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Drastic Decrease in Isoflurane Minimum Alveolar Concentration and Limb Movement Forces after Thoracic Spinal Cooling and Chronic Spinal Transection in Rats

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Background: **Individuals with spinal cord injury may undergo multiple surgical procedures; however, it is not clear how spinal cord injury affects anesthetic requirements and movement force under anesthesia during both acute and chronic stages of the injury.**

Methods: **The authors determined the isoflurane minimum alveolar concentration (MAC) necessary to block movement in response to supramaximal noxious stimulation, as well as tail-flick and hind paw withdrawal latencies, before and up to 28 days after thoracic spinal transection. Tail-flick and hind paw withdrawal latencies were measured in the awake state to test for the presence of spinal shock or hyperreflexia. The authors measured limb forces elicited by noxious mechanical stimulation of a paw or the tail at 28 days after transection. Limb force experiments were also conducted in other animals that received a reversible spinal conduction block by cooling the spinal cord at the level of the eighth thoracic vertebra.**

Results: **A large decrease in MAC (to ≤ 40% of pretransection values) occurred after spinal transection, with partial recovery (to approximately 60% of control) at 14–28 days after transection. Awake tail-flick and hind paw withdrawal latencies were facilitated or unchanged, whereas reflex latencies under isoflurane were depressed or absent. However, at 80–90% of MAC, noxious stimulation of the hind paw elicited ipsilateral limb withdrawals in all animals. Hind limb forces were reduced (by ≥ 90%) in both chronic and acute cold-block spinal animals.**

Conclusions: **The immobilizing potency of isoflurane increases substantially after spinal transection, despite the absence of a baseline motor depression, or "spinal shock." Therefore, isoflurane MAC is determined by a spinal depressant action, possibly counteracted by a supraspinal facilitatory action. The partial recovery in MAC at later time points suggests that neuronal plasticity after spinal cord injury influences anesthetic requirements.**

VOLATILE anesthetics act predominantly in the spinal cord to abolish movement in response to noxious stimulation.[1,2] Spinal cord injury results in an initial state of "spinal shock," accompanied by flaccidity and areflexia; the recovery of reflex activity is followed by disinhibition and reorganization of spinal cord circuitry, resulting in hyperreflexia and spasticity.[3,4] These changes might influence anesthetic requirements; however, few studies have assessed how removal of descending supraspinal influences on the spinal cord affect anesthetic requirements, and no studies have assessed changes in anesthetic requirements over time after spinal cord injury. Furthermore, changes in the force and pattern of "gross and purposeful" movement after spinal transection have not been quantified.

Several studies have indicated that volatile and barbiturate anesthetics have a supraspinally mediated facilitatory action on noxious stimulus–evoked movement or nociceptive reflexes.[5–7] In preferentially anesthetized goats, the minimum alveolar concentration (MAC) necessary to block movement in response to supramaximal noxious stimulation decreases to approximately 60% of baseline MAC values when cranial isoflurane is decreased to approximately 0.3%.[5] In rats, the antinociceptive action of isoflurane on the tail-flick (TF) reflex is greatly enhanced in spinal animals, which is at least partially due to removal of a supraspinal α_1 adrenoceptor–mediated pronociceptive action.[6] The above studies taken together suggest that isoflurane MAC is determined by an increased descending facilitation of spinal nociceptive sensorimotor circuits that opposes a direct spinal depressant action.

We currently tested how acute reversible spinal transection, performed by spinal cold block in rats, changes the force of noxious stimulus–evoked movement under isoflurane anesthesia. Experiments were also conducted on chronic spinal rats up to 28 days after transection to determine (1) nociceptive reflexes in the awake state to verify that any change in MAC values (or movement force) were due to anesthesia alone and not to a baseline motor depression (spinal shock) and (2) changes in MAC values over time and their correspondence to recovery of nociceptive reflexes and hyperreflexia. Because spinal transection might remove a presumed descending facilitation of gross and purposeful movement under isoflurane anesthesia, we hypothesized that forces of limb movements would reversibly decrease during spinal cold block. In chronic spinal animals, we hypothesized that MAC values would decrease despite the absence of spinal shock in the awake state, with a partial recovery coinciding with hyperreflexia at later time points.

Materials and Methods

The University of California, Davis Animal Care and Use Committee approved this study. Chronic and acute

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terminal experiments were conducted on 16 adult Sprague-Dawley rats (10 male and 6 female; weight, 340–620 g). Animals were given free access to food and water and were maintained on a 12 h–12 h light–dark cycle with lights on at 07:00.

Surgery and Caretaking in Chronic Spinally Transected Animals

Chronic experiments were conducted on eight rats (five male and three female) with a T8 spinal transection and three female sham-operated adult Sprague-Dawley rats (weight, 350–450 g). Anesthesia was induced in an acrylic box with isoflurane (5%), and animals were intubated with a 14-gauge arterial catheter and mechanically ventilated with 100% oxygen and 1.5% isoflurane. Body temperature was monitored and maintained at 37 ± 1°C with an electric heating pad. End-tidal carbon dioxide and anesthetic concentration were also monitored during surgery and each testing period with a calibrated Ohmeda Rascal II analyzer (Helsinki, Finland). Under aseptic conditions, a midline incision was made at the T7–T9 level, and a laminectomy was performed on T8. The cord was then completely transected at T8 with a No. 11 scalpel blade, and after visual inspection to verify complete transection, gel foam was packed between the cut ends of the cord. The muscle and overlying fascia were closed with absorbable sutures, and the skin was closed with 2-0 silk sutures. Immediately before surgery and for the entire 28-day testing period, animals were given the antibiotic enrofloxacin (10 $mg \cdot kg^{-1} \cdot day^{-1}$ subcutaneously; Bayer, Shawnee Mission, KS). Immediately after surgery, rats were given buprenorphine (0.08 mg/kg). Urine was expressed two to three times daily by applying manual pressure to the bladder, and the rats' hindquarters were thoroughly washed. Saline injections (10 ml subcutaneously) were given during surgery and twice daily during the next week. The animals' diets were supplemented with Nutri-Cal (Evsco Pharmaceuticals, Buena, NJ) for the first 3 days after surgery, and the animals were given food mash daily (by adding water to dry rat chow) for the entire 28-day postoperative period. Body weight, grooming, appetite, bowel movements, hydration, and urine color were monitored closely each day. For sham-operated animals, surgery was performed as described above, except gel foam was placed in the laminectomized site, over the intact spinal cord at T8. Sham animals were given saline, Nutri-Cal, mash, and enrofloxacin for 5 days, after which special caretaking needs were discontinued.

