Anesthesiology 2003; 99:1125–31

Cholinergic Reversal of Isoflurane Anesthesia in Rats as Measured by Cross-approximate Entropy of the Electroencephalogram

Anthony G. Hudetz, B.M.D., Ph.D., James D. Wood, R.L.A.T.,† John P. Kampine, M.D., Ph.D.‡*

Background: **Pharmacologic modulation of the state of consciousness is of interest for clinical practice and for a better understanding of anesthetic mechanisms. The cholinergic activating system is an important regulator of the state of consciousness during general anesthesia. Entropy of the electroencephalogram has been proposed as a promising measure of anesthetic depth. The authors have shown that volatile anesthetics decrease cross-approximate entropy (C-ApEn) of the bihemispheric frontal electroencephalogram in rats. The effect of cholinergic agents on C-ApEn has not been examined. Here, the authors test the hypothesis that cholinergic activation reverses the effect of isoflurane anesthesia on C-ApEn.**

Methods: **An electroencephalogram in the 1- to 100-Hz range was recorded bipolarly, with epidural leads from the frontal cortex of both hemispheres, and used to calculate C-ApEn, which reflects statistical independence of bihemispheric electroencephalographic activity. Cholinesterase inhibitor, neostigmine (25 μg), or the muscarinic agonist oxotremorine (25 μg) were infused intracerebroventricularly while the rats were inhaling 1.0% (0.7 minimum alveolar concentration) isoflurane. In other animals, isoflurane was lowered to 0.4% (0.3 minimum alveolar concentration) to assess the electroencephalogram in a sedated, waking state.**

Results: **At 1.0% isoflurane, C-ApEn decreased by 54% compared with that at 0.4%, but the motor reflex response to tail pinch was still present. Cholinergic agents reversed the electroencephalogram-depressant effect of isoflurane, *i.e.*, C-ApEn rose to the level measured at 0.4% isoflurane. The rise in C-ApEn was paralleled by the appearance of spontaneous limb and orofacial explorative movements, suggesting a return of consciousness. In contrast, cholinergic agents fully blocked the motor reflex to tail pinch.**

Conclusions: **C-ApEn of the bihemispheric electroencephalogram correlates with the return of spontaneous motor signs but not with the nociceptive reflex. Cerebral cholinergic activation dissociates central and peripheral anesthetic effects. C-ApEn, a novel measure of interhemispheric electroencephalogram independence, is a promising correlate of depth of sedation and state of consciousness.**

UNDERSTANDING the neural mechanism of anesthetic modulation of consciousness is of interest for clinical practice as well as for neuroscience. Cholinergic pathways of the ascending activating system have been known to be important regulators of the state of consciousness during the natural sleep–wake cycle and during general anesthesia.[1] Cholinergic activation by the administration of selective agonists or cholinesterase inhibitors has been shown to produce cortical activation[2–9] and increase the anesthetic requirement to produce unconsciousness.[10] The cholinesterase inhibitor physostigmine has been suggested as a reversal agent for neuroleptanesthesia[11] and has been shown to promote the reversal of the anesthetic state, as indicated by the return of response to verbal commands, waking electroencephalogram, and auditory evoked response.[12]

An objective assessment of the anesthetic state from the electroencephalogram has been of interest for more than half a century.[13–16] Assessment of the state of hypnosis should be based on a suitable electrophysiologic parameter of cerebral function[17] rather than the nociceptive reflex, because general anesthetic–induced areflexia to peripheral nociceptive stimulation is mediated in large part at the spinal level.[18,19] Although several commercial instruments designed to monitor anesthetic depth are now available,[20–23] an understanding of the neurophysiologic basis of anesthetic-induced unconsciousness is incomplete and requires further exploration.

Electroencephalogram entropy has recently been tested as a promising measure of anesthetic drug effect on the central nervous system (CNS) with propofol,[24] desflurane,[25] isoflurane,[26] and sevoflurane.[21] To date, various definitions of entropy have been used[21]: most commonly, spectral entropy, approximate entropy (ApEn),[24] and recently, cross-approximate entropy (C-ApEn).[27] C-ApEn measures the statistical dissimilarity or independence of two concurrent biologic signals.[28] In this sense, C-ApEn describes both spatial and temporal independence, whereas ApEn reflects only temporal irregularity. Because conscious cognitive processes involve large-scale distributed networks of the brain,[29–31] an electroencephalographic index that incorporates both spatial and temporal dynamics may in principle be a more suitable indicator of the state of consciousness or depth of sedation than an electroencephalographic index based on temporal properties alone. In particular, prominent studies have suggested the importance of interhemispheric coherence of cortical electromagnetic signals during conscious perception[31–35] and anesthesia.[16] C-ApEn could be a suitable parameter to quantitatively describe the interhemispheric relationship of neuroelectric signals underlying conscious perception and

* Professor of Anesthesiology, Physiology, and Biophysics, † Research Technician, ‡ Professor of Anesthesiology and Physiology and Chairman.

Received from the Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin. Submitted for publication March 13, 2003. Accepted for publication June 30, 2003. Supported in part by the National Institutes of Health, Bethesda, Maryland, grants GM-56398 and MH-51358, and the National Science Foundation, Arlington, Virginia, grant BES-0002945. Presented in part at the 5th International Conference on Memory, Anesthesia, and Consciousness, New York, New York, June 1–3, 2001.

