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Supraspinal Anesthesia

Behavioral and Electroencephalographic Effects of Intracerebroventricularly Infused Pentobarbital, Propofol, Fentanyl, and Midazolam

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Background: **Anesthetic endpoints of unconsciousness and immobility result from agent effects on both brain and spinal cord that are difficult to separate during systemic administration. To investigate cerebral mechanism of anesthetic-induced unconsciousness, the authors studied behavioral and electrophysiologic effects of four anesthetics infused intracerebroventricularly to conscious rats. The authors aimed to produce progressively increasing anesthetic depths, indicated by electroencephalographic synchronization and behavioral change.**

Methods: **During anesthesia, rats were equipped with intracerebroventricular infusion catheters, hind-paw stimulation, and epidural electrodes to record the electroencephalogram from the somatosensory cortex. Silicone bolus was injected into the fourth ventricle to minimize drug distribution to the spinal cord. 60 min later, 50-min infusion of pentobarbital (6.0 mg/h), fentanyl (0.75 μg/h), propofol (3.0 mg/h), or midazolam (0.24 mg/h) was initiated. Vibrissal, olfactory, corneal, and tail-pinch responses were tested every 10 min.**

Results: **All agents depressed vibrissal, olfactory, and corneal responses; propofol and pentobarbital produced the strongest effect. All agents except propofol depressed tail-pinch response; fentanyl and pentobarbital produced the strongest effect. All agents except midazolam increased δ power. Pentobarbital enhanced θ power. All agents except fentanyl enhanced α and β power. Pentobarbital and midazolam slightly increased, whereas fentanyl decreased, γ power. Pentobarbital increased and midazolam decreased somatosensory evoked potential; these changes were small and apparently unrelated to behavior.**

Conclusions: **Alpha and β power increase may reflect sedative component of anesthesia. Simultaneous δ, α, and β power increase may correlate with loss of consciousness. Theta and δ power increase may reflect surgical anesthesia. Opioid-induced γ power decrease may reflect suppression of pain perception. Pentobarbital-, fentanyl-, and midazolam-induced immobility to noxious stimulation may be mediated supraspinally.**

DESPITE decades of research into the molecular mechanism of anesthetic action, the mechanisms by which general anesthetics produce their behavioral effects, such as immobility, analgesia, amnesia, and loss of consciousness, remain unclear. Scientific evidence suggests that various agents affect different, well-described regions of the central nervous system by acting on various receptors and receptor subunits,[1–3] producing highly agent-specific effects. Such differences in action may clearly underlie the varied clinical effects of different anesthetics.

The brain has been assumed to be the primary site for anesthetic action with respect to unconsciousness and amnesia,[4] whereas immobility in response to noxious stimulation has been ascribed to an anesthetic effect on both spinal and supraspinal sites.[5–7] Therefore, an interaction of spinal and supraspinal mechanisms must be considered in the explanation of anesthetic effects. For example, systemically administered anesthetic agents would suppress sensory afferents originating in the spinal cord and may therefore augment their supraspinal effect by reducing the ascending arousal signals *via* the reticular activating system.[8,9]

To study the neural mechanism of the anesthetic-induced unconsciousness, one depends on a suitable battery of electrophysiologic and behavioral assessment of the state of consciousness. An assessment of unconsciousness from behavioral signs becomes particularly difficult when spinal motor systems are inhibited by the anesthetic or neuromuscular agent. In addition, anesthetic alterations in ascending spinal nerve traffic present a confounding factor of the direct affect of anesthetics on the brain.[8,10] These difficulties have led investigators to develop animal models in which agent delivery to the brain and spinal cord could be independently controlled.[11–14] Others have infused agents supraspinally to demonstrate preferential anesthetic effects on the brain,[6,15] and only a few have assessed both behavioral and electroencephalographic changes using such models.[16,17]

In this study, we compared the effects of four commonly used intravenous agents, propofol, fentanyl, pentobarbital, and midazolam, on the electroencephalographic activity, somatosensory evoked potential (SEP), and a battery of sensorimotor responses before and at various times during continuous intracerebroventricular infusion of the agents. To minimize the cerebrospinal spread of the drugs and thereby to focus the anesthetic effect on the brain, we also introduced a block of cerebrospinal fluid outflow at the level of the fourth ventricle—a technique that, to our knowledge, has not been used before.

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The primary purpose of this study was to characterize and compare the anesthetic states that resulted from the intracerebroventricular infusion of four agents by electrophysiologic and behavioral indices. To identify differential effects, we chose four representative agents with known preferential hypnotic, analgesic, and sedative–amnesic properties. We hypothesized that the intracerebroventricular infusion of the anesthetic agents would produce a gradual electroencephalographic synchronization effect typical to a sleeping state in clinical settings, which would correlate with behavioral suppression. We expected that the agents' observed differential effects would, in the long run, provide valuable insights into their specific mechanisms of action and would help us to better understand the neural mechanisms underlying anesthetic-induced immobility, areflexia, and unconsciousness.

Materials and Methods

The experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin, Milwaukee, Wisconsin. All procedures conformed to the Guiding Principles in the Care and Use of Animals of the American Physiologic Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (Washington, D.C., National Academy Press, 1996).

Surgery

Forty-two male Sprague-Dawley rats weighing 200–250 g were used in the study. All animals were housed on a 12-h light–dark cycle at a constant temperature of 23° ± 1°C with free access to food and water for 2 weeks before physiologic experiments. Before surgery, the animal was briefly anesthetized in an anesthesia box with 5% isoflurane (Abbot Laboratories, Chicago, IL). The animal was then placed into a stereotaxic frame where the head was secured in a flexed-forward position. A gas anesthesia mask was placed over the snout to continue the anesthesia at 1.9% isoflurane. Isoflurane concentration and the levels of oxygen and carbon dioxide were monitored through a sampling line connected to the anesthesia mask using a gas analyzer (POET II; Criticare Systems, Inc., Waukesha, WI). A rectal temperature probe was inserted, and the temperature was maintained at 37°C with a thermostat-controlled water-circulated heating pad. Sterile 1% Xylocaine (Astra Zeneca LP, Wilmington, DE) was injected under the scalp, and a midline incision was made. The skin was laterally reflected, and the exposed cranium was gently scraped of connective tissue and bleeding was cauterized. A hole for the intracerebroventricular anesthetic agent infusion was bored at coordinates overlaying the right cerebral ventricle (anterior–posterior −0.8 mm from bregma, lateral ±1.5 mm, and 3.5 mm depth from the skull surface) according to the atlas of Paxinos and Watson.[18] Another hole for viscous silicon infusion was bored at coordinates overlaying the fourth cerebral ventricle (anterior–posterior −11.6 mm, lateral ±0.0 mm).[18] Through the latter hole, a 30-gauge stainless steel cannula (2 cm long) was stereotaxically inserted into the fourth cerebral ventricle (−8.0 mm depth from bregma). Before insertion, 30-cm-long polyethylene tubing (PE-10, 0.28-mm ID, Intramedic; Becton Dickinson, Franklin Lakes, NJ) was used to connect the infusion syringe containing viscous silicone (1-ml syringe with Luer-Lok) to the intracerebroventricular cannula. Viscous silicone was prepared by heating hydrogen functional polydimethyl siloxane (RT604A; Wacker Silicone Corp., Adrian, MI) with a platinum catalyst (×84; Wacker Silicone Corp.), mixed at a ratio of 9:1, and blue dye (LR CM-340) at 37°C water bath with continual manual agitation for 90 s. The tubing and the intracerebroventricular cannula were prefilled with the viscous silicone from the infusion syringe. The intracerebroventricular cannula was then inserted into the fourth ventricle and fixed to the skull with dental acrylic (Lang Dental MFG, Wheeling, IL) and was allowed to dry for approximately 30 min. One microliter silicone mixture was then infused into the fourth ventricle to restrict the anesthetic distribution into the spinal cord.

