Brainstem Regions Affecting Minimum Alveolar Concentration and Movement Pattern during Isoflurane Anesthesia

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ABSTRACT

Background: Spinal transection or selective delivery of volatile anesthetics to the spinal cord reduces minimum alveolar concentration (MAC), whereas precollicular decerebration does not. The authors sought to determine which brainstem regions influence anesthetic requirements and movement responses with isoflurane.

Methods: Movement (biceps femoris electromyogram) and MAC were measured in adult rats before and after decerebration at the precollicular, mid-collicular, pontine or medullary level, or decerebellation. Additional experiments assessed the effects of lidocaine inactivation of the mesencephalic locomotor region on MAC and the effects of isoflurane on nociceptive neuronal responses in this region.

Results: Transections placed at the level of the mid-colliculus, rostral pons, and pontomedullary junction significantly reduced MAC by approximately 10, 40, and 45%, respectively. MAC was decreased 9% after mid-medullary transections that were placed caudal to the nucleus raphe magnus but rostral to the dorsal reticular nucleus; however, only weak, single movements occurred. Caudal medullary transections at the obex decreased MAC by 60%. Bilateral inactivation of the mesencephalic locomotor region with lidocaine caused a reversible, 32% decrease in MAC and reduced the number and amplitude of movements at sub-MAC isoflurane concentrations. Neuronal responses of mesencephalic locomotor region neurons to supramaximal noxious tail clamp were reduced by 87% by 1.2 MAC isoflurane.

Conclusions: The authors conclude that the mesencephalic locomotor region influences anesthetic requirements and promotes repetitive movement with sub-MAC isoflurane by facilitating ventral spinal locomotor circuits, where anesthetics seem to exert their key immobilizing effects. However, net brainstem influences on MAC seem to result from interaction among descending nociceptive and locomotor modulatory pathways.

What We Already Know about This Topic

❖ Minimum alveolar concentration (MAC) is defined by lack of movement to incision and largely reflects anesthetic inhibition in the spinal cord
❖ Brainstem–spinal circuits facilitate movement to incision, but the sites relevant to MAC are not well known

What This Article Tells Us That Is New

❖ In normal rats, MAC is reduced by injury or inhibition of the mesencephalic locomotor region, suggesting that this motor regulatory site counteracts anesthetic actions in the spinal cord for immobilization to surgery

VOLATILE anesthetics act primarily in the spinal cord to abolish movement in response to noxious stimulation.1–3 However, selective delivery of isoflurane to the spinal cord (keeping cranial isoflurane concentration low) reduces isoflurane minimum alveolar concentration (MAC) by more than 30%.4 Moreover, in rats, chronic spinal transection reduces MAC by approximately 50% in the absence of spinal shock.3 This means that the supraspinal regions contribute to determining anesthetic immobilizing requirements, apparently by counteracting a direct depressant action in the spinal cord through descending facilitation. Because precollicular decerebration does not significantly change MAC or the type of movement elicited by a noxious stimulus at sub-MAC isoflurane concentrations,5,6 the important supraspinal sites responsible for determining anesthetic requirements in an intact animal lie in the brainstem.

One candidate site is the mesencephalic locomotor region (MLR), based on its ability to initiate locomotion through descending facilitation of locomotor circuit in the ventral horn,7 where anesthetics predominantly act to produce immobility.5,8–10 Furthermore, it has been shown that noxious stimulation sufficient to elicit motor reflexes evokes neuronal responses in mesencephalic areas associated with the MLR,11 namely the cuneiform and pedunculopontine nuclei.12

We hypothesized that MAC values would decrease on removal of descending locomotor facilitation: after brainstem transections associated with removal of the MLR or during local inactivation of the MLR with lidocaine micro-

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pronociceptive area, should then decrease MAC to values seen in spinally transected animals. Finally, MTTs were referenced to bregma at coordinates of 1/1000 to 1/11000.

sections that remove the dorsal reticular nucleus (MdD), a pronociceptive area, should then decrease MAC to values seen in spinally transected animals.

transections were based on viewing the colliculi. Pontomedullary and mid-medullary transections were based on the rostral reticulotegmental area (RRT), where perijMAC isoflurane produces a net increase in descending inhibition. Finally, MTTs were referenced to bregma at coordinates of 1/1000 to 1/11000.

view subcortical structures, and transectioning the brain with a scalpel blade. Precollicular, mid-collicular, and rostral pontine transections were based on removal of the rostral ventromedial medulla (RVM), where perijMAC isoflurane produces a net increase in descending inhibition.