Address reprint requests to Dr. Hudetz: Department of Anesthesiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226. Address electronic mail to: ahudetz@mcw.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

its depression during anesthesia. We postulate that either disconnection or hypersynchronization of the hemispheres by anesthetics may interfere with conscious perception and may be detected by a change in C-ApEn.

We[27] previously used C-ApEn to characterize the effect of volatile anesthetics on the electroencephalogram in the rat and showed that C-ApEn was decreased by both anesthetics as the animals lost the righting reflex, a behavioral index of consciousness. In the present work, we examined whether the effect of isoflurane on C-ApEn of the electroencephalogram may be modulated by the administration of cholinergic agents. We hypothesized that if a reduction in C-ApEn correctly reflected deepening anesthesia,[27] then it should increase on cholinergic arousal of the CNS. We chose to use neostigmine, a potent cholinesterase inhibitor, and oxotremorine, a potent muscarinic receptor agonist, for cholinergic activation. Muscarinic receptors have been implicated in cholinergic electroencephalogram activation.[36,37] Neostigmine does not cross the blood-brain barrier; therefore, both agents were administered *via* the intracerebroventricular route.

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* (National Academy Press, Washington, D.C., 1996).

Experiments were performed in 26 adult, male, Sprague-Dawley rats. The animals were anesthetized with 1.5% isoflurane and prepared for bihemispheric electroencephalogram recording. The anesthetics were vaporized into a gas mixture of 30% O_2, 70% N_2 that the animals were breathing spontaneously through a gas anesthesia mask (model 51610, Stoelting, Wood Dale, IL). Anesthetic concentration was monitored (POET II monitor; Criticare Systems, Inc., Waukesha, WI) through a sampling line connected to the anesthesia mask. The body temperature was maintained at 37°C with a thermostat-controlled (model 73A, YSI, Yellow Springs, OH), water-circulated (K-mod 100, Baxter Healthcare Corp., Chicago, IL) heating mat. For epidural electroencephalogram recording, two pairs of stainless steel screw electrodes were placed through burr holes in the cranium symmetrically over the left and right frontal cortices (coordinates: 3.5 mm lateral and 3.2 mm rostral or 0.7 mm caudal from bregma). According to the stereotaxic atlas of Paxinos and Watson,[38] these coordinates corresponded to primary motor and forelimb somatosensory regions, respectively. For the infusion of cholinergic agents, a 30-gauge needle was implanted into the lateral ventricle (coordinates: 1.2 mm lateral, 0.8 mm caudal, and 3.5 mm deep from bregma). The screws were advanced to the level of the dura. In some animals, the femoral arteries were cannulated for the measurement of blood pressure and for the withdrawal of blood samples. All surgical sites were treated with bupivacaine.

After surgery, the rats were stabilized for 1 h at 1.0% inspired isoflurane, equivalent to 0.7 minimum alveolar concentration (MAC) in the rat. In two separate groups of rats (n = 7 and 8), the cholinesterase inhibitor neostigmine (25 μg) or the muscarinic agonist oxotremorine (25 μg) was infused for 60 min at rates of 5 and 2.5 μl/min, respectively. The doses and infusion rates were established in preliminary experiments to attain maximum electroencephalographic effect and minimum systemic (blood pressure, heart rate) effect, respectively. Each animal received only one type of drug. In a third group of rats (n = 5), the vehicle, artificial cerebrospinal fluid, was infused. In all groups, the electroencephalogram was recorded continuously, starting 10 min before the infusion started. The motor reflex to tail pinch was tested before and after the infusion of agents. Arterial samples were taken at the same time points. Tail pinch was performed with a hemostat, applying a gentle force for a few seconds at approximately mid-tail, with the exact location varied at random. If no response was seen, the pinch was repeated up to three times at slightly different locations.

A fourth group of animals (n = 6) received no drug infusion; these rats were instead allowed to emerge to an awake, sedated state by reducing the isoflurane concentration to 0.4%. Although bupivacaine was applied to all surgical sites, we chose not to remove isoflurane completely so as to minimize stress to the animal. At the beginning of the experiment, the anesthetized animals were placed in a cylindrical, plastic restrainer of 6-cm diameter (Harvard rodent restrainer, model AH-52-0292; Harvard Apparatus, Holliston, MA) that gave them limited movement of their head and limbs but prevented them from crawling out of the cylinder. The assembly was then placed in a transparent Plexiglas anesthesia box. The electroencephalogram was recorded continuously. After a baseline had been established at 1.0% isoflurane, the concentration was lowered to 0.4%. The rats were observed continuously for alertness and the absence of signs of discomfort. An orientation response to gentle knocking on their housing and the presence of spontaneous, calm snouting, sniffing, and whisking was taken as an indication of normal waking state. Potential signs of discomfort would be shaking, tremor, vocalization, or persistent or fierce escape attempt. A final behavioral assessment was done at 1 h.

Bipolar electroencephalographic signals recorded from the two hemispheres were analog-filtered for 1–100 Hz and digitized at 200 Hz using the WinDaq data

acquisition software (DATAQ Instruments, Akron, Ohio). We previously tried higher sampling frequencies, such as 400 Hz, but found that C-ApEn worked best with 200-Hz data. This sampling rate makes C-ApEn relatively sensitive to γ-frequencies (30–80 Hz) but excludes high-frequency noise. Although anesthetic agents may influence sub-δ (<1 Hz) rhythms of the electroencephalogram, these waves are often the most contaminated by motion artifacts and therefore were filtered out. We also theorized that the dominant electroencephalographic features that may change with the anesthetic dose near the threshold for loss of consciousness would be the high-frequency rhythms.