For epidural recording of electroencephalographic activity, two stainless steel screw electrodes were inserted in the skull periosteum, one in the hind-paw area of the left somatosensory cortex (anterior–posterior −0.3 mm from bregma, lateral −2.5 mm, 2.5 mm depth from the skull surface)[18] and the second one in the indifferent site of the posterior parietal cortex in the left cerebral hemisphere (anterior–posterior −6 mm from bregma, lateral −2.5, and 2.5 mm depth from the skull surface).[18] The reference electrode was placed in posterior parietal cortex of the right cerebral hemisphere. After the electrode implantation, a 30-gauge stainless steel cannula (2 cm long) for the intracerebroventricular infusion was stereotaxically inserted into the right lateral brain ventricle. Polyethylene tubing (PE-10, 0.28-mm ID, Intramedic), 30-cm-long, was used to connect the infusion syringe containing the anesthetic agent to the intracerebroventricular cannula. The tubing and the intracerebroventricular cannula were then prefilled with the anesthetic agent from the infusion syringe. The intracerebroventricular cannula was fixed to the skull with dental acrylic (Lang Dental MFG) and was allowed to dry for approximately 30 min.

For sensory stimulation, a pair of 25-gauge needle electrodes was inserted into the skin of the right hind paw between the second and fourth fingers and was secured in place with a scotch tape.

After surgery was completed, bupivacaine (0.02–0.05 mg/kg subcutaneous) was applied to all surgical sites to minimize discomfort, and isoflurane anesthesia was discontinued. The still-anesthetized rat was quickly placed into a rodent torso sling suit, stationed inside the sling frame. This suit allows limited movements of the head and limbs but prevents the animal from crawling out of the sling. One hour was allowed for isoflurane to wash out.

Experimental Protocol

Four groups of animals, each receiving one of four anesthetic agents or artificial cerebrospinal fluid (aCSF), were used. Concentrations and infusion rates of each agent were as follows: pentobarbital (9 animals): 25 mg/ml, 4.0 μl/min; midazolam (8 animals): 1 mg/ml, 4.0 μl/min; propofol (9 animals): 10 mg/ml, 5.0 μl/min; fentanyl (11 animals): 0.05 mg/ml, 2.5 μl/min. The pentobarbital concentration of 25 mg/ml was achieved by diluting the standard agent concentration of 50 mg/ml with the aCSF at 1:1 ratio to avoid the suppression of electroencephalographic activity at higher doses. The control group of 5 animals received dyed artificial cerebrospinal fluid (1% Evans blue solution) only at a rate of 5.0 μl/min. For the anesthetic agent intracerebroventricular infusion, we chose to use these anesthetic concentrations and the slow rates to facilitate the distribution of drugs in the cerebrospinal fluid space, and to produce a gradual and maximal increase in the amplitude of electroencephalographic activity within a 50-min period. We estimated that 50 min of infusion time at the rate of 2.5–5 μl/min would suffice to fill most of the cerebrospinal fluid space of approximately 200 μl.

One hour after the termination of isoflurane anesthesia, when the animal showed signs of complete recovery from anesthesia, judged by spontaneous purposeful movements, infusion of pentobarbital, fentanyl, propofol, or midazolam was initiated. The right hind paw was electrically stimulated (1 mA) with single pulses repeated every 5 s for a period of 5 min to produce SEP. The stimulation was initiated 10 min before the intracerebroventricular infusion and was repeated every 10 min for a total duration of 50 min. Figure 1 shows the timeline of the experiment along with an example of electroencephalographic activity and SEP recorded during infusion of pentobarbital. Graded responses to tail pinching (TPR), vibrissal stroking, exposure to an offensive odor, and corneal stimulation were tested 15 min before the agent intracerebroventricular infusion (EEG-1 control block in fig. 1) and between the hind-paw stimulation periods after the intracerebroventricular infusion (consecutive electroencephalogram blocks in fig. 1) twice in each testing period at the second and fourth minutes.

Tail pinching was performed using a spring-loaded instrument with an applied force of approximately 500 g (80 g/mm^2). To prevent nerve damage and consequently an altered response, tail pinching was performed at the tip of the tail in the wakeful rat and was then gradually moved toward the root of the tail at each consecutive anesthetic dose. A cotton-tipped applicator was used to test the corneal and vibrissal responses. The reaction to offensive odor was tested using a highlighting pen (Sharpie permanent marker, fine point; Sanford, Bellwood, IL). The TPR and vibrissal, olfactory, and corneal responses (VOCRs) were graded on the scale of 0–2, where 2 and 0 signified fully preserved and completely abolished responses, respectively. The detailed description of the graded scale of behavioral assessment is presented in table 1.

The intracerebroventricular infusion of the anesthetic agent was discontinued after 50 min, and Evans blue dye mixed with aCSF (1% solution) was then intracerebroventricularly infused for additional 50 min at the same infusion rate. The animal was then taken out of the restrainer and placed into the anesthesia box, where it was exposed to 5% isoflurane for euthanasia. The brain and the proximal one third of the spinal cord were removed from the skull and the vertebral canal and were placed into the aCSF for 3 min and then sliced into seven coronal sections 1.5–2.0 mm wide. The sections were examined under the light microscope to assess the presence or absence of the dye in the left and right brain ventricles, the third ventricle, and the cerebral aqueduct and to verify the position of the intracerebroventricular cannula. The fourth ventricle was examined for the presence or absence of the blue silicone drop. The spinal cord was examined for the presence or absence of the Evans blue. Data from rats with dye-free spinal cord canals only were used in this study. The control group of animals infused with the dyed aCSF only did not receive an additional 50-min infusion of Evans blue dye solution. These animals were killed after 50 min of intracerebroventricular aCSF infusion, and the postmortem examination was performed as described above.

Measurement of ICP

In a subset of five animals, the effect of the intracerebroventricular infusion on intracranial pressure (ICP) and mean arterial blood pressure was studied. In preparation for the experiment, each animal was anesthetized with 60 mg/kg intraperitoneal sodium pentobarbital, and an arterial catheter was placed in the right femoral artery for the measurement of mean arterial blood pressure and heart rate. The anesthetized animal was then placed into a stereotaxic frame. Rectal temperature probe was inserted, and the temperature was maintained at 37°C with a thermostat-controlled water-circulated heating pad. The animal was breathing spontaneously. The intracerebroventricular cannula was then inserted into the right lateral brain ventricle using the procedure described above. A "Y" cannula was created by soldering