Behavioral Testing

Before data collection and surgery, rats were acclimated to the TF restrainers and the hind paw withdrawal (HPW) apparatus during three daily 45-min sessions. Two awake baseline TF and HPW latency measurements were taken at 3 days and 1 day before surgery and at postoperative days 3, 7, 14, and 28. Reflex latencies tested under isoflurane (box induction and intubation as described above) were performed immediately before spinal transection surgery and at postoperative days 3, 7, 14, and 28. Body temperature, end-tidal carbon dioxide, and anesthetic concentration were monitored and maintained as described in the section Surgery and Caretaking in Chronic Spinally Transected Animals.

To measure TF latency, rats were placed in a cylindrical acrylic restrainer with the tail protruding out of a hole in the rear gate. The ventral surface of the distal third of the tail was placed over a 0.5-cm hole in an aluminum box, with a 300-W halogen projector bulb housed beneath the hole. Electrical current from a variable alternating current power supply lighted the bulb so that it heated the overlying tail until the rat moved its tail away from the light. The variable power supply was set to yield a TF latency of approximately 4 s. During formal testing, the TF latency was measured by a timer that was started at the onset of tail heating and stopped at the moment of the tail flick. An 8-s cutoff was set to prevent tissue damage. Five TF measurements were made in each rat at each session with a 2- to 4-min time interval between trials. The average of the numeric middle three TF latency measurements was taken for each rat per session.

Thermal HPW latency was measured by placing the rat on a clear glass surface heated to 30 ± 1°C.[8] An infrared light beam (Plantar test 7370; Ugo-Basile, Verese, Italy) was directed onto the middle portion of the plantar surface of the rat's hind paw, and the time until the paw reflexively moved away from the beam was measured. The infrared intensity was set to produce a HPW of approximately 6 s. The stimulus was terminated at 18 s to prevent tissue damage. Compared with the TF stimulus, a longer cutoff time for HPW was permitted because the apparatus produces a thermal stimulus that reaches the pain tolerance level more slowly over time (as judged by the experimenter placing his fingertip over the stimulus). Five latency measurements were made per rat per session, with at least 2- to 4-min time intervals between trials. The average of the numeric middle three latency measurements was taken for each rat per session.

Initially, TF and HPW latencies were measured under isoflurane anesthesia at 0.8 MAC. However, it was found that spinally transected animals never exhibited TFs at 0.8 MAC. Therefore, after transection in five animals, we progressively decreased isoflurane concentrations by 0.2% increments until we observed TF latencies within the 8-s cutoff. We discontinued testing if no TF occurred down to 0.5% isoflurane (approximately 0.35–0.45 MAC).

MAC Measurement

Minimum alveolar concentration determinations were performed immediately before spinal transection or sham surgery and at postoperative days 1, 3, 7, 14, and

28. Anesthesia induction and intubation and monitoring of body temperature, end-tidal carbon dioxide, and anesthetic concentration were conducted as described in the section Surgery and Caretaking in Chronic Spinally Transected Animals.

The MAC values for both tail and forepaw stimulation were determined. For tail stimulation, we applied 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.[9] For forepaw stimulation, we applied a supramaximal electrical stimulus (60 mA, 100 Hz; model NS252J; Fisher and Paykel Healthcare, Auckland, New Zealand) delivered through platinum needle electrodes (Grass Instruments, West Warwick, RI) inserted into the ventral forepaw skin. Multilimb movement was interpreted as a positive response, whereas single-limb withdrawals and tonic limb or neck extensions were interpreted as a negative response.[9] In spinally transected animals, a positive response consisted of bilateral hind limb or forelimb movements in response to tail clamp or forepaw electrical stimulation, respectively, whereas unilateral limb withdrawals and tonic limb extensions were not considered positive movement responses. Depending on the response, the anesthetic concentration was increased or decreased by 0.2%. After an equilibration time of 15–20 min, the clamp or electrical stimulus 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 the MAC.[9]

Tail stimulation was usually ineffective in eliciting movement in spinal transected animals, even at isoflurane concentrations at or below 0.6 MAC. Therefore, in addition, we determined MAC values for hind paw clamping (n = 8; using the same mechanical stimulus used for tail stimulation). If no movement was observed down to 0.5% isoflurane, testing was discontinued, and the rat's MAC value was designated as 0.4%.

Acute Reversible Spinal Transection Using Spinal Cold Block

Acute terminal experiments were conducted on five adult male Sprague-Dawley rats (weight, 450–600 g) to investigate the effects of spinal cold block on movement elicited by supramaximal mechanical stimulation. Animals were anesthetized and prepared for surgery as described in the section Surgery and Caretaking in Chronic Spinally Transected Animals. In addition, cannulations of the carotid artery and jugular vein were performed to monitor blood pressure (model PB-240; Puritan-Bennett Corp., Hazelwood, MO) and for fluid administration, respectively. Blood pressure was always maintained above a mean arterial pressure of 75 mmHg, with lactated Ringer's solution (Abbott Laboratories, Chicago, IL) administered intravenously when necessary. A laminectomy was performed at T8 to permit placement of a cooling probe to the cord. The spinal cold-block method was performed in the same manner as in our previous study,[10] using a custom-built cooling probe machined from a solid aluminum block. Ethanol was circulated through a copper coil embedded in dry ice and then to the probe using a variable-speed roller pump. We continuously monitored the temperatures of the dorsal and ventral surfaces of the spinal cord (directly below the probe) using two small thermistors (Physitemp, Clifton, NJ). After surgery was completed, the rat's MAC value was determined before placing the rat in a stereotaxic frame for recording limb forces.[11] The cord was slowly cooled over a time period of 10–15 min. During spinal cold-block testing, the temperature of the dorsal surface of the cord was maintained between 0° and 3°C.

Limb Force Measurement

In chronic spinally transected animals at postoperative day 28 and in acute spinal cold-block experiments, the isometric forces of all four limbs in response to supramaximal tail, hind paw, and forepaw stimulation were measured by attaching each limb to a force transducer (model FT-03; Grass Instruments) *via* a 1-0 silk suture. Noxious supramaximal mechanical stimulation (10-s duration) was applied every 3–4 min, and resulting responses were recorded at 0.6 and 0.8 MAC. In cold-block experiments, limb force measurements were taken before spinal cooling (at 0.8 MAC), during spinal cooling (at 0.6 MAC), and after rewarming the spinal cord (at 0.6 MAC). Limb force data were digitized at a rate of 200 Hz and collected on a personal computer using a Powerlab with Chart software (AD Instruments, Grand Junction, CO).

