

## Local distribution and toxicity of prolonged hippocampal infusion of muscimol

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**Object.** The activity of  $\gamma$ -aminobutyric acid (GABA), the principal inhibitory neurotransmitter, is reduced in the hippocampus in patients with complex partial seizures from mesial temporal sclerosis. To provide preliminary safety and distribution data on using convection-enhanced delivery of agents to treat complex partial seizures and to test the efficacy and safety of regional selective neuronal suppression, the authors infused muscimol, a GABA-A receptor agonist, directly into the hippocampus of nonhuman primates using an integrated catheter electrode.

**Methods.** Ten rhesus monkeys were divided into three groups: 1) use of catheter electrode alone (four monkeys); 2) infusion of escalating concentrations of muscimol followed by vehicle (three monkeys); and 3) infusion of vehicle and subsequent muscimol mixed with muscimol tracer (three monkeys). Infusions were begun 5 days after catheter electrode placement and continued for 5.6 days before switching to the other agent. Head magnetic resonance (MR) images and electroencephalography recordings were obtained before and during the infusions. Brain histological studies and quantitative autoradiography were performed.

Neurological function was normal in controls and when muscimol concentrations were 0.125 mM or less, whereas higher concentrations (0.5 and 1 mM) produced reversible apathy and somnolence. Fluid distribution was demonstrated on MR images and muscimol distribution was demonstrated on autoradiographs throughout the hippocampus and adjacent white matter.

**Conclusions.** Targeted modulation of neuronal activity is a reasonable research strategy for the investigation and treatment of medically intractable epilepsy.

**KEY WORDS** • epilepsy • infusion test • magnetic resonance imaging • drug delivery system • *Macaca mulatta*

**M**EDICALLY intractable epilepsy develops in approximately 15,000 to 30,000 people each year in the US. Epilepsy is considered intractable when it significantly reduces the quality of a person's life, either because frequent seizures persist despite appropriate doses and types of antiepilepsy medications or because the control of seizures is possible only with medication dosages that produce excessive sedation.<sup>34</sup> In most cases of surgically remediable medically intractable epilepsy, the pathological disorder is mesial temporal sclerosis, a condition in which the seizure focus is located in one of the hippocampi. Standard treatment for this condition requires frontotemporal craniotomy for removal of the affected hippocampus. Such surgery is successful in eliminating seizures in two of three patients, in preventing cerebral degeneration and death from repeated seizures, and in avoiding the detrimental effects

of toxic doses of medication on learning and job performance.<sup>45</sup>

In most patients with epilepsy, oral medication provides seizure control because it produces drug levels in the brain that are sufficient to suppress the epileptic focus but insufficient to suppress cognitive activity. For patients with medically intractable epilepsy, however, moderate drug levels are incapable of suppressing the epileptic focus; efforts to further increase the drug dose generate levels that suppress neural activity throughout the brain and cause cognitive side effects. Medication delivered by mouth or vein results in drug levels in the epileptic focus that are approximately equal to those in the rest of the brain; because medication is not specifically targeted to the epileptic focus, improved seizure control comes with the cost of increased cognitive side effects. The link between improved control of seizures and cognitive side effects could be broken if a method were developed to preferentially deliver drugs directly to the seizure focus. Because the seizure focus in many patients with medically intractable epilepsy is located in the hippocampus, selective suppression of neuronal activity in this discrete anatomical structure could potentially control this type of epilepsy and avoid cognitive side effects. Although the

*Abbreviations used in this paper:* AP = anteroposterior; CED = convection-enhanced delivery; CSF = cerebrospinal fluid; EEG = electroencephalography; FLAIR = fluid-attenuated inversion-recovery; GABA =  $\gamma$ -aminobutyric acid; GPI = globus pallidus internus; IC<sub>50</sub> = 50% inhibitory concentration; MR = magnetic resonance; SD = standard deviation.

hippocampus could be targeted for direct infusion, even if medication spread from this structure but remained within the same cerebral hemisphere, sedation would not occur.<sup>46</sup> Furthermore, monitoring the clinical effects of muscimol infusion into a discrete anatomical region may provide a new approach for the precise localization of the seizure origin and might predict the clinical outcome of selective excision or selective neuronal ablation by the infusion of drugs with selective neuronal cytotoxicity.