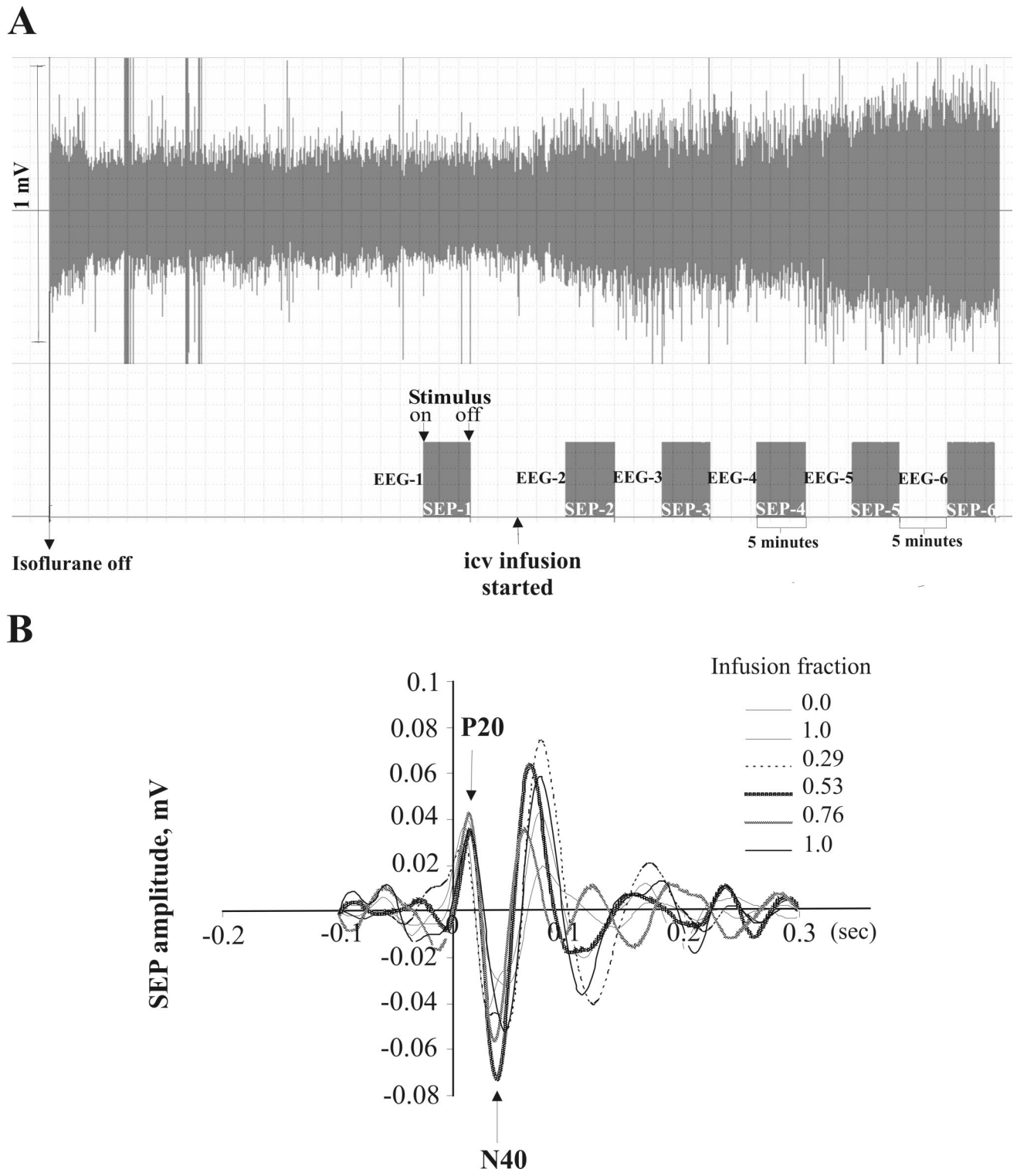

Fig. 1. Experimental time course and a typical example of recorded spontaneous electroencephalographic activity (EEG; *A*) and of the somatosensory evoked potential (SEP; *B*) in one experiment with pentobarbital. Blocks labeled EEG-1 through EEG-6 represent periods of spontaneous electroencephalographic activity. Blocks labeled SEP-1 through SEP-6 represent periods of electrical hind-paw stimulation. Vibrissal, olfactory, and corneal responses and tail-pinch responses were tested before each SEP block. The hind-paw stimulation was repeated every 10 min, beginning 10 min before the start of the intracerebroventricular (icv) infusion. For every stimulation block, single-trial SEPs comprising 300-ms poststimulus periods were extracted from the record using a threshold peak-detection algorithm. The amplitude of the average SEP was determined as the difference from the first positive (P20) to the following negative (N40) peak. The change in SEP amplitude is expressed in terms of infusion fraction, *i.e.*, the fraction of total volume of drug infused by 50 min. As shown in this example, pentobarbital exerted a dose-dependent enhancement of the electroencephalographic activity and the SEP amplitude.

Table 1. Criteria for Scoring Depth of Anesthesia in Rats

Test/Score	Definition
Tail pinch	
0	No response to tail pinch
0.5	Weak tail-flick response, no paw movements
1	Tail flick and hind-paw movements only
1.5	Tail flick and alternating paw movements
2	Brisk tail flick and gross motor movements
Corneal stimulation	
0	No blink response
0.5	Very slow, incomplete blink of the stimulated eye only
1	Bilateral eye blink; no head movements
1.5	Bilateral eye blink; turns head in the opposite direction from the stimulus but less brisk
2	Brisk bilateral eye blink accompanied with head movements in the opposite direction from the stimulus, and gross motor movements
Vibrissal stroking	
0	No response to whisker stroking
0.5	Delayed and very slow whisker movements after stimulation
1	Bilateral whisker movement; no accompanied head movements
1.5	Bilateral whisker movements accompanied by delayed head movements toward the stimulated side
2	Movement of the head toward the stimulated side
Olfaction	
0	No whisker movements
0.5	Delayed and slow bilateral whisker movements *after* exposure to the odor; no head movements
1	Whisker movements during exposure to the odor and head movements in the opposite direction but noticeably less brisk
1.5	Fast whisker movements *during* exposure to the odor; turns head away from the odor; alternating paw movements only
2	Brisk whisker movements *during* exposure to the odor; turns head away from the odor; gross motor movements

two 15-mm-long 30-gauge stainless steel tubing sections to one end and one 15-mm-long 30-gauge stainless steel tubing section to the other end of an 18-mm-long 23-gauge stainless steel tubing. The single-ended side of the Y cannula was connected to the intracerebroventricular cannula using 7-mm-long polyethylene tubing (PE-50, 0.58-mm ID, Intramedic). Using 30-cm long polyethylene tubing (PE-50, 0.58-mm ID), one of the double-ended sides of the Y cannula was connected to the DTX Plus DT-12 pressure transducer (Becton Dickinson) for the ICP measurement. The same type of tubing was used to connect the second side of the double-ended Y cannula to the infusion syringe containing the aCSF. The tubing and the intracerebroventricular cannula were then prefilled with the aCSF from the infusion syringe.

With all connections in place, baseline ICP, mean arterial blood pressure, and heart rate were measured for 10 min. The infusion of the aCSF at the rate of 5 μl/min was then initiated and was maintained for 50 min. ICP, mean arterial blood pressure, and heart rate were continuously measured during the intracerebroventricular infusion. Throughout the experiment, anesthesia was maintained with additional injections of 15 mg/kg intraperitoneal sodium pentobarbital as needed. The intracerebroventricular infusion of the aCSF was discontinued after 50 min, and the animal was killed with an injection of 100 mg/kg intraperitoneal sodium pentobarbital.

Data Analysis

The electroencephalographic activity was analog band-pass-filtered at 1–100 Hz and digitally sampled at 500 Hz (WINDAQ Data Acquisition Software; DATAQ Instruments, Akron, OH). Only artifact-free recordings were used in the analysis.

For every anesthetic dose in each experiment, 5 min of spontaneous electroencephalographic activity was band-pass-filtered to δ (2–4 Hz), θ (4–8 Hz), α (8–12 Hz), and γ (25–60 Hz) frequencies using a bidirectional Butterworth digital filter (n = 2). Variance of the filtered signal was determined as an estimate of power in each frequency band.[19]

For every stimulation block, single-trial SEPs comprising 300-ms poststimulus periods were extracted from the record using a threshold peak-detection algorithm. Because SEP had little variability from trial to trial, all SEPs were averaged. The amplitude of the average SEP was determined as the difference from the first positive (P20) to the following negative (N40) peak.

To identify the anesthetic agent-invariant metric, dose-dependent effects on electroencephalographic activity, band power, SEP amplitude, VOCR, and TPR were compared as a function of infusion fraction rather than an actual anesthetic dose. The infusion fraction at each block was calculated by dividing the cumulative volume given by the total volume infused over the period of 50 min.