Statistics

Changes in MAC values across pretransection and posttransection time points were compared using two-factor analysis of variance followed by *post hoc* Tukey multiple comparisons. TF and HPW latencies were each compared across time points and between awake and anesthetized states using three-factor analysis of variance followed by *post hoc* Tukey multiple comparisons. Limb forces (area under the curve and peak force) were normalized as percent of maximum for each animal and were compared using two-factor analysis of variance with *post hoc* Tukey multiple comparisons. Single pairwise comparisons were made using a two-tailed, paired or unpaired *t* test where indicated. *P* values of less than 0.05 were considered statistically significant.

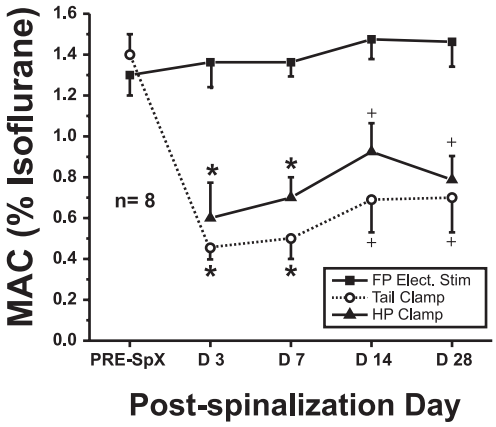

Fig. 1. Effect of spinal transection on isoflurane mean alveolar concentration (MAC). Mean MAC values obtained from tail clamp (*open circles*) and hind paw (HP) clamp (*filled triangles*) and forepaw (FP) electrical stimulation (*filled squares*) before (BL) and up to 28 days after spinal transection. Mean MAC values for both tail and hind paw clamp decreased significantly after transection and remained significantly reduced during the entire 28-day period, although there were significant increases in MAC values at 14 and 28 days after transection (D14 and D28). Mean MAC values obtained from forepaw stimulation remained unchanged after transection. * Significantly decreased compared with pretransection values. + Significantly decreased from pretransection values, but significantly increased from 3-day values. *Error bars* = mean ± SD.

Results

Chronic Spinal Rats

Changes in Isoflurane Requirements after Spinal Transection. Before spinal transection, the mean MAC for tail clamping was 1.4 ± 0.1%. Three days after transection, the mean MAC value for tail clamping decreased to less than 0.5% isoflurane. That is, only three of eight rats showed movement in response to tail clamping at 0.5% isoflurane (*i.e.*, MAC = 0.6%), with the remaining five rats showing no movement down to 0.5%. This was followed by a small but significant partial recovery to mean MAC values of 0.7 ± 0.2% at both 14 days (P = 0.02) and 28 days (P = 0.014) after transection (fig. 1). During the posttransection period, mean hind paw clamp MAC values also showed a partial recovery over the 28-day posttransection period, with increases in MAC values from 0.6 ± 0.2% at 3 days, to 0.9 ± 0.1% at 14 days ($P < 0.001$), and 0.8 ± 0.1% at 28 days (P = 0.015) after transection (fig. 1). However, the partial recovery of MAC values for tail clamp remained significantly decreased from pretransection values ($P <$ 0.00001). This comparison could not be made for hind paw clamping because pretransection MAC determinations were not conducted.

Mean MAC values for forepaw stimulation (1.4 ± 0.2%) in spinally transected animals remained unchanged throughout the 28-day testing period. In three sham-operated animals, MAC values for both forepaw stimulation (1.3 ± 0.2%) and tail clamping (1.4 ± 0.2%) remained unchanged during the 28-day period.

Tail-flick and Hind Paw Withdrawal Latencies

Despite a large decrease in tail clamp MAC values (to < 36% of control) at 3 days after transection, the TF reflex in awake rats was enhanced, with a significant decrease in latency from 4.2 ± 0.5 s before transection to 2.9 ± 1.0 s ($P < 0.015$) at 3 days after transection, followed by a significant recovery to 4.4 ± 0.8 s at 28 days after transection ($P < 0.008$; fig. 2A). Under 0.8 MAC isoflurane (using the pretransection MAC value for tail clamping), TF latency before transection was significantly delayed to 5.7 ± 1.5 s ($P <$ 0.014) compared with latencies measured in awake rats. After spinal transection and under 0.8 MAC isoflurane, only one of eight rats exhibited a TF reflex, which only occurred at 3 days after transection. The remaining animals showed no TF up to the 8-s cutoff at all posttransection time points (fig. 2A). When isoflurane was decreased to 0.5% (approximately 0.35–0.45 MAC) and TF was tested in five animals at 28 days after transection, only two of the five animals exhibited TF reflexes, with latencies of 5.1 and 4.3 s.

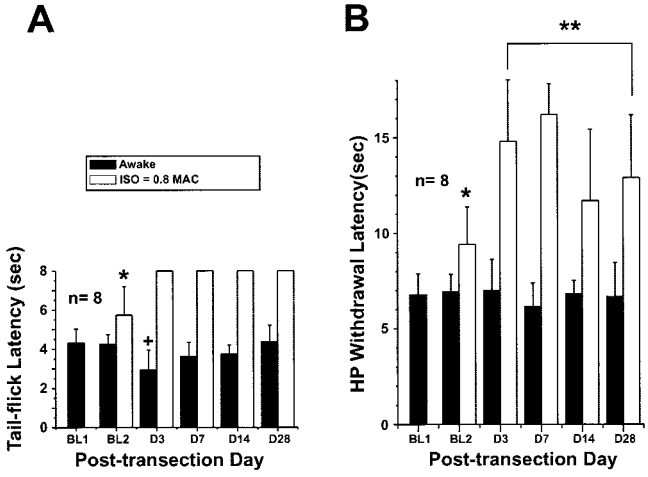

Fig. 2. Effects of spinal transection and isoflurane on tail-flick and hind paw (HP) withdrawal latencies. (*A*) Mean latencies for the tail-flick reflex in awake (*filled bars*) and isoflurane-anesthetized (*open bars*) rats. Mean awake tail-flick latencies were significantly reduced from baseline (BL) latencies at 3 days after transection (D3), followed by a recovery at 14 days (D14). Before transection, isoflurane (ISO) significantly increased mean tail-flick latencies. After spinal transection and under 0.8 isoflurane, the heat stimulus no longer elicited a tail-flick reflex up to the cutoff latency of 8.0 s. (*B*) Mean latencies for thermal hind paw withdrawal in awake (*filled bars*) and isoflurane-anesthetized (*open bars*) rats. Awake withdrawal latencies remained unchanged from baseline after transection. Isoflurane significantly increased withdrawal latency in intact animals before transection. After transection, withdrawal latencies under isoflurane were further increased during the 28-day period. MAC = minimum alveolar concentration. * Significantly increased latencies under isoflurane compared with awake baseline. + Significantly reduced awake latencies compared with awake baseline. ** Significantly increased isoflurane latencies compared with pretransection isoflurane latencies. *Error bars* = mean ± SD.

