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Anesthetic Potency (MAC) Is Independent of Forebrain Structures in the Rat

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Background: The ability of general anesthetics to suppress somatomotor responses to surgical incision and other noxious stimuli is of particular clinical relevance. When the blockade is due to inhaled agents, this effect can be quantified as the minimum alveolar concentration (MAC), *i.e.*, that concentration that blocks movement evoked by a noxious stimulus (ED₅₀).

Methods: To identify the neural structures that subtend this somatomotor response, we anesthetized 14 rats with isoflurane in oxygen and performed bilateral parietal-temporal craniotomies. In each rat, MAC was repeatedly tested using tail-clamping and Dixon's up-down concentration technique. After determination of baseline MAC, seven rats underwent aspiration decerebration, after which MAC was repeatedly measured.

Results: In the control group (N = 7), MAC (mean ± SD) remained constant at 1.30 ± 0.25% for more than 6 h. In the seven rats that underwent aspiration decerebration, baseline MAC was 1.26 ± 0.14%. These seven rats with histologically validated precollicular decerebration demonstrated no change in MAC relative to control rats, as much as 11 h after decerebration (P = 0.14).

Conclusions: These findings suggest that the anesthetic-induced unresponsiveness to noxious stimuli measured by MAC testing does not depend on cortical or forebrain structures in the rat. (Key words: Anesthesia; mechanisms. Anesthetics, volatile: isoflurane. Brain decerebration; nociception. Potency: minimum alveolar concentration.)

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§ Dwyer R, Rampil IJ, Eger EI II, Bennett H: EEG in surgical patients does not predict movement in response to incision. Unpublished data. 1991.

|| Neural structures and behaviors related to pain.

BLOCKING purposeful movement following a supra-maximal noxious stimulus is a fundamental property of adequate anesthesia¹ and has been accepted as a standard point of reference in the spectrum of anesthetic action.² More than 150 yr after the introduction of clinical anesthesia, the mechanisms by which general anesthetics produce surgical unresponsiveness remain unknown. It is possible that anesthetics achieve this desirable effect by depressing site(s) within the cerebral cortex and thalamus. Alternatively, it is possible that general anesthetics suppress noxious-evoked movement through actions in the hindbrain or spinal cord.

One method of investigation into the role of cortical activity in the anesthetic state has been to measure the cortical electrical (electroencephalographic [EEG]) activity while testing minimum alveolar concentration (MAC). Although quantitative EEG measures may reveal anesthetic dose-related changes, they fail to predict accurately the movement response to supramaximal noxious stimulation in rats³ and patients.⁴ § For example, rats may exhibit cortical burst suppression phenomena at end-tidal isoflurane concentrations at which they continue to withdraw, in a complex and coordinated fashion, from noxious stimuli.³ Humans undergoing thiopental anesthesia⁴ demonstrate the same responsiveness during EEG burst suppression, indicating that the unresponsiveness produced by barbiturates is also independent of cortical activity. To evaluate the contribution of telencephalic activity to this aspect of anesthesia, we tested the effect of acute decerebration on the potency of isoflurane in suppression of nocifensive|| motor activity due to noxious stimuli in rats.

Methods

With the approval of the Committee on Animal Research of the University of California, San Francisco, we studied 14 male Sprague-Dawley rats of similar age (≈120 days) and weight (315 ± 34 g). Animals were

allowed food and water *ad libitum* until the day of surgery.