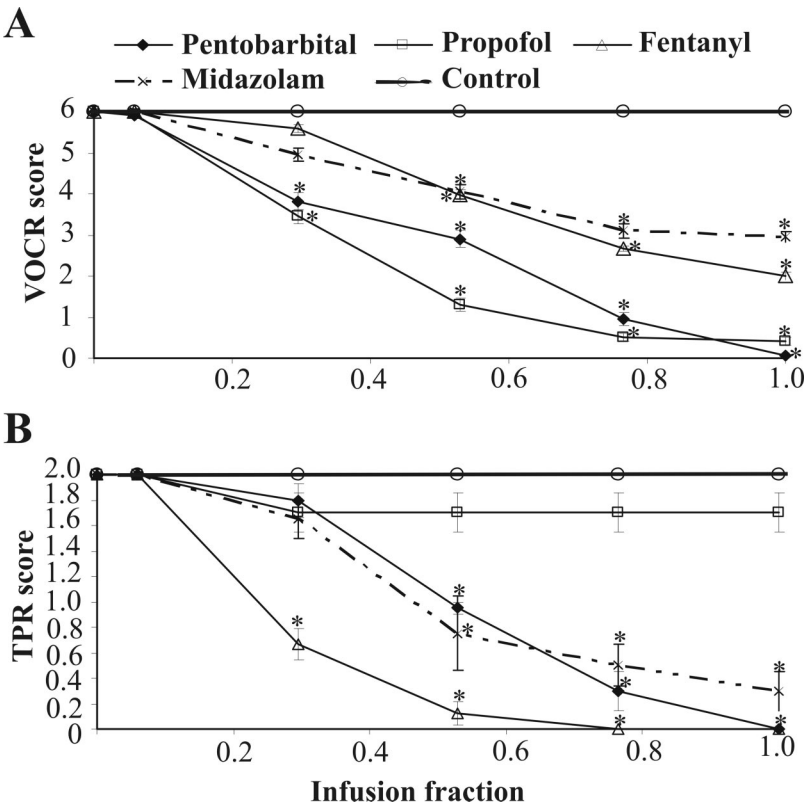

Fig. 2. Dose-dependent group-average effects of pentobarbital, propofol, fentanyl, midazolam, and artificial cerebrospinal fluid on vibrissal, olfactory, and corneal responses (VOCRs; *A*) and tail-pinch responses (TPRs; *B*). The change in the TPR and VOCR is expressed in terms of infusion fraction. All anesthetics depressed the VOCR in a dose-dependent manner. Propofol and pentobarbital exerted the greatest effect in suppressing the VOCR at all anesthetic doses, followed in order by fentanyl and midazolam. All anesthetic agents except propofol depressed the TPR in a dose-dependent manner. Fentanyl at all anesthetic doses and pentobarbital at high doses exerted the greatest suppression of the TPR, followed by midazolam. Neither the VOCR nor the TPR was affected by the artificial cerebrospinal fluid infusion. * $P \leq 0.05$ level of significance.

Statistical Analysis

As customary for the analysis of electroencephalographic data,[20] all-band power and SEP amplitude data in each experiment were z-scored using the means and SDs of the corresponding waking baseline. We observed that VOCR changed largely in parallel with all four agents. Therefore, for statistical analysis, the three scores were summed in each experiment to form a single index.

To test for a significant effect of anesthetic concentration on behavioral responses, SEP amplitude, and band power, repeated-measures analysis was used with the anesthetic concentration as a fixed factor and the rat as a subject variable. In this test, $P \leq 0.05$ was accepted for statistical significance. The Bonferroni comparison was used to test for a significant difference in observations at different anesthetic doses from waking control. For the Bonferroni test, $P \leq 0.01$ was accepted for statistical significance. Statistical analyses were performed using NCSS (NCSS, Kaysville, UT).

Results

Spontaneous Behavior

Forty to 50 min after isoflurane was terminated, the rats appeared completely awake as shown by a variety of spontaneous movements and brisk responses to sensory stimulation. All four intracerebroventricularly infused anesthetic agents produced readily observable dose-dependent changes in spontaneous and reflexive behavior, although the particular response pattern and extent of change differed from agent to agent as described in detail in the subsequent sections. In general, all agents produced a *biphasic* effect on spontaneous behavior. The first phase was seen as the excitation phase, which started 5–10 min after the start of intracerebroventricular infusion and lasted for 10–30 min. It consisted of behavioral activation, including gross motor movements that could be characterized as disinhibition. The excitation phase was the most evident with propofol and fentanyl; it was only brief with pentobarbital and mild with midazolam. The sedation and anesthesia phase followed the excitation phase and was generally characterized by the absence of spontaneous movement and increasing synchronization of electroencephalographic activity with all agents as detailed below.

Behavioral Responses to Sensory Stimulation

Responses to the stimulation of the cranial nerve (VOCR) and somatosensory (TPR) pathways showed dose-dependent, agent-specific decrements from control. The vibrissal, olfactory, and corneal responses changed largely in parallel with all four agents, and therefore, they were combined to form a single index. Figure 2 shows dose-dependent group-average effects of the four anesthetic agents and the aCSF on the VOCR and TPR scores. All anesthetic agents significantly depressed the VOCR in a dose-dependent manner. Propofol and pentobarbital exerted the greatest effect in sup-

pressing VOCR, followed in order by fentanyl and midazolam. All anesthetic agents except propofol significantly depressed the TPR in a dose-dependent manner. Fentanyl at all anesthetic doses and pentobarbital at high doses produced the strongest suppression of TPR, followed by midazolam. Neither the VOCR nor the TPR was affected by the aCSF infusion.

Comparing the characteristic effects of the four agents at their given concentrations on both the cranial and somatic sensory stimulation responses, one comes to the following observations. Pentobarbital was the only one of the four agents that fully blocked both VOCR and TPR. Propofol was exceptional in that it blocked the VOCR while exerting essentially no effect on TPR. Fentanyl was the strongest in suppressing the TPR, but it was only moderately effective in suppressing the VOCR. Midazolam exerted a moderate effect on both VOCR and TPR.

Baseline Electroencephalographic Activity and Excitation Phase

At baseline control conditions, the electroencephalographic activity showed a typical waking, desynchronized pattern, occasionally interspersed with transient motion artifacts, but otherwise normal. Infusion of aCSF did not alter this pattern, suggesting absent affects of the small infused fluid volume, and no abnormal ICP. After infusing the anesthetic agents, the first changes in the electroencephalographic power were noticeable at 5–8 min after starting the intracerebroventricular infusion. Propofol and fentanyl were exceptional in that they often induced transient periods of epileptiform activity with characteristic electroencephalographic and behavioral manifestations. Figure 3 shows examples of propofol- and fentanyl-induced epileptiform electroencephalographic activity and corresponding power spectra during the excitation phase from one animal. Propofol-induced epileptiform electroencephalographic activity was characterized by short, approximately 25-s-long periods of rhythmic, low-frequency, high-amplitude spiking-wave complexes that appeared approximately four to six times during the excitation phase. The epileptiform behavior consisted of forceful body twitching, facial muscle fibrillation, teeth chattering, and rhythmic extension of limbs, often culminating in opisthotonus, vocalization, and/or urination. Fentanyl-induced epileptiform electroencephalographic activity was characterized by short, approximately 20-s-long periods of paroxysmal shaking of the head, neck, and trunk that appeared approximately six to eight times during the excitation phase. The head shaking was often preceded by staring and gustatory automatisms. Electroencephalographic alterations consisted of high-voltage fast-activity spiking bursts.

Electroencephalographic Power and SEP

A progressive, dose-dependent increase in the electroencephalographic power was observed in most cases. Subsequent analysis confirmed that this increase was a result of an increase in the power of δ waves. Figure 4 illustrates dose-dependent changes in spontaneous δ electroencephalographic activity together with the peak-to-peak amplitude of SEP during intracerebroventricular infusion of four anesthetic agents and aCSF. Pentobarbital produced the strongest dose-dependent increase in δ power, followed in order by fentanyl, propofol, and midazolam.

Pentobarbital significantly increased and midazolam significantly decreased the SEP amplitude in a dose-dependent manner. However, these effects were small. Propofol and fentanyl had no significant effect on SEP. The aCSF infusion had no significant effect on either δ power or SEP amplitude.