Selective targeting of specific brain regions is possible with CED, a means of directly infusing substances into the extracellular space of a targeted brain region.<sup>5,33</sup> After placing a catheter tip into the region to be treated, the substance in solution is slowly pumped into the extracellular fluid space where it is carried through the extracellular space by a combination of bulk flow and diffusion. This method has the potential to provide more widespread distribution of effective and nontoxic antiepilepsy drug levels compared with purely diffusion-based approaches, which require much higher drug concentrations at the point of injection and distribute molecules on the basis of concentration gradients.<sup>5,33</sup> The hippocampus presents a challenging therapeutic target for CED because of its proximity to the temporal horn of the lateral ventricle and the basal subarachnoid cisterns.

Many antiepilepsy drugs (phenobarbital, valproic acid, and benzodiazepine agents) suppress seizures by acting through the GABA-A receptor. Muscimol (3-hydroxy-5-aminomethylisoxazole), a GABA-A receptor agonist, has been used to study the role of GABA within the brain. Present in 60 to 70% of all brain synapses, GABA, the primary inhibitory neurotransmitter in the central nervous system, produces hyperpolarization of the postsynaptic neuron by increasing Cl<sup>-</sup> conductance. Because muscimol in the brain is degraded much more slowly than GABA, it has a much more sustained effect than GABA itself.<sup>19</sup>

In this study we examined the distribution, gross behavioral effects, and toxicity of chronic unilateral infusion of muscimol into the hippocampus of rhesus monkeys. This work was performed to acquire preliminary data that would be relevant to the feasibility of using CED of muscimol to suppress epileptic activity in patients with medically intractable epilepsy. To better predict the spread of an agent infused into the hippocampus in humans, rhesus monkeys were selected for these experiments because their brains are relatively large (80–100 g) and provide a greater volume for muscimol distribution within the hippocampus and temporal lobe than a rodent brain. The infusion rate could not be scaled to reflect the smaller mass of the rodent brain because slower infusion rates would increase the amount of muscimol distributed by diffusion, not convection.

## Materials and Methods

### *Surgical Procedure*

The study was conducted in accordance with the National Institutes of Health guidelines on the use of animals in research and was approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke. The study was performed using 10 adult rhesus monkeys, four male and six female, each weighing from 5.2 to 13.4 kg (mean  $\pm$  SD  $8 \pm 2.6$  kg). After the intramuscular administration of ketamine (3 mg/kg), insertion of an intravenous catheter, endotracheal intubation, and the induction of general anesthesia with isoflurane, the animal was placed prone in a

primate stereotactic frame (Kopf Instruments, Tujunga, CA) and we performed surgery in a dedicated surgical suite using sterile instruments. Stereotactic coordinates and catheter trajectory were determined from a preliminary MR image.<sup>1,17</sup> A posterior rather than a lateral approach to the hippocampus was chosen because it avoids trauma to the temporalis muscle and resultant pain with mastication, prevents leakage of muscimol into the ventricle because it increases the distance from the catheter tip to the point at which the catheter may traverse the ventricular wall, and allows a longer length of catheter to be placed within the hippocampus, which could improve the spread of muscimol throughout this structure. The electrode guide (Kopf Instruments) of the stereotactic device was used to direct the catheter electrode from the outer table of the occipital skull anteriorly and 20 to 30° inferiorly to the target, to a depth of 40 to 43 mm. The catheter electrode was attached to the skull with dental cement and its distal end was tunneled subcutaneously and brought through a skin puncture on the dorsal thorax. The catheter was filled with vehicle and capped. In the last three animals, electrode leads were connected to an EEG transponder (TL10M3-D70-EEE; Data Sciences International, Inc., St. Paul, MN) placed in the left flank. Scalp and flank wounds were closed with sutures. The external part of the catheter electrode was placed in the pocket of a monkey vest. Isoflurane was withdrawn and the first dose of analgesic agent was given before the animal recovered from anesthesia.

### *Postoperative Care and Neurological Monitoring*

Behavior and activity levels, food and water intake, and wound healing were assessed daily. A seven-point scale of neurological function was used daily, scoring 1 point each for alertness, avoidance of eye contact, normal body posturing, facial symmetry, startle response to hand clap, symmetrical limb movement, and ability to stand normally.<sup>16</sup> Mild postoperative pain or discomfort was prevented or allayed with the administration of Ketoprofen (2 mg/kg intramuscularly) or buprenorphine (0.01–0.03 mg/kg intramuscularly) every 12 hours. Skin sutures were removed 7 days after surgery.

