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Isoflurane Differentially Modulates Medullary ON and OFF Neurons While Suppressing Hind-limb Motor Withdrawals

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Background: **Isoflurane acts primarily in the spinal cord to block movement; however, it is unclear how supraspinal sites might contribute to anesthetic effects on quantified parameters of movement such as force.**

Methods: **The authors investigated the effects of isoflurane on spontaneous and noxious heat–evoked activity of nociceptive reflex–modulating ON and OFF cells in the rostral ventromedial medulla of rats. Single ON or OFF neurons were recorded simultaneously with hind-limb withdrawal force elicited by graded noxious thermal hind paw stimulation. Isoflurane concentrations were administered in reference to each animal's minimum alveolar concentration (MAC) of isoflurane.**

Results: **From 0.65 to 1.15 MAC, isoflurane dose-dependently reduced spontaneous activity of ON cells by 70% ($P < 0.001$). OFF-cell spontaneous activity was dose-dependently increased 138% ($P < 0.001$). ON-cell heat-evoked activity was depressed 95% by isoflurane from 0.65 to 1.15 MAC ($P < 0.001$). Isoflurane-induced changes in ON- and OFF-cell activity paralleled similar reductions in withdrawal force, with the largest change in both neuronal activity and withdrawal force occurring between 0.85 and 1.15 MAC. For the lowest stimulus temperature, excitatory responses of ON cells and inhibitory responses of OFF cells were significantly greater for trials in which withdrawals occurred than for trials in which no withdrawal occurred, suggesting that responses in both classes of neurons were related to movement rather than the stimulus alone.**

Conclusions: **The results show that isoflurane modulation of ON- and OFF-cell activity corresponds to anesthetic-induced reductions in hind-limb withdrawal force, and therefore, the effects of isoflurane on these classes of neurons in rostral ventromedial medulla might contribute to motor depression.**

DESPITE widespread use of anesthetics in clinical practice and basic neuroscience research, the mechanisms by which anesthetics affect sensorimotor processes to cause immobility, a desired anesthetic endpoint, are poorly understood. Moreover, anesthetic disruption of responses to noxious stimuli may be used as a tool to investigate the roles of brain and spinal cord sites in nociceptive sensorimotor processing.

Previous studies suggest anesthetics block movement largely *via* a spinal action.[1–3] Results were based on the minimum alveolar concentration (MAC) of anesthetic required to block gross and purposeful movement in response to a supramaximal noxious stimulus. However, MAC determination is a nonquantitative "all-or-none" measure of motor output, which does not account for graded anesthetic effects on movement parameters such as force. While a direct spinal action plays a pivotal role in anesthetic-induced immobility, both spinal and supraspinal anesthetic effects could impede nociceptive processes that are dependent on spinal–bulbospinal feedback circuits.

A supraspinal site that might contribute to anesthetic depression of movement is the rostral ventromedial medulla (RVM), a relatively well-studied area implicated in the modulation of nociceptive reflexes. The nucleus raphe magnus and nucleus reticularis magnocellularis of the RVM contain two classes of reflex-modulating neurons, ON cells and OFF cells, which have projections to the spinal cord and are believed to facilitate and to suppress nociceptive reflexes, respectively.[4,5] A noxious stimulus sufficient to elicit a tail flick or a paw withdrawal causes ON cells to increase their activity and OFF cells to decrease their activity in close association with the occurrence of the reflex.[6] Furthermore, opiates blunt nociceptive responsiveness while appropriately modulating RVM neurons, such that ON cells are depressed and OFF cells are facilitated,[7,8] whereas hyperalgesic states are associated with increased ON cell activity.[9]

The current study sought to determine how isoflurane modulation of ON- and OFF-cell activity relates to anesthetic depression of movement. This was achieved by simultaneously recording neuronal and hind-limb withdrawal responses to graded noxious thermal hind-paw stimulation, using small changes in isoflurane concentration related to individual MAC (iMAC) values of each animal. We hypothesized that isoflurane would depress ON-cell activity and facilitate OFF-cell activity in conjunction with anesthetic depression of a withdrawal reflex.

Materials and Methods

This study was approved by the University of California, Davis, animal care and use committee. Experiments were conducted in 19 isoflurane-anesthetized adult male Sprague-Dawley rats (weight, 375–550 g), housed individually on a 12-h light–dark cycle (lights on at 07:30) and given free access to food and water. Animals were not given neuromuscular blocking drugs at any time.

Surgery and Monitoring

Rats were placed in an acrylic box, and anesthesia was induced with 4% isoflurane (Minrad Inc., Buffalo, NY).

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The rat was then placed on mask anesthesia (2.0%), and a tracheostomy was performed. A 14-gauge catheter was inserted into the trachea, and the animal was mechanically ventilated with isoflurane mixed in 100% O_2 for the remainder of the experiment. Jugular vein and carotid artery cannulations were performed for fluid administration and blood pressure monitoring, respectively. We continuously monitored rectal body temperature and blood pressure (model PB-240; Puritan-Bennett Corp., Hazelwood, MO). End-tidal carbon dioxide and anesthetic concentration were also monitored throughout the experiment 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 80 mmHg with lactated Ringer's solution (Abbott Laboratories, Chicago, IL) when necessary. Rectal temperature was maintained at 36.5 ± 1°C with a lamp connected to a variable power supply.

A midline craniotomy 2–3 mm in diameter was performed immediately caudal to the lambda to permit electrode insertion into the RVM. Two midline incisions were made to expose the C2 and S1–S2 spinous processes, to which vertebral clamps were later applied to stabilize the animal during electrophysiologic recording. After the animal's iMAC determination (see MAC Measurement), the rat was then placed in the stereotaxic frame (Trent H. Wells, South Gate, CA) and affixed *via* the vertebral clamps and ear bars.

MAC Measurement

Each animal's iMAC was determined after all surgical procedures, before electrophysiologic recording. We allowed the animal to equilibrate to an anesthetic concentration that corresponded to approximate MAC values for isoflurane (1.1–1.3%). The iMAC value was determined by applying a supramaximal mechanical stimulus (30 cm hemostat that delivered 1.7 N/mm^2) midway down the length of the tail. The clamp was applied and oscillated (rotated back and forth approximately ±30°) at approximately 2 Hz for 1 min or until gross purposeful movement was observed during the 1 min of clamping.[10] Head turning toward the stimulus, multilimb movement, or both were interpreted as a positive response, whereas single limb withdrawals and tonic limb or neck extensions were interpreted as a negative response.[10] Depending on the response, the anesthetic concentration was increased or decreased by 0.2%. 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.[10]

Electrophysiologic Recording

After the animal was fixed in the stereotaxic frame, a 10- to 13-MΩ tungsten microelectrode (FHC Inc., Bowdoinham, ME) was positioned on the midline of the cerebellar surface 10–12 mm caudal to bregma, then advanced manually 8 mm ventral, and then advanced in 5-μm increments with a microdrive (D. Kopf Instruments, Tujunga, CA) until a neuron of interest was isolated. All neurons were searched, isolated, and tested for their responsiveness to noxious pinch and heat at an anesthetic depth of 0.8–0.9 MAC. Only neurons that exhibited a discernable increase (approximately 200%) or decrease (approximately 50%) in firing rate to noxious pinch applied to the tail and hind paw and that in addition responded to noxious heat (50°C, 10 s) were selected for further testing. Noxious pinch was not applied to the hind paw used for formal testing of neuronal/withdrawal responses to heat.