Anesthetic Management

Anesthesia was induced by inhalation of isoflurane. Following induction, the animal's airway was secured, either by tracheal intubation under direct vision or by tracheostomy, and the endotracheal catheter was positioned to obtain bilaterally symmetric thoracic excursions during spontaneous ventilation. Anesthesia was maintained with 1.5–2.0% isoflurane in oxygen. The endotracheal catheter was connected to an open breathing circuit (Y-piece with dead space) that was designed to allow continuous on-line monitoring of inspired and end-tidal concentrations of isoflurane, carbon dioxide, and oxygen. An infrared multichannel (isoflurane, carbon dioxide, oxygen) analyzer (Capnomac Ultima, Datex, Helsinki, Finland) was used for the gas analysis; a two- or three-point calibration was performed before each study. In a few cases, a femoral arterial cannula was placed for additional monitoring. The animals were restrained in a rodent stereotaxic frame. Rectal temperature was thermostatically controlled to $37.5 \pm 0.5^\circ\text{C}$ using an infrared lamp. Lactated Ringer's solution was administered subcutaneously or intravenously to maintain hydration.

Surgical Preparation

A wide excision of the scalp exposed the full dorsal surface of the rat's skull. Burr holes were made in the skull above each hemisphere using a high-speed drill, and the remainder of the parietal bones and squamous portions of the temporal bones were removed by rongeur, with care not to invade the sagittal sinus. The dura was reflected bilaterally, which widely exposed the pial surface, over which a film of warm mineral oil was applied. The animals were allowed to equilibrate at an end-tidal concentration of 1.3–1.5% isoflurane for at least 30 min.

MAC Studies

After completion of the craniotomy, the MAC for each rat was determined using a modification of Dixon's up-down method described previously.³ Administration of isoflurane was adjusted in steps of 0.2%, and 30 min was allowed for equilibration once a stable end-tidal concentration had been obtained at each step. Noxious stimulation was applied *via* an alligator clip clamped to the proximal third of the tail and oscillated $\pm 45^\circ$ at approximately 1 Hz. The criteria for a positive move-

ment included purposeful movement of the four extremities, excluding, for the purposes of this study, isolated simple withdrawal of a single rear limb. If the animal had a positive response, the isoflurane concentration was increased; if there was no response, the concentration was decreased until movement was observed. Baseline MAC determination used two independent move-no move crossovers.

After baseline MAC determination, the entire forebrain was removed by gentle aspiration in the study group of seven rats. An effort was made to restrict the lesion to the precollicular plane. Bleeding was controlled with oxidized cellulose fibers and topical thrombin solution, and the craniotomy sites closed or covered to preserve normothermia in the cranium. The control group (N = 7) received a sham operation (craniotomy with dural incision), but no aspiration of tissue.

Move-no move crossover concentrations were assessed in the study group using the up-down method, beginning 1 h after decerebration, for at least 8 h. Minimum alveolar concentration was assessed in the same fashion in the control group. Groups were compared using analysis of variance with Scheffe's test for *posthoc* comparisons (Statview 4, Abacus Concepts, Berkeley). A $P \leq 0.05$ was considered significant.

After completion of MAC studies, the inspired isoflurane concentration was increased to kill the animals, which were then perfused *via* the left ventricle with 4% paraformaldehyde and 7% sucrose in 0.1 M phosphate-buffered saline. After fixation, the remaining central nervous system rostral of the cervical spinal cord was removed and stored in 30% sucrose. Serial coronal sections (100–120 μm) were cut on a freezing microtome, mounted, and stained with cresyl violet. All sections were examined under a microscope, and the extent of the aspiration lesions was recorded by examination of five structures: the ventral and dorsal periaqueductal gray (PAG), the red nucleus, the cerebral peduncles, and the superior colliculus. Histologic grading of these tissues was provided by an experienced observer blinded to the identity of the individual animal. Each structure was judged for damage and assigned a number as follows: 0 = no damage, 1 = minimal damage, 2 = moderate damage, and 3 = severe damage.