### *Drug Delivery System*

A depth electrode with an outside diameter of 1.3 mm (AdTech Medical, Racine, WI) and modified to accommodate a central perfusion catheter was placed in the right or left hippocampus. The catheter remained in place for 5 days and infusion was then started, with muscimol or vehicle given for 5.6 days before switching to the other agent for another 5.6 days. The perfusion catheter (AdTech Medical) was made of synthetic fused silica with a polyamide coating and had an inner diameter of 75  $\mu$ m and an outer diameter of 355  $\mu$ m. The catheter was flexible with curvature qualities similar to 32-gauge stainless-steel hypodermic stock. The tip of the perfusion catheter extended 4 mm beyond the deepest electrode. The perfusion catheter had a connector for the attachment of plastic tubing from an infusion pump (model 404-SP; MiniMed, Sylmar, CA) in the pocket of a monkey jacket (Lomir, Inc., Malone, NY). The infusion pump held a 3-ml syringe, which was loaded with infusate daily. The infusion rate (volumetric flow rate) was chosen so that fluid would backflow along the catheter, creating a fluid anulus to deliver muscimol to the surrounding hippocampus. The volumetric flow rate (Q expressed in microliters per minute) required to achieve a particular flow rate is related to backflow length ( $x_m$  expressed in centimeters) and catheter radius ( $r_c$  expressed in centimeters) by the scaling relationship,<sup>32</sup>  $Q = 0.018 x_m^{5/3} r_c^{-4/3}$ . Because the radius of the catheter over most of the desired backflow length is that of the electrode portion, catheter radius was taken as 0.065 cm. The chosen infusion rate of 84  $\mu$ l/hour (1.4  $\mu$ l/minute) should yield a backflow length of 1.5 cm.

The infusion concentration for these toxicity studies was selected initially as 1 mM, later broadened to a range of 0.125 to 1 mM, well below the 8.8 mM previously used in humans to inhibit the GPI and thalamus.<sup>36,38</sup> Concentration distributions resulting from infusion of such muscimol solutions were estimated from a convection-diffusion-permeation-reaction model in temporal lobe tissue.<sup>33</sup> Solved initially in spherical geometry—that is, in the absence of backflow—an order of magnitude estimate of the penetration depth was obtained as 9 mm by using an inflow rate of 1.4  $\mu$ l/minute, a concentration of 0.125 mM, a muscimol extracellular diffusion coefficient of  $5.9 \times$

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$10^{-6}$  cm<sup>2</sup>/second (molecular weight scaled from sucrose value using a  $-0.6$  exponent),<sup>30,37</sup> a permeation-reaction rate constant of 0.008 minute<sup>-1</sup> (upper bound estimate derived from the low flow-rate intracortical infusions of Martin<sup>27</sup>), an extracellular volume fraction of 0.22,<sup>8</sup> and a minimum effective concentration of 150 nM muscimol, equivalent to five times the IC<sub>50</sub> of GABA receptor *in vitro*.<sup>15</sup> This penetration depth exceeded the radial dimension of the hippocampus in the coronal plane of the primate and approximated the human dimension, suggesting that the chosen combinations of flow rate and infusate concentration would be sufficient to dose the majority of the hippocampal target in both animal species. Later computations (see *Results*) were performed in cylindrical symmetry to account for backflow. Numerical software used routines from Mathematica, version 4.1 (Wolfram Research, Inc., Champaign, IL) and IMSL, version 4 (Absoft Corp., Rochester Hills, MI). Muscimol solution was prepared by diluting muscimol (National Institutes of Health Compound 773) with Elliotts B solution (artificial CSF) to a concentration of 14 to 114  $\mu$ g/ml (0.125–1 mM). In animals receiving H3-muscimol for the autoradiography studies, 1 mCi/ml H3-muscimol, (PerkinElmer Life Sciences, Inc., Boston, MA) was diluted in unlabeled muscimol solution to 20  $\mu$ Ci/ml (Animals 8 and 9) or 40  $\mu$ Ci/ml (Animal 10). If the animal broke the catheter electrode, it was occluded and left in place. To prevent breakage of the infusion catheter in the last three animals, the catheter electrode was modified so that the silica portion of the catheter extended only from the hippocampus to the burr hole, where it attached to flexible plastic tubing.