Figure 5 shows dose-dependent group-average effects of the anesthetic agents on the electroencephalographic power in various frequency bands. Only significant changes in power with respect to waking baseline are discussed. All anesthetics except midazolam increased δ power in a dose-dependent manner; this effect was most pronounced for pentobarbital. The maximum increase in δ power produced with pentobarbital was 2 times that of fentanyl and 2.3 times that of propofol. Theta power was enhanced by pentobarbital only. Pentobarbital, propofol, and midazolam but not fentanyl enhanced α and β power. Small increases in γ power were seen with pentobarbital and midazolam, whereas a dose-dependent suppression of γ power was observed with fentanyl.

Histologic Brain and Spinal Cord Examination

Histologic examination of six coronal sections of the brain and spinal cord revealed the presence of Evans blue in both left and right ventricles, in the third ventricle, and in the cerebral aqueduct. Inspection of the spinal cord revealed absence of the dye.

Effect of aCSF Intracerebroventricular Infusion on ICP

In five animals tested under pentobarbital anesthesia, baseline ICP and mean arterial blood pressure were 1.0–3.0 mmHg and 80 mmHg, respectively. Both ICP and mean arterial blood pressure remained within the normal range during the 50-min aCSF intracerebroventricular infusion at a rate of 5 μl/min. Specifically, ICP and blood pressure ranged between 3 and 5 mmHg and between 80 and 90 mmHg, respectively.

Discussion

The purpose of this study was to characterize and compare the behaviorally and electrophysiologically

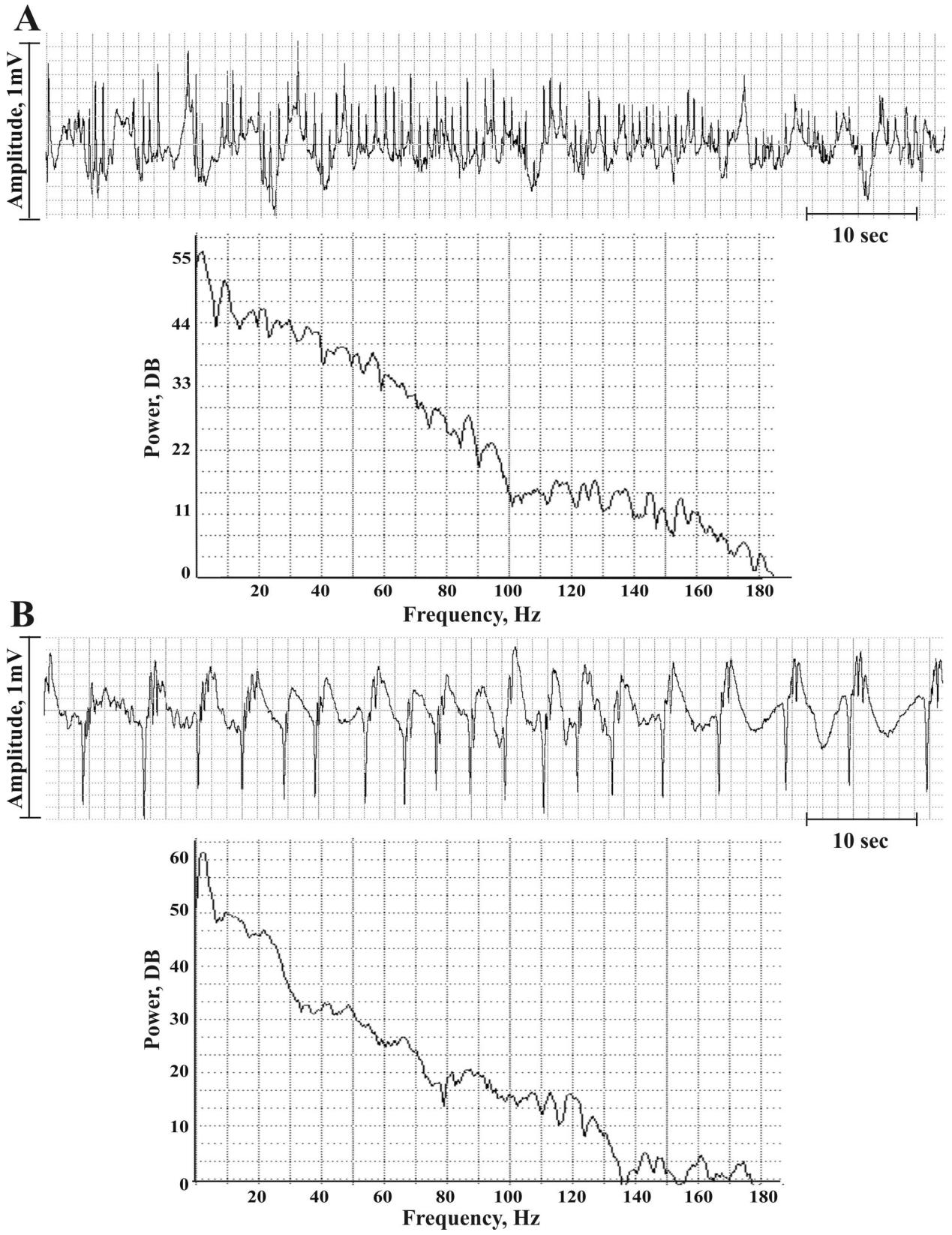

Fig. 3. Two examples of 10-s epileptiform electroencephalographic activity and the corresponding power spectra during propofol (*A*) and fentanyl (*B*) infusion. Records were obtained during the 15th and 20th minutes of infusion, respectively.

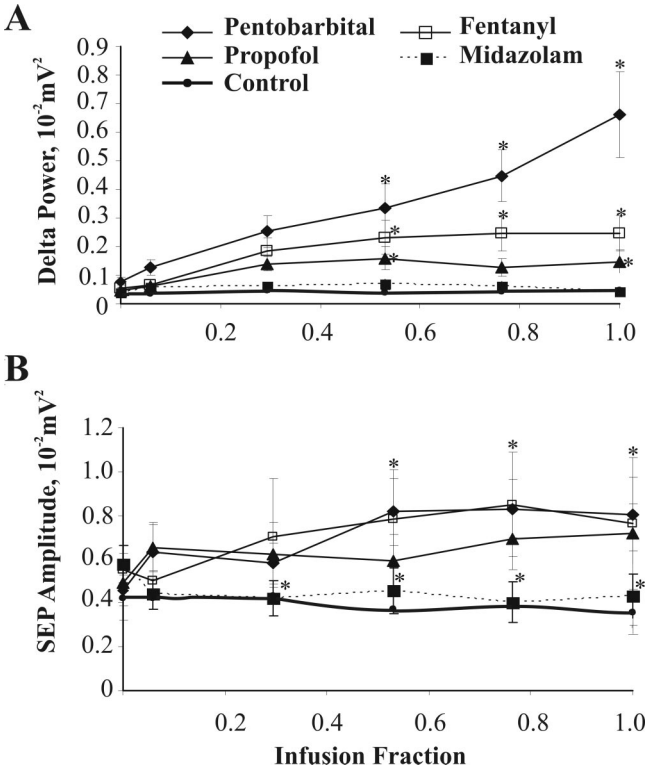

Fig. 4. Dose-dependent effects of pentobarbital, fentanyl, propofol, and midazolam and artificial cerebrospinal fluid on electroencephalographic δ power (*A*) and on somatosensory evoked potential (SEP) (*B*). The change in band power and somatosensory evoked potential amplitude is expressed in terms of infusion fraction. Pentobarbital produced the strongest dose-dependent increase in δ power, followed in order by fentanyl, propofol, and midazolam. Pentobarbital increased and midazolam decreased the somatosensory evoked potential amplitude. The artificial cerebrospinal fluid infusion had no significant effect on either δ power or somatosensory evoked potential amplitude. * $P \leq 0.01$ level of significance.

identified states of anesthesia produced by intracerebroventricular infusion of four common injectable anesthetic agents. Intracerebroventricular administration was chosen to target the drug action to the brain and thus avoid the potential confounding spinal cord effects. Our principal interest has been in the mechanisms of anesthetic-induced unconsciousness, and much of the observed effects are discussed from that point of view.

