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The Influence of Cryogenic Brain Injury on Nociception in the Rat

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Background: Previous studies have suggested that focal cryogenic brain lesions that cause functional cerebral depression may increase anesthetic potency. To determine whether this effect was caused by changes in nociception, this study prospectively evaluated the influence of an experimental focal brain injury on the analgesic effects of the opioids, fentanyl and alfentanil, in rats.

Methods: The cortical freezing lesion was made with a brass probe cooled to -50°C , applied through a craniotomy to the intact dura for 5 s. The analgesic effects of the opioids were quantified by tail-flick latency 3 days after the injury. The prolongation of tail-flick latency by infusions of each opioid in animals injured with a standardized cortical freezing lesion was compared with the results obtained from sham-operated control animals.

Results: At the endpoint of the experiment, prolongation of the tail-flick latency to 10 s, the mean serum concentrations (EC_{50}) of both fentanyl and alfentanil were approximately 25% less in the brain-injured animals than in the controls (EC_{50} fentanyl; injured: 10.2 ± 2.6 ng/ml, controls: 13.6 ± 5.2 ng/ml [$P < 0.02$]; EC_{50} alfentanil; injured: 54.7 ± 9.2 ng/ml, controls: 74.3 ± 18.4 ng/ml [$P < 0.02$]). For alfentanil, no significant differences in pharmacokinetics between injured and control animals were observed.

Conclusions: These results support the hypothesis that reductions in anesthetic requirements in this animal model of brain injury may be caused, in part, by alterations in nociception. (Key words: Analgesics, opioids: alfentanil; fentanyl. Measurement techniques: gas chromatography; tail-flick latency; tail-flick response. Pharmacodynamics, brain: injury.)

ALTHOUGH it is reasonable to postulate that the effect of anesthetics or analgesics may be altered by injury or functional changes in the central nervous system (CNS), few investigations have focused on the effects of brain injury on the pharmacodynamics of these drugs. Recently, the influence of psychoactive drugs on the recovery of function after CNS injury has been reviewed,

emphasizing that agents that interact with central cholinergic and monoaminergic (catecholamine, dopamine, serotonin) neurotransmitter systems can profoundly alter outcome after experimental neurologic injury.¹ Studies of brain metabolism and brain function after focal cold injury in the rat indicate that functional cerebral depression and behavioral changes observed after the injury may be caused by increased activity in cortical serotonergic and/or catecholaminergic neurons.^{2,3} Recently, our laboratory reported that the brain concentration of pentobarbital associated with lack of response to tail clamp was reduced 30% after cold injury.⁴ This change in anesthetic requirement was not observed in serotonin-depleted animals, consistent with a role for serotonin in the effects of cold injury on the pharmacodynamics of pentobarbital. Because the major opioid-mediated analgesia systems that modulate nociception in the spinal cord project serotonergic fibers from the periaqueductal gray and the nucleus raphe magnus to the dorsal horn,⁵ we speculated that pharmacodynamics of opioids would be altered after focal cryogenic injury.

Accordingly, this investigation tests the hypothesis that, during administration of opioids by continuous infusion, rats with brain injury will reach specified levels of analgesia at lower doses and plasma concentrations of opioid than control animals.

Materials and Methods

The study was approved by the University of Calgary Animal Care Committee. Male Sprague-Dawley rats, 200–350 g, were randomly assigned to the sham-operated and injured groups, and all testing was done by a single observer blinded to the injury status of the animals.

Brain Injury

A standardized focal cortical injury was produced in the manner described by Pappius² and previously used in this laboratory.⁴ In all animals, under general anes-

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thetia with halothane (2–2.5%) in oxygen, a left parietal craniectomy sufficient to allow penetration of a 3-mm diameter brass probe, was made with a dental drill, 2 mm to the left of midline immediately behind the coronal suture. For sham-operated controls, the wound was then closed. For injured animals, a brass probe, cooled to -50°C , with a slurry of dry/acetone was gently applied to the intact dura for 5 s. After wound closure, all animals were allowed to recover in their cages without further handling until tail-flick latency testing on the third postinjury day, the time at which previous studies have demonstrated maximal functional cerebral depression after this experimental injury.²