### Magnetic Resonance Imaging Procedures

Magnetic resonance imaging of the brain in the sagittal and coronal planes was performed in a 1.5-tesla MR unit (General Electric Medical Systems, Milwaukee, WI) while the animals were in a state of general anesthesia. The MR images were obtained before and after placement of the catheter electrode and during infusion of muscimol and vehicle. The imaging sequences included the following: 1) three-dimensional spoiled gradient-recalled acquisition; 2) T<sub>1</sub>-weighted; 3) T<sub>2</sub>-weighted; and 4) FLAIR images. The neuroradiologist (J.A.B.) evaluated the T<sub>2</sub>-weighted and FLAIR images for an increased water signal within the brain, resulting from placement of the catheter electrode or distribution of the infused solution. The increased water signal was measured in three planes in the temporal and occipital lobes, and the affected volume was calculated using the formula for volume of an ellipsoid (length  $\times$  width  $\times$  height  $\times$   $\pi/6$ ).<sup>10</sup>

### Electroencephalography Monitoring

The first seven animals were trained to sit in a chair before surgery and underwent scalp and depth electrode EEG recordings before infusion and during the two infusion periods (Grass EEG and Polygraph Data Recording System, model 78D; Grass Instrument Co., Quincy, MA). The EEG recordings were traced on paper and interpreted by an electroencephalographer (S.S.). To reduce motion artifact, a biopotential and physical activity transponder (TL10M3-D70-EEE; Data Sciences International) was implanted in the final three animals and its signal was received (RMC-1; Data Sciences), stored, and analyzed (ART 2.2 Silver with Computer; Data Sciences) beginning 4 days after surgery. Mean EEG frequency and power was recorded during the various conditions (no infusion, vehicle, and muscimol). The range of predominant frequencies (hertz) and the mean frequency for each recording were determined by the electroencephalographer (S.S.). The difference in mean frequency between vehicle and muscimol infusion was evaluated using a paired *t*-test (StatView; SAS Institute, Inc., Cary, NC).

### Gross Pathological Study

At the end of the infusion, the animals were killed with a 90-mg/kg intravenous infusion of a pentobarbital solution followed by intracardiac perfusion with phosphate-buffered saline followed by 4% paraformaldehyde in PBS. The brain was removed rapidly and analyzed by the neuropathologist (A.V.). In the animals that had received an infusion of 3H-muscimol, the brain was immediately cut in the coronal plane into 5-mm-thick slabs, which were embedded in

optimal cutting temperature compound (Tissue-Tek; Sakura Finetek, Torrance, CA) and frozen in isopentane cooled with dry ice. Brains that had not been infused with 3H-muscimol were fixed in 4% paraformaldehyde for 2 months and then cut by the neuropathologist. Tissue slabs were digitally photographed before histological processing. A veterinarian (J.O.) or a veterinary pathologist performed the postmortem examination of other organs.

### Histological Study

In animals that had received an infusion of 3H-muscimol, frozen brain tissue sections (20- $\mu$ m thick) were cut in the coronal plane by using a cryostat and thaw-mounted on slides. Sections were taken at 1-mm intervals for histological and autoradiography studies. Histological sections were stained with H & E. From the brains of animals that did not receive a 3H-muscimol infusion, paraffin-embedded sections were obtained. Staining with H & E was performed for general morphological studies.

### Autoradiography Studies

Quantitative autoradiograms for 3H-muscimol were prepared from coronal sections of the entire brain, spaced 1 mm apart; the distance in the AP direction from each section to the infusion point was recorded.<sup>27</sup> Tritium radioactive standards (American Radiolabeled Chemicals, Inc., St. Louis, MO) and tissue sections on glass slides were exposed to 20  $\times$  25-cm tritium imaging plates (Fujifilm Medical Systems USA, Inc., Stamford, CT) in film cassettes. After a 3-week exposure, the plates were analyzed using a BAS5000 bioimaging analyzer and imaging software (both from Fujifilm Medical Systems).

The autoradiograms were assessed for the concentration and distribution of 3H-muscimol within each section. The mean concentration of muscimol within each tissue section was measured between concentric circles placed at 1-mm intervals around the path of the catheter electrode. This measurement gave the radius of distribution, *r*, of a specific muscimol concentration, *c*, within each section located at an AP distance, *z* (millimeters), from the section containing the infusion point. A positive *z* direction points anteriorly along the catheter axis. Distance was measured by section number and confirmed by comparing sections with T<sub>2</sub>-weighted MR images. The 3H-muscimol concentration in the ventricles and subarachnoid space was measured. Cerebrospinal fluid from the cerebellomedullary cistern was obtained when the animals were killed, and was analyzed by scintillation counting.<sup>2</sup>

## Results

### Postoperative Care and Neurological Monitoring

All animals survived surgery and remained physiologically stable. Neurological function remained normal (7 points on the seven-point scale) after instrument placement and during infusions of vehicle and muscimol at a concentration of 0.125 mM (six animals; Table 1). Sedation and somnolence occurred within 2 to 6 hours of administering the higher concentrations of muscimol (0.5 and 1 mM, three of three animals; 0.25 mM, one of two animals) and resolved within the same interval after the switch to vehicle.