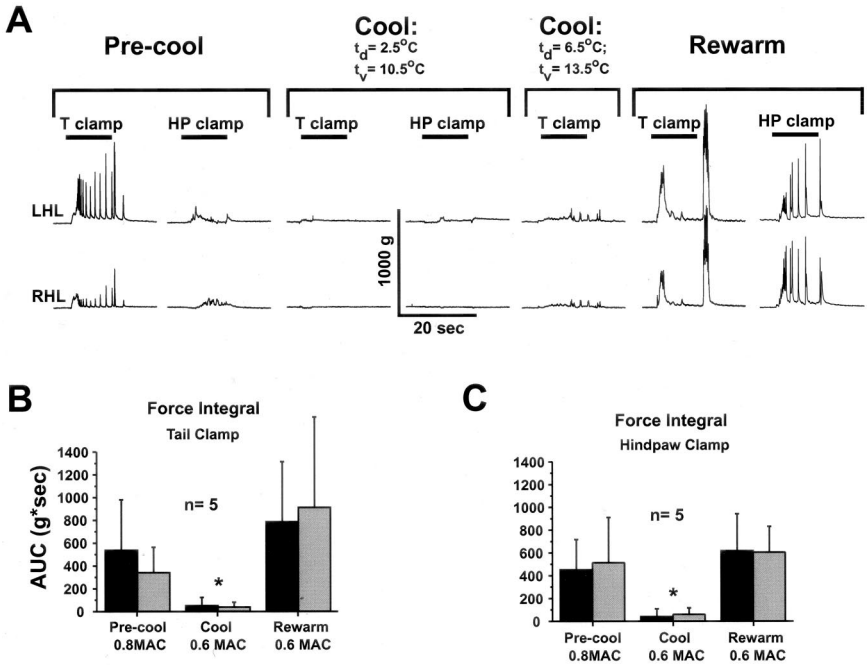

Fig. 3. Effect of spinal cold block on hind limb movement force under isoflurane. (*A*) Individual example of hind limb movement forces elicited by supramaximal noxious mechanical stimulation of the tail (T) and hind paw (HP) before (*left traces*) and during (*middle traces*) spinal cooling and on rewarming (*right traces*) of the spinal cord. Spinal cooling nearly abolished hind limb forces, with a partial recovery occurring when cord temperatures increased slightly (*middle right*) and a full recovery when the cord was rewarmed to precooling temperatures. *Thick horizontal bars* over force traces indicate when the clamp was applied. (*B*) Mean force integrals, or area under the curve (AUC), before, during, and on rewarming of the spinal cord. *Bar graph* shows mean hind limb forces elicited by tail clamp. (*C*) Mean hind limb forces elicited by hind paw clamp. Hind limb force in response to both tail and hind paw stimulation were significantly reduced by spinal cooling, followed by recovery on rewarming of the spinal cord. *Solid black bars* represent mean left hind limb (LHL) force integral, and *shaded bars* represent mean right hind limb (RHL) force integral. MAC = minimum alveolar concentration; t_d = dorsal cord temperature; t_v = ventral cord temperature. * Significantly reduced hind limb forces compared with precooling and postcooling ("rewarm") forces. *Error bars* = mean ± SD.

In awake animals, HPW latencies remained unchanged throughout all pretransection and posttransection time points. HPW latencies before transection were significantly increased from 6.7 ± 1.0 s in the awake state to 9.4 ± 2.0 s under 0.8 MAC isoflurane ($P < 0.021$; fig. 2B). Spinal transection caused mean HPW latencies under isoflurane to further increase to 14.8 ± 3.4 s at 3 days ($P < 0.001$) and 16.2 ± 1.6 s at 7 days after transection ($P < 0.0001$) compared with pretransection HPW latencies under isoflurane (fig. 2B). This increase was followed by a partial but significant decrease in HPW latency at 14 days (to 11.7 ± 3.7 s; $P < 0.003$) and 28 days (to 12.9 ± 3.3 s; $P < 0.05$) compared with latencies measured at 7 days after transection (fig. 2B). Although we did not quantitatively measure the force of thermal HPWs in this testing procedure, we observed that under isoflurane, HPWs were clearly of much lesser magnitude at all posttransection time points compared with those measured before spinal transection. In fact, it was rare that the rat fully displaced its hind paw away from the infrared beam.

Spasticity

By 7 days after transection, all awake rats exhibited clear signs of spasticity, which included movements of tail and hind limbs, as well as flexion of the digits in response to innocuous stroking or manipulation of these appendages. This behavior persisted though the 28-day posttransection time point. Under isoflurane anesthesia, the hind limbs and tail became flaccid despite the presence of spasticity in awake animals.

Changes in Multilimb Movement Forces after Acute Reversible and Chronic Spinal Transection

In acute cold-block experiments, rats exhibited robust and synchronous movement of all four limbs in response to supramaximal noxious stimulation of the hind paw or tail, before cooling the spinal cord (individual example in fig. 3A, left traces). During spinal cold block, the force of hind limb movements in response to hind paw or tail clamping was nearly abolished at isoflurane concentrations down to 0.6 MAC (fig. 3A, middle traces). In these experiments, we could not perform testing at isoflurane concentrations below 0.6 MAC because of ethical concerns. We did not observe spontaneous movements, indicating that these animals were not conscious or perceiving pain. After rewarming of the spinal cord (to 34–36°C), vigorous limb movements in response to noxious tail and hind paw stimulation returned (fig. 3A, right traces). Mean limb forces were significantly depressed under spinal cold block, in response to both noxious tail ($P < 0.001$; fig. 3B) and hind paw stimulation ($P < 0.004$; fig. 3C).

