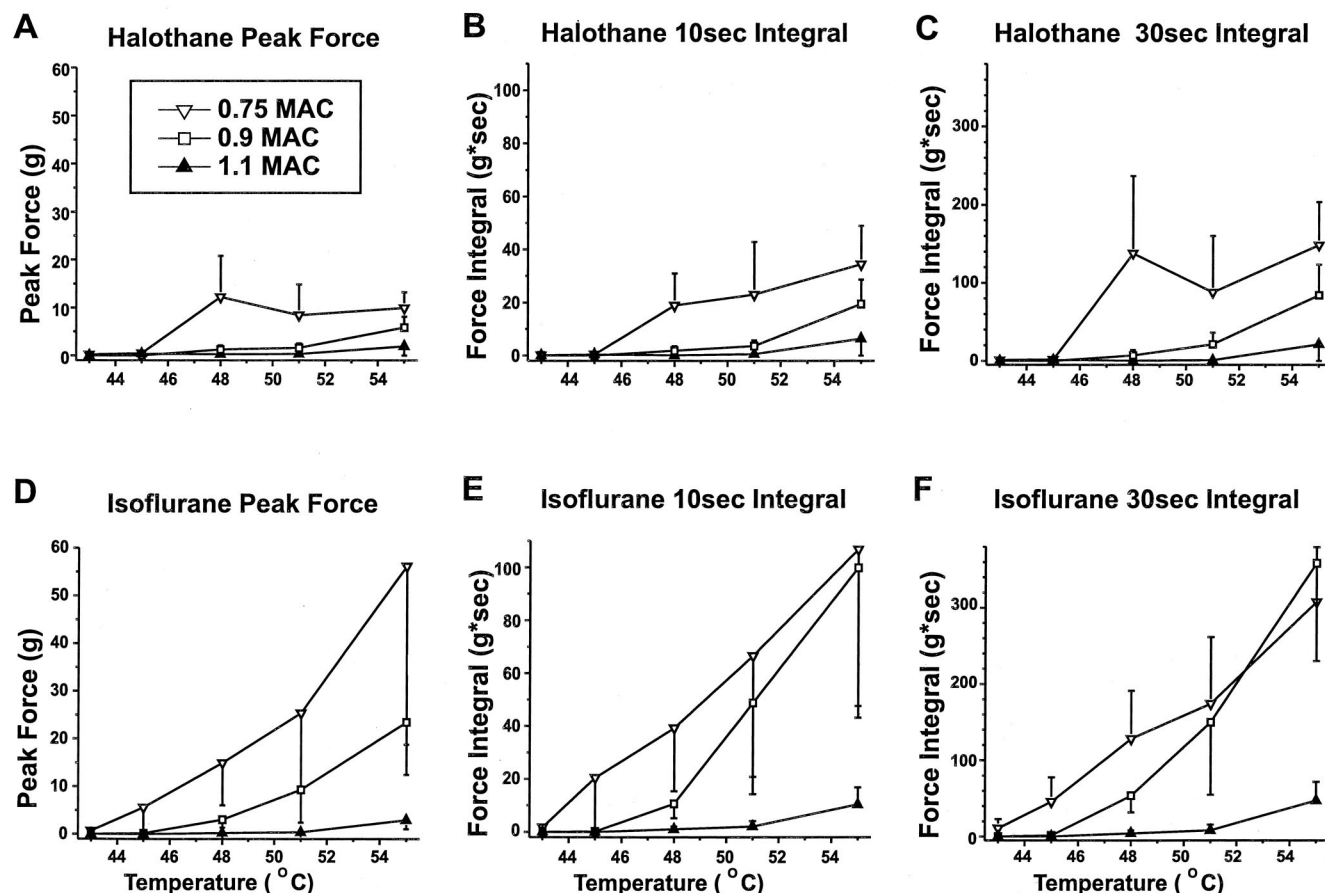
Fig. 5. Line graphs showing stimulus–response functions for mean withdrawal forces elicited by graded noxious heat under halothane (*A-C*; n = 6) or isoflurane (*D-F*; n = 9) anesthesia. Mean peak withdrawal forces are shown for halothane (*A*) and isoflurane (*B*). Mean integrated forces generated during the 10-s heat stimulus are shown for halothane (*B*) and isoflurane (*E*), and integrated forces generated during 30-s following the onset of heat are shown in *C* and *F*, for halothane and isoflurane, respectively. Increases in concentration of either anesthetic were accompanied by increases in withdrawal thresholds ($P < 0.001$) and large reductions in, or absence of, withdrawal responses at 1.1 of the minimum alveolar concentration (MAC), compared to 0.9 MAC ($P < 0.015$). Mean withdrawal force was greater under isoflurane compared to halothane anesthesia ($P < 0.02$). Error bars = SEM.