For analysis, the electroencephalographic records were divided into epochs of 2-s duration. Epochs containing movement-related artifacts were dropped from the analysis. A computer subroutine designed to detect transient, large-amplitude deflections in the electroencephalogram was used for this purpose. For each valid epoch and hemispheric lead, the SD of the signal was calculated and used for normalization. C-ApEn was calculated as introduced by Pincus *et al.*[28] We present the definition in a simpler, but mathematically equivalent form:

$$\text{C-ApEn} = (n - m + 1)^{-1}\Sigma\ln[C_i^m(r)/C_1^{m+1}(r)].$$

Here, $C_i^m(r)$ is the relative frequency of finding the same brief signal pattern of length m in both electroencephalogram channels within the epoch. $C_i^{m+1}(r)$ is the relative frequency of finding in both electroencephalogram channels the same signal pattern extended to length m + 1. The sum is for i = 1 to n − m + 1, where n is the number of samples used for comparison. The parameter r is the noise threshold; two samples differing by less than r are considered equal. We chose parameter values n = 20, m = 3, and r = 0.25 × SD, where SD is the SD of the signal within each epoch. Using n = 20, the temporal window of C-ApEn calculation was 100 ms. We previously examined[27] the dependence of the C-ApEn–anesthetic concentration relationship on window duration between 0.05 and 2 s and found that 100 ms, equivalent to 20 points, produced optimal results in terms of dynamic range and variance. We have also evaluated the effect of choice of m between 1 and 4. The most reproducible results were obtained with m = 3. Another consideration to use m = 3 was to confer a greater sensitivity of C-ApEn to high-frequency components of the electroencephalogram (*i.e.*, γ). The strong dependence of C-ApEn on electroencephalogram components greater than 20 Hz was demonstrated previously.[27] Note that the very-low-frequency electroencephalogram components (less than 1 Hz) were attenuated at the time of recording.

Calculations were performed using a program written in QuickBASIC (Microsoft, Redmond, WA). In essence, recorded electroencephalographic data were read from disk in packets of 400 × 2 (2 s of data times two channels), normalized, and processed to derive one C-ApEn value for each epoch. The resulting data were plotted as a function of time or averaged over 1-minute intervals at selected time points. Statistical analysis was carried out using Analysis of Variance of Microsoft Excel, Office 2000, and NCSS 2001 Statistical Software (NCSS, Kaysville, UT). For *post hoc* comparison of C-ApEn group means, the Tukey-Kramer test was applied. The data were tested for normality using the Shapiro-Wilk test, which yielded no reason to reject the normality assumption. Physiologic data means before and after cholinergic activation were compared using a *t* test assuming unequal variances.

Results

Behavioral Observations

Animals receiving 0.4% (0.3 MAC) isoflurane produced frequent movements of the snout, such as sniffing, chewing, licking, and occasional gross limb movements, but the animals showed no sign of stress or discomfort. Their eyelid reflex to touch was present, and a motor response to gentle tail pinch could invariably be elicited. In separate, noninstrumented animals, this state of sedation was associated with preserved righting reflex, suggesting a conscious state.[27]

Animals anesthetized with 1.0% (0.7 MAC) isoflurane showed no spontaneous motor signs. Animals subjected to this dose of anesthesia invariably lose their righting reflex,[27] suggesting loss of consciousness. However, the motor response to tail pinch was present in all animals, as expected on the basis of the applied MAC fraction.

After the infusion of neostigmine or oxotremorine, animals showed motor signs similar to those under 0.4% isoflurane. In some animals, gross motor behavior was observed (in this case, the isoflurane concentration was immediately raised, and the experiment was terminated). Although the righting reflex was not tested in these animals, their behavioral signs indicated a capability and motivation to right themselves, thus suggesting that they were in the conscious state. In surprising contrast, the motor reflex to tail pinch was absent with no exception, and the response could not be elicited on any repeated trial. Cerebroventricular infusion of artificial cerebrospinal fluid at 1.0% isoflurane anesthesia produced no change in behavior.

Physiologic Parameters

Systemic physiologic parameters obtained in three states are displayed in Table 1. The P_{CO_2} was moderately elevated, with a corresponding decrease in pH, during 1.0% isoflurane administration before cholinergic drug administration, most likely because of depressed ventilation of the spontaneously breathing animals. This difference was diminished after cholinergic activation, sug-

Table 1. Systemic Physiologic Parameters

	Po_2	$PoCO_2$	pH	MAP
0.4% Isoflurane	156 ± 28	44 ± 8	7.39 ± 0.04	132 ± 11
1% Isoflurane	124 ± 15	58 ± 10*	7.31 ± 0.06*	88 ± 8*
1% Isoflurane + cholinergic agent	140 ± 28	44 ± 7	7.39 ± 0.05	117 ± 23

* $P < 0.05$ *vs.* 1% isoflurane + cholinergic agent.
MAP = mean arterial pressure, mmHg.

gesting restored respiratory drive. Similarly, MAP was lower at 1.0% isoflurane (before cholinergic activation) than at 0.4% isoflurane, but it was increased after cholinergic activation. In all three states, Po_2 and MAP remained within the physiologic limits and within the range of cerebral blood flow autoregulation.