Test For Analgesia

The time between exposure of the tail to noxious heat stimulus and initiation of the flick of the tail (tail-flick latency) was used to measure the analgesic effects of the injected opioids, fentanyl and alfentanil. The tail-flick response has been considered to be a spinal cord reflex that is modulated by supraspinal influences. Raphe-spinal serotonergic terminals have been thought to contribute to inhibition of nociception,⁶ although the role of tonic serotonergic inhibition has recently been questioned.⁷ This reflex has been used to quantify the analgesic effects of opioids in rodents,⁸ and the version of the tail-flick test used in our laboratory has been previously described.⁹

Briefly, on the third day after cold injury, the animals were weighed and then anesthetized with halothane (2–2.5%) in oxygen for insertion of vascular catheters into a femoral artery for measurement of blood pressure and withdrawal of blood samples and into a femoral vein for infusion of one of the opioids. The animals were then partially restrained in a plaster spica cast on a wooden block, placed under a warming lamp, and allowed to eliminate the halothane for two hours. Rectal and tail temperatures were measured with a thermistor probe (Model 43TD, Yellow Springs Instruments, Yellow Springs, OH). Rectal temperature was measured every 15 min and adjusted to $36\text{--}37^{\circ}\text{C}$ using a warming lamp. Physiologic variables measured included mean arterial pressure (MAP), hematocrit (Hct), arterial blood gas tensions and pH, and tail temperatures at the onset and at the end of the tail-flick test.

To measure tail-flick latency, the distal 2 cm of the animal's tail was gently placed in a container of water heated to 54°C . The time from immersion to first flick movement (tail-flick latency) was measured with a

stopwatch to the nearest 0.5 s. Tail-flick latency was measured before and at 3, 5, 7, 10, 13, and 15 min after the beginning of the opioid infusion and each 5 min thereafter. The endpoint for the experiment was prolongation of the tail-flick latency to 10 s. Values for the time from the onset of the infusion to the 10-s endpoint were used to calculate the total dose of opioid infused for each animal.

Opioid Infusion

To determine the relationship between plasma opioid concentration, C_p , and the tail-flick latency, infusions of the drugs were administered. In this way, we hoped that the plasma concentration of opioid would change slowly enough to allow us to demonstrate a relationship between concentration and effect.¹⁰ For each opioid, the infusion rate, adjusted for body weight, was the same for the injured and the control animals. A difference in analgesic potency between the groups would thus be identified by differences in infusion times, doses infused, and plasma concentration of opioid associated with the defined endpoint.

Based on preliminary studies (results not shown), rates for intravenous infusion of fentanyl and alfentanil were selected that would achieve the endpoint of prolongation of tail-flick latency to 10 s after 15–30 min of infusion in the uninjured animals. The infusion rate that achieved this result was $0.6\text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of a solution containing either $1\text{ }\mu\text{g/ml}$ fentanyl or $5\text{ }\mu\text{g/ml}$ alfentanil.

The effects of repeated tail-flick latency testing and blood sampling were examined in five normal, unoperated animals. On the day of the experiment, these animals were prepared and handled in the same way as the experimental groups described above except that no opioid was infused.

Fentanyl Sampling

When the tail-flick latency had prolonged to 10 s, blood (2–3 ml) for fentanyl assay was withdrawn from the arterial cannula into a heparinized tube. The plasma was separated from the blood sample by centrifugation, frozen immediately, and stored at -70°C .

Alfentanil Sampling

In the alfentanil group, two blood samples were drawn according to the tail-flick latency, 1 ml when the tail-flick latency had prolonged to 6–7 s, and 2–3 ml at a prolongation of 10 s. The initial sample time was chosen as the half-response, while the sample at

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tail-flick latency of 10 s was the same endpoint as for fentanyl. In addition, samples (1 ml) were drawn at approximately 3, 5, 10, 15, 20, and 25 min after the start of the infusion. An equal volume of saline was injected intravenously after each sample was drawn. The number of samples drawn from each animal was recorded.