In chronic spinal rats, hind limb movement forces elicited by noxious stimuli were nearly abolished, although weak ipsilateral hind limb withdrawals in response to hind paw clamping were still present at 0.8 MAC (individual example in fig. 4A, top traces). However, forelimb movement forces in response to noxious forepaw stimulation were bilateral and robust (fig. 4A, bottom traces). Mean force integrals for tail, hind paw, and forepaw clamping are shown in figure 4B. Because we could not obtain limb force data in these same

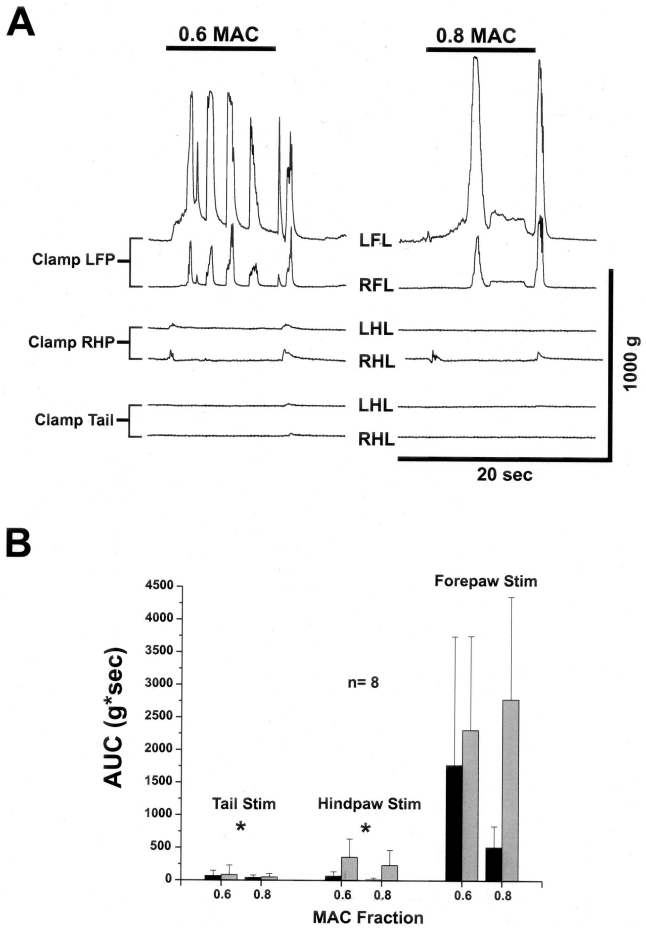

Fig. 4. Effect of chronic spinal transection on hind limb movement forces under isoflurane. (*A*) Individual example of hind limb movement forces elicited by supramaximal noxious mechanical stimulation of the forepaw (*top two traces*), hind paw (*middle two traces*), and tail (*bottom two traces*) in a 28-day chronic spinal animal. Responses to forepaw clamp were repetitive and robust; however, responses to hind paw and tail clamp consisted of only single weak hind limb withdrawals at the onset or offset of clamp removal. (*B*) Mean force integrals, or area under the curve (AUC), elicited by tail, hind paw, and forepaw clamping at 0.6 and 0.8 minimum alveolar concentration (MAC). *Solid black bars* represent mean left limb force integral, and *shaded bars* represent mean right limb force integral. LFL = left forelimb; = right forelimb; RFL = right forelimb; LHL = left hind limb; RHL = right hind limb. * Significantly reduced hind limb forces compared with hind limb forces in cold-block animals after cooling (see "rewarm," fig. 3B). *Error bars* = mean ± SD.

animals before transection, mean hind limb forces in chronic spinal animals were compared with hind limb forces measured in animals used for the spinal cold-block experiments. In 28-day chronic animals, hind limb forces elicited by hind paw and tail clamp, at both 0.6 and 0.8 MAC, were significantly lower than in separate intact animals ($P < 0.007$ in all cases, unpaired two-tailed t test). However, the hind limb forces elicited by tail and hind paw clamping in chronic animals were not significantly different from those exhibited by animals during acute spinal cold block ($P = 0.78$).

Discussion

The current results extend previous studies regarding supraspinal *versus* spinal contributions to the immobilizing action of volatile anesthetics.[2,5–7] Spinal transection in chronic rats drastically reduced MAC values to 40% or less at 3 days after transection, followed by a partial recovery of MAC (to 60% of control) occurring between day 3 and day 14 after transection (fig. 1) During a 28-day period, TF and HPW latencies were either facilitated or unchanged (fig. 2), indicating that the decreases in isoflurane requirements were not due to a baseline motor depression such as that seen during spinal shock. By 7 days after transection, all animals showed signs of spasticity. However, under isoflurane anesthesia, TF and HPW were greatly depressed or absent. The parallel depression in both MAC and spinal reflexes under isoflurane anesthesia suggest that these two types of motor responses might have some degree of overlap in their respective circuitry or at least share a common site of anesthetic action. This is consistent with our previous studies,[12,13] which show that hind limb withdrawal force is greatly reduced or abolished in each animal's immediate isoflurane peri-MAC range. Therefore, hind limb withdrawal seems to be a strong predictor of MAC. However, because reflex latencies remained unchanged over time while MAC showed partial recovery, anesthetic effects on the neuronal plasticity underlying the two types of motor responses might differ. Finally, forces generated by limb movements were profoundly depressed (by ≥ 90%) during acute reversible spinal cold block (fig. 3) and in 28-day chronic spinal rats (fig. 4). During spinal cooling, the forces of limb movements were nearly abolished at 0.6 MAC, and none of these movements would be considered a positive response for the purposes of MAC determination.

Although the current data are consistent with removal of descending supraspinal influences, we cannot exclude the possibility that neuronal plasticity below the transection could have rendered the spinal cord more sensitive to anesthesia, while leaving the reflexes intact in the awake state. Such a change could result in decreased activity of spinal neurons through increased glycinergic activity[14] or decreased alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptor expression.[15] However, reflex latencies remained unchanged in the awake state, and movement suppression by spinal cooling under anesthesia recovered immediately after rewarming the spinal cord. This would suggest that loss of descending projections might play a relatively greater role in decreasing anesthetic requirements and movement force in the very early stages after spinal cord trauma, with a possibly greater role for plasticity at later stages.

The decreases in MAC and limb forces after spinal transection or cooling were not likely attributed to the

animal's inability to exhibit bilateral limb movements in the awake state. We noted that all awake spinal-transected animals were capable of exhibiting robust, bilateral hind limb movements in response to tail pinching (usually alternating but sometimes a synchronous "kicking" movement). At the 7-day and later time points, all animals exhibited bilateral limb movement in response to nonnoxious manipulation of the limbs, stroking the hindquarters, and general handling. Hence, all rats were quite capable of exhibiting vigorous, bilateral movements in the awake state. Many previous studies have indicated that central pattern generators controlling hind limb stepping are distributed within the lower thoracic–lumbar segments,[16–18] well below the T8 segment transected or cooled in the current study.