Electrophysiologic Findings

Electroencephalogram signals obtained from one animal are shown in figure 1. The electroencephalogram at 0.4% isoflurane was typical of the awake, sedated rat, essentially desynchronized, with high-frequency activity superimposed on runs of slower θ waves. At 1.0% isoflurane, synchronized δ waves dominated the electroencephalogram. After neostigmine or oxotremorine infusion, the desynchronized electroencephalogram pattern typical to the waking state returned.

With the chosen infusion rate, C-ApEn increased gradually, reaching a maximum within 60 min. The effect time courses for the two agents were similar and are illustrated for neostigmine in figure 2. The maximum effect was an approximately two-fold increase.

Figure 3 summarizes the finding that C-ApEn was significantly lower at 1.0% isoflurane than at 0.4% isoflurane, consistent with previous findings.[27] Both neostigmine and oxotremorine, administered in the presence of 1.0% isoflurane, increased C-ApEn to approximately the same degree. Postinfusion C-ApEn values were not significantly different from those measured in animals receiving 0.4% isoflurane alone. Cerebroventricular infusion of artificial cerebrospinal fluid in animals anesthetized with 1.0% isoflurane did not change C-ApEn.

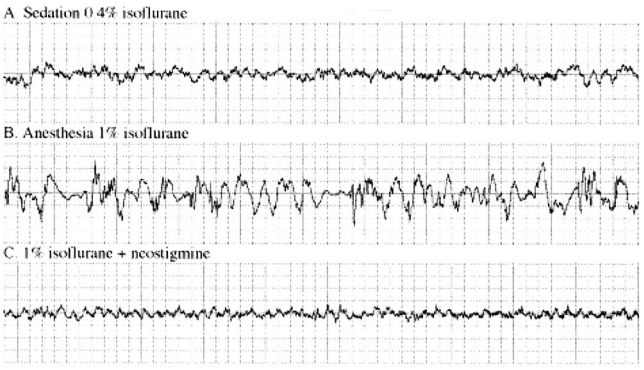

Fig. 1. Examples of rat frontal electroencephalogram in three states: (*A*) sedation with 0.4% isoflurane, (*B*) anesthesia with 1.0% isoflurane, (*C*) after neostigmine administration in the presence of 1.0% isoflurane. *B* and *C* were obtained in the same animal before and after neostigmine administration. Note the similarity between traces *A* and *C*. *Vertical scale* = 0.1 mV per division; *horizontal scale* = 0.2 s per division.

Discussion

Recent years have seen a growing interest in applying novel electroencephalogram-derived indices, particularly various entropies and complexity measures, to assess the depth of anesthesia and loss of consciousness.[21,24–26] Although visual inspection of raw electroencephalogram traces can be informative of the anesthetic state, the use of a suitable electroencephalogram-derived index could greatly improve the accuracy, speed, and consistency of the assessment of anesthetic depth.[13–15] In addition, an examination of specific properties of the electroencephalogram, such as its complexity[39] or interhemispheric synchrony, may help us to better understand the neurophysiologic basis of conscious perception[32,34,35,40] and anesthetic action on the CNS.[16]

Most previous studies calculated ApEn from single-channel recordings of the electroencephalogram. A nonlinear, inverse relation between ApEn and the anesthetic dose in human subjects has been established for various anesthetics.[24–26] Whereas the single-channel ApEn measures the temporal complexity of the electroencephalo-

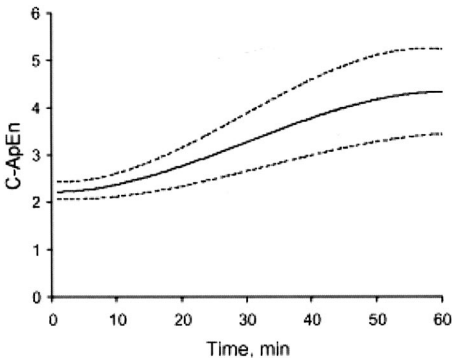

Fig. 2. Time course of the effect of intracerebroventricular infusion of neostigmine on cross-approximate entropy (C-ApEn) of the electroencephalogram in rats anesthetized with 1.0% isoflurane. *Solid curve* shows mean values from seven experiments; *dotted lines* show mean ± SD. Neostigmine infusion started at time zero. A maximum effect was reached within 1 h.