In brief, we found that pentobarbital, propofol, fentanyl, and midazolam produced substantial changes in spontaneous behavior, sensory responses, and electroencephalographic activity. Nevertheless, the agents differed, not unexpectedly, in the degree the various measured parameters were affected. These differential actions can be interpreted in the context of the agents' known behavioral and electroencephalographic effects and highlight the uniqueness of neuronal changes produced by each agent.

Supraspinal Anesthesia

The brain has been traditionally recognized as a primary site of anesthetic action to suppress perception, voluntary action, memory, and consciousness. To study the neuronal mechanism of anesthetic-induced unconsciousness, one desires to deliver the anesthetic agent selectively to the brain as much as possible. There are two major reasons for supraspinal as opposed to systemic administration. First, when anesthetic agents are administered systemically, spinoreticular and spinothalamic nerve traffic and therefore cortical arousal are attenuated,[7] which makes the direct cerebral effects of the anesthetic agents more difficult to discern. Second, systemic drug delivery produces a suppression of spinal sensory and motor neurons,[11] and therefore, consequent changes in behavior cannot be ascribed exclusively to a suppression of consciousness.

The need for studying anesthetic effects on the brain and spinal cord in isolation has previously been recognized. Early attempts were made to perfuse the brain of animals selectively, but incomplete isolation, crude oxygenators, and nonphysiologic perfusates complicated experimental procedures.[8] Using a more elaborate animal preparation, Antognini *et al.*[5,11,13,14,21] successfully separated the systemic arterial circulation from the cranial circulation at the level of the caudal medulla and upper cervical spinal cord in the goat, and showed that by selective delivery of anesthetic agents to the cranium or torso, various anesthetic endpoints, such as hypnosis and areflexia, could be selectively modulated. In mice, Taira *et al.*[22] infused midazolam intracerebroventricularly, and Narita *et al.*[23] administered fentanyl intracerebroventricular to produce antinociception. Wang *et al.*[24] injected intracerebroventricular propofol that produced hyperalgesia in rats. Wang and Fujimoto[25] administered intracerebroventricular pentobarbital to mice to antagonize antinociception to intrathecal morphine. None of these studies, however, assessed the loss of consciousness by either behavioral or electrophysiologic measures.

In this work, we applied a slow rate of intracerebroventricular infusion to distribute the anesthetic agents in the cranial cerebrospinal fluid space. Previous studies suggest that intracerebroventricularly administered drugs remain in the cranial compartment. For example, Taira *et al.*[22] confirmed the supraspinal localization of 5 μl intracerebroventricularly injected Evans blue dye. Stabernack *et al.*[15] showed that after slow-rate intracerebroventricular infusion of 25 μg/min thiopental, its concentration in the brain was approximately 300 times higher than that in the spinal cord. To further delimit the chance of spread of the anesthetic agents to the spinal compartment, we supplemented the intracerebroventricular infusion technique with an injection of silicone bolus into the fourth ventricle. Although we do not know the extent to which the silicone bolus contributed to the prevention of cerebrospinal drug leakage, both the histologic examination and the physiologic results support the contention that the anesthetics were con-

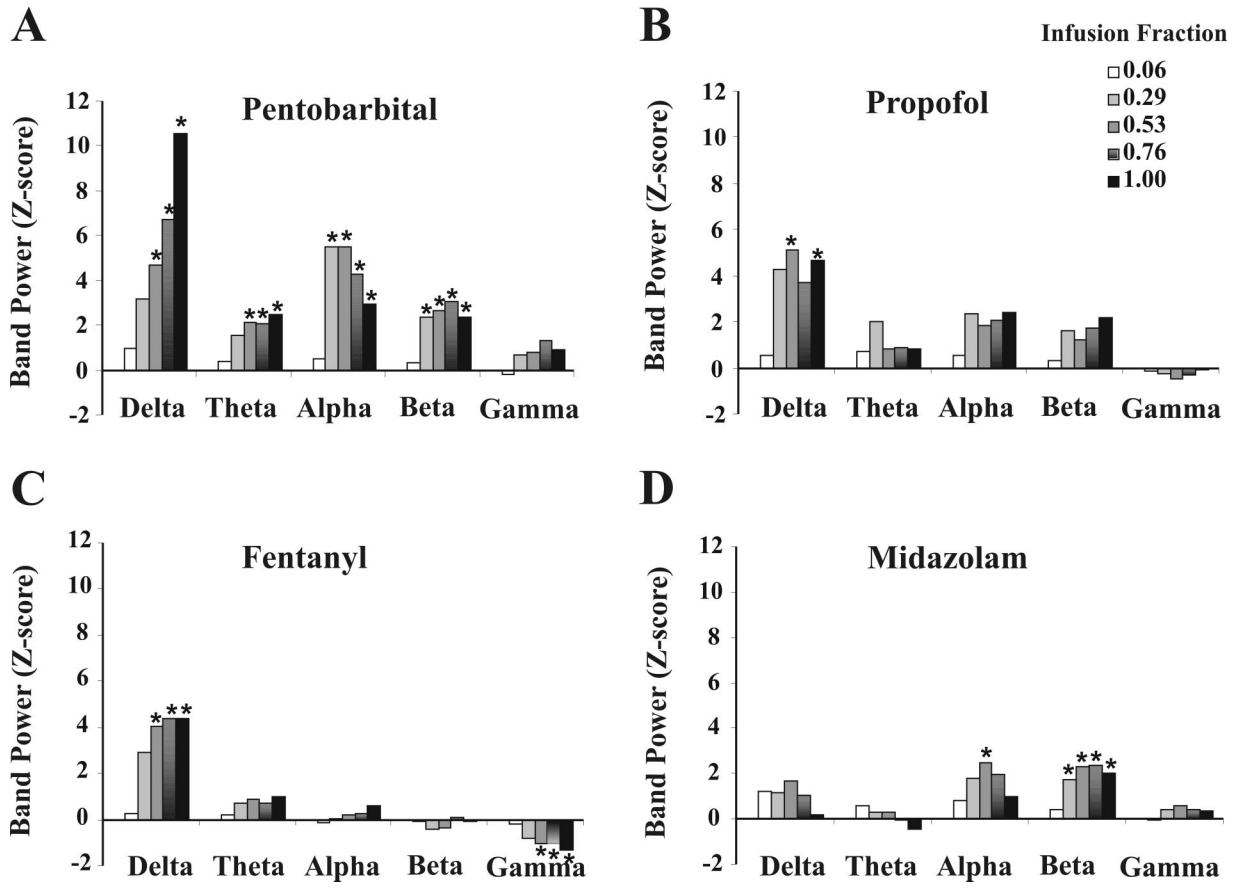

Fig. 5. Dose-dependent group-average effects of pentobarbital (*A*), propofol (*B*), fentanyl (*C*), and midazolam (*D*) on the electroencephalographic power in δ, θ, α, β, and γ frequency bands. Band power is expressed in terms of z score values, computed using means and SDs of the waking baseline. The change in band power is shown in terms of infusion fraction. All anesthetics except midazolam increased δ power in a dose-dependent manner; this effect was more pronounced for pentobarbital. Theta power was enhanced by pentobarbital only. Pentobarbital, propofol, and midazolam but not fentanyl enhanced α and β power. Small increases in γ power were seen with pentobarbital and midazolam, whereas a dose-dependent suppression of γ power was observed with fentanyl. * $P \leq 0.01$ level of significance.

tained supraspinally. For example, with propofol infusion, the spinal nociceptive motor response was preserved in the presence of marked cortical depression, suggesting that the drug was confined to supraspinal targets. Furthermore, the electroencephalographic changes were not due to a nonspecific volume effect of the intracerebroventricular infusion, because the aCSF infusion caused no alterations in ICP, spontaneous electroencephalographic activity, SEP, or behavior.

Behavioral Measure of the State of Consciousness

In this study, we used the three-component VOCR as a behavioral measure of anesthetic-induced loss of consciousness. The validity of this measure depends in part on what we mean by *consciousness*.