Single-unit activity was amplified and displayed on an oscilloscope, as well as digitized using two personal computer systems. On one computer, single-unit activity was discriminated and saved for use with off-line template-matching software.[11] On another computer, raw single-unit activity was digitized and displayed simultaneously with hind-limb force and thermode temperature, using Powerlab and Chart software (AD Instruments, Grand Junction, CO). Firing rate histograms were constructed on the Powerlab system using the Histogram spike discrimination software plug-in for Chart (AD Instruments, Grand Junction, CO). At the end of the experiment, recording sites were marked with electrolytic lesions by passing anodal DC current through the electrode. The brain was then excised, placed in 10% formalin, cut transversely in 40-μm-thick sections, counterstained with cresyl violet, mounted, and coverslipped.

Hind-limb Withdrawal Force Measurement

The method for isometric hind-limb withdrawal force measurement was identical to our previous study.[12] After a neuron was selected for analysis, the hind paw was strapped with tape to a feedback-regulated Peltier thermode (Thermal Devices Inc., Golden Valley, MN) with a heated surface area of 0.42 cm^2. The midportion of the plantar hind-paw surface was in contact with the thermode, which was left in place throughout the testing period. The Peltier device was attached to one end of a horizontally oriented, counterbalanced lever, the midpoint of which had a freely moving swivel. The other end of the lever was attached to a force transducer (model FT03; Grass Instruments, West Warwick, RI) *via* a piece of thread. The thread and the center of the hind paw were attached to opposite ends of the lever at equal distances from the pivoting point of the lever (the swivel), and the suture was in vertical alignment with the action of the force transducer. Thus, when the animal pulled its hind limb up with the attached thermode (withdrew), the other end of the lever pulled down on the force transducer. Before testing began, 50–60 g passive tension was imposed on the hind limb by adjust-

ing the height of the lever. Force transducer output (calibrated in grams) was digitized and recorded on a personal computer, along with thermode temperature and single-unit activity (AD Instruments). After collection of neuronal and withdrawal responses to noxious heat, some animals had one or more limbs each attached to a force transducer to record neuronal and withdrawal responses to pinch, clamping, or both of the hind paw or tail. However, responses to mechanical stimuli were used for diagnostic purposes only and were not routinely recorded and formerly tested.

Experimental Design and Data Analysis

At least 15 min was allowed to lapse between changes in anesthetic concentration before retesting. The order in which different anesthetic concentrations were tested was randomized. We waited at least 10 min after any type of noxious stimulus before recording spontaneous activity for 7–15 min at 0.65, 0.85, and 1.15 iMAC. The isoflurane concentrations used for testing were chosen because they span a concentration range that begins where motor withdrawals to graded noxious thermal stimuli are reliably elicited on nearly every trial (0.65 MAC) and ends at an isoflurane concentration where withdrawals to noxious heat are abolished on nearly every trial (1.15 MAC). At each of these isoflurane concentrations, graded noxious thermal stimuli (48°, 51°, and 54°C; 10 s) were then delivered to the hind paw in ascending order at 5-min intervals, from a baseline temperature of 35°C. For each stimulus, simultaneous neuronal and withdrawal responses to heat were measured. Spontaneous activity was recorded 1 min before each heat stimulus. Neuronal responses were quantified by summing the number of impulses during the 60 s after the onset of the heat stimulus. Hind-limb withdrawals were detected if there was an increase of 0.5 g or more in tension. Peak force and force integrated over time (area under the curve) were calculated for each withdrawal. Spontaneous activity was analyzed using a two-factor analysis of variance (MAC × unit). Neuronal or withdrawal responses at different stimulus temperatures and different MAC concentrations were analyzed by a three-factor analysis of variance (MAC × temperature × unit, or animal) with *post hoc* Tukey multicomparisons. Statistical comparisons were made using commercial software (SPSS, Inc., Chicago, IL). In all cases, a P value of less than 0.05 was considered statistically significant.

Results

Results were obtained from a total of 21 units (10 ON cells and 11 OFF cells) in animals that had a mean isoflurane MAC value of 1.2 ± 0.1% (SD) (n = 19). Histologic verification of 8 ON-cell and 9 OFF-cell recording sites showed that ON and OFF cells were located in the nucleus raphe magnus and surrounding nucleus reticularis magnocellularis and were depicted in section templates from a rat brain atlas[13] (fig. 1).

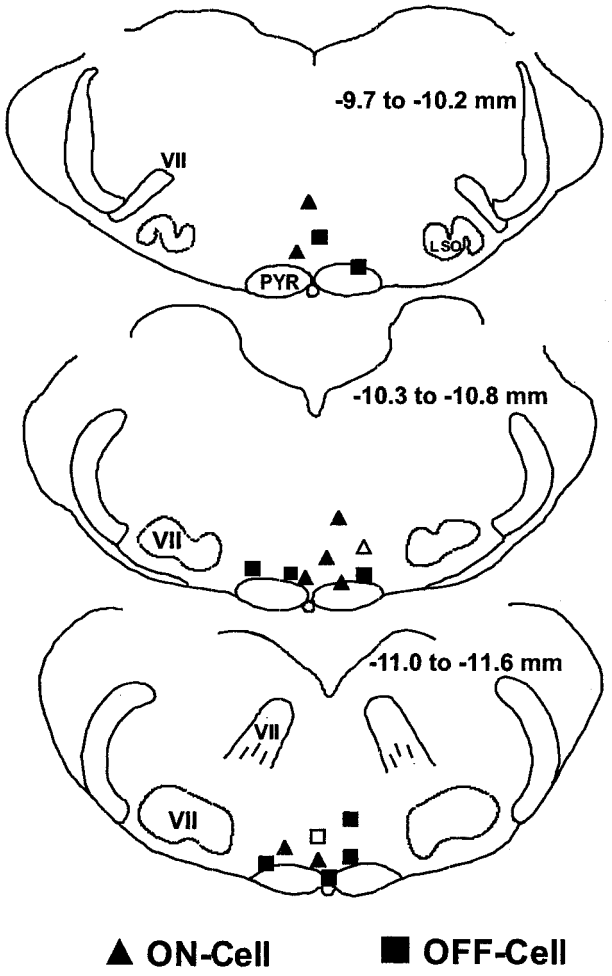

Fig. 1. Histologically identified recording sites of ON cells (*triangles*) and OFF cells (*squares*). Electrolytic lesions were made by passing DC current through the recording electrode. Cross-section templates are in reference to bregma. All sites were located in the nucleus raphe magnus and surrounding nucleus reticularis magnocellularis. Sites indicated by *open symbols* were interpolated using an identified lesion site in the same tract as a reference. LSO = lateral superior olive; PYR = pyramidal tract; VII = facial nerve/nucleus.