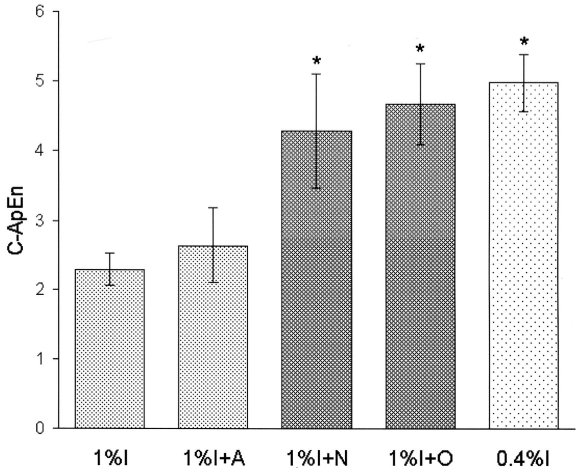

Fig. 3. Effects of cholinesterase inhibitor neostigmine and muscarinic agonist oxotremorine on cross-approximate entropy (C-ApEn) of the electroencephalogram in isoflurane-anesthetized rats. Both drugs were administered at constant 1.0% isoflurane concentration. Both agents increased C-ApEn approximately twofold, to a level comparable to that measured in control animals receiving 0.4% isoflurane and no cholinergic agent. Infusion of vehicle, artificial cerebrospinal fluid, had no significant effect. 1%I = 1% isoflurane; 1%I+A = 1.0% isoflurane plus artificial cerebrospinal fluid infusion; 1%I+N = 1.0% isoflurane plus neostigmine; 1%I+O = 1.0% isoflurane plus oxotremorine; 0.4%I = 0.4% isoflurane. *$P < 0.001$ *versus* 1%I and 1%I+A. Data shown are mean ± SD. Means marked with * are not significantly different from one another. Means without * are not different from one another.

gram, the two-channel C-ApEn reflects the spatial and temporal independence of cortical potentials from two remote sites. The temporal relationship between the electroencephalograms of the two hemispheres has traditionally been assessed in terms of cross-correlation or coherence.[31–34] Anesthetic-induced changes in interhemispheric electroencephalogram coherence have been demonstrated in surgical patients[16] and in healthy adults during drowsiness.[41] The application of cross-correlation and coherence techniques requires stationarity and linearity of signals, conditions not typical of the electroencephalogram. Entropy measures, conversely, can be reliably calculated for relatively short runs of data and are less sensitive to nonstationarity.

We recently argued[27] that C-ApEn may be interpreted as a statistical measure of the number of neural states independently accessible by the two hemispheres. Thus, a decrease in C-ApEn during anesthesia would reflect a decrease in the number of independent hemispheric states, which may interfere with perceptual information processing. This interpretation is consistent with the strong dependence of C-ApEn on high-frequency electroencephalogram components[27] that reflect fast cortical state transitions.

We previously showed that C-ApEn was reduced by isoflurane in rats[27] and that this reduction occurred at an agent concentration that abolished the rats' righting reflex. The righting reflex in rats is lost at a MAC fraction similar to that which produces a loss of response to verbal commands in humans.[42] We hypothesized that if C-ApEn correctly reflects the level of sedation, then cholinergic activation that restores wakeful behavioral signs should also reverse the anesthetic effect on C-ApEn. We indeed found that cholinergic agents administered to isoflurane-anesthetized animals increased C-ApEn to a value measured in a lightly sedated, possibly conscious state at 0.4% isoflurane concentration. The level of sedation at 0.4% isoflurane is significantly lighter than that which would ablate the righting reflex.[27] Although the return of spontaneous motor signs after cholinergic activation does not guarantee the return of consciousness, the comparably high value of C-ApEN after cholinergic activation suggests that it may have in fact reflected a conscious state.

Cholinergic mechanisms have been known to be important regulators of the state of consciousness[43] and to play a key role in conscious information processing.[44] The important role of cholinergic systems in general anesthesia has also been recognized.[1] Halothane and enflurane have been shown to decrease acetylcholine turnover in the rat cerebral cortex.[45] Isoflurane and halothane dose-dependently depress acetylcholine release in cortex and striatum.[46,47] Halothane, isoflurane, and enflurane decrease acetylcholine concentration in the pontine reticular formation,[48] a source of cortical arousal. In human subjects, isoflurane-induced reductions in regional cerebral metabolism are correlated with muscarinic receptor density, suggesting an involvement of cholinergic antagonism in isoflurane anesthesia.[49]

The behavioral and electroencephalogram-activating effects of cholinergic agents have been studied in freely moving animals. Infusion of the cholinergic agonist carbachol into the pontine reticular formation of rats increased the time spent in rapid eye movement sleep.[7,8] Microinjection of neostigmine into the dorsal pontine tegmentum of freely moving cats produced electroencephalogram desynchronization.[4] Intracerebroventricular administration of oxotremorine in rats suppressed neocortical sleep spindles.[9] The systemic, blood-brain–permeable cholinesterase inhibitor physostigmine produced hemispheric electroencephalogram asymmetry in rats.[50] This result is consistent with a greater hemispheric independence, as indicated by the rise in C-ApEn after cholinergic activation in our study.

Cholinergic agents have also been shown to exert an anesthetic sparing or reversal effect. In humans, pretreatment with physostigmine increased the anesthetic requirement to produce unconsciousness in human subjects.[10] In a recent study, a close correlation between the return of response to verbal commands, bispectral index, and auditory steady-state response after physostigmine infusion and continued administration of propofol at hypnotic dose was found.[12] The antimuscarinic agent scopolamine reduced interhemispheric electroencepha-

logram coherence in human volunteers.[51] In rats, both physostigmine and oxotremorine reduced the duration of ketamine anesthesia.[5,6] In dogs, acetylcholinesterase inhibitors were found to antagonize halothane anesthesia and produce an awake-like electroencephalogram.[2,3] In rats, injection of carbachol into the pontine reticular formation antagonized sleep spindles during halothane anesthesia.[52]

In our experiments, cholinergic agents produced antinociception concurrently with their arousing effect on the electroencephalogram. Roy and Stullken[3] and others have noted this divergence between CNS arousal and nociceptive depression by cholinesterase inhibitors in dogs. In rats, Hartvig *et al.*[53] showed that physostigmine infusion produced analgesia, as indicated by an increase in latency of the tail-flick response to radiant heat. These results are consistent with the differing mechanisms of action for hypnosis and areflexia by general anesthetics.[18,19]