Measurement of Opioids

Plasma concentrations of fentanyl and alfentanil were determined after extraction of the opioids from the serum samples using a modification (toluene was used instead of hexane as extracting solvent) of the technique described by Gillespie *et al.*¹¹

The absolute limit of detection for both fentanyl and alfentanil was estimated to be 0.2 ng opioid base/ml. For the alfentanil assay, the coefficient of variation (for repeated analyses, 1 standard deviation/mean value $\times 100\%$, determined for the highest value on the standard curves, 100 ng/ml for alfentanil and 30 ng/ml for fentanyl) was $\pm 6.3\%$ and the accuracy (determined from the standard curves) was $\pm 4.59\%$.

All values are expressed as mean \pm standard deviation. In the study of the effects of repeated blood sampling on blood pressure and hematocrit, the Pearson product-moment correlation coefficients for the relationships between the cumulative blood volume exchanged and the respective physiologic variables were calculated. The correlation between the tail-flick latency and the number of trials was also evaluated in this way.

In the operated animals, the values of physiologic variables and the opioid infusion and serum concentration data observed in the injured animals were compared with those obtained in the control group for each opioid separately using an unpaired *t* test. For all tests, statistical significance was inferred when $P < 0.05$.

Results

Consequences of Blood Sampling and Repeated TFL Testing

Five animals (body weights 380 ± 10 g) underwent tail-flick latency testing and blood sampling according to the analgesia testing protocol described above without any opioid infusion. The results, summarized in table 1, show that, although blood pressure and hematocrit decreased significantly during the blood sampling protocol, the changes were small. No correlation was found between the tail-flick latency and the number of trials.

Physiologic State of the Animals

None of the animals was noted to have suffered any obvious neurological deficit and none of the animals died or became noticeably impaired after cryogenic brain injury or during analgesia testing.

As shown in table 2, the only physiologic value that differed between the injured and the control animals in either of the opioid groups was the tail temperature. In the fentanyl group, the initial tail temperature was 1°C less in the injured animals than in the sham-operated rats, while, in the alfentanil group, the final tail temperature was 1.1°C greater in the injured animals than in the controls. Arterial pH decreased significantly during the opioid infusion in both groups of alfentanil-treated animals, without any significant difference between the injured animals and the controls. The effect of the partial restraint on cerebral activity and analgesia was not studied. Cerebral function, as reflected by cerebral glucose utilization, was not altered by this technique,¹² and stress-induced hyperglycemia is not usually seen using this model.^{2,18}

Table 1. Consequences of Blood Sampling and Repeated Tail Flick Latency Testing

Vol	0	4	8	11	15	19	23	26	<i>r</i>
Trial no.	1	2	3	4	5	6	7	8	—
MAP (mmHg)	149 \pm 6	148 \pm 9	147 \pm 6	144 \pm 7	142 \pm 9	139 \pm 16	137 \pm 15	137 \pm 9	0.98*
Hct (%)	46 \pm 2	43 \pm 4	45 \pm 3	45 \pm 3	42 \pm 2	41 \pm 4	39 \pm 4	37 \pm 6	0.92†
TFL (s)	2.8 \pm 0.7	3.2 \pm 0.8	3.5 \pm 0.5	3.6 \pm 0.9	3.8 \pm 0.7	3.7 \pm 0.6	3.7 \pm 0.8	3.3 \pm 0.8	0.588

Values are mean \pm SD.

Vol = cumulative volume, in milliliters/killigram of body weight, of blood withdrawn in equal exchange with normal saline; MAP = mean arterial pressure; Hct = blood hematocrit; TFL = tail flick latency; *r* = Pearson product-moment correlation coefficient for (1) the relationship between the exchanged volume and MAP or Hct or (2) the relationship between the number of trials and the tail flick latency.

* $P < 0.001$.

† $P < 0.01$.