Caudal medullary transections were based on viewing the obex. Brain structures rostral to the transection were aspirated, and gelfoam was gently placed against the cut end of the transection. We performed only one transection in each animal.

Biceps femoris electromyogram signals were amplified and band-pass filtered (10 Hz to 2 kHz) with a Tektronix differential amplifier (model 2601; Beaverton, OR). Electromyogram was fed to a Cambridge Electronic Design, Power 1401 data acquisition system with Spike 2 software (Cambridge Electronic Design, Cambridge, United Kingdom).

MAC Determination We recorded electromyogram responses and determined MAC in each animal before and more than 90 min after a decerebration at the level of the mid-colliculus, rostral pons, pontomedullary junction, mid-medulla, and caudal medulla or after decerebellation. MAC was determined using a tail clamp. Animals were anesthetized with isoflurane, and the clamp was applied for up to 1 min or until movement was observed. A positive response was based on observation of multisegmentally mediated movement (movement of a limb or limbs in response to tail clamp). Typically, head turning is also considered a positive response in MAC testing, which was not assessed in the current study because the experiments necessitated fixing the head to the stereotaxic frame. However, in our experience, head turning in the absence of limb movement is less common, and our MAC testing criteria yielded typical baseline MAC values (see Results). Depending on the response, the anesthetic concentration was changed in 0.2% increments with an intervening 15–20 min equilibration period. The average of the two values that just permitted and just prevented movement was MAC.

MLR Identification and Lidocaine Microinjection In precollicular decerebrate animals, an Ag-AgCl stimulating electrode was placed inside a glass pipette (outer tip diameter: 50–100 μm) filled with saline alone or 4% lidocaine (Sigma, St. Louis, MO) in saline. The injection pipette/electrode was lowered into the midbrain to search for the MLR using electrical stimulation. By using the center of the intercollicular crux as a zero reference point, we positioned the stimulating electrode 1.8–2.0 mm lateral to the midline and ±0.5 mm anterior-posterior from this point. Constant-current electrical pulses (0.5 ms pulse duration, 60 Hz) were passed through the electrode using a PSIU6 stimulus isolation unit connected to an S88 stimulator (Grass-Telefactor, Warwick, RI). When a site was found to elicit locomotion, we decreased and increased the stimulus intensity and finely adjusted the position of the electrode to determine the lowest threshold site for four-limb galloping (threshold range: 20–60 μA).

After MLR identification on each side of the midbrain, lidocaine (4%, 1.0 μl) was injected into the MLR bilaterally at 0.8 MAC, and tail clamp was applied every 5–10 min until
recovery was observed. If the animal failed to respond to the tail clamp (negative MAC test), after recovery, the isoflurane concentration was decreased by 0.2 MAC, and the lidocaine injections were repeated. In pilot studies, a 0.5-μl volume of lidocaine (4%) decreased MAC in 50% of animals only, whereas a 1.0-μl volume decreased MAC to more than 20% in all animals tested.