The mechanism of antinociception by neostigmine is not quite clear and may be caused by either a central or a peripheral effect. In the study by Horrigan,[2] both neostigmine and physostigmine reduced anesthetic MAC requirement in halothane-anesthetized dogs. Because only physostigmine, not neostigmine, crosses the blood–brain barrier, this suggests a mode of peripheral action. However, the effectiveness of neostigmine administered *via* the intracerebroventricular route implicates a central mechanism of antinociception. The antinociceptive and sedative effects of neostigmine injected into the pontine reticular formation support a mechanism of central modulation.[54,55]

The similar nociceptive and electroencephalographic effects of oxotremorine and neostigmine further implicate the involvement of muscarinic receptors.[56] Antinociception produced by carbachol injection into the brainstem reticular formation was blocked by the muscarinic antagonist atropine[55] or a specific M_2 antagonist.[57] Some investigators[55,58] found the MAC sparing of oxotremorine but not of physostigmine in isoflurane-anesthetized rats.[58] This difference may be a result of a lower dose or a different route of administration of the cholinesterase inhibitor.

Cholinergic agents may activate the electroencephalogram by reversing an effect of isoflurane on the cholinergic system, or they may work through an independent pathway. General anesthetics depress cholinergic transmission in the CNS[59] and have been suggested to affect consciousness through nicotinic receptors.[60] A recent study by Flood *et al.*[61] suggests that nicotinic acetylcholine receptors are unlikely to be involved in isoflurane-induced immobility and hypnosis, although they may play a role in amnesia and analgesia. Conversely, the involvement of M_1 and M_2 muscarinic receptors in prefrontal electroencephalogram activation in the rat has been supported.[36,37] Thus, it is likely that the electroencephalographic effects of cholinergic activation reflect the reversal of a muscarinic receptor–mediated anesthetic action.

Certain limitations of the present study should be recognized. First, the rats were restrained, which may have contributed to their arousal after cholinergic activation. Second, because of the restraint, we were not able to record an electroencephalogram at zero percent isoflurane. For the same reason, the righting reflex could not be assessed in the same animal in which the electroencephalogram was recorded. Future experiments should be performed in chronically instrumented, freely moving rats to verify and extend the present findings. Also, more specific cholinergic agonists and antagonists could be used and injected at multiple doses at selected cortical and subcortical sites to further delineate the neurophysiologic mechanisms that contribute to the observed changes in the electroencephalogram and C-ApEn. With respect to the electroencephalogram, we derived C-ApEn from frontal leads only. To obtain a fuller characterization of functional connectivity throughout the cortex that may affect consciousness, it would be interesting to examine the electroencephalogram from posterior sites as well and to analyze their intrahemispheric relationship as a function of anesthetic dose. Intracortical electrodes could be used for a better localization of the recorded field potentials. In addition to spontaneous activity, the effect of anesthesia on functional connectivity derived from cortical evoked potentials would be informative for an assessment of cortical sensory processes. Such a study is in progress in our laboratory.

In summary, our present data are consistent with the cortical arousing and antinociceptive effect of cholinesterase inhibitors and cholinergic agonists as found in humans and most mammalian species. They lend further support to the previously recognized mechanistic divergence of anesthetic actions on consciousness and the nociceptive reflex. They suggest that the degree of independence of the frontal hemispheric electroencephalogram, as measured by the interhemispheric C-ApEn, correlates with conscious behavior but not with the nociceptive response. C-ApEn promises to be a suitable indicator of anesthetic hypnosis.

References

1. Lydic R, Biebuyck JF: Sleep neurobiology: Relevance for mechanistic studies of anaesthesia. Br J Anaesth 1994; 72:506–8

2. Horrigan RW: Physostigmine and anesthetic requirement for halothane in dogs. Anesth Analg 1978; 57:180–5

3. Roy RC, Stullken EH: Electroencephalographic evidence of arousal in dogs from halothane after doxapram, physostigmine, or naloxone. ANESTHESIOLOGY 1981; 55:392–7

4. Baghdoyan HA, Monaco AP, Rodrigo-Angulo ML, Assens F, McCarley RW, Hobson JA: Microinjection of neostigmine into the pontine reticular formation of cats enhances desynchronized sleep signs. J Pharmacol Exp Ther 1984; 231: 173–80

5. Mimura M, Namiki A, Kishi R, Ikeda T, Miyake H, Iwasaki H: Central cholinergic action produces antagonism to ketamine anesthesia. Acta Anaesthesiol Scand 1992; 36:460–2