The current results seem to be at odds with one study in which low cervical (C7) hypothermic transection (freeze-lesioning) of the spinal cord caused no significant changes in isoflurane MAC values.[2] However, single hind limb withdrawals in that study were considered "purposeful" movement, whereas the typical methodology for MAC determination, used in the current study, does not include single limb withdrawals as a positive response. All animals had thermal HPWs (albeit of weak magnitude) at 0.8 MAC, and furthermore, during MAC determination with hind paw clamping, all rats had weak to moderate withdrawals at 80–90% of pretransection MAC values (also see force traces showing hind limb withdrawals at 0.8 MAC in figs. 3A and 4A). Therefore, the current data seem consistent with those of Rampil.[2] However, the movements in that study were "less vigorous but of the same type," and it is therefore unclear what types of movements were observed and how they compare with movements typically observed in a MAC study and in the current study. This confusion could stem from the limitations of MAC determination, which classifies movement into an "all-or-none" response based on subjective evaluation of limb and neck movements. The current study has the added advantage of multilimb force measurement, which objectively demonstrates how spinal transection changes the magnitude and pattern of limb movements elicited by a supramaximal noxious stimulus.

Role of Descending Modulation in Anesthetic Immobilizing Action

The current results are consistent with previous studies from our laboratory and of others' studies addressing the role of supraspinal sites in anesthetic-induced immobility.[5–7] In goats, isoflurane MAC was reversibly reduced from 1.4% to 0.8% (in the torso) when isoflurane concentration was selectively decreased in the cranial circulation.[5] In our recent study,[13] isoflurane caused a large depression of spontaneous and noxious heat–evoked activity of rostral ventromedial medullary ON cells, which are believed to facilitate nociceptive reflexes through descending projections to the spinal cord.[19–21] A previous study reported that increases in TF latency in isoflurane-anesthetized spinal animals results from removal of a supraspinal α_1 adrenoceptor–mediated descending facilitation.[6] Because α_1-adrenoceptor activation in the rostral ventromedial medulla excites ON cells, decreases TF latency, and mediates certain states of hyperalgesia,[22–25] isoflurane could engage supraspinal adrenergic pathways that relay in the rostral ventromedial medulla to excite ON-cells and thus facilitate spinal nociceptive sensorimotor circuits. However, the effects of isoflurane on other descending facilitatory pathways cannot be excluded.

Although the current study did not assess the role of specific descending pathways in the spinal cord, the decrease in movement after spinal transection and cooling would more likely be attributed to interruption of descending fibers in the ventral cord, which primarily mediate facilitation of nociceptive reflexes and locomotion.[26–31] However, a smaller contribution from dorsolateral pathways cannot be excluded.[29] For the cold-block experiments conducted in the current study, we found that approximate ventral cord temperatures below approximately 12°C were necessary to prevent movement of the forelimbs in response to noxious mechanical stimulation, indicating that ascending nociceptive transmission in ventrolateral pathways were blocked. When responses to noxious stimuli were tested at slightly higher ventral cord temperatures (fig. 3A), not only did forelimbs exhibit movement, but the pattern of movement changed from none or tonic flexion to the larger, repetitive "gross and purposeful" movements, suggesting that activity descending along ventral pathways was largely responsible for generating this type of movement.

Spinal Targets of Supraspinal Facilitation and Anesthetic Action

Although previous studies have shown depressant actions of volatile anesthetics on dorsal horn neurons,[32–38] an increase in the immobilizing potency of isoflurane after spinal transection was probably not a result of changes in dorsal horn neuronal activity. First, spinal transection or selective dorsolateral funiculus blockade causes an increase rather than a decrease in noxious stimulus–evoked discharges in dorsal horn neurons,[39–42] and we recently confirmed this same effect on dorsal horn neurons under isoflurane anesthesia during spinal cold block.[10] Second, several of our previous studies have shown that isoflurane has little to no effect on nociceptor-driven responses of dorsal horn neurons in the peri-MAC (approximately 0.8–1.2 MAC) concentration range where noxious stimulus–evoked movement is abolished.[10,12,43,44] Therefore, both the isoflurane-induced descending facilitation and the direct spinal depressant action of isoflurane seem to target

more ventrally located spinal interneurons or motoneurons. Several previous studies have shown depressive effects of volatile anesthetics on motoneurons.[45–48]

Long-term Changes in Spinal Sensorimotor Function and Clinical Implications

Changes in spinal cord function after spinal cord injury occur not only from immediate removal of descending influences but also as a result of longer-term neural plasticity that underlies states of motor hyperreflexia/spasticity[3,4] and, if the lesion is above T7, autonomic hyperreflexia.[49] In the current study, we found that although MAC values remained significantly reduced during the entire 28-day posttransection period, they nonetheless showed partial recovery between the 3- and 28-day time points. However, TF and HPW latencies in awake animals were unchanged from baseline levels, and TF latencies were only facilitated at the 3-day time point when MAC values were at their lowest. This mismatch might be explained by the two different types of stimuli used for reflex latency measurement (thermal stimulus) and MAC determination (mechanical stimulus) or by differences in stimulus intensity (near-threshold noxious *vs.* supramaximal noxious, respectively). Whereas noxious thermal stimuli primarily activate cutaneous C fibers, with a lesser contribution from Aδ fibers,[50,51] the mechanical stimulus would tend to recruit both Aδ and C nociceptors as well as low-threshold Aβ fibers that innervate both cutaneous and deep tissues. The onset of spasticity in awake animals coincided with partial recovery of MAC values, and thus sensitization of reflex pathways receiving low-threshold primary afferent input might have contributed to MAC increases over time in spinally transected animals. The increases in spinal excitability and MAC values could have resulted from increases in interneuronal or motoneuron excitability[52–54] or primary afferent sprouting[55] that coincides with spasticity/hyperreflexia in chronic spinal rats. There are a plethora of molecular and cellular changes that occur after spinal cord injury that could potentially lead to sensitization of spinal pathways, including altered expression of *N*-methyl-D-aspartate–type glutamate receptors,[56] neurotrophins,[57] inflammatory mediators, and voltage-gated sodium channels.[58]

It is possible that some of the partial recovery in MAC was attributed to the presence of hyperalgesia after repeated stimulation of the tail and hind paw, because there was a slight albeit insignificant increase in forepaw MAC values over time. However, the lack of changes in reflex latencies and the relatively long testing intervals (1–2 weeks) suggest that confounding influences from repeated stimulation, if any, were minimal.