Table 2. Physiologic State of the Animals

	Fentanyl Group		Alfentanil Group	
	Sham	Injured	Sham	Injured
No. of animals	15	19	8	7
Weight (g)	283 ± 19	287 ± 19	262 ± 19	259 ± 14
Rectal temperature (° C)	36.7 ± 0.7	36.4 ± 0.7	36.1 ± 0.7	36.4 ± 1.1
Blood exchanged (ml/kg)	3	3	9.3 ± 0.7	8.9 ± 0.7
Hematocrit (%)	42.5 ± 3	42.7 ± 3	45.4 ± 4	45.5 ± 5
MAP (mmHg)	130 ± 8	132 ± 8	120 ± 9	130 ± 17
Tail temperature (° C)				
Initial	24.5 ± 0.8	25.5 ± 1.2*	25.6 ± 0.8	25.8 ± 1.1
Final	25.3 ± 1.0	26.0 ± 1.4	26.2 ± 1.0	25.1 ± 1.3*
No. of TFL tests	8 ± 1	9 ± 2	6 ± 1	7 ± 2
pH (arterial)				
Initial	7.42 ± 0.02	7.41 ± 0.01	7.39 ± 0.03	7.41 ± 0.01
Final			7.31 ± 0.03†	7.31 ± 0.05†
Pa _{CO₂} (mmHg)				
Initial	34.3 ± 2	35.6 ± 0.6	35.0 ± 3	33.9 ± 4
Final			35.6 ± 6	36.5 ± 6
Pa _{O₂} (mmHg)				
Initial	78 ± 6	78 ± 8	95 ± 22	93 ± 12
Final			103 ± 8	102 ± 13

Values are mean ± SD.

MAP = mean arterial pressure; Pa_{CO₂} = carbon dioxide tension; Pa_{O₂} = arterial oxygen tension; TFL = tail flick latency.

* $P < 0.05$ versus sham-operated animals.

† $P < 0.05$ versus initial values.

Pharmacokinetics of Alfentanil

The relationship between the Cp of alfentanil and the dose of the drug infused is summarized in figure 1. Because of the vagaries of arterial cannula function, the number of subjects contributing to the mean value changes with time. Only at the 9- μ g/kg dose did the injured animals differ significantly from the controls ($P = 0.013$). From this data, we concluded that there was no significant difference in the pharmacokinetics of alfentanil between the injured and the sham-operated animals.

The relationship between the endpoints (EC₅₀ and ED₅₀) and the plasma concentration curves are also shown in figure 1. In summary, the endpoint of prolongation of TFL to 10 s was achieved after a shorter infusion time and, therefore, opioid dose (table 3) in the injured group than in the control animals, resulting in lower values for EC₅₀ and ED₅₀ in the injured animals.

Plasma Concentration-Response Relationship

The relationship between serum alfentanil concentration and tail-flick latency is shown in figure 2. There is no difference in the tail-flick latencies between the injured and the control animals at the beginning of the

infusion; however, the concentration-response curve of the injured rats is shifted significantly to the left of the curve of the control animals.

Table 3 summarizes the data concerning the opioid infusions. Although the mean serum concentrations of both fentanyl and alfentanil at the endpoint were less in the injured than in the control animals (fentanyl reduced by 25%, alfentanil by 26%; for both, $P < 0.02$), only in the alfentanil-treated animals was there a significant difference in the dose of opioid infused.

Discussion

This study shows that the cryogenic brain injury altered the antinociceptive effects of two opioids, fentanyl and alfentanil, with regard to a commonly used measure of analgesia, the tail-flick response in rats. This result is consistent with our previous study in this model, which showed that: 1) anesthetic requirements for pentobarbital were reduced in brain-injured rats when compared with unoperated controls, and 2) that the effects of the lesion on anesthetic requirements may have been mediated by serotonin. In the current study,