At sub-MAC isoflurane concentrations, ON cells were characterized by an excitatory response to hind paw and tail pinch and to noxious thermal hind paw stimulation, whereas OFF cells were inhibited by these stimuli. Individual neuronal and withdrawal responses to noxious mechanical and thermal stimuli are shown for an ON cell at 0.65 MAC isoflurane in figures 2A–C and for an OFF cell at 0.85 MAC isoflurane in figures 2D and E.

Effects of Isoflurane on Spontaneous Activity of ON and OFF Cells

Spontaneous activity was recorded in 10 ON cells and 9 OFF cells for at least 7 min. From 0.65 to 1.15 MAC, isoflurane significantly inhibited spontaneous activity of

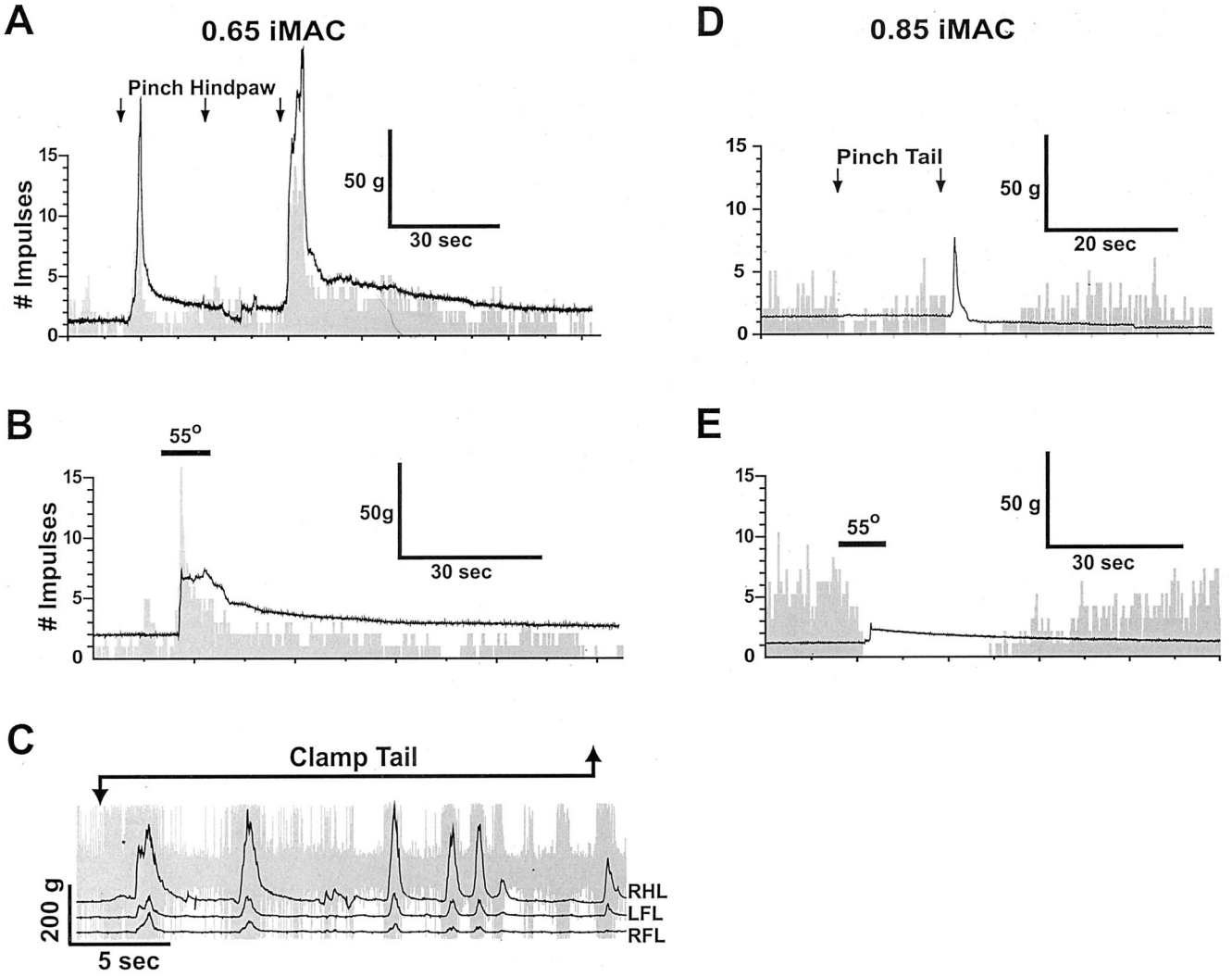

Fig. 2. Individual responses of an ON cell (*A–C*) and an OFF cell (*D*, *E*) to noxious mechanical and thermal stimuli. (*A*) Simultaneous ON-cell responses (*gray histogram*; bin width = 200 ms) and withdrawal responses (*black trace*) to pinching the right hind paw three consecutive times with forceps under 0.65 minimum alveolar concentration (MAC) isoflurane. During the second pinch (*middle arrow*), there was little discernable ON-cell response, which corresponded to only a subtle movement. iMAC = individual MAC value. (*B*) ON-cell excitatory response (*gray histogram*; bin width = 200 ms) to a 10-s noxious thermal stimulus (*thick black horizontal bar*) and corresponding heat-evoked withdrawal (*black trace*) under 0.65 MAC isoflurane. (*C*) ON-cell discharge pattern (*gray, raw trace*) in relation to synchronous multilimb withdrawal forces (*black traces*) elicited by a supramaximal tail clamp under 0.65 MAC isoflurane. ON-cell bursts usually coincided with synchronous, multilimb withdrawals during a continuous 25-s tail clamp. LFL = left forelimb; RHL = right hind limb; RFL = right forelimb. (*D*) Simultaneous OFF-cell inhibitory responses (*gray histogram*; bin width = 200 ms) and withdrawal responses (*black trace*) to pinching the tail two consecutive times with forceps under 0.85 MAC isoflurane. Note that the lesser inhibitory response during the first pinch is associated with a much smaller movement. (*E*) OFF-cell inhibitory response (*gray histogram*; bin width = 200 ms) to a 10-s noxious thermal stimulus (*thick black horizontal bar*) and corresponding heat-evoked withdrawal (*black trace*) under 0.85 MAC isoflurane.