decreases in both neuronal responses and withdrawal force (fig. 7A). Under isoflurane, however, the reduction in withdrawal force observed when the concentration was raised from 0.9 to 1.1 MAC was not accompanied by reduced dorsal horn neuronal firing (fig. 7B). Under sub-MAC concentrations of halothane or isoflurane (0.75 and 0.9 MAC), neuronal responses were significantly correlated with integrated withdrawal force ($r = 0.63$–0.74; $P < 0.005$ in all cases).

Discussion

Several findings emerge from this study. Halothane depressed heat-evoked responses of dorsal horn neurons in a concentration-dependent manner, mainly from 0.9 to 1.1 MAC, confirming and extending previous findings.[10–12] This reduction corresponded to a reduction in reflexive limb withdrawal force, suggesting that depression of nociceptive transmission through the dorsal horn

contributes at least partly to halothane's immobilizing effect. However, halothane may also act at a more ventral site, as indicated by F and H wave studies (see Isoflurane section). In contrast, isoflurane did not depress dorsal horn neuronal responses, even above 1 MAC, where withdrawals were depressed or abolished. Therefore, isoflurane's immobilizing action must depress more ventral sites in the reflex pathway. Agent-specific actions of volatile anesthetics at other central nervous system sites (*e.g.*, hippocampal neurons) have been reported.[19] The present results, and numerous other studies, cast further doubts on the unitary hypothesis of anesthetic action.[20] We further discuss these findings, along with methodological limitations.