Results

All rats survived the craniotomy and two independent baseline MAC determinations. Rats in both groups re-

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Table 1. Individual Rat Data

Groups	MAC (% isoflurane)				Histologic Damage Grading				
	Baseline	0-2 h	2-6 h	6-12 h	RN	SC	CP	dPAG	vPAG
Control	1.27 ± 0.17	1.28 ± 0.29	1.29 ± 0.25	1.33 ± 0.23					
PrColl	1.26 ± 0.14	1.03 ± 0.23	1.20 ± 0.19	1.16 ± 0.13					
1	1.45	0.85	1.20	1.16	1	3	3	0	1
2	1.25	1.10	1.07	1.13	0	1	0	0	1
3	1.25	0.96	1.11	1.10	0	1	2	0	0
4	1.23	1.00	1.20	—	0	1	0	0	0
5	1.10	>1.3	1.30	1.17	0	0	1	1	0
6	1.30	0.81	0.80	—	0	1	0	0	0
7	1.41	1.40	1.48	1.40	0	0	0	0	0

RN = red nucleus; SC = superior colliculus; CP = cerebral peduncles; dPAG = dorsal periaqueductal gray; vPAG = ventral periaqueductal gray; nc = no crossing (unresponsive at any concentration during this period); — = premature mortality.

mained stable, and their responses to tail clamps were vigorous following surgery, except for two rats in the experimental group that died prematurely, having developed mucous plugging of the endotracheal catheter, which was unrecognized *ante mortem*.

Histology

On the basis of blinded postmortem microscopic examination of the experimental lesions, the precollicular margins of the decerebrations were verified (table 1). The decerebrate lesions were made anterior to the posterior commissure, at a level 4.5–5.0 mm rostral to interaural zero (fig. 1).⁵ In these animals, there was little or no damage in the PAG, red nucleus, ventral tegmentum, or deep layers of the superior colliculus. In two animals, there was significant damage in the cerebral peduncles; and in one animal, the superior colliculus was severely damaged. In one precollicular animal, nucleus cuneiformis was damaged unilaterally as well as lateral portions of the midbrain tegmentum rostral to the trochlear nucleus.

Anesthetic Potency

The mean baseline MAC value (after dural incision; mean ± SD) was 1.25 ± 0.18% and did not differ between the control and study group, nor between the study subgroups. In the first 2 h following decerebration, MAC declined (not significant) to 1.06 ± 0.22% in the decerebrated group, but did not differ from MAC in the control group during the same time interval ($P = 0.08$). One animal did not complete a MAC crossover in this measurement interval (0–2 h), because testing had begun with a positive movement at 0.9% isoflurane,

and positive responses also occurred at 1.1% and 1.3%. A negative response occurred in the next testing interval (2–6 h) at 1.5%; therefore, this animal was considered to have an estimated MAC (worst case) of 1.3% isoflurane in the 0–2-h interval. In the next measurement interval (2–6 h), MAC returned to essentially “normal” values (1.20 ± 0.19%) in the decerebrate rats. The lack of difference in MAC continued through the 6–12-h interval following decerebration, when MAC was 1.16 ± 0.13% in the decerebrate group. In the control group, MAC did not vary with time, despite the presence of an acute craniotomy wound (fig. 2).

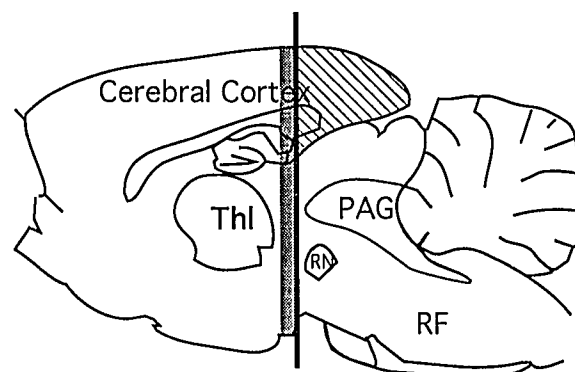


Fig. 1. A schematic (based on Paxinos and Watson's plate 79,⁵ 0.9 mm lateral from midline) representation of the extent of the lesions created. The heavy black line represents the target lesion, with the decerebrate lesions falling within the heavily stippled region rostral to the target line. Cortical regions that remain following decerebration (diagonal rule) were physiologically and anatomically isolated from the midbrain and brainstem. Th1 = thalamus; RN = red nucleus; PAG = periaqueductal gray; RF = reticular formation.