ON cells by 70% (from 15.0 ± 10.0 [SD] impulses/s to 4.5 ± 6.8 impulses/s; $P < 0.001$). In contrast, from 0.65 to 1.15 MAC, isoflurane significantly enhanced spontaneous activity of OFF cells by 138% (from 18.2 ± 14.7 to 43.4 ± 21.7 impulses/s; $P < 0.001$). The most substantial changes in both ON- and OFF-cell spontaneous activity occurred between 0.85 and 1.15 MAC, where immobility occurred. Effects of isoflurane on spontaneous activity are shown in figure 3A for ON cells (n = 10) and in figure 3B for OFF cells (n = 9). From 0.65 to 0.85 MAC, mean ON-cell spontaneous activity was depressed by 36% (from 15.0 ± 10.0 impulses/s to 10.2 ± 9.0 impulses/s), and OFF-cell spontaneous activity was increased by 31% (from 18.2 ± 14.7 to 23.9 ± 19.1 impulses/s. Between 0.65 and 0.85 MAC, changes in ON- and OFF-cell spontaneous activity were insignificant when each class of neuron was analyzed separately. However, when ON and OFF cells were pooled together and the absolute value of changes in spontaneous activity between 0.65 and 0.85 MAC were compared, a significant change was found ($P < 0.003$, paired *t* test). We felt justified in pooling these data because the amount of reflex facilitation or depression is determined by both ON- and OFF-cell activity combined.[14,15]

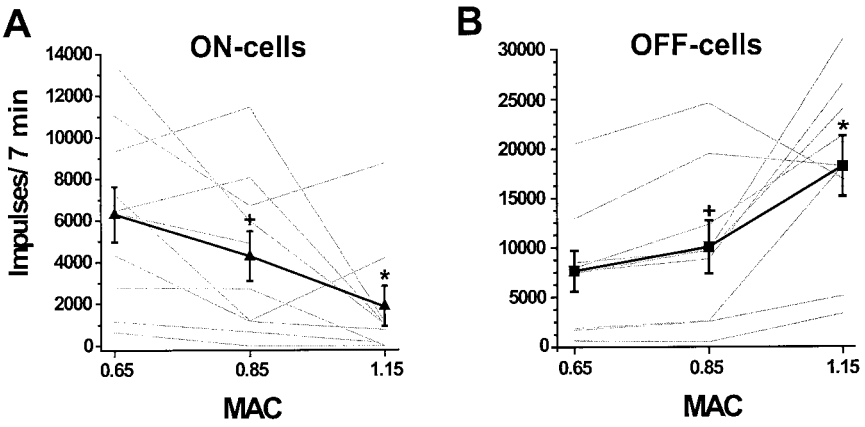

Fig. 3. Line graphs showing mean spontaneous activity of ON cells (*A*) and OFF cells (*B*) at different isoflurane concentrations relative to each animal's minimum alveolar concentration (MAC) value. Each *thin gray line* represents a single neuron's spontaneous activity summed over 7 min at each isoflurane concentration. The *thick black line* represents the mean spontaneous activity at each isoflurane concentration. Isoflurane depressed spontaneous activity of ON cells and facilitated spontaneous activity of OFF cells. *Error bars* = SEM. * Significantly different from 0.65 and 0.85 MAC ($P < 0.001$). + Significantly different from 0.65 MAC when ON and OFF cells were pooled ($P < 0.003$).

Effects of Isoflurane on ON- and OFF-cell Responses to Noxious Heat

Noxious heat–evoked responses of ON cells were significantly depressed by isoflurane at 1.15 MAC compared with 0.65 and 0.85 MAC ($P < 0.001$), whereas OFF-cell responses were significantly enhanced ($P < 0.001$). When the change in activity evoked by heat was calculated by subtracting spontaneous activity recorded during 60 s before the stimulus from the response (number of total impulses fired over 60 s after the onset of heat), isoflurane depressed the heat-evoked change in activity for ON cells but not for OFF cells over all stimulus temperatures. Thus, heat caused a decrease in OFF cell activity at 1.15 MAC that was not significantly different from 0.65 and 0.85 MAC, except for responses to the lowest stimulus intensity of 48° ($P < 0.01$). Individual examples of isoflurane effects are shown for an ON cell in figure 4A and an OFF cell in figure 4B, with raw traces of withdrawal force shown above the responses where withdrawals occurred. Mean heat-evoked changes in activity (spontaneous activity subtracted) are shown in figure 5A for ON cells (n = 10) and in figure 5B for OFF cells (n = 11).

Effects of Isoflurane on Withdrawal Force

Withdrawal force (both peak force and area under the curve) was significantly depressed by isoflurane from 0.65 to 0.85 MAC ($P < 0.001$). At 0.85 MAC, the stimulus–response function showed a rightward shift compared with withdrawals at 0.65 MAC, which together with a decrease in withdrawal incidence indicated an increase in withdrawal threshold by isoflurane (fig. 6). From 0.65 to 0.85 MAC, the smaller changes in ON- and OFF-cell activity were accompanied by a 55% decrease in withdrawal force across all stimulus temperatures. At 1.15 MAC, withdrawals occurred in only 2 of 21 trials (in 19 animals), in which the two withdrawals were small and only occurred at the highest stimulus intensity of 54°C. Thus, at 1.15 MAC, the large changes in ON- and OFF-cell spontaneous activity (and heat-evoked activity of ON cells) were accompanied by a nearly complete absence of motor withdrawals to noxious thermal stimuli.

Relation of ON- and OFF-cell Activity to Noxious Stimulus–evoked Movement

ON- and OFF-cell responses were associated better with the motor withdrawal than the heat stimulus *per se*. Figure 7 shows an individual example in which the animal was subjected to a 54°C stimulus in three consecutive trials. The two trials that elicited a withdrawal also evoked an ON-cell response. In the trial in which no withdrawal occurred, the ON cell did not respond. Raw traces of single-unit activity and withdrawal force for the first and third trials are shown on expanded time scales. Note that the pattern of ON-cell discharge reflects the pattern of withdrawal force and that the magnitude of the response corresponds to the magnitude of the withdrawal from trial to trial. For the first 30 s after a 48°C heat stimulus, the mean excitatory response of ON cells was significantly greater in animals that withdrew from the stimulus (315 ± 377 [SD] impulses/30 s) than for animals that had no detectable withdrawal (38 ± 84 [SD] impulses/30 s; $P < 0.05$, unpaired *t* test). The mean inhibitory response of OFF cells was also greater in animals that showed a withdrawal response (−425 ± 382 [SD] impulses/30 s) than for animals that did not (−107 ± 203 [SD] impulses/30 s; $P < 0.05$, unpaired *t* test). Mean responses of ON cells are shown for animals that had a withdrawal response (fig. 8A) and animals that did not (fig. 8B). Mean inhibitory responses of OFF cells are shown for animals that had a withdrawal response (fig. 8C) and for animals that did not (fig. 8D).