Halothane

Halothane significantly depressed spontaneous and evoked activity of dorsal horn neurons in the 0.75–1.4 MAC range (figs. 2A, 4A,C). The greatest depression occurred between 0.9 and 1.1 MAC, suggesting this
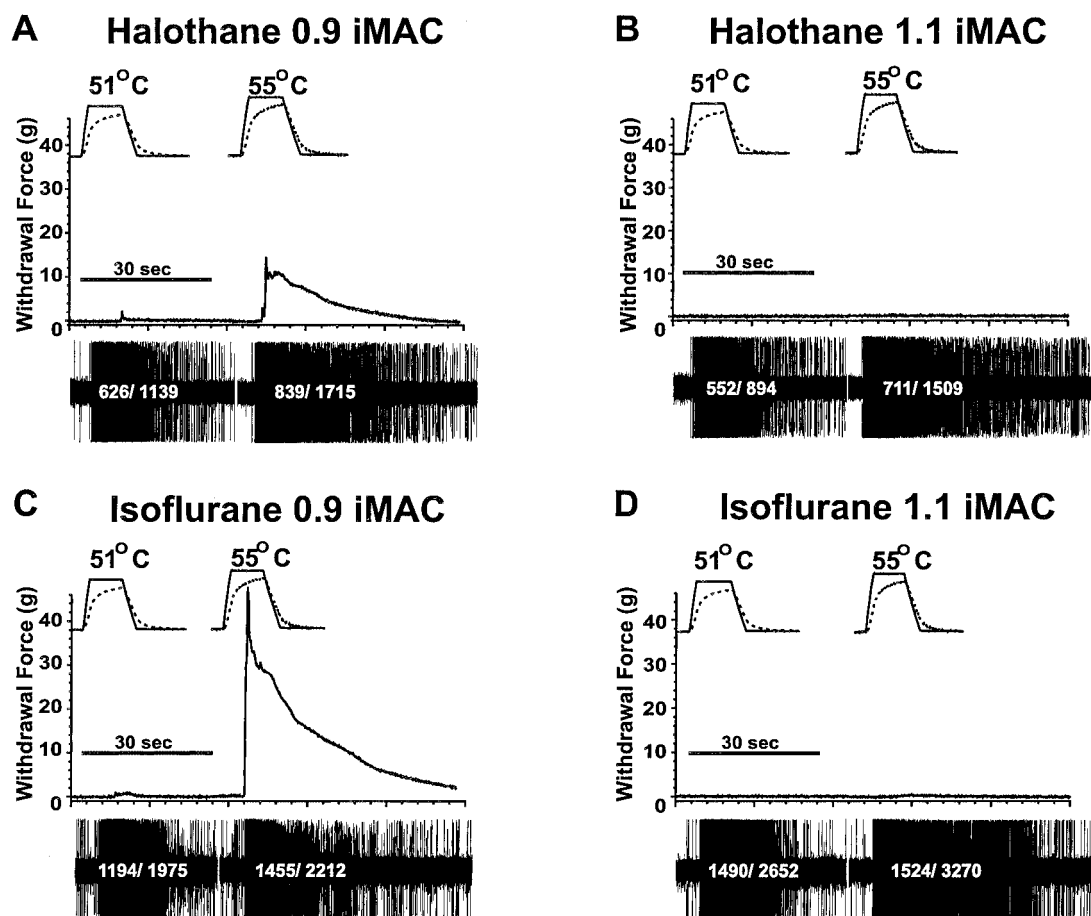
Fig. 6. Individual examples showing relation among simultaneously-recorded stimulus temperature (*top*), withdrawal force (*middle*) and unit responses (*bottom*, raw trace) for one unit recorded under halothane anesthesia (*A, B*) and another unit recorded under isoflurane anesthesia (*C, D*). Unit responses to 51 and 55°C stimuli are shown at 0.9 of the animal's individual minimum alveolar concentration (iMAC) (*A, C*) and at 1.1 iMAC (*B, D*) for halothane and isoflurane, respectively. Absence of a withdrawal response was accompanied by a reduction in the number of impulses discharged under halothane (*A, B*), but not for isoflurane (*C, D*). Note that spike amplitude remained stable throughout withdrawal responses. Temperature tracings at top show thermode temperatures (*solid line*) and intradermal temperatures (*dotted line*), rising from a baseline temperature of 35°C. Intradermal temperatures were taken from pilot experiments in another animal. The number of impulses discharged during the heat stimulus and during the 60-s period following the onset of heat, for each response, is superimposed over the raw unit traces (*bottom*).

decline contributed to large decreases in noxious stimulus-evoked movement within this concentration range.

Halothane's action was characterized by a concentration-dependent reduction in the slope of stimulus–response functions for graded heat (fig. 4), consistent with a previous study employing a similar range, but larger increments, of halothane concentrations.[12] The slope reduction implies a reduction in the gain of spinal nociceptive transmission, which might be accounted for by presynaptic inhibition[21,22] or reduced spatial recruitment of nociceptive dorsal horn neurons *via* a documented halothane-induced reduction in receptive field size.[23] Unfortunately, we were presently unable to assess receptive field changes, since the paw had to remain affixed to the thermode.

Anesthetic effects on dorsal horn neurons and withdrawals were likely attributed to a direct spinal action. Using differential anesthetic delivery to goats, isoflurane

or halothane MAC requirements were much lower when administered to the torso (*i.e.*, spinal cord) circulation than when administered to the cerebral circulation.[2,3] Others reported that spinalization does not affect isoflurane MAC or halothane-induced reductions in receptive fields of dorsal horn neurons,[23] consistent with a spinal action. We conclude that halothane exerts its immobilizing effect largely *via* a spinal inhibitory action on dorsal horn and possibly ventral horn neuronal processing.