**Electrophysiology**

During isoflurane anesthesia (1.5–1.8%), we performed a precollicular decerebration and a lumbar laminectomy to dissect free L4–5 ventral roots. After a 90-min postdecerebration recovery period and verification that MAC had returned to more than 90% of control, we searched for the MLR using low-threshold electrical stimulation as previously described.6 We then paralyzed the animal with pancuronium bromide (0.6 mg · kg⁻¹ · h⁻¹), cut ventral roots distally, and placed them bilaterally on platinum hook electrodes that were insulated with a vaseline and mineral oil mixture for electroneurogram recording. Ventral root electroneurogram activity was recorded to monitor motor output while recording single-unit activity in the MLR. The tungsten MLR microelectrode (8–10 MΩ, FHC, Bowdoinham, ME) was switched from stimulation to recording, and we isolated a single neuron that responded to noxious mechanical tail stimulation. Single-unit activity was band-pass filtered (300 Hz to 5 kHz) with a Grass p511 amplifier (Grass-Telefactor, Warwick, RI) and acquired on a PC using a Power 1401 system with Spike 2 software. After a MLR unit was isolated, supramaximal noxious mechanical stimulation (tail clamp) was applied for 30 s with 0.0, 0.4, 0.8, and 1.2 MAC isoflurane. The total number of action potentials and peak firing rate during tail stimulation were measured at each isoflurane concentration.

**Histology**

At the end of experiments, animals were killed with saturated KCl with isoflurane, and the remaining brainstem was removed and placed in formalin for at least 24 h, followed by 30% sucrose. Brainstems were cut sagittally, and sections from several mediolateral levels on each side were mounted, counterstained with cresyl violet, and coverslipped. These sections were used to verify the level of transection. If the intended transection was actually placed at the level of another group, that animal was reassigned to the appropriate transection group. In three cases, transections were found to be at a level not consistent with any of the transection groups, and these animals were excluded from the study.

**Statistics**

Changes in MAC for each transection group were assessed by comparing posttransection MAC with each group’s respective intact MAC value using a two-tailed paired t test with a Bonferroni correction for multiple comparisons. Within-group comparisons of movement number and peak movement amplitudes were made for the MLR lidocaine injection group and for the mid-medullary transection group using a two-tailed paired t test. Comparisons between groups of animals were made using a two-tailed unpaired t test. A one-way ANOVA with “neuron” as a random effects factor, followed by Tukey multicomparsions, was used to compare changes in MLR neuronal activity across isoflurane concentrations. A P value less than 0.05 was considered statistically significant. Data analyses were performed using SPSS (Chicago, IL).

**Results**

**Effects of Brainstem Transection and MLR Inactivation on Isoflurane MAC**

Pretransection (intact) isoflurane MAC values were 1.3% atm ± 0.1% SD. Transections placed at the mid-collicular level (n = 9) caused a small but significant decrease in MAC to 90 ± 5% SD of intact MAC values (P < 0.006). Transections placed in the pons, immediately caudal to the inferior colliculus (n = 8) or near the pontomedullary junction (n = 9), substantially decreased MAC to 60 ± 9% SD and 55 ± 5% SD of intact MAC values, respectively (P < 0.0006 in both cases). However, when transections were placed caudal to the nucleus raphe magnus/gigantocellularis pars alpha, but rostral to the MdD (n = 15), MAC decreased to only 91 ± 8% SD of intact MAC values (P < 0.03). Isoflurane MAC dramatically decreased to 40 ± 3% SD (P < 0.0006) in animals that received obex-level transections, at the caudal edge of the MdD and within the trigeminal nucleus (n = 6). Animals that received a complete cerebellectomy did not exhibit a significant change in their MAC values (95 ± 9% SD of intact MAC; n = 7). Figure 1 shows individual examples of movement/MAC changes after a rostral transection of the pons (fig. 1A) and after a mid-medullary transection (fig. 1B). Figure 2A shows mean MAC values in separate groups of animals receiving different levels of brainstem transections, lidocaine microinjection into the MLR, or cerebellectomy. Figure 2B shows the range of transection levels of each group that we histologically examined.

Bilateral inactivation of the MLR by lidocaine microinjection (n = 10) caused a 32 ± 6% decrease in MAC (P < 0.0001) that recovered 20–60 min after injection. The line graph in figure 2 shows mean MAC values after lidocaine microinjection into the MLR. An individual example showing the reversible effect of MLR lidocaine microinjection on tail clamp-evoked hindlimb electromyogram activity is shown in figure 3. As a control for lidocaine spread, six animals received 0.5 μl lidocaine (4%) injections 700 μm dorsal and 700 μm ventral to the site producing locomotion in response to low-threshold electrical stimulation, and in no cases did these injections change MAC (n = 6).