Discussion

The current results provide additional evidence for the role of RVM ON and OFF cells in nociceptive reflex modulation and further suggest that the effects of isoflurane on these classes of RVM neurons might contribute to isoflurane-induced motor depression. Previous studies show that episodes of increased OFF-cell activity, de-

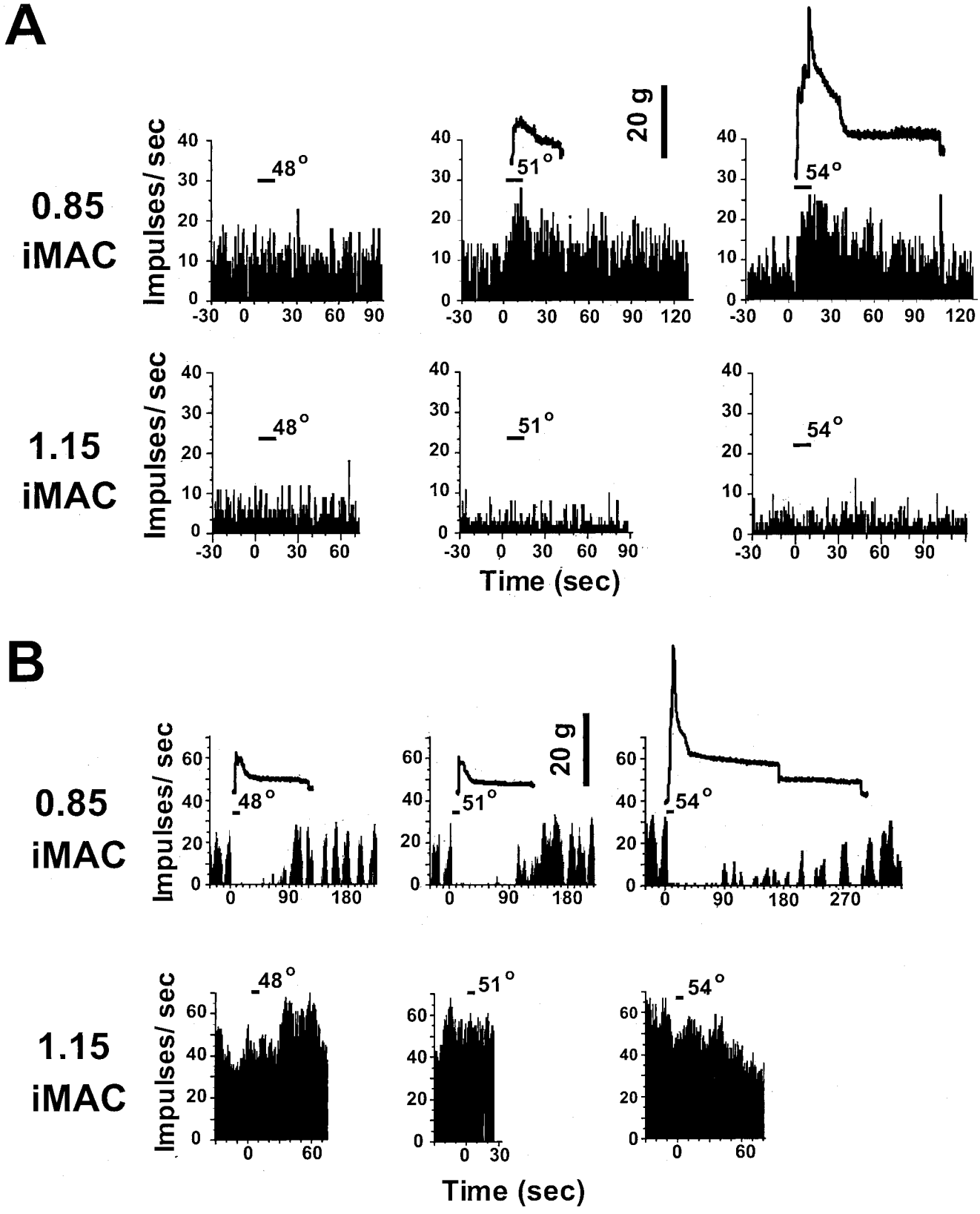

Fig. 4. Peristimulus histograms (bin width = 1 s) of individual unit responses to graded noxious heat. In *A*, ON cell's spontaneous activity and excitatory response to heat are suppressed by isoflurane, with a corresponding abolishment of hind-limb withdrawals at 1.15 times the animal's individual minimum alveolar concentration (iMAC). In *B*, an OFF cell's spontaneous activity is enhanced by isoflurane, while the hind-limb withdrawals are absent at 1.15 iMAC. The 10-s heat stimuli (onset = 0 s) are indicated by the *thick black horizontal bars* placed underneath the stimulus temperature.