Isoflurane requirements and limb movement forces are drastically reduced by transecting or cooling the spinal cord, in the absence of spinal shock, and are thus consistent with the notion that the immobilizing potency of isoflurane in rats is determined by the net result of opposing actions from spinal and supraspinal sites. Therefore, future studies should focus on both spinal and supraspinal anesthetic actions and interactions. The potent spinal depressant effect found in the current study invites the development of anesthetics that target the spinal cord without effect on supraspinal facilitatory centers.

The results further suggest that anesthetic requirements are much lower in patients with spinal cord injuries if surgery is performed below the level of the lesion. However, the exact requirements for a spinal cord injured individual probably depend on the extent and location of the spinal injury and the amount of time since the injury. In humans, changes in motor and autonomic reflex excitability can occur months to more than a year after spinal cord injury,[59,60] far later than the 28-day testing period used in the current study. Therefore, it is not known how these later changes might further influence anesthetic requirements. Future studies are necessary to unveil the specific neuronal populations and molecular mechanisms involved in both supraspinal and spinal actions of volatile anesthetics and how reorganization of neural pathways after spinal cord injury influences anesthetic requirements.

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References

1. Antognini JF, Schwartz K: Exaggerated anesthetic requirements in the preferentially anesthetized brain. ANESTHESIOLOGY 1993; 79:1244–9

2. Rampil IJ: Anesthetic potency is not altered after hypothermic spinal cord transection in rats. ANESTHESIOLOGY 1994; 80:606–10

3. Atkinson PP, Atkinson JL: Spinal shock. Mayo Clin Proc 1996; 71:384–9

4. Edgerton VR, Tillakaratne NJ, Bigbee AJ, de Leon RD, Roy RR: Plasticity of the spinal neural circuitry after injury. Annu Rev Neurosci 2004; 27:145–67

5. Borges M, Antognini JF: Does the brain influence somatic responses to noxious stimuli during isoflurane anesthesia? ANESTHESIOLOGY 1994; 81:1511–5

6. Kingery WS, Agashe GS, Guo TZ, Sawamura S, Frances DM, David CJ, Kobilka BK, Maze M: Isoflurane and nociception: Spinal α_{2A} adrenoceptors mediate antinociception while supraspinal α_1 adrenoceptors mediate pronociception. ANESTHESIOLOGY 2002; 96:367–74

7. Stein C, Morgan MM, Liebeskind JC: Barbiturate-induced inhibition of a spinal nociceptive reflex: Role of GABA mechanisms and descending modulation. Brain Res 1987; 407:307–11

8. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988; 32:77–88

9. Quasha AL, Eger EI, Tinker JH: Determination and applications of MAC. ANESTHESIOLOGY 1980; 53:315–34

10. Jinks SL, Antognini JF, Carstens E: Isoflurane depresses diffuse noxious inhibitory controls in rats between 0.8 and 1.2 minimum alveolar anesthetic concentration. Anesth Analg 2003; 97:111–6

11. Antognini JF, Wang XW, Carstens E: Quantitative and qualitative effects of isoflurane on movement occurring after noxious stimulation. ANESTHESIOLOGY 1999; 91:1064–71

12. Jinks SL, Martin JT, Carstens E, Jung SW, Antognini JF: Peri-MAC depression of a nociceptive withdrawal reflex is accompanied by reduced dorsal horn activity with halothane but not isoflurane. ANESTHESIOLOGY 2003; 98:1128–38

13. Jinks SL, Carstens E, Antognini JF: Isoflurane differentially modulates medullary ON and OFF neurons while suppressing hind-limb motor withdrawals. ANESTHESIOLOGY 2004; 100:1224–34

14. Simpson RK Jr, Robertson CS, Goodman JC: The role of glycine in spinal shock. J Spinal Cord Med 1996; 19:215–24

15. Grossman SD, Wolfe BB, Yasuda RP, Wrathall JR: Alterations in AMPA receptor subunit expression after experimental spinal cord contusion injury. J Neurosci 1999; 19:5711–20

16. Barbeau H, McCrea DA, O'Donovan MJ, Rossignol S, Grill WM, Lemay MA: Tapping into spinal circuits to restore motor function. Brain Res Brain Res Rev 1999; 30:27–51

17. Kiehn O, Butt SJ: Physiological, anatomical and genetic identification of CPG neurons in the developing mammalian spinal cord. Prog Neurobiol 2003; 70:347–61

18. Fulton JF, Sherrington CS: State of the flexor reflex in paraplegic dog and monkey respectively. J Physiol 1932; 17–22

19. Fields HL, Malick A, Burstein R: Dorsal horn projection targets of ON and OFF cells in the rostral ventromedial medulla. J Neurophysiol 1995; 74:1742–59

20. Vanegas H, Barbaro NM, Fields HL: Tail-flick related activity in medullospinal neurons. Brain Res 1984; 321:135–41

21. Foo H, Mason P: Discharge of raphe magnus ON and OFF cells is predictive of the motor facilitation evoked by repeated laser stimulation. J Neurosci 2003; 23:1933–40

22. Bie B, Fields HL, Williams JT, Pan ZZ: Roles of alpha1- and alpha2-adrenoceptors in the nucleus raphe magnus in opioid analgesia and opioid abstinence-induced hyperalgesia. J Neurosci 2003; 23:7950–7

23. Hirakawa N, Tershner SA, Fields HL, Manning BH: Bi-directional changes in affective state elicited by manipulation of medullary pain-modulatory circuitry. Neuroscience 2000; 100:861–71

24. Fields HL, Heinricher MM, Mason P: Neurotransmitters in nociceptive modulatory circuits. Annu Rev Neurosci 1991; 14:219–45

25. Heinricher, MM, Haws, CM, and Fields, HL: Opposing actions of norepinephrine and clonidine on single pain-modulating neurons in the rostral ventromedial medulla, Pain Research and Clinical Management. Vol 3. Edited by Dubner R, Gebhart GF, Bond MR. Amsterdam, Elsevier, 1988, pp 590–4

26. Zhuo M, Gebhart GF: Biphasic modulation of spinal nociceptive transmission from the medullary raphe nuclei in the rat. J Neurophysiol 1997; 78:746–58

27. Zhuo M, Gebhart GF: Modulation of noxious and non-noxious spinal mechanical transmission from the rostral medial medulla in the rat. J Neurophysiol 2002; 88:2928–41

28. Basbaum AI, Fields HL: Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci 1984; 7:309–38

29. Whelan PJ: Control of locomotion in the decerebrate cat. Prog Neurobiol 1996; 49:481–515

30. Noga BR, Kriellaars DJ, Brownstone RM, Jordan LM: Mechanism for activation of locomotor centers in the spinal cord by stimulation of the mesencephalic locomotor region. J Neurophysiol 2003; 90:1464–78