**Effects of Brainstem Transection and MLR Inactivation on Noxious Stimulus-evoked Movement Pattern**

Under 0.6 MAC (pre-lidocaine injection MAC), microinjection of lidocaine into the MLR significantly reduced the...
amplitude ($P < 0.012$) and number of movements ($P < 0.02$) elicited by a supramaximal noxious mechanical tail stimulus (fig. 4). No animals displayed positive movement at 0.8 MAC after lidocaine injection. Low levels of tonic electromyogram activity were often detectable, but this did not translate to observable movement (criterion for a positive response in MAC testing). Effects of MLR lidocaine microinjection on mean amplitude and number of movements are shown in figure 4.

Although MAC was only modestly reduced after mid-medullary transections, movement was weaker and non-repetitive, where animals only displayed a single movement at the onset and/or offset of tail clamp application. Both movement number and amplitude with 0.8 MAC isoflurane were significantly reduced in this group ($n = 6$) compared with intact control values ($P < 0.03$ in both cases).

**Effects of Isoflurane on Neuronal Activity in the MLR**

Neuronal responses to supramaximal noxious mechanical tail stimulation exhibited bursting behavior that was associated with movement bouts detected in ventral root recordings under sub-MAC isoflurane concentrations. Isoflurane significantly reduced the total action potentials and peak firing rate of MLR neuronal responses ($n = 11$) to tail clamp at 1.2 MAC, compared with responses recorded under anesthetic-free baseline (0.0 MAC), 0.4 MAC, and 0.8 MAC ($P < 0.001$ for all comparisons). With 1.2 MAC isoflurane, MLR total action potentials were reduced to $13 \pm 9\%$ SD of control (0.0 MAC), and peak firing rate was reduced to $34 \pm 14\%$ SD. The total number of action potentials with 1.2 MAC isoflurane was significantly reduced to $16 \pm 15\%$ SD of 0.8 MAC values, and peak firing rate was reduced to $49 \pm 22\%$ SD (fig. 5). From 0.0 to 0.8 MAC, the total number of spikes was not changed significantly; however, peak firing rate was significantly decreased to $74 \pm 8\%$ SD of control ($P < 0.034$). An individual example of isoflurane effects on tail clamp-evoked responses of an MLR neuron is shown in figure 5A. Mean isoflurane effects on MLR neurons ($n = 11$) are shown in figure 5B, and histologically identified recording sites are illustrated in figure 5C.

**Discussion**

We investigated the influences that different brainstem regions had on isoflurane MAC and noxious stimulus-evoked movement patterns and isoflurane effects on movement-related neurons in the MLR. Although volatile anesthetic-induced immobility is explained by a direct anesthetic action in the spinal cord, the literature collectively suggests that the brainstem, but not forebrain regions, indeed plays a critical role in establishing the precise anesthetic immobilizing requirements in an intact animal. This was shown in previous studies reporting that precollicular decerebration does not change MAC,\(^5,\(^6\) whereas chronic spinal transection, reversible spinal cold block, and selective perfusion of the spinal cord (in goats with an intact nervous system) all decrease MAC by 30–50%.\(^3,\(^4\) Overall, our data suggest that facilitation of spinal locomotor networks from the MLR, nociceptive inhibition from the RVM, and nociceptive facilitation from the
Brainstem Influences on MAC and Movement Pattern