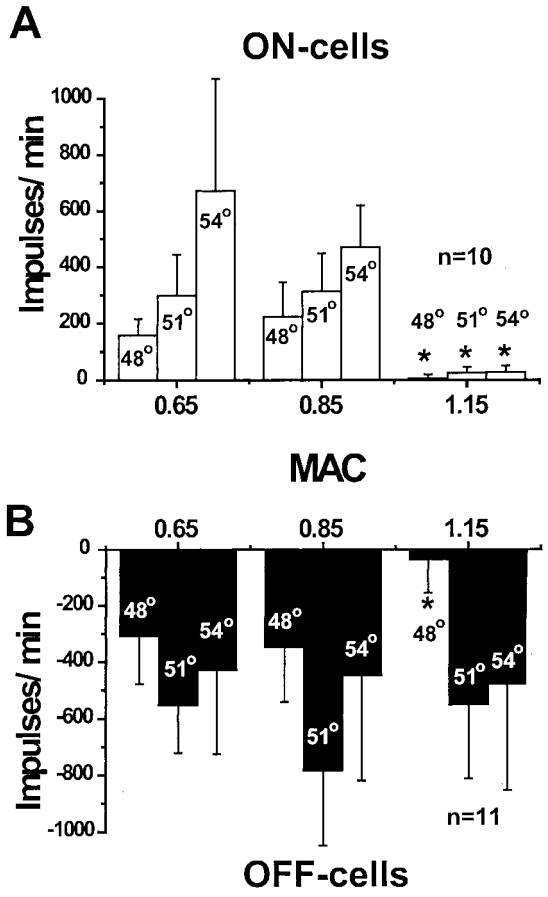

Fig. 5. Mean neuronal responses to graded noxious heat 60 s after the onset of the 10-s heat stimulus for ON cells (*A*) and OFF cells (*B*). Spontaneous activity 60 s before the heat stimulus was subtracted from the total number of impulses during the 60-s response period. ON-cell excitatory responses are substantially reduced, whereas OFF cell inhibitory responses evoked by noxious heat are not significantly different over the 0.65–1.15 minimum alveolar concentration (MAC) isoflurane concentration range, except for the 48°C stimulus at 1.15 MAC. *Error bars* = SEM. * Significantly different from 0.65 and 0.85 MAC ($P < 0.001$).

creased ON-cell activity, or both were coupled with decreases in the efficacy of a noxious stimulus to elicit a nociceptive reflex, and *vice versa*.[16] This is consistent with the current results, in which isoflurane suppressed ON cells (figs. 3A, 4A, and 5A) and facilitated OFF cells (figs. 3B and 4B) while depressing withdrawal responses to noxious heat (fig. 6). Previous studies suggest OFF-cell responses are reflex related because they correspond better to tail-flick latency than to the stimulus *per se*,[17] as confirmed currently. When animals were subjected to the same heat stimulus at sub-MAC anesthetic concentrations, excitatory ON-cell responses and inhibitory OFF-cell responses were significantly larger for those animals that exhibited a withdrawal (fig. 7). Furthermore, increases in both ON-cell responses and withdrawal force occurred with increases in the thermal stimulus intensity.

Role of RVM Neurons in Nociceptive Reflex Modulation

The relative amounts of ON- and OFF-cell spontaneous activity have been shown to be important in determining nociceptive responsiveness, such that ON-cell firing facilitates and OFF-cell firing inhibits a nociceptive reflex.[14] Moreover, hyperalgesic states are marked by increased ON-cell activity,[9,18] whereas increased OFF-cell activity occurs during analgesia.[7] A recent study used a double-pulse laser stimulation paradigm,[15] which showed that the amount of reflex facilitation corresponded to the combined amount of both ON- and OFF-cell activity at the time of the second pulse. Because ON and OFF cells seem to function in concert to determine the efficacy of a nociceptive reflex, the small changes in ON- and OFF-cell spontaneous activity we observed between 0.65 and 0.85 MAC, when combined (67%), could have accounted for much of the 55% decrease in force production seen between these two anesthetic concentrations. Larger changes in spontaneous activity that occurred in both ON and OFF cells from 0.85 to 1.15 MAC (fig. 3) were accompanied by a nearly complete absence of withdrawals to noxious heat (fig. 6). Although we found that the absolute amount of heat-evoked decrease in mean OFF-cell activity remained more or less unchanged by isoflurane (fig. 5B), it is

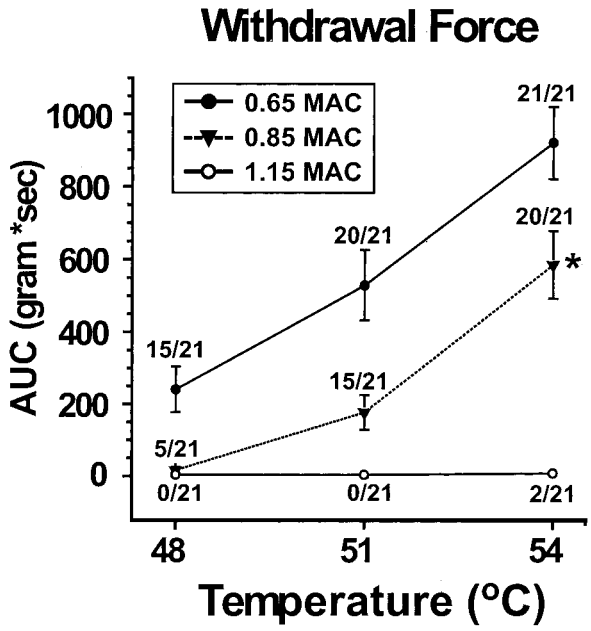

Fig. 6. Mean stimulus–response functions for the area under the curve (AUC) of withdrawal force. Withdrawal force was significantly depressed by isoflurane from 0.65 to 0.85 minimum alveolar concentration (MAC) ($P < 0.001$) and abolished at 1.15 MAC, except for two animals that showed small withdrawals to the highest stimulus intensity. *Fractions* indicate the proportion of trials in which an animal exhibited a withdrawal (for each of 21 neurons in 19 animals), at each stimulus temperature and each MAC concentration. Withdrawal incidence was dose-dependently decreased by isoflurane. *Error bars* = SEM. * Significantly less than withdrawal force at 0.65 MAC across all stimulus temperatures.