Human consciousness has been naively defined as the awareness of oneself and one's environment.[26] Awareness requires cortical representations and is therefore dependent on the functional integrity of the cerebral cortex and its subcortical connections.[27] One of the criteria proposed to be a clear sign of awareness is a purposeful response to stimuli that is reproducible and is not due to a simple reflex.[28] Following this criterion, we set out to assess the state of consciousness by testing three cranial nerve–mediated responses that involve a cortical component (VOCR). These responses can be graded, reflecting a continuum from an elaborate, complex cortically mediated behavioral response to a simple reflexive one. We hypothesized that only an alert and aware mind can elicit a complex purposeful response to a specific stimulus. The TPR, on the other hand, is thought to be reflexive and mediated at the spinal level.[5,29] However, in unanesthetized animals, a more complex behavioral response is seen. The ascending spinothalamic and spinoreticular pathways convey nociceptive information and may elicit a more complex avoidance response. The chosen tests are analogous with the current neurologic scales for the assessment of the state of awareness in human patients[30] as well as with those for assessing depth of anesthesia in rats.[16,31,32]

Epileptiform Activity

Propofol and fentanyl were observed to induce periods of epileptiform activity. Propofol has been previously shown to produce neuroexcitation expressed in abnor-

mal patient movements and seizure-like activity.[33] In our study, propofol-induced epileptiform activity resembled a grand mal attack. The pathophysiologic mechanism underlying the neuroexcitatory symptoms associated with propofol are unknown, but several hypotheses have been proposed. These include drug-induced decerebrate rigidity, strychnine-like effect on glycinergic and γ-aminobutyric acidergic pathways, and an imbalance between cholinergic and dopaminergic activity at the level of the basal ganglia[34] with an increase in excitatory cholinergic output.[35] The seizure-like activity observed with propofol may also be due to uneven distribution of the anesthetic in the brain resulting in higher concentrations of propofol in brain areas adjacent to cerebral ventricles. It may also indicate the combined effect of propofol and intralipid, which under standard systemic administration does not enter the central nervous system. A concentration-dependent study designed to investigate the effects of intracerebroventricularly infused anesthetic agents and their comparison with the effects of systemically administered anesthetics may help to answer these questions in the future.

The seizure-like behavior after fentanyl administration has been described in the literature as "wet dog" shakes.[36,37] This behavior is typically accompanied by paroxysmal bursts of high-voltage spike and wave complexes particularly at high fentanyl doses.[36,38,39] Epileptiform activity with increased metabolic rate in hippocampal foci has also been reported in rats after large intracerebroventricular doses of fentanyl[36,38] opiate receptor agonists.[40,41] It is assumed that opioids produce seizures by inhibiting cortical γ-aminobutyric acid interneurons and consequently disinhibiting glutamatergic excitatory neurons.[42–44] The ventral hippocampus is one of the regions where interneuronal γ-aminobutyric acid–containing fibers terminate,[45] and this region is adjacent to the intracerebroventricular injection site. Therefore, μ agonists affecting this region may produce excitation and epileptiform activity originating in the ventral hippocampus. This seizure activity from the ventral hippocampus when radiated to the motor area of the brain may lead to the expression of wet dog shakes and convulsions.[46]

Sensory-evoked Behavioral Responses

The response to tail pinch was suppressed by three of the tested agents. Fentanyl had the greatest effect, followed by pentobarbital and midazolam, whereas propofol had essentially no effect. It is clear that with intracerebroventricular drug administration, the suppression of the tail-pinch response could only be mediated by a modulation of descending cerebrospinal nerve pathways. The strongest analgesic effect of fentanyl was not unexpected. Microinjection of opioids into the cerebral ventricles has been shown to produce dose-dependent antinociception in rats and mice,[47–51] likely though the stimulation of μ-opioid receptors in the periaqueductal gray matter—the source of descending inhibitory control of spinal nociceptive inputs.[52] Other possible targets are the medial thalamic nuclei,[9] caudate–putamen,[53] and cerebral cortex.[54]

Similar to fentanyl, intracerebroventricular pentobarbital at the applied dose significantly suppressed the tail-pinch response, again implying the role of a supraspinal mechanism. Until now, the mechanism of nociceptive areflexia produced by pentobarbital has been controversial. Sudo *et al.*[12] found, based on differential delivery of thiopental to spinal cord and brain in goats, that thiopental produced immobility by directly depressing lumbar dorsal horn neuronal responses in the spinal cord. In contrast, Stabernack *et al.*[15] argued that the effect of thiopental producing immobility was supraspinal based on the data showing significant minimum alveolar concentration–sparing effect of supraspinal thiopental added to isoflurane in rats. The mesopontine tegmentum has been suggested as a specific target site for pentobarbital for both immobility and loss of consciousness.[16] This area is a source of multiple descending and ascending projections to various subcortical structures and spinal cord.[55] Another possible mechanism that may contribute to pentobarbital's strong antinociceptive effect could be its affinity to inhibit nicotinic acetylcholine receptors[56–59]—an affinity not seen with other anesthetic agents.[3]

Midazolam attenuated the TPR but fell short of producing complete immobility. One could argue that higher concentrations of midazolam may have produced a complete suppression of the TPR; however, our final dose of midazolam was relatively high (200 μg). Also, at the lower doses, midazolam and pentobarbital were nearly equipotent, suggesting that the dose itself was not a limiting factor of midazolam's effect. It is known that systemic administration of benzodiazepines produces sedation and muscle relaxation but not analgesia.[60,61] When administered intrathecally, midazolam produces analgesia by a spinal mechanism.[60–65] However, spinal analgesia can be overcome by supraspinal antianalgesia when the drug has access to the brain.[60] When midazolam is given intracerebroventricularly, only central mechanisms are activated that can produce both motor depression (catalepsy)[62] and hyperalgesia[61,62,66–68] at the same time. Because we saw a partial suppression of the motor response to tail pinching—an arousing but only moderately painful stimulus—we surmise that this behavioral effect may have been caused by a central sedative effect of midazolam in opposition to the more common hyperalgesic response to a more painful stimulus.

The absent effect of propofol on the tail-pinch response is interesting and may be a product of the selective, intracerebroventricular route of administration. At least one study confirmed that when administered *via*

the usual intravenous route, propofol exerts its antinociceptive effect by an inhibition of dorsal root neurons in the spinal cord.[11] In rats, intrathecal propofol has also been shown to produce antinociception.[24] It follows that intracerebroventricular propofol probably did not engage the central sedative or descending antinociceptive systems, in contrast to the other three anesthetics that likely did. It has been suggested that anesthetics that do not inhibit nicotinic acetylcholine receptors in the clinical range, such as propofol, are not antinociceptive.[69] Consistent with this theory is our observation that after intracerebroventricular propofol infusion, tail-pinch stimulation continued to produce nocifensive movements that consisted of alternating proximal flexion and extension, resembling ambulation in at least two limbs simultaneously.

As to the three cranial nerve–mediated sensory responses (VOCR), all four agents suppressed the response, although the degree of suppression was varied. Pentobarbital and propofol caused a complete disappearance of the VOCR, whereas fentanyl and midazolam exerted a partial effect. Pentobarbital and propofol are principally hypnotic agents, whereas fentanyl and midazolam are analgesic and sedative, respectively. The agents' relative potencies are thus consistent with the assumption that the VOCR suppression reflects mainly a hypnotic effect.