Fig. 2. Isoflurane minimum alveolar concentration (MAC) after different lesions of the brainstem and inactivation of the midbrain locomotor region (MLR) by lidocaine. Data are shown as mean and SEM (A) Mean isoflurane MAC values. Dashed lines denote transections. Precollular decerebration did not significantly change MAC. MAC was slightly but significantly reduced to 90% of control (+, P < 0.006) after mid-collicular transections that began to encroach on or removed part of the pedunculopontine nucleus (PPN) associated with the MLR. Inactivation of the MLR with bilateral lidocaine microinjections (shaded) decreased MAC to 68% of control (*, P < 0.001), whereas transections ranging from the rostral pons to the pontomedullary junction reduced MAC to 60% (**, P < 0.0006) of control (all pontine transection data pooled in figure). Mid-medullary transection only reduced MAC to 90% of control (%P < 0.03). Caudal medullary transections at the level of the obex caused MAC to decrease to approximately 50% of control ($, P < 0.0006) (B) The same sagittal template as in (A) depicting the actual range of transection levels (shaded) in each group, verified histologically. 1 = mid-collicular transection range; 2 = rostral pons transection range; 3 = pontomedullary transection range; 4 = mid-medullary transection range. Precollular decerebrations (gray dashed line) and obex-level transections (black dashed line) were made by viewing the superior colliculus and obex, respectively, but they were not histologically examined. CN = cuneiform nucleus; 7 = facial nucleus; Vc = trigeminal subnucleus caudalis. * = significantly different from intact MAC (paired t test).

MdD contribute to modulating MAC (see proposed model in fig. 6). We discuss our current findings in the context of the role and relative importance of different brainstem regions in modulating isoflurane MAC, effects of isoflurane on these regions, and the limitations of the study.

Descending Locomotor Command and MAC

In particular, we were interested to study the influence of the MLR on MAC and isoflurane effects on movement-related neurons in the MLR that were activated by supramaximal noxious mechanical stimulation. We found that brainstem transections that encroached on the MLR (i.e., mid-collicular transections) caused a small decrease in MAC, and transections immediately caudal to the MLR caused substantial decreases in MAC. Moreover, a 32% decrease in MAC occurred during local MLR inactivation with lidocaine. We also found that peri-MAC isoflurane significantly suppresses noxious stimulus-evoked neuronal responses of MLR neurons. Combined spinal depressant and supraspinal facilitatory actions may underlie immobilizing requirements for other anesthetic classes, as this was previously implicated for barbiturates16,17 (although tail flick, not MAC, was assessed).

Fig. 3. Midbrain locomotor region (MLR) inactivation reduces minimum alveolar concentration (MAC). (A) Electrical stimulation was used to perform a functional search for the MLR, and lidocaine was injected bilaterally at sites producing the low-threshold hindlimb locomotion (right). (B) Normal electromyogram hindlimb motor response to noxious tail clamp. (C) The same animal was immobile during tail clamp with 0.8 MAC isoflurane after bilateral lidocaine microinjection into the MLR. (D) Robust and repetitive tail clamp-elicited movement recovered 60 min after MLR lidocaine microinjection. R.BF = right biceps femoris; L.BF = left biceps femoris.
Significantly different from control (*). ** = significantly different from control (P < 0.02; paired t test).

Peak firing rate of MLR neurons was significantly suppressed by 26% at 0.8 MAC, suggesting that this was perhaps a more sensitive measure of sub-MAC motor depression that occurs between 0.4 and 0.8 MAC. Effects of isoflurane on the overall activity of MLR neurons were predictive of MAC, because responses were significantly decreased only between 0.8 and 1.2 MAC, but by 87%. This suggests that isoflurane-induced disfacilitation from the MLR plays a role in the brainstem-mediated changes that finally lead to immobility. However, isoflurane suppression of MLR activity is perhaps a necessary, but not sufficient, condition for the final transition to immobility, because we found that mid-medullary transections removed an inhibitory influence on MAC (see Descending Nociceptive Modulation and MAC).

The current study was limited in addressing some factors to consider. Because noxious stimuli activated MLR neurons, isoflurane action in the spinal cord could have indirectly affected facilitation from the MLR. In addition, an increase in MAC from descending facilitation does not necessarily imply that MLR neurons are resistant to isoflurane. Therefore, it is conceivable that anesthetic effects on mutual interactions between the brainstem and the spinal cord ultimately result in net effects of isoflurane on brainstem or spinal neurons, and MAC being greater in intact compared with spinalized animals.