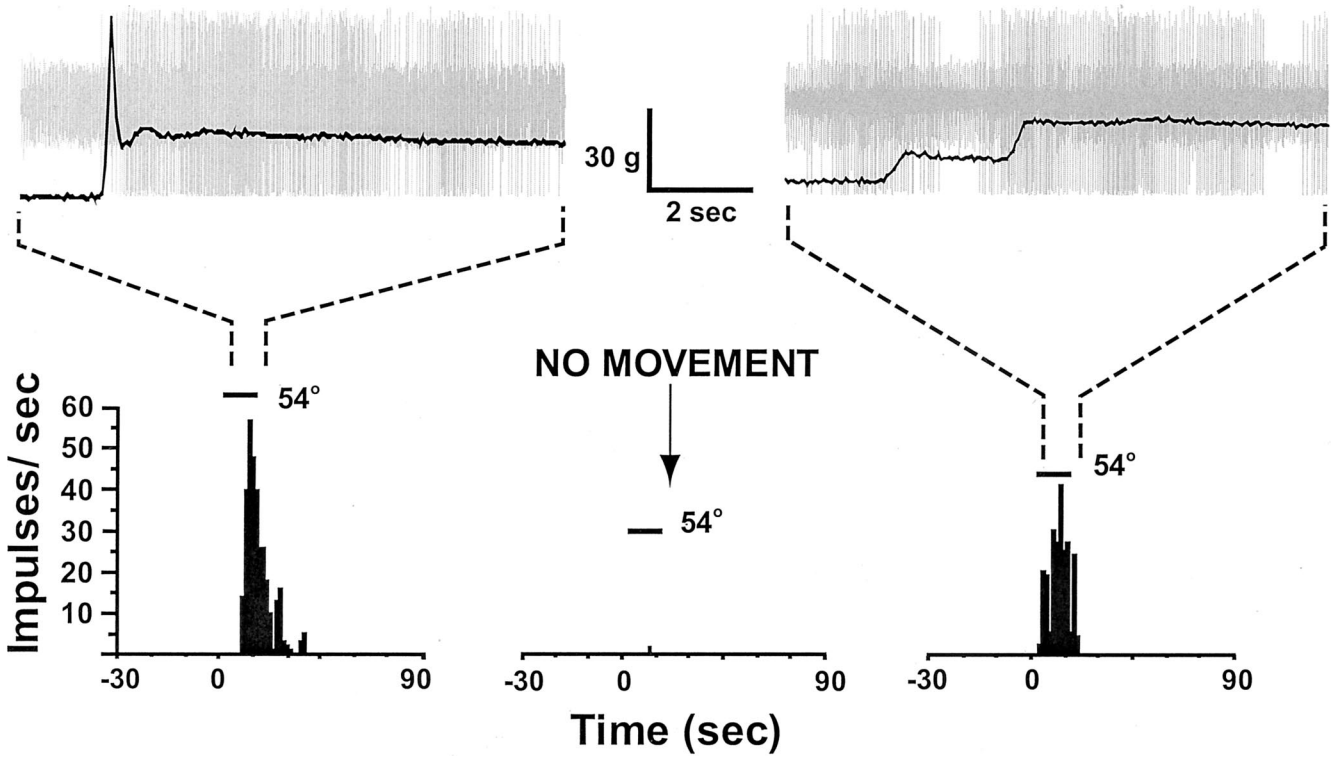

Fig. 7. An ON cell's response corresponded better to the presence or absence and magnitude of a withdrawal response rather than the stimulus *per se*. Responses to three identical thermal stimulus presentations, in which little to no response occurred during the trial where no withdrawal was detected (*middle*, *bottom*). For the two withdrawals, the larger ON-cell response is associated with a larger withdrawal (first *vs.* third trial). *Raw single-unit* and *force traces* are shown on an expanded time scale above the peristimulus histograms (bin width = 1 s) for the two trials during which a withdrawal occurred. Note that the pattern of the response reflects the pattern of the withdrawal.

possible that at 1.15 MAC, isoflurane increased OFF-cell spontaneous activity to a point at which heat-evoked decreases in tonic firing are insufficient to permit disinhibition of the reflex (fig. 4B).

Increases in ON-cell activity and decreases in OFF-cell activity evoked by noxious stimuli usually occur shortly before the onset of a nociceptive reflex, and thus the RVM was initially proposed to have a permissive, or

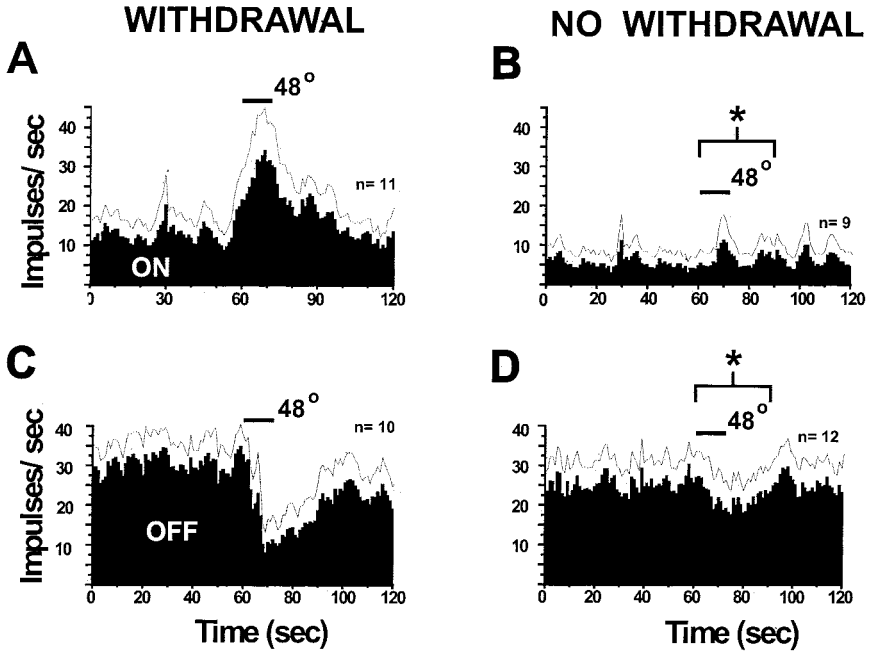

Fig. 8. Mean histograms (bin width = 1 s) showing responses of ON cells (*A*, *B*) and OFF cells (*C*, *D*) to a 48°C stimulus at sub–minimum alveolar concentration (MAC) (0.65 and 0.85 MAC, data pooled) concentrations. Unit responses were segregated according to whether the animal exhibited a withdrawal (*left*) or had no detectable movement (*right*). Excitatory ON-cell responses and inhibitory OFF-cell responses to heat were significantly greater in animals that exhibited a withdrawal than in those that were not accompanied by a withdrawal ($P < 0.05$ in both cases, unpaired *t* test). In contrast, mean responses for ON and OFF cells to 48°C were not significantly different when the same responses were segregated according to anesthetic concentration (fig. 5). *Thin broken line* = SEM. * Significantly decreased response compared with trials in which withdrawals occurred.

"gating," role in reflex initiation. However, with rapid thermal laser stimulation, ON-cell bursts and OFF-cell pauses usually begin shortly after the onset of tail-flick electromyographic activity.[15] This suggests that ON and OFF cells are not likely to play a role in reflex initiation, but rather they may play a role in modulating the reflex after its initiation or in response to subsequent noxious stimuli. In the current study, the more slowly rising contact heat stimuli (12°C/s) elicited ON- and OFF-cell responses that usually (although not always) preceded withdrawals (figs. 2 and 7). Given that noxious stimuli encountered by an animal in its natural environment are of variable intensity and speed of onset, it is possible that ON cells, OFF cells, or both might have a permissive (reflex-initiating) role as well as the capability to modulate nocifensive behavior after its initiation.