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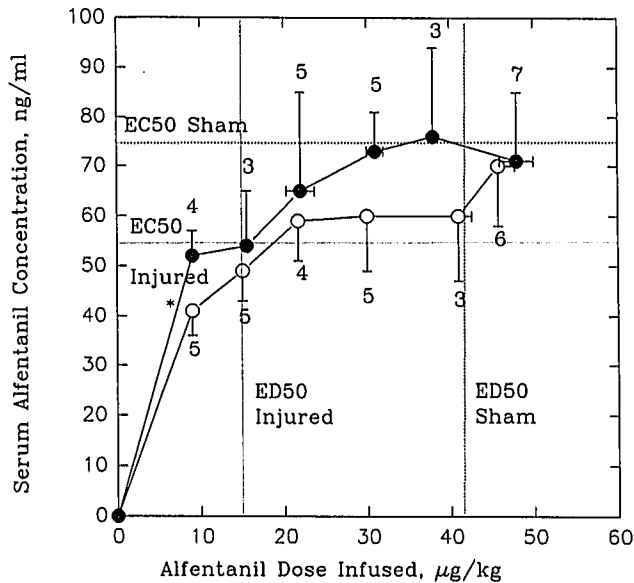


Fig. 1. Plasma alfentanil concentrations during continuous intravenous infusion of alfentanil. Open circles represent animals with cryogenic lesions, and filled circles represent sham-operated control animals. The numbers of animals at each time is shown adjacent to the mean value. Error bars represent the standard deviations. * $P < 0.05$ versus sham value at a cumulative dose of 9 µg/kg.

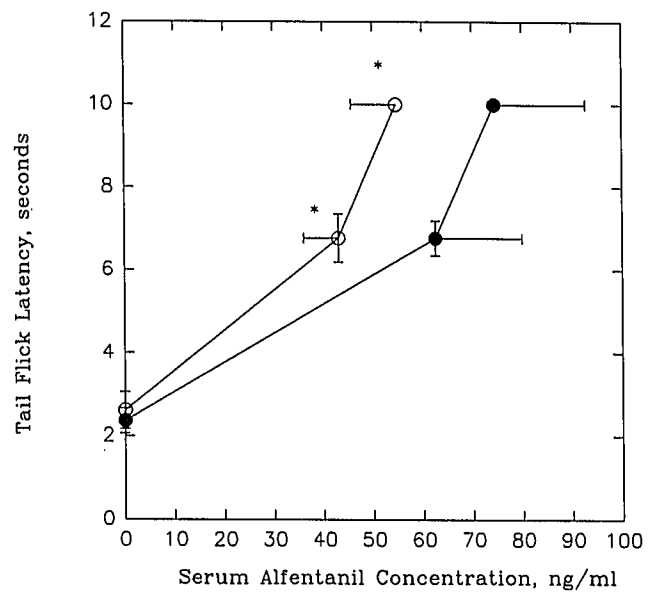


Fig. 2. Mean values of tail-flick latency and serum alfentanil concentrations measured during a continuous intravenous infusion of alfentanil. Open circles represent animals with cryogenic lesions, and filled circles represent sham-operated control animals. Error bars represent the standard deviations. * $P < 0.02$ versus uninjured animals.

the alteration in the relationship between C_p for these opioids and tail-flick latency indicates that the focal brain injury may interact in some way with the modulation of nociception at the spinal cord level.

The clinical equivalent of the lesion used in this study is probably a focal cortical contusion with necrosis, with little evidence of injury below the cortex.¹³ This freezing lesion is similar to the cytochemical lesion shown by Ginsberg *et al.*¹⁴ to cause diaschisis in the rat (WD Dietrich, personal communication). Using Evan's blue, Pappius² has shown that the blood-brain barrier is pervious to plasma proteins at 4 and 24 h after the lesion, but is intact by the third day after the

injury. The lesion produces no easily detectable neurological sequelae, but a detailed study¹⁵ has shown that reductions in performance in specific behavioral tests correlate with the alterations in cortical glucose utilization after the cryogenic lesion. These authors¹⁵ have interpreted the behavioral changes to have occurred on the basis of widespread functional depression of the cortex rather than focal parietal damage. Many of the investigations using the focal freezing lesion model have focused on the widespread depression of local cerebral glucose utilization that occurs in the days after the lesion, most marked in the cortical regions ipsilateral to the lesion.^{2,16} This decrease in metabolic

Table 3. Effect of Focal Cold Injury on the Total Dose and Concentration of Opioids at Prolongation of Tail Flick Latency to 10 s in Rats Receiving an Opioid Infusion

	Fentanyl Group		Alfentanil Group	
	Sham	Injured	Sham	Injured
Infusion time (min)	18.4 ± 5.8	16.9 ± 5.8	21.8 ± 22.9	7.14 ± 3.4
Total dose (µg/kg)	10.0 ± 3.4	8.6 ± 3.1	41.8 ± 8.7	14.3 ± 3.6†
Plasma concentration (ng/ml)	13.6 ± 5.2	10.0 ± 2.6*	74.3 ± 18.4	54.7 ± 9.2*

* $P < 0.02$, sham versus injured.