One potential concern with the current study and with previous studies on cranial-bypassed goats is trauma alone might influence MAC. A recent study using emulsified isoflurane in goats avoided potential traumatic effects by isoflurane injection into the aorta to achieve a preferential body delivery, which showed that MAC was unchanged. Although data from a nontraumatic preparation are informative, there was no evidence in the current study or in previous cranial-bypassed goat studies that trauma appreciably influenced MAC. MAC values of goats recover postbypass, and a MAC increase, not decrease, occurs when isoflurane concentration to the brain is selectively increased. In the latter study, Antognini and Borges found that MAC was decreased when isoflurane was selectively administered to the torso and at low brain concentration (~0.2 MAC), whereas the study by Yang et al. found that MAC was unchanged, but the separation was limited (lowest achievable isoflurane brain concentration was 0.5 MAC). This difference is more likely explained by low isoflurane concentrations producing an increase in supraspinal facilitation that is reversed somewhere between 0.2 and 0.5 MAC, rather than trauma. In our current study, any trauma associated with removal of all forebrain structures after precoccius decerebration did not change MAC nor did complete removal of the cerebellum. Furthermore, although MAC decreased after pontomedullary transection, it increased to 90% of control after more caudal transections were made. Finally, isoflurane microinjection into the MLR presumably caused minimal trauma compared with transections but produced most of the MAC decrease as transections immediately caudal to the MLR. Thus, all these points indicate that MAC changes were primarily the result of effects on descending modulation and not from general trauma-related issues.

Another concern relates to the issue of lidocaine spread outside the MLR. We based our lidocaine concentration and volume on a previous study, which found that 0.5 μl lidocaine (4%) inactivated a medullary region with a radius of 0.5 mm. As we noted, 0.5 μl MLR injections decreased MAC in 50% of animals tested. We then switched to 1 μl injections, which by spherical volume would inactivate an area with a radius of 0.63 mm. This corresponds in a temporal lobe with that both the PPN and the cuneiform nuclei (both form the MLR) need to be inactivated to see a consistent decrease in MAC. We cannot be certain that lidocaine did not spread to other brain sites that may have influenced MAC. However, this was unlikely for several reasons. First, we tested a group of animals that received 0.5 μl injections dorsal and ventral to the site where we could elicit locomotion with low-threshold electrical stimulation and never did this affect MAC in any animals. Second, mid-collicular transections, which often removed a rostral portion of the MLR, minimally reduced MAC by 10%. Furthermore, on the basis of our pontine/medullary transection data, lidocaine inactivation of regions caudal to the MLR would remove inhibition from these areas and thus tend to increase MAC not decrease it. On the basis of these findings, it is likely that the effect of lidocaine injection on MAC was primarily, if not exclusively, due to inactivation of the MLR.

**Descending Nociceptive Modulation and MAC**

The current data suggest that there is a modulation of anesthetic requirements from brainstem sites involved in descending nociceptive modulation. Transections ranging from the rostral pons (immediately caudal to the MLR) to...
the pontomedullary junction caused a 40% decrease in MAC. These transections left intact the RVM, an area well known to mediate descending facilitatory and inhibitory modulation of nociception (see Refs. 20–23 for reviews). The results indicate that when the MLR is compromised, a robust inhibition from the rostral medulla is unmasked that is capable of decreasing MAC. This net inhibition could arise from the ability of isoflurane to both facilitate nociceptive inhibitory “off” cells and inhibit nociceptive facilitatory “on” cells located in the RVM.13 One limitation is that we did not selectively lesion the RVM, and therefore, other rostral medullary sites could have contributed to MAC changes. However, the RVM is the medullary component of a part of a major descending nociceptive modulatory circuit receiving input from the periaqueductal gray. Furthermore, MAC decreases and increases corresponded with the presence or absence of the medullary level where RVM on and off reside.