It is possible that ON-cell and OFF-cell activity reflects, rather than causes, modulation of nociceptive motor output. However, microinjection of opioids into the RVM causes both ON-cell inhibition and OFF-cell facilitation while inhibiting the tail-flick reflex,[19] arguing in favor of a direct role for the RVM in modulating nociceptive reflexes. Furthermore, when RVM neurons were recorded through the electrode used for stimulation, OFF cells were most frequently recorded close to sites at which electrical stimulation elicited the greatest degree of tail-flick reflex inhibition.[20] Moreover, local microinjection of α_1-noradrenergic agonists excites ON cells[21] while producing hyperalgesia.[22] However, a role for ON cells in modulation of nociceptive reflexes is still somewhat less clear than for OFF cells. Kynurenate microinjection into the RVM (to block excitatory amino acid transmission) selectively blocks ON-cell activity without changing tail-flick latency, while OFF-cell activity and its inhibition by noxious heat remains unaffected.[23] Overall, the majority of evidence from previous studies suggests that the current changes in ON- and OFF-cell activity by isoflurane contributed to the depression of limb withdrawal force.

Nearly all previous studies of RVM ON and OFF neurons measured reflex latencies alone (but see Foo and Mason[15]). However, the precise latency of the neuronal response is inherently difficult to determine because of the cyclic spontaneous firing patterns in ON and OFF cells. We currently opted for a paradigm that addressed the role of ON- and OFF-cell activity in relation to the magnitude and pattern of motor withdrawals. Using this model, we found that ON- and OFF-cell responses correspond to the presence and magnitude of the withdrawal (figs. 5, 7, and 8).

Anesthetic Sites of Immobilizing Action

The specific neuronal populations involved in depression of sensorimotor processing by anesthetics are largely unknown. Using MAC determination, several anesthetics have been shown to block movement *via* a spinal action, albeit with varying degrees of supraspinal effects.[1–3] We currently tested graded isoflurane effects on descending modulation by quantifying the motor output in terms of withdrawal force, in lieu of using an "all-or-none" MAC determination. We have recently validated the thermal hind-limb withdrawal force method as relevant to MAC while permitting quantification of graded anesthetic effects on motor and neuronal responses simultaneously.[12] The current results confirm the model's clinical relevance in that withdrawals were greatly reduced or abolished just above each animal's MAC value.

The current data support our earlier hypothesis[24] that under conditions of high anesthetic concentration in the brain, ON cells are relatively more depressed than OFF cells, leading to a net balance favoring descending inhibition in the spinal cord. Additional limited evidence in support of this hypothesis derives from a previous study,[25] which showed that isoflurane at presumed peri-MAC concentrations depressed ON cells to a greater extent than OFF cells, which were variably affected. The current study, which showed a dose-dependent increase in OFF-cell spontaneous activity, had the added advantage of determining each animal's iMAC value before testing to control for individual differences in sensitivity to isoflurane.

Barbiturate anesthetics have been shown to drastically change response characteristics of RVM neurons compared to the awake state, in which only low-threshold excitatory ON neurons are found, whereas high-threshold neurons and OFF cells are not found until the animal is anesthetized.[26] In unstimulated awake animals, others have found OFF cells, which were characterized by facilitatory effects of morphine.[27] These previous studies suggest that OFF cells may become profoundly inhibited by noxious stimuli in the awake state and are more resistant to inhibitory effects of noxious stimuli in the anesthetized or analgesic state.

From the current results, it is not possible to conclude whether isoflurane exerted its effects directly in the RVM. However, changes in RVM activity by isoflurane cannot be accounted for entirely by a spinal effect. That isoflurane has little to no effect on evoked responses of dorsal horn neurons in the peri-MAC range[12,24,28,29] argues against a spinally mediated action of isoflurane on blocking ascending input to the RVM from the spinal cord. It is possible that reductions in dorsal horn spontaneous activity by isoflurane could account for reductions in withdrawal force. However, in our previous study,[12] dorsal horn neurons exhibited higher spontaneous firing rates under supra-MAC concentrations of halothane, where little to no movement occurs, than under sub-MAC concentrations of isoflurane, where movement does occur. Also, one would expect that any spinal action would depress ON- and OFF-cell responses to

noxious heat equally, which was not the case. However, because OFF-cell spontaneous activity was lower under sub-MAC compared with a supra-MAC isoflurane concentration, the effects of isoflurane on OFF-cell responses to heat may have been underestimated because of a possible saturation of the OFF-cell inhibitory response at lower isoflurane concentrations.

Isoflurane may act at other supraspinal sites to mediate its effect on RVM neurons. One such site is the midbrain periaqueductal gray, which projects to the RVM as part of a descending antinociceptive circuit to mediate opiate-induced analgesia.[30] Interestingly, there are also ON and OFF cells in the periaqueductal gray with properties similar to those in the RVM.[31] The similar actions of isoflurane and morphine on ON and OFF cells raise the possibility that isoflurane could act on endogenous opioidergic systems in the periaqueductal gray, the RVM, or both to decrease the force of movement elicited by a noxious stimulus. MAC *per se* is not affected by naloxone for several volatile anesthetics,[32,33] but sevoflurane MAC increases by approximately 20% in μ-opioid receptor knockout mice compared with wild types.[34] However, another study showed that μ-opioid receptor knockout mice exhibit significant increases in *N*-methyl-D-aspartate glutamate receptor messenger RNA.[35] It is therefore unclear whether the MAC increase in knockout mice was due to absence of opiate receptors or to an *N*-methyl-D-aspartate receptor–mediated increase in neuronal excitability. The effect of isoflurane on noradrenergic systems is also a possibility. Norepinephrine excites ON cells, although it is without effect on OFF cells.[21] It was reported that isoflurane (up to approximately 1 MAC) caused a supraspinally mediated facilitation of tail-flick latencies, which was partially reversed by selective ablation of noradrenergic neurons.[36]

Currently, when spontaneous firing was subtracted from heat-evoked responses, there was a marked, concentration-dependent reduction in the ON-cell burst, whereas there was a much lesser reduction of the OFF-cell pause that occurred in relation to reflex movement (fig. 5). Because ON-cell activity is dependent on glutamatergic transmission[23] and OFF-cell inhibition is γ-aminobutyric acid type A receptor mediated,[16] peri-MAC concentrations of isoflurane might therefore have a more pronounced glutamate-depressant action than a γ-aminobutyric acid–enhancing action. This is consistent with a recent study, which shows relatively greater effects of halothane on glutamatergic transmission than on γ-aminobutyric acid–mediated transmission.[37]

In general, results from the current study as well as our previous study[29] suggest that descending modulation of nociceptive processing remains functionally intact at sub-MAC isoflurane concentrations but becomes depressed or abolished along with noxious stimulus–evoked movement within the immediate peri-MAC concentration range (*i.e.*, approximately 0.8–1.2 MAC). Further studies are necessary to fully understand brainstem–spinal cord interactions involved in nociceptive sensorimotor processing and the specific sites and mechanisms by which anesthetics act in this circuitry to ablate movement.

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