6. Mimura M, Namiki A, Kishi R, Ikeda T, Miyake H: Antagonistic effect of

physostigmine on ketamine-induced anesthesia. Psychopharmacology (Berl) 1990; 102:399–403
7. Marks GA, Birabil CG: Enhancement of rapid eye movement sleep in the rat by cholinergic and adenosinergic agonists infused into the pontine reticular formation. Neuroscience 1998; 86:29–37
8. Horner RL, Kubin L: Pontine carbachol elicits multiple rapid eye movement sleep-like neural events in urethane-anaesthetized rats. Neuroscience 1999; 93: 215–26
9. Puolivali J, Jakala P, Koivisto E, Riekkinen P Jr.: Oxotremorine suppresses thalamocortical oscillations via thalamic muscarinic acetylcholine receptors. Psychopharmacology (Berl) 1998; 140:285–92
10. Fassoulaki A, Sarantopoulos C, Derveniotis C: Physostigmine increases the dose of propofol required to induce anaesthesia. Can J Anaesth 1997; 44: 1148–51
11. Thompson DE: Physostigmine as an adjuvant to neuroleptanaesthesia in neurosurgical procedures. Can Anaesth Soc J 1976; 23:582–6
12. Meuret P, Backman SB, Bonhomme V, Plourde G, Fiset P: Physostigmine reverses propofol-induced unconsciousness and attenuation of the auditory steady state response and Bispectral Index in human volunteers. ANESTHESIOLOGY 2000; 93:708–17
13. Pomfrett CJ: Focus on: Electrical signals: The EEG during anaesthesia. Curr Anaesth Crit Care 1998; 9:117–22
14. Drummond JC: Monitoring depth of anesthesia: With emphasis on the application of the Bispectral Index and the middle latency auditory evoked response to the prevention of recall. ANESTHESIOLOGY 2000; 93:876–82
15. Schneider G, Sebel PS: Monitoring depth of anaesthesia. Eur J Anaesthesiol Suppl 1997; 15:21–8
16. John ER, Prichep LS, Kox W, Valdes-Sosa P, Bosch-Bayard J, Aubert E, Tom M, diMichele F, Gugino LD: Invariant reversible QEEG effects of anesthetics. Conscious Cogn 2001; 10:165–83
17. Rampil IJ: A primer for EEG signal processing in anesthesia. ANESTHESIOLOGY 1998; 89:980–1002
18. Rampil IJ, Mason P, Singh H: Anesthetic potency (MAC) is independent of forebrain structures in the rat. ANESTHESIOLOGY 1993; 78:707–12
19. 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
20. Struys MM, Jensen EW, Smith W, Smith NT, Rampil I, Dumortier FJ, Mestach C, Mortier EP: Performance of the ARX-derived auditory evoked potential index as an indicator of anesthetic depth: A comparison with Bispectral Index and hemodynamic measures during propofol administration. ANESTHESIOLOGY 2002; 96:803–16
21. Muncaster AR, Sleigh JW, Williams M: Changes in consciousness, conceptual memory, and quantitative electroencephalographical measures during recovery from sevoflurane- and remifentanil-based anesthesia. Anesth Analg 2003; 96:720–5
22. Drover DR, Lemmens HJ, Pierce ET, Plourde G, Loyd G, Ornstein E, Prichep LS, Chabot RJ, Gugino L: Patient State Index: Titration of delivery and recovery from propofol, alfentanil, and nitrous oxide anesthesia. ANESTHESIOLOGY 2002; 97:82–9
23. Kreuer S, Biedler A, Larsen R, Schoth S, Altmann S, Wilhelm W: The Narcotrend: A new EEG monitor designed to measure the depth of anaesthesia: A comparison with bispectral index monitoring during propofol-remifentanil-anaesthesia. Anaesthesist 2001; 50:921–5
24. Sleigh JW, Donovan J: Comparison of bispectral index, 95% spectral edge frequency and approximate entropy of the EEG, with changes in heart rate variability during induction of general anaesthesia. Br J Anaesth 1999; 82:666–71
25. Bruhn J, Ropcke H, Hoeft A: Approximate entropy as an electroencephalographic measure of anesthetic drug effect during desflurane anesthesia. ANESTHESIOLOGY 2000; 92:715–26
26. Bruhn J, Ropcke H, Rehberg B, Bouillon T, Hoeft A: Electroencephalogram approximate entropy correctly classifies the occurrence of burst suppression pattern as increasing anesthetic drug effect. ANESTHESIOLOGY 2000; 93:981–5
27. Hudetz AG: Effect of volatile anesthetics on interhemispheric EEG cross-approximate entropy in the rat. Brain Res 2002; 954:123–31
28. Pincus SM, Mulligan T, Iranmanesh A, Gheorghiu S, Godschalk M, Veldhuis JD: Older males secrete luteinizing hormone and testosterone more irregularly, and jointly more asynchronously, than younger males. Proc Natl Acad Sci U S A 1996; 93:14100–5
29. Desmedt JE, Tomberg C: Transient phase-locking of 40 Hz electrical oscillations in prefrontal and parietal human cortex reflects the process of conscious somatic perception. Neurosci Lett 1994; 168:126–9
30. Gazzaniga MS: Brain and conscious experience. Adv Neurol 1998; 77: 181–92
31. Varela F, Lachaux JP, Rodriguez E, Martinerie J: The brainweb: Phase synchronization and large-scale integration. Nat Rev Neurosci 2001; 2:229–39
32. Srinivasan R, Russell DP, Edelman GM, Tononi G: Increased synchronization of neuromagnetic responses during conscious perception. J Neurosci 1999; 19:5435–48