### Drug Delivery System

The six animals in the two infusion groups received hippocampal infusions of muscimol for 5.6 days and vehicle for 5.6 days, except in one case in which vehicle infusion was shortened to 3 days when the animal tore the catheter electrode. Three of four animals in the instrument control group tore the external part of the catheter electrode. The infusion system remained intact in the last three animals in which the extracranial portion of the catheter was flexible plastic tubing (Table 2).

TABLE 1  
Neurological evaluation scores in 10 animals that did or did not receive muscimol infusion\*

Animal No.	Experimental Group	Vehicle Infusion	Muscimol Infusion (mM)			
			1.0	0.5	0.25	0.125
1	instrument alone	NA				NA
2	muscimol & vehicle	7	3			7
3	muscimol & vehicle	7		3	7	7
4	muscimol & vehicle	7				7
5	instrument alone	NA				NA
6	instrument alone	NA				NA
7	instrument alone	NA				NA
8	vehicle & muscimol	7				7
9	vehicle & muscimol	7				7
10	vehicle & muscimol	7	6	6	6	7

\* Scores are based on a seven-point scale; see *Materials and Methods* for details. Abbreviation: NA = not applicable because there were no infusions in the instrument control group.

### Magnetic Resonance Imaging

The T<sub>1</sub>-weighted images and three-dimensional spoiled gradient-recalled acquisition images confirmed placement of the catheter electrode tip within the hippocampus (Fig. 1a). Fluid-attenuated inversion-recovery and T<sub>2</sub>-weighted images demonstrated regions of increased water signal within the brain parenchyma (Fig. 1b). Analysis of FLAIR and T<sub>2</sub>-weighted images (Figs. 1b and 2) revealed that muscimol and vehicle infusion increased water signal in significant volumes of the brain. In the one animal (Animal 4) in which it was evaluated, increased parenchymal water signal from hippocampal infusion on Day 10 postintervention resolved completely within 24 hours after ending the infusion (Fig. 3).

### Electroencephalography Monitoring

Muscimol had no discernible effect on surface EEG recordings. Telemetric recordings from the hippocampal electrode had less motion artifact than surface recordings. Muscimol infusion reduced the power (voltage) of depth electrode recordings from the hippocampus, but the mean frequency (muscimol 11.4 ± 3 Hz, vehicle 9.7 ± 6.4 Hz; *p* = 0.45, paired *t*-test) was largely unaffected (Fig. 4). Seizures did not occur and epileptiform activity was not noted during the study.

TABLE 2  
Catheter electrode infusion and recording system\*

Animal No.	Catheter Segment			Electrode Segment		
	Intra-cranial	Skull Interface	External	Intra-cranial	Skull Attachment	External
2	I	I	B†	I	I	I
3	I	I	I	I	I	I
4	I	B‡	I	I	I	I
8	I	I	I	I	I	§
9	I	I	I	I	I	§
10	I	I	I	I	I	§

\* B = broken; I = intact and functional.

† Broken on Day 13 postintervention.

‡ Broken on Day 16 postintervention.

§ Electrode entirely internal because of transponder.

### Pathological Studies

Brain weight was 92 ± 10 g (mean ± SD). The catheter tract within the occipital and temporal lobes was evident after the brain was cut (Fig. 5a). There was no gross evidence of a reaction to the catheter electrode or the infusion, except for minimal enlargement of the temporal lobe white matter in Animal 9. On histological examination, there was no difference between the brains of animals that had received an infusion (infusion group) and those that had undergone placement of the catheter electrode (instrument control group). Placement of the catheter electrode within the brain caused gliosis and hemosiderin deposition limited to four to five cell layers immediately around the catheter (Fig. 5b). No abnormal finding was evident on postmortem examination of the other organs.