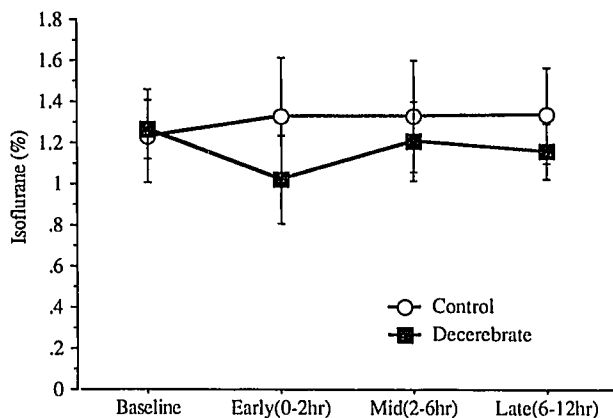


Fig. 2. Change in minimum alveolar concentration (MAC) after decerebration. Group values of MAC (mean \pm SD) did not change over time in the control (sham-operated) group. In the decerebration group, there was a nonsignificant trend toward decreased MAC immediately following the decerebration; otherwise MAC in this group did not differ from control.

The nocifensive movements observed in all groups in this study consisted of alternating proximal flexion and extension, resembling ambulation in at least two (generally more) limbs simultaneously. The movements were considerably more complex than, and did not resemble, the simple flexion or flexion-crossed-extension responses seen in animals with spinal cord transections.^{6, #}

All rats maintained adequate spontaneous ventilation. Both control rats and those with decerebration lesions maintained relatively normocapnic end-tidal carbon dioxide levels (fig. 3).

Discussion

The MAC of isoflurane that inhibits nocifensive responses is not different in precollicular decerebrate and sham-operated animals. Therefore, the mechanism by which isoflurane produces surgical unresponsiveness appears independent of forebrain structures. These results support past studies^{3, 4, §} that failed to find quantitative EEG or evoked potential measures that accurately predict surgical immobility. Furthermore, in light of the present results, it is unlikely that current technologies that analyze cortical activity will yield correlates with surgical levels of anesthesia. Instead, surgical unresponsiveness appears to be supported, and may be determined by, subcortical structures.

The midbrain is an important region in the generation of surgical anesthesia.^{7, 8} Cortical response to anesthetics, as measured by EEG, is thought to be mediated, in part, by ascending projections from the midbrain. An intact midbrain is required for generation of the normal EEG response to anesthetic administration, evidence that anesthetics act on the cerebral cortex through a relay in the midbrain.⁷ We found no change in isoflurane's capacity to suppress nocifensive activity due to noxious stimuli (*i.e.*, no change in isoflurane MAC) following acute decerebration with midbrain intact. Our results appear to support the hypothesis that anesthetics have a primary action within the midbrain or lower.

Anesthetics may produce surgical immobility in several ways. It is possible that anesthetics directly inhibit a hypothetical "nocifensive movement generator." It is also possible that anesthetics act to facilitate endogenous analgesia systems (see below), which, in turn, inhibit nocifensive movement either within the brainstem or at the level of the spinal cord. Finally, it is possible that both of these actions occur.

Despite the destruction of motor cortex in all our decerebrate animals, complex nocifensive movement persisted. Therefore, organized nocifensive movements do not depend on cortical control in the rat. These results are consistent with previous studies of chronic decerebrate rats, in which the motor reactions evoked by noxious stimuli were indistinguishable in precollicular decerebrate and intact rats.^{9, 10} Thus, it appears that nocifensive movement generators in the rat exist in subcortical regions.