Isoflurane

In contrast to halothane, isoflurane did not depress single-unit responses and actually enhanced responses at 1.1 MAC (fig. 7). Isoflurane depressed spontaneous firing in a concentration-dependent manner. Although changes were small, isoflurane depression of spontaneous activity might have reduced tonic facilitation of motoneurons to depress the reflex. However, spontane-
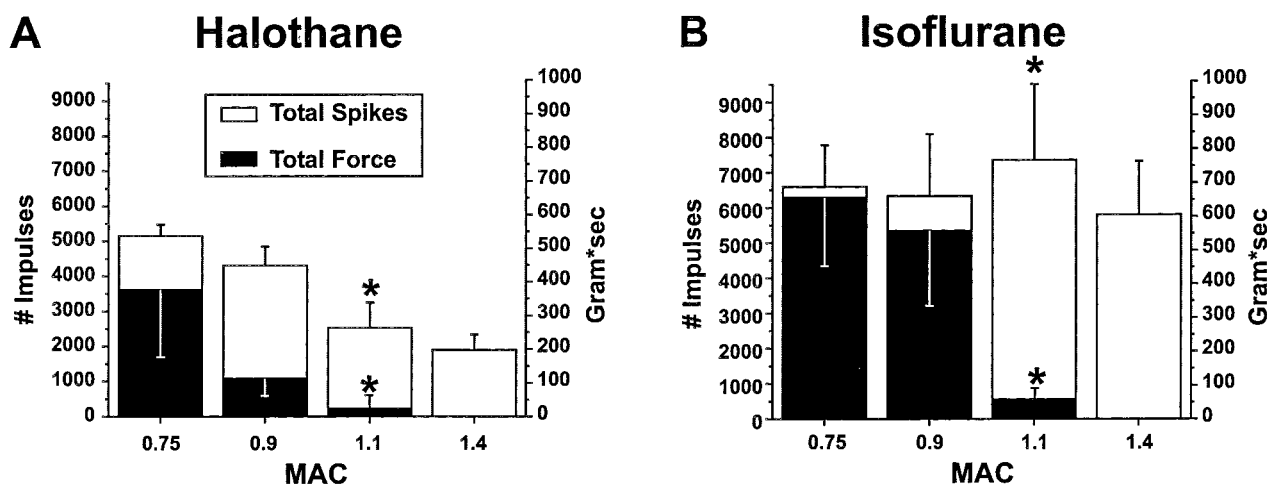
Fig. 7. Bar graphs showing the relation between area under the curve for unit responses (*open bars*) and area under the curve for withdrawal forces (*filled bars*) over different minimum alveolar concentrations (MAC) for halothane (*A*; n = 6) and isoflurane (*B*; n = 9). At sub-MAC anesthetic concentrations, for halothane and isoflurane, neuronal responses correlated significantly with the withdrawal force (r = 0.63–0.74; P < 0.005 in all cases). Halothane caused a dose-related reduction in unit responses, with corresponding reductions in withdrawal force. Under isoflurane anesthesia, withdrawals were substantially reduced or absent at 1.1 MAC despite the failure of isoflurane to reduce responses of dorsal horn neurons. Error bars = SEM. *Significantly different from 0.9 MAC (P < 0.05).

ous activity under isoflurane at 0.75 and 0.9 MAC (where movement occurred) was lower than that under halothane at 1.1 and 1.4 MAC (where little or no movement occurred), suggesting that reduced spontaneous activity did not play a significant role in decreasing withdrawal force.

In a previous study, responses of cat dorsal horn neurons were depressed when isoflurane concentration increased from 0.5% to 1.5%, approximately 0.4–1.2 MAC.[15] However, most of this depression may have occurred in the 0.4–0.75 MAC range, as supported by the following observations. First, in the present study, spontaneous firing was already very low at 0.75 MAC isoflurane, compared to units recorded under halothane. Second, our previous studies show that isoflurane depresses responses of dorsal horn neurons to mechanical stimuli in the 0.3–0.8% range, with little or no effect at peri-MAC isoflurane concentrations, where noxious-evoked movement ceases.[13,14] The data suggest that depression of dorsal horn neurons by isoflurane occurs mainly well below 1 MAC, and therefore isoflurane's immobilizing effect is not mediated by a depression of nociceptive transmission through the dorsal horn, but rather by depression at a more ventral location. Micro-stimulation of the dorsal horn is one method to investigate this possibility. If halothane and isoflurane differ as to their site of action (dorsal *vs.* ventral horn), micro-stimulation might differentially affect movement responses in halothane- *versus* isoflurane-anesthetized animals. Although reductions of dorsal horn activity by isoflurane do not correspond to the abolishment of gross and purposeful movement seen at and above MAC, inhibition of dorsal horn neurons in the 0.4–0.75 MAC range

may contribute to reducing the duration, frequency and/or intensity of gross and purposeful movement evoked by a noxious stimulus.