### Autoradiography Studies

Muscimol spread from the catheter tip to structures within the medial aspect of the temporal lobe, including the hippocampus and amygdala. The gray matter of the hippocampus, amygdala, and cortex of the ipsilateral temporal lobe were preferential sites of distribution compared with adjacent white matter tracts (Fig. 6). In all tissue sections, the muscimol concentration was greater in the medial than in the lateral temporal lobe structures. Muscimol did not spread to the adjacent frontal and parietal lobes. Relative to an infusate concentration of 1 mM, ipsilateral (mean ± SD, 0.005 ± 0.003 mM) and contralateral (0.004 ± 0.001 mM) lateral ventricles and the convexity of the ipsilateral hemisphere (0.004 ± 0.001 mM) contained low concentrations of muscimol. Using scintillation counting we measured low concentrations of radiolabeled muscimol in the cisternal CSF (0.0046 ± 0.0036 mM) and in venous blood (0.0004 ± 0.0005 mM). Muscimol was not detected within the parenchyma of the contralateral hemisphere.

Muscimol concentrations were distributed along the catheter and hippocampal axis, extending from approximately 18 mm posterior to 6 mm anterior to the catheter tip. Distribution was consistent with backflow at 1.4 μl/minute and a fluid anulus around the catheter, which was 3 mm longer than expected from the scaling relationship. The data pattern indicates that the delivery of muscimol to tissue occurs by fluid and solute movement across the surface of this anu-

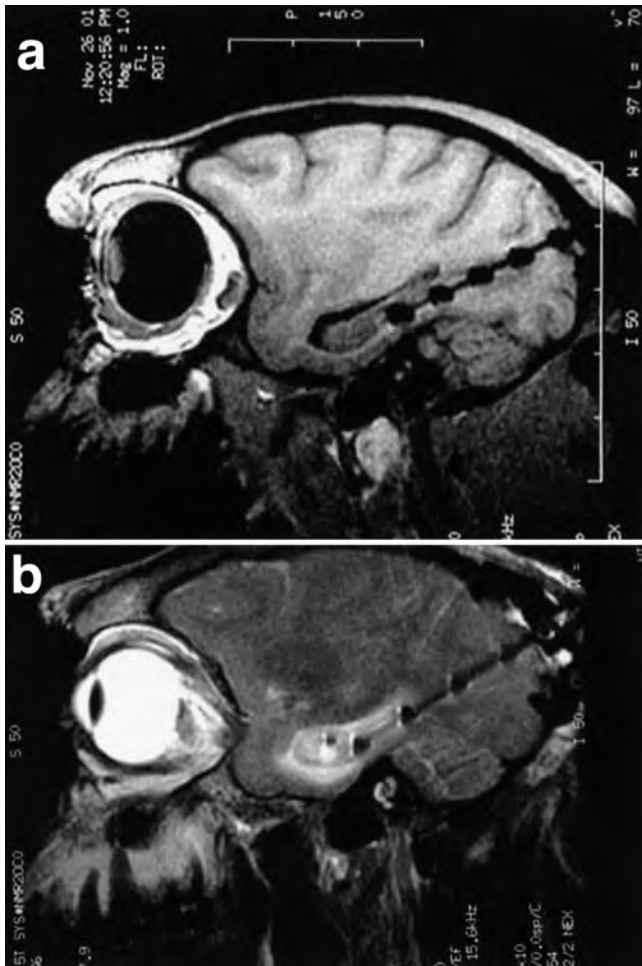


FIG. 1. a: Parasagittal T<sub>1</sub>-weighted MR image demonstrating the catheter electrode as a black line with nodal enlargements at the electrode contacts. The apparent size of the electrodes is magnified on these images because of the metal within them. The tip of the perfusion catheter extends 4 mm beyond the deepest electrode and is not seen because of its small size (outer diameter 0.355 mm). b: Parasagittal T<sub>2</sub>-weighted image exhibiting the increased signal from the infusate (originating at the tip of the catheter), which displays the distribution of the infusate in the hippocampus and the adjacent white matter.