† $P < 0.05$, sham versus injured.

rate for glucose has been interpreted to represent functional cerebral depression,^{17,18} and evidence has been presented to support a role for serotonin and noradrenaline metabolism in this phenomenon. Clinical evidence consistent with posttraumatic functional cerebral depression in humans has been presented.¹⁹ Our current results suggest that, in addition to functional cerebral depression, the focal cortical injury alters nociception at the level of the spinal cord.

Recently, Shapira *et al.* have studied the effects of a concussive closed head injury on halothane MAC in rats.²⁰ They reported little or no change in MAC for the mildly injured animals; however, in the more severely injured rats, MAC decreased as the severity of the injury increased. The injury produced in this model²¹ was a much larger lesion than our focal freezing lesion and caused many animals to be neurologically impaired. The freezing lesion introduced by Pappius and used in our laboratory is histologically much smaller, with a zone of necrosis restricted to the cortex, and caused no easily detectable neurological sequelae.

The results of Shapira *et al.* are not necessarily inconsistent with our findings. Shapira *et al.* were not specifically investigating mild brain injury and many of their animals were severely impaired. This may have rendered their study less sensitive to alterations in MAC in the mildly injured animals. However, others have also failed to detect changes in MAC after freezing lesions of the type used in the current study (MM Todd, personal communication). We suspect that, in the latter case, the lesions are similar to those produced in our laboratory and, at present, we cannot account for the difference in the findings.

In this study, we used a constant infusion to gradually increase the plasma concentration of opioid while we were concomitantly measuring the tail-flick latency to determine the relationship between the tail-flick latency and the plasma opioid concentration. This method has been used previously to investigate alterations in the pharmacokinetics and pharmacodynamics of pentobarbital.²² The technique assumes that the opioid in the plasma equilibrates rapidly with effector sites in the nervous system. For alfentanil, this assumption is well supported by human studies,^{23,24,25} although, to our knowledge, it is not documented in the rat. The main advantage of this technique is that the Cp-response relationship can be determined in each subject over a full range of concentrations. The principal disadvantage is that, in the nonsteady-state conditions during the onset of an infusion, the Cp asso-

ciated with a given response will be greater than the plasma concentration for that response at steady state, Cpss.²⁶ The magnitude of this difference will be related to the rate of change of the plasma concentration and the equilibration time. For the alfentanil infusion in the injured animals, using the equilibration time (k_{co}) of 1.5 min estimated for humans,²⁵ and the rate of rise of Cp at the EC₅₀ calculated from figure 1, we would estimate that the Cpss would be approximately 5 ng/ml less than the Cp ($1.5 \text{ min} \times 3.6 \text{ ng/min}$ rate of increase of [alfentanil]_{plasma}). In the sham-operated group, the difference would probably be less (Cp approximately 1.5 ng/ml greater than Cpss) because the rate of rise of the [alfentanil]_{plasma} at the EC₅₀ for that group is only 1 ng/min. Thus, the error associated with the technique tends to bias the study against our findings, and the difference in EC₅₀ using a Cpss technique may, in fact, be greater than we report here.

The assumption for rapid equilibration between plasma and site of effect does not hold nearly as well for fentanyl, as evidenced by the k_{co} of 5.6 min in humans.²⁵ This problem, as well as the fact that only one point on the concentration-response curve was evaluated in the fentanyl protocol, makes the results we report for fentanyl much weaker. Although the infusion time and dose were numerically less in the injured group, the difference was not statistically different. This may have been because, as outlined above, the temporal disparity between plasma concentration and effect obscured the differences between the groups for these variables. For fentanyl, a technique using Cpss would be more suitable.

Plasma protein binding has an important role in the Cp-response relationship for alfentanil.²⁷ If the plasma protein binding of alfentanil was altered by brain injury, it would not be detected in our study, because the bound and free fractions were not measured separately.

The alteration in the Cp-response relationship for the opioids in the injured animals may be caused by: 1) a decrease in the gradient between the plasma and the effector site, resulting in higher effector site concentrations; 2) an increase in opioid receptor number or sensitivity; 3) a primary sensory deficit cause by the lesion; 4) an increase in the concentration of free opioid in the plasma of the injured animals; and 5) an increase in antinociceptive modulatory input related to the injury. We feel that the first possibility is unlikely because of the high lipid solubility of fentanyl and alfentanil,²⁸ and because the blood-brain barrier has been reported to be intact in this experimental model.²

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Although some preliminary data suggest that monoamine receptor numbers in the cortex may be altered in this model (HM Pappius, personal communication), no evidence currently supports alterations in number or sensitivity of opioid receptors. We believe the third possibility is unlikely given the spinal nature of the reflex being tested in this study and the observation that the baseline TFL values were not altered in the injured group. The results of this study are consistent with the hypothesis that the cortical injury alters neuromodulation of nociception. (Neuromodulator is the term given to a neurotransmitter that is released from a nonsynaptic site and diffuses to target cells to modulate the release of neurotransmitters.²⁹) In the case of the antinociceptive effects of opioids in the current study, one would expect that, initially, when opioid levels and, therefore, neurotransmitter release was low, the alteration in neuromodulation by the lesion would be small or absent, resulting in the similar baseline values for TFL. As neurotransmitter release increased, provoked by higher opioid concentrations, the difference between injured and control animals appeared.

The lack of significant correlation between the tail-flick latency and the number of trials (table 1) indicates that damage to the tail by the hot water was not a major cause for the increase in tail-flick latency during the nociception study, confirming previous observations using this model.⁹ The animals used in the pilot study were larger (and therefore older) than those used in the nociception study. This may account for the slightly higher blood pressure in that group.

The tail temperatures differed between the control and injured animals in both the fentanyl- and alfentanil-treated groups. In the fentanyl group, initial tail temperature was 1° C less in the injured animals than in controls, while, in the alfentanil group, final tail temperature was 1.1° C more in the injured animals than in the controls. From a previous report, the change in tail-flick latency with change in tail temperature can be estimated to be 6% of baseline latency per ° C change in temperature. There does not appear to have been an effect of brain injury on baseline tail temperature or tail-flick latency (table 2), and there does not appear to have been any effect of brain injury on tail temperature during the opioid infusion. Consequently, we do not feel that the differences in tail temperature observed in these experiments had any significant role in altering tail-flick latency. Similarly, although the arterial pH was significantly less at the end of the alfentanil infusions (see table 2) than initially, we did not determine any

difference in this expected consequence of opioid administration between the injured rats and the control animals, and we do not feel that the pH change had a physiologically significant effect on the tail-flick latency.

The results of this study support the hypothesis that the reduction in anesthetic requirements for pentobarbital observed previously⁴ was, in part, caused by an alteration of nociception. One possible mechanism for this effect of brain injury on nociception may be that the injury activates bulbospinal serotonergic pathways. Interneurons in layers I, II_o, and II_i of the dorsal horn are the targets of descending inhibitory serotonergic projections from the periaqueductal gray, dorsal raphe nucleus, and mesencephalic reticular formation (mesencephalon) and the nucleus raphe magnus (medulla), which, together, are thought to be the main components of the system that mediates the effects of systemically administered opioids.³⁰ If these pathways are activated by the focal freezing lesion in a manner similar to the cortical serotonergic pathways³ (which derive largely from the raphe nuclei³¹), alterations in the pharmacodynamics of the opioids, such as those observed in this study, would be anticipated.

In summary, this study shows that a unilateral focal freezing lesion alters the analgesic response to two opioids, fentanyl and alfentanil, reducing the EC₅₀ for the tail-flick response by approximately 25% for both drugs.

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