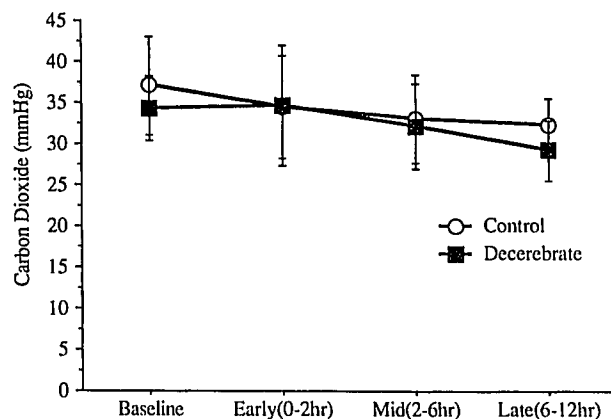


Fig. 3. Changes in ventilation over time. End-tidal carbon dioxide concentrations were essentially stable in the control and decerebrate groups.

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The midbrain PAG may be important in the production of nocifensive movement. Electrical stimulation of or excitatory amino acid microinjection into the dorsolateral PAG elicits a variety of defense and escape reactions in experimental animals.¹¹ These somatomotor reactions typically are accompanied by changes in cardiovascular measures¹² similar to those seen in response to high-intensity noxious stimuli.¹³ If noxious tail-clamp elicits movement, at least in part, through a relay in the dorsal PAG, then animals with lesions in this region might have a lower nociceptive responsiveness and thus a lower MAC. It is possible that this "motor effect" explains the decrease in MAC in the animals with intercollicular decerebration.

In addition to its role in defense behavior, the midbrain PAG plays an important role in the endogenous modulation of nociceptive transmission.¹⁴ Stimulation of the PAG relieves pain in humans and inhibits motor and neuronal responses to noxious stimuli in animals and humans. Anesthetics may facilitate the nociceptive suppressive output of the PAG. Consistent with this idea, PAG stimulation increases anesthetic potency in humans,¹⁵ perhaps, in part, *via* inhibition of regions in the central nervous system that produce nocifensive movement.

Interpretation of the results of this study requires acknowledgment of some of its limitations. To track changes in MAC over time, we used relatively short periods of equilibration. Although 30 min at a steady state end-tidal concentration is not enough time to ensure true equilibration, it is enough to attain a close approximation in the vessel-rich group of organs, like the central nervous system. Another limitation might be spontaneous variability in MAC. In the present study, MAC did not change with time in control animals that received extensive surgical manipulation. This confirms the findings of Eger *et al.*, who initially described MAC as stable over time.¹⁶ Minimum alveolar concentration also has been reported as independent of concurrent surgical stimulation.¹⁷

As designed, the present study examined acute changes in MAC values following the creation of brain lesions. It is possible that our results may be confounded by the presence of acute injury, beyond the simple ablation of descending forebrain control. Our results demonstrate an initial, brief decline in MAC after

precollicular brain resection that, in most cases, resolved within two h, resulting in a stable MAC value for up to 12 h. This decline may be due to a wide combination of factors, including cortical spreading depression of Leao, direct excess neurotransmitter release, and transient hypotension. Longer observation or chronic experiments may be possible but might be subject to other confounding variations, including remarkable central nervous system plasticity in lower mammals (*i.e.*, rats are able to right and groom within 24–48 h after decerebration.)^{9,10} It is also important to note that rats are an imperfect model of the effects of decerebration in higher animals; for example, chronically decerebrated rats retain the ability to perform complex behavioral patterns including a coordinated motor response to pain, whereas cats and monkeys do not.^{9,10} Two recent reports describe a decline in anesthetic requirement following acute, severe head injury.^{17,18} Comparison of the present results with those studies is difficult because, while head injury induces a partial decerebration, the extent of injuries, edema formation, or blood-brain barrier disruption was not documented in those studies. It is possible that structures outside the forebrain, particularly the midbrain and brainstem structures, may have been compromised by increased intracranial pressure following the closed dura injuries, and that subprosencephalic damage was responsible for the increased anesthetic potency.

In summary, anesthetic potency, relative to movement evoked by noxious stimulation, is unchanged in acutely decerebrated rats.

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