lus and the hemispherical surface at the tip of the catheter (Fig. 7). Analysis of prior research data<sup>32</sup> has shown that pressure in the anulus drops almost linearly with the distance from the catheter tip. Because differential volume flow across any surface is proportional to the differential area normal to the flux and local pressure, this linear pressure relationship predicts that only approximately 4% of the 1.4 μl/minute inflow volume, that is, 0.06 μl/minute, flows radially across the 2 mm-long segment of the anular surface most distant from the tip. Results of Peclet analysis<sup>42</sup> indicate that this flow is too low to support significant convection in the tissue, and thus these radial concentration profiles (in cylindrical coordinates with the z axis coincident with the catheter axis) should be closely described in steady state by solution of the diffusion-permeation-reaction equation in cylindrical coordinates:  $0 = \nabla^2 c - kc/(\phi D_e)$ , where k is the permeation-reaction rate,  $\phi$  is the extracellular vol-

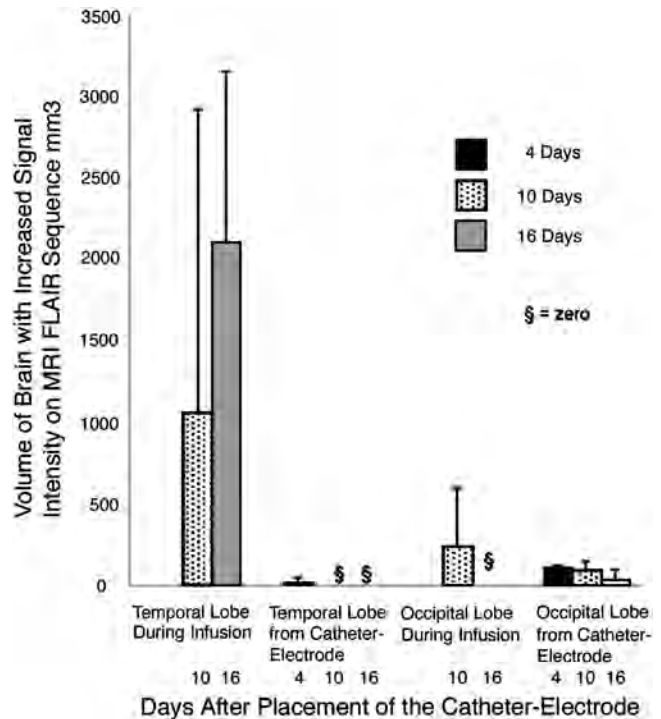


FIG. 2. Bar graph depicting the results of FLAIR MR images used to evaluate the effects of the catheter electrode and infusion on brain volume containing an increased water signal. The volume of brain affected by infusion in the temporal lobe far exceeded the volume affected by placement of the catheter electrode ( $p = 0.001$ , Day 10;  $p = 0.001$ , Day 16; analysis of variance). The infusion groups do not include a measurement on Day 4 because infusions began 5 days after the catheter electrode was implanted. The § symbol indicates that the water signal was not increased in that brain region during the indicated period.

ume fraction, and  $D_e$  is the extracellular diffusion constant. With the radial concentration at the AP axial position z denoted by  $c(r,z)$  and the concentration at the outer radius of the catheter presented as  $c(\zeta,z)$ , the solution to this equation is

$$c(r,z) = c(\zeta,z)[K_0(\alpha r)/K_0(\alpha \zeta)] \quad \text{eq. 1}$$

where  $K_0$  is a modified Bessel function of the second kind, and  $\alpha$  is a parameter equal to

$$\sqrt{k/(\phi D_e)} \quad \text{eq. 2}$$

Reexpressed as a relative concentration,  $\hat{c}(r,z)$ , in which the concentration of each radial profile is referenced to its value at a particular r value,  $r^*$ , the solution becomes

$$\hat{c}(r,z) = K_0(\alpha r)/K_0(\alpha r^*) \quad \text{eq. 2}$$

Accordingly, the three observed radial concentration profiles at AP positions -15, -16.5, and -18 mm (Fig. 7) were all referenced to their values at  $r = 1$  mm (the location of the first experimental radial measurement) and fit by Equation 2, with  $\phi$  and  $D_e$  held constant to yield a permeation-reaction rate constant of  $k = (9 \pm 0.1) \times 10^{-4}$  minute<sup>-1</sup> (Fig. 8). The data were well fit by the cylindrical model ( $R^2 = 0.989$ ), supporting the presence of a tapered cylindrical anulus serving as a fluid and muscimol source along the axis of the catheter and confirming diffusion-