One possibility is that isoflurane directly depresses motoneurons in the peri-MAC concentration range. Volatile anesthetics, such as desflurane, enflurane and halothane, depress H reflexes,[24–26] suggesting effects on motoneurons and/or Ia primary afferents. The F wave, an indirect measure of motoneuron excitability, is depressed by inhaled anesthetics. Isoflurane significantly decreased F-wave amplitude in the 0.8–1.2 MAC range,[7,27] but halothane did not significantly depress the F wave in this range.[28] It is not known if isoflurane also depresses premotor interneurons.

Differential effects on distinct receptors that exhibit lamina-specific expression might explain the contrasting effects of halothane and isoflurane on dorsal horn neurons. Halothane depressed neurons in spinal laminae I and V, but not in IV.[10] Indeed, isoflurane and halothane have differential effects on receptors involved in neuronal inhibition, such as γ-aminobutyric acid receptor type A (GABA$_A$) and glycine,[29] as well as on excitatory receptors, such as substance P and glutamate.[30,31] Laminar differences in expression are reported for these receptors,[32] but site-specific pharmacologic actions of anesthetics need more thorough investigation. In the present study, however, neurons in each group responded consistently, regardless of recording depth, making laminar-specific effects in the dorsal horn unlikely. Intravenous anesthetics, such as thiopental and propofol, appear similar to halothane in that these drugs depress dorsal horn neuronal responses to noxious stimuli.[33–35]

Effects of Halothane and Isoflurane on Hind Limb Withdrawal Force

Halothane and isoflurane both decreased the force of reflexive limb withdrawals in a concentration-dependent manner, with the largest reduction between 0.9 and 1.1 MAC. However, withdrawal forces were greater under isoflurane compared to equipotent halothane concentrations (figs. 5 and 7). In our previous study, the force of multiple limb and head movements in response to a supramaximal mechanical stimulus decreased substantially from 0.9 to 1.1 MAC under both halothane and isoflurane.[36] Limb movements were weaker under halothane compared to equipotent isoflurane, consistent with the present findings. The present data, showing anesthetic-induced reductions in reflexive withdrawals elicited by graded noxious thermal stimuli, are consistent with previous studies that used the more conventional assessment of MAC according to the presence of gross purposeful movement in response to supramaximal stimuli. While it remains unclear how spinal circuitry mediating simple limb withdrawal reflexes relates to the more complex circuits generating coordinated multi-limb movements, the generally similar anesthetic effects on these two types of movement suggest a degree of overlap in the underlying circuits.

Spinal Nociceptive Reflex Circuitry

Primary afferent nociceptors terminate in the dorsal horn[8,9] and, therefore, some dorsal horn neurons must presumably participate in the reflex. Responses of some nociceptive dorsal horn neurons preceded and correlate with the withdrawal magnitude or motor unit discharge,[37–39] providing necessary but not absolute proof of their involvement in the reflex. While it is possible to functionally identify last-order interneurons connected to motoneurons using spike-triggered averaging,[40] it is not currently possible to unequivocally identify earlier-order interneurons, hence limiting our understanding of nociceptive reflex circuitry.

The available evidence suggests that spinal interneurons and ascending sensory neurons may constitute separate neuronal populations.[41] The present study is limited in that it was not possible to functionally identify the early-order neurons from which we recorded. Further work is required to determine if functionally diverse spinal cord neurons are similarly affected by anesthetics. However, the effects of halothane or isoflurane were homogeneous for each respective unit sample, suggesting that these anesthetics affect dorsal horn neurons similarly, regardless of function. Halothane had its greatest effect on both neuronal and withdrawal responses between 0.9 and 1.1 MAC where gross and purposeful movement was abolished. Additional studies are needed to unveil the organization of nociceptive sensorimotor integration and to further address the relative anesthetic effects on individual components of the underlying circuits.

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