33. Joliot M, Ribary U, Llinas R: Human oscillatory brain activity near 40 Hz coexists with cognitive temporal binding. Proc Natl Acad Sci U S A 1994; 91:11748–51
34. Knyazeva MG, Kiper DC, Vildavski VY, Despland PA, Maeder-Ingvar M, Innocenti GM: Visual stimulus-dependent changes in interhemispheric EEG coherence in humans. J Neurophysiol 1999; 82:3095–107
35. Mima T, Oluwatimilehin T, Hiraoka T, Hallett M: Transient interhemispheric neuronal synchrony correlates with object recognition. J Neurosci 2001; 21:3942–8
36. Douglas CL, Baghdoyan HA, Lydic R: Postsynaptic muscarinic M1 receptors activate prefrontal cortical EEG of C57BL/6J mouse. J Neurophysiol 2002; 88:3003–9
37. Douglas CL, Baghdoyan HA, Lydic R: Prefrontal cortex acetylcholine release, EEG slow waves, and spindles are modulated by M2 autoreceptors in C57BL/6J mouse. J Neurophysiol 2002; 87:2817–22
38. Paxinos G, Watson C: The Rat Brain in Stereotaxic Coordinates, 4th Edition. San Diego, Academic Press, 1998
39. Tononi G, Edelman GM: Consciousness and complexity. Science 1998; 282:1846–51
40. Meador KJ, Ray PG, Echauz JR, Loring DW, Vachtsevanos GJ: Gamma coherence and conscious perception. Neurology 2002; 59:847–54
41. Wada Y, Nanbu Y, Koshino Y, Shimada Y, Hashimoto T: Inter- and intrahemispheric EEG coherence during light drowsiness. Clin Electroencephalogr 1996; 27:84–8
42. Kissin I, Morgan PL, Smith LR: Anesthetic potencies of isoflurane, halothane, and diethyl ether for various end points of anesthesia. ANESTHESIOLOGY 1983; 58:88–92
43. Jones BE: The neural basis of consciousness across the sleep-waking cycle. Adv Neurol 1998; 77:75–94
44. Perry E, Walker M, Grace J, Perry R: Acetylcholine in mind: A neurotransmitter correlate of consciousness? Trends Neurosci 1999; 22:273–80
45. Ngai SH, Cheney DL, Finck AD: Acetylcholine concentrations and turnover in rat brain structures during anesthesia with halothane, enflurane, and ketamine. ANESTHESIOLOGY 1978; 48:4–10
46. Damsma G, Fibiger HC: The effects of anaesthesia and hypothermia on interstitial concentrations of acetylcholine and choline in rat striatum. Life Sci 1991; 48:2469–74
47. Shichino T, Murakawa M, Adachi T, Nakao S, Shinomura T, Kurata J, Mori K: Effects of isoflurane on in vivo release of acetylcholine in the rat cerebral cortex and striatum. Acta Anaesthesiol Scand 1997; 41:1335–40
48. Keifer JC, Baghdoyan HA, Becker L, Lydic R: Halothane decreases pontine acetylcholine release and increases EEG spindles. Neuroreport 1994; 5:577–80
49. Alkire MT, Haier RJ: Correlating in vivo anaesthetic effects with ex vivo receptor density data supports a GABAergic mechanism of action for propofol, but not for isoflurane. Br J Anaesth 2001; 86:618–26
50. Vorobyov VV, Ahmetova ER: Muscarinic elicitation of EEG asymmetry in freely moving rats. Brain Res 1998; 794:299–303
51. Kikuchi M, Wada Y, Koshino Y, Nanbu Y, Hashimoto T: Effects of scopolamine on interhemispheric EEG coherence in healthy subjects: Analysis during rest and photic stimulation. Clin Electroencephalogr 2000; 31:109–15
52. Keifer JC, Baghdoyan HA, Lydic R: Pontine cholinergic mechanisms modulate the cortical electroencephalographic spindles of halothane anesthesia. ANESTHESIOLOGY 1996; 84:945–54
53. Hartvig P, Gillberg PG, Gordh T Jr, Post C: Cholinergic mechanisms in pain and analgesia. Trends Pharmacol Sci 1989; (suppl):75–9
54. Kshatri AM, Baghdoyan HA, Lydic R: Cholinomimetics, but not morphine, increase antinociceptive behavior from pontine reticular regions regulating rapid-eye-movement sleep. Sleep 1998; 21:677–85
55. Ishizawa Y, Ma HC, Dohi S, Shimonaka H: Effects of cholinomimetic injection into the brain stem reticular formation on halothane anesthesia and antinociception in rats. J Pharmacol Exp Ther 2000; 293:845–51
56. Yaksh TL, Dirksen R, Harty GJ: Antinociceptive effects of intrathecally injected cholinomimetic drugs in the rat and cat. Eur J Pharmacol 1985; 117:81–8
57. Ma HC, Dohi S, Wang YF, Ishizawa Y, Yanagidate F: The antinociceptive and sedative effects of carbachol and oxycodone administered into brainstem pontine reticular formation and spinal subarachnoid space in rats. Anesth Analg 2001; 92:1307–15
58. Zucker J: Central cholinergic depression reduces MAC for isoflurane in rats. Anesth Analg 1991; 72:790–5
59. Bosnjak ZJ, Seagard JL, Wu A, Kampine JP: The effects of halothane on sympathetic ganglionic transmission. ANESTHESIOLOGY 1982; 57:473–9
60. Forman SA, Miller KW, Yellen G: A discrete site for general anesthetics on a postsynaptic receptor. Mol Pharmacol 1995; 48:574–81
61. Flood P, Sonner JM, Gong D, Coates KM: Heteromeric nicotinic inhibition by isoflurane does not mediate MAC or loss of righting reflex. ANESTHESIOLOGY 2002; 97:902–5