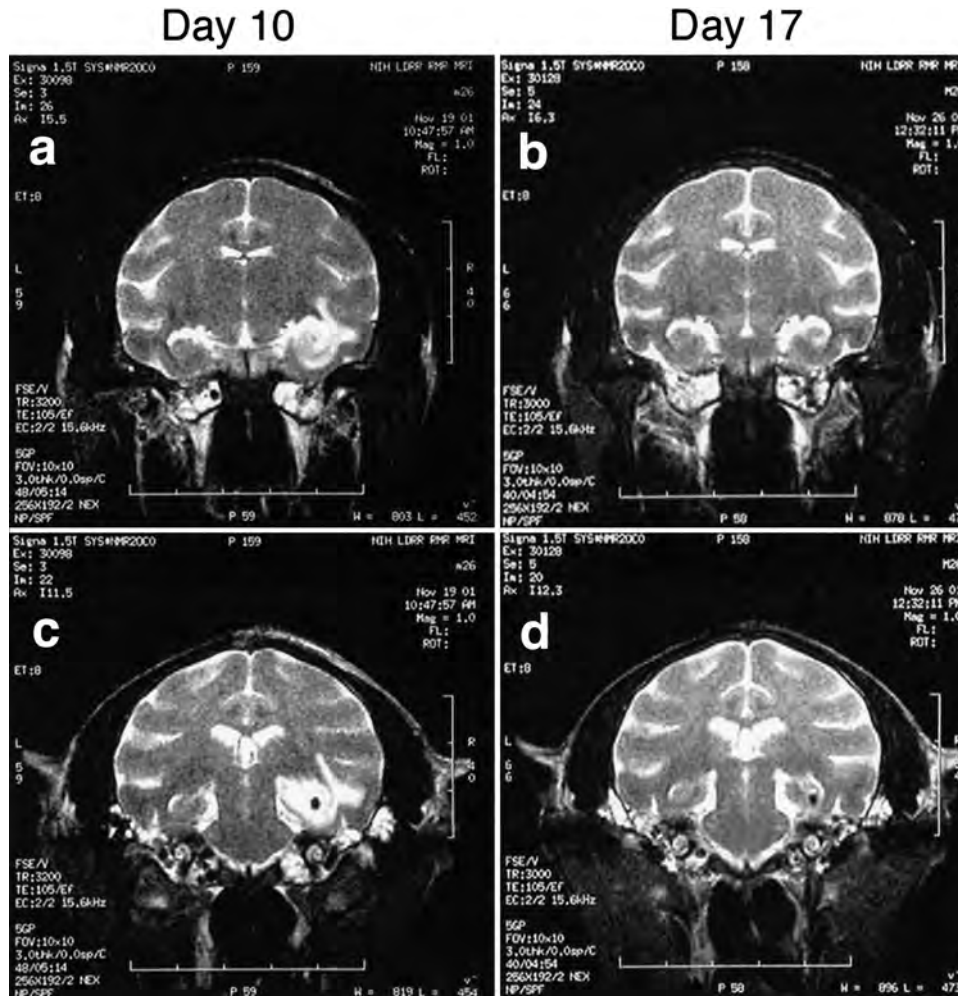


FIG. 3. Animal 4. Coronal T<sub>2</sub>-weighted MR images of the brain in an animal whose infusion was stopped 16 days after surgery. During infusion (a and c), the water signal produced by infusion of the temporal lobe parenchyma was increased. By 24 hours after the end of the infusion (b and d) the brain appears normal and the increase in the water signal has resolved completely.

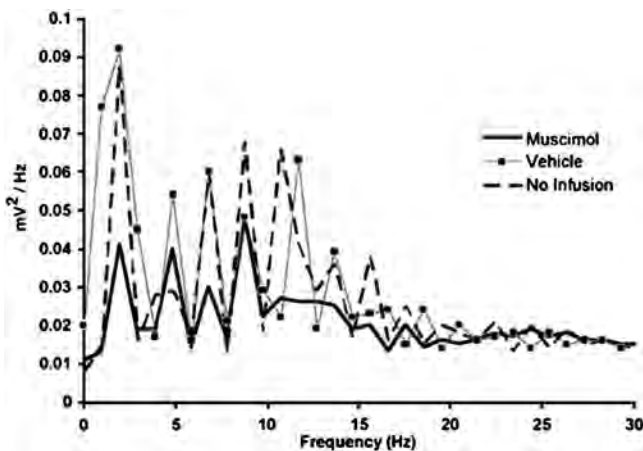


FIG. 4. Graph depicting the power spectral EEG recordings obtained on different nights during infusion of muscimol, during infusion of vehicle, or before infusion. Muscimol reduces EEG power compared with the power with either vehicle or no infusion