31. Noga BR, Kriellaars DJ, Jordan LM: The effect of selective brainstem or spinal cord lesions on treadmill locomotion evoked by stimulation of the mesencephalic or pontomedullary locomotor regions. J Neurosci 1991; 11:1691–700

32. De Jong RH, Robles R, Heavner JE: Suppression of impulse transmission in the cat's dorsal horn by inhalation anesthetics. ANESTHESIOLOGY 1970; 32:440–5

33. Asai T, Kusudo K, Ikeda H, Takenoshita M, Murase K: Effect of halothane on neuronal excitation in the superficial dorsal horn of rat spinal cord slices: evidence for a presynaptic action. Eur J Neurosci 2002; 15:1278–90

34. Ota K, Yanagidani T, Kishikawa K, Yamamori Y, Collins JG: Cutaneous responsiveness of lumbar spinal dorsal horn neurons is reduced by general anesthesia, an effect dependent in part on GABAA mechanisms. J Neurophysiol 1998; 80:1383–90

35. Nagasaka H, Hayashi K, Genda T, Miyazaki T, Matsumoto N, Matsumoto I, Hori T, Sato I: Effect of isoflurane on spinal dorsal horn WDR neuronal activity in cats [in Japanese]. Masui 1994; 43:1015–9

36. Nagasaka H, Sugai M, Genda T, Aikawa K, Matsumoto N, Matsumoto I, Hori T, Sato I: Halothane reduces the inhibition of dorsal horn lamina V type neuronal activity induced by bradykinin injection into the femoral artery contralateral to the recording site [in Japanese]. Masui 1995; 44:221–6

37. Yanagidani T, Ota K, Collins JG: Complex effects of general anesthesia on sensory processing in the spinal dorsal horn. Brain Res 1998; 812:301–4

38. Namiki A, Collins JG, Kitahata LM, Kikuchi H, Homma E, Thalhammer JG: Effects of halothane on spinal neuronal responses to graded noxious heat stimulation in the cat. ANESTHESIOLOGY 1980; 53:475–80

39. Wall PD: The laminar organization of dorsal horn and effects of descending impulses. J Physiol (Lond) 1967; 188:403–23

40. Sandkuhler J, Fu QG, Zimmermann M: Spinal pathways mediating tonic or stimulation-produced descending inhibition from the periaqueductal gray or nucleus raphe magnus are separate in the cat. J Neurophysiol 1987; 58:327–41

41. Pubols LM, Simone DA, Bernau NA, Atkinson JD: Anesthetic blockade of the dorsolateral funiculus enhances evoked activity of spinal cord dorsal horn neurons. J Neurophysiol 1991; 66:140–52

42. Cervero F, Iggo A, Ogawa H: Nociceptor-driven dorsal horn neurones in the lumbar spinal cord of the cat. Pain 1976; 2:5–24

43. Antognini JF, Carstens E: Increasing isoflurane from 0.9 to 1.1 minimum alveolar concentration minimally affects dorsal horn cell responses to noxious stimulation. ANESTHESIOLOGY 1999; 90:208–14

44. Jinks S, Antognini JF, Carstens E, Buzin V, Simons C: Isoflurane can indirectly depress lumbar dorsal horn activity in the goat via action within the brain. Br J Anaesth 1999; 82:244–9

45. Cheng G, Kendig JJ: Enflurane directly depresses glutamate AMPA and NMDA currents in mouse spinal cord motor neurons independent of actions on $GABA_A$ or glycine receptors. ANESTHESIOLOGY 2000; 93:1075–84

46. King BS, Rampil IJ: Anesthetic depression of spinal motor neurons may contribute to lack of movement in response to noxious stimuli. ANESTHESIOLOGY 1994; 81:1484–92

47. Rampil IJ, King BS: Volatile anesthetics depress spinal motor neurons. ANESTHESIOLOGY 1996; 85:129–34

48. Antognini JF, Carstens E, Buzin V: Isoflurane depresses motoneuron excitability by a direct spinal action: An F-wave study. Anesth Analg 1999; 88:681–5

49. Amzallag M: Autonomic hyperreflexia. Int Anesthesiol Clin 1993; 31:87–102

50. LaMotte RH, Thalhammer JG, Torebjork HE, Robinson CJ: Peripheral neural mechanisms of cutaneous hyperalgesia following mild injury by heat. J Neurosci 1982; 2:765–81

51. Yeomans DC, Proudfit HK: Nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different nociceptors in the rat: Electrophysiological evidence. Pain 1996; 68:141–50

52. Bennett DJ, Li Y, Harvey PJ, Gorassini M: Evidence for plateau potentials in tail motoneurons of awake chronic spinal rats with spasticity. J Neurophysiol 2001; 86:1972–82

53. Bennett DJ, Li Y, Siu M: Plateau potentials in sacrocaudal motoneurons of chronic spinal rats, recorded in vitro. J Neurophysiol 2001; 86:1955–71

54. Jankowska E, Hammar I: Spinal interneurons: How can studies in animals contribute to the understanding of spinal interneuronal systems in man? Brain Res Brain Res Rev 2002; 40:19–28

55. Krenz NR, Weaver LC: Sprouting of primary afferent fibers after spinal cord transection in the rat. Neuroscience 1998; 85:443–58

56. Grossman SD, Wolfe BB, Yasuda RP, Wrathall JR: Changes in NMDA receptor subunit expression in response to contusive spinal cord injury. J Neurochem 2000; 75:174–84

57. Uchida K, Baba H, Maezawa Y, Furukawa S, Omiya M, Kokubo Y, Kubota C, Nakajima H: Increased expression of neurotrophins and their receptors in the mechanically compressed spinal cord of the spinal hyperostotic mouse (twy/twy). Acta Neuropathol (Berl) 2003; 106:29–36

58. Resnick DK, Schmitt C, Miranpuri GS, Dhodda VK, Isaacson J, Vemuganti R: Molecular evidence of repair and plasticity following spinal cord injury. Neuroreport 2004; 15:837–9

59. Ditunno JF, Little JW, Tessler A, Burns AS: Spinal shock revisited: A four-phase model. Spinal Cord 2004; 42:383–95

60. Helkowski WM, Ditunno JF Jr, Boninger M: Autonomic dysreflexia: Incidence in persons with neurologically complete and incomplete tetraplegia. J Spinal Cord Med 2003; 26:244–7