Transections placed caudal to the RVM (nucleus raphe magnus and gigantocellularis pars alpha), but rostral to the MdD, caused MAC to increase to near baseline values (compared with pontine transections). Slightly more caudal transections that removed the MdD caused MAC to drop dramatically to 50% of control (similar to spinalized animals). This suggests that the removal of inhibition from the RVM unmasks descending pronociceptive actions from the caudal medulla. The MdD in the caudal medulla facilitates nociceptive transmission by directly enhancing dorsal horn activity24 and by mandating diffuse noxious inhibitory controls (DNICs). DNIC is a spinal-bulbospinal process through which a noxious stimulus at one location suppresses dorsal

Fig. 5. Effects of isoflurane on supramaximal noxious mechanically evoked responses of midbrain locomotor region (MLR) neurons. Data are shown as mean and SEM. (A) Tail clamp-evoked responses in an MLR neuron (firing rate histogram; bin, 50 ms) that matched movement bouts monitored by recording bilateral L5 ventral root activity (bottom two raw traces). MLR neuronal responses to tail clamp were suppressed by peri-minimum alveolar concentration (MAC) isoflurane. (B) Bar graph showing mean effects of isoflurane on total responses of MLR neurons (n = 11). * Significantly decreased compared with responses under all sub-MAC isoflurane concentrations (P < 0.001 in all cases). (C) Bar graph showing mean effects of isoflurane on peak firing rate of MLR neurons (n = 11). ** Significantly decreased compared with 0.0 MAC values (P < 0.034); + significantly decreased compared with all sub-MAC concentrations (P < 0.001 in all cases). (D) Sagittal template (lateral 1.9 mm) adapted from Paxinos and Watson31 depicting locations of six histologically identified MLR recording sites. CN = cuneiform nucleus; IC = inferior colliculus; PPN = pedunculopontine nucleus.
horns in all outlying segments, thereby enhancing nociceptive signal contrast (see Ref. 15 for review). A reduction in DNIC is thought to contribute to analgesia, as others have found morphine to suppress DNIC in rodents and humans.25,26 One limitation was that currently we did not assess the effects of isoflurane on dorsal MdD neurons. However, we previously found that DNIC is ablated by isoflurane between 0.8 and 1.2 MAC,27 suggesting that isoflurane profoundly inhibits neuronal activity in the MdD or at least its ability to modulate nociceptive dorsal horn activity.

**Relative Importance of MAC-modulating Brainstem Sites to Immobility**

Our current focus on the MLR was warranted by previous studies demonstrating that volatile anesthetics produce immobility mainly by affecting ventral spinal circuitry,8 possibly locomotor networks6,28 and not by an action on sensory dorsal horn neurons.9,27,29–31 Thus, the main brainstem influence on MAC in intact animals must be derived from effects on descending motor commands, more so than from descending nociceptive modulation. However, we previously found that approximately 10% of isoflurane’s immobilizing properties seem to be attributed to dorsal horn effects,5 which could result from such anesthetic effects on descending nociceptive modulation.

When the MLR was removed or inactivated, inhibitory influences from the rostral medulla and facilitation from the caudal medulla decreased and increased MAC, respectively. Thus, it is possible that these nociceptive modulatory sites play a much larger role in situations that compromise brainstem locomotor regions. Although MAC was 90% of control after mid-medullary transections, movement during sub-MAC isoflurane was nonrepetitive and weaker compared with conditions in which the MLR was intact. Furthermore, selective inactivation of the MLR reduced the number and amplitude of movements at 0.6 MAC (at 0.8 MAC, movement did not occur after lidocaine injection that decreased MAC by 32%). Thus, the MLR promotes robust and repetitive movement with isoflurane, conceivably of clinical relevance regardless of MAC changes.

**Conclusions**

In summary, we found that the substantially increased isoflurane immobilizing requirements of the neuraxis-intact or decerebrate versus spinalized animal are due to certain regions located in the brainstem. Activity of the MLR likely increases MAC requirements and sub-MAC movements through facilitation of ventral spinal locomotor circuits, whereas more caudal brainstem areas, possibly the RVM and the MdD, may affect the motor response via modulation of nociceptive dorsal horn activity. Thus, the precise brainstem contribution to the immobilizing requirements of isoflurane seems to result from interplay among descending locomotor command and descending nociceptive inhibitory and facilitatory modulation.
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