Propofol and pentobarbital are both known as prominent γ-aminobutyric acid type A ($GABA_A$)–positive modulators in the brain. $GABA_A$ receptors are widely distributed in the central nervous system, including the olfactory and trigeminal systems[70]; the latter participates in the vibrissal sensory pathway. Propofol[1,71] and pentobarbital[72] produce similar, selective decreases in the regional cerebral blood flow response in various cortical and subcortical structures involved in olfaction and in the thalamic relay of the vibrissal somatosensory pathways. Both propofol and pentobarbital show high affinity for α_3 subunits of the $GABA_A$ receptors, whereas propofol has also been shown to act on α_2 subunits.[3,73] A high concentration of α_3-containing $GABA_A$ receptors has been found in the forebrain, the basal ganglia,[74] and the septohippocampal system.[73] The reticular thalamic nucleus may also be a target for both anesthetics because it expresses the α_3 subunit[75] and has been reported to play a major role in regulating transitions between waking and sleep and may play a role in anesthetic-induced unconsciousness.[76–78]

The moderate effect of fentanyl on the VOCR is consistent with the sedative effect of opioids. Mortazavi *et al.*[79] suggested that fentanyl inhibits acetylcholine release in the medial pontine reticular formation, which may reduce the level of arousal but allow a certain degree of responsiveness as observed in our rats throughout the period of fentanyl infusion.

Midazolam also had a moderate effect on the VOCR. Although midazolam is also a $GABA_A$ agonist, it differs from pentobarbital and propofol in that it has a stronger affinity for the α_1 subunit of the $GABA_A$ receptor,[80] through which it may produce a different, essentially sedative effect. A high expression of α_1 subunit has been found in the cerebral and cerebellar cortex, thalamus,[3,81] limbic system, amygdala, hippocampus, and striatum.[75]

Electroencephalogram, SEP, and Their Behavioral Correlates

One of the design goals of our experiment was to produce a gradual electroencephalographic synchronization effect during anesthetic infusion and then compare this effect with the resultant behavioral changes. Within the confines of maximum drug concentrations and maximum intracerebroventricular infusion rates, this goal was essentially achieved as indicated by the significant increase in δ power with three of the anesthetics. The exception was midazolam, which did not increase δ power but caused significant power change in other bands. In addition, the four agents differed in their effects on power in distinct frequency bands.

When barbiturates or propofol are administered systemically, they induce a biphasic change in the electroencephalographic activity, consisting of an increase in α and β activity followed by a decrease in α and β activity and an increase in δ activity.[82] In our study, both, pentobarbital and propofol increased α, β, and δ power and suppressed the VOCR. However, biphasic changes in the α band were only observed with pentobarbital and midazolam. The reason for this difference is unclear. It could be due to a species difference or to the difference in the route and time course of administration (intracerebroventricular *vs.* intravenous). Also, in our experiment, agent infusion was slow compared with the normally rapid clinical induction; the presence of excitation phase may also depend on how rapidly the agent concentration changes. All four agents produced behavioral disinhibition during the excitation phase, but the duration and intensity of gross movements varied among them. The excitation phase was the most evident with propofol and fentanyl but was only brief with pentobarbital and mild with midazolam. Although the electrophysiologic expression of excitation was apparent in individual electroencephalographic records, changes in the electroencephalographic band power did not consistently reflect this because of the brevity and temporal variance of excitation events.

The dominant effect of midazolam was an increase in α and especially β power; they were accompanied by an attenuation of the VOCR to a certain degree. Our findings are consistent with those of Feshchenko *et al.*,[83] who showed that sedative doses of midazolam produced maximum β power in the central and parietal areas analogous to the region where we recorded the electroencephalographic activity. An increase in α and particu-

larly β power may be associated with the sedative effect of midazolam.

The electroencephalographic effects of opioids are known to differ from those of the hypnotic agents. When opioids are administered systemically, they produce a steady decline in high-frequency content and a shift of the frequency spectrum to the δ band.[84] There is no further change in electroencephalographic activity with increasing dose, and burst suppression does not occur.[85] Consistent with this, in our study, fentanyl produced a dose-dependent increase in δ power and decrease in γ power. Fentanyl was in fact the only one of four agents that decreased the γ power. Gamma oscillations may play a major role in memory and cognitive functions.[86] Studies in rats showed that opioids can disrupt synchronous γ oscillation in hippocampal slices.[87]

The natural question that arises is if any of the observed band power changes could be associated with a particular behavioral effect in a way that could be generalized across the four anesthetic agents. We saw that midazolam increased α and β power while it produced sedation. Propofol in addition increased δ power and produced unconsciousness (suppressed VOCR). One could surmise that an increase in α and β power may reflect a sedative action and, when it occurs together with δ synchronization, may predict unconsciousness. Pentobarbital fits this scheme because it increased α, β, and δ and fully blocked the VOCR. Pentobarbital also increased θ power, which was not seen with the other three agents, and may indicate deep, surgical level of anesthesia with complete irresponsiveness. Finally, fentanyl produced an increase in δ power only but, like midazolam, did not produce unconsciousness. However, it was the most effective in suppressing the TPR, and it was the only agent that suppressed γ power. Taken together, this comparison suggests that an increase in δ power alone correlates with neither antinociception nor loss of consciousness. The suppression of γ power may reflect diminished pain perception[88] at preserved consciousness.

A recognized limitation of this study is that it did not compare the effects of the anesthetic agents in a strict concentration-dependent manner. Although the dose of anesthetic in the brain accumulated during the continuous intracerebroventricular infusion, only one concentration per agent was used. Three of the agents were used in their standard pharmacologic concentrations. Decreasing these concentrations would have diminished the desired effect, and we were obviously not able to increase these concentrations. Pentobarbital at its standard concentration produced an abrupt effect leading to complete electroencephalographic suppression within minutes. Therefore, in preliminary studies, we selected a lower concentration of pentobarbital that produced a gradual electroencephalographic synchronization effect over a period of 50 min. Another possible limitation is that the distribution of the intracerebroventricularly infused drugs in the brain is not known and may not be uniform. For example, a gradient may exist from the infusion site to more peripheral areas in the brain resulting in lower anesthetic concentrations in more distal sites. This limitation applies to all intracerebroventricular studies and could only be overcome by intracerebral drug distribution studies in the future. We believe that slow and prolonged injection protocols as applied in our study are superior to intracerebroventricular bolus injection techniques in that respect. Given these limitations, we cannot exclude the possibility that the particular agent concentrations used and a possibly nonuniform agent distribution in the brain may have contributed to the observed agent-specific differences. Therefore, it may be of interest in the future to repeat the current studies with multiple agent concentrations and infusion rates. Finally, one cannot completely rule out that some residual isoflurane from the preparatory surgery may have augmented the effect of the intracerebroventricularly infused anesthetic agents.

In additional control animals, we compared the effects of systemically delivered to those of intracerebroventricularly infused pentobarbital on behavioral responses. We found that unlike the intracerebroventricular infusion, the intravenous infusion of pentobarbital at the same slow rate of 0.1 mg/min produced a 50% suppression of the tail-pinch response but had only a minimal effect on the VOCR by the end of the 50-min infusion. This observation confirms that given systemically, 5 mg pentobarbital may exert some effect on the spinally mediated reflexes but is not enough to produce a significant supraspinal effect. This dose likely overestimates the amount of drug that would be absorbed systemically after intracerebroventricular injection. Taken together, these findings suggest that electroencephalographic and behavioral changes seen with the intracerebroventricular protocol reflect mainly supraspinal drug effects and are unlikely due to the reabsorption and redistribution of the drug.

In conclusion, we found that intracerebroventricular infusion of each anesthetic agent at standard concentrations produced a distinct pattern of behavioral and electroencephalographic changes. The findings with all four agents suggest that an increase in α and β power may reflect the sedative component of anesthesia. A simultaneous increase in δ, α, and β power may correlate with loss of consciousness. Increases in both θ and δ power may be associated with surgical depth of anesthesia. Opioid-induced decreases in γ power may correlate with suppression of pain perception. These results also support that immobility to noxious stimulation produced by pentobarbital, fentanyl, and midazolam is mediated by supraspinal mechanisms. Propofol at hypnotic dose did not produce areflexia, suggesting the need for spinal

mediation of the latter. These findings are admittedly preliminary and require further studies with additional anesthetic agents and concentration-dependent protocols. More elaborate electroencephalographic parameters, such as bispectrum, entropy, and complexity, should be examined to determine whether a consistent, agent-invariant association between changes in behavior and the electroencephalographic activity during intracerebroventricular infusion of anesthetic agents could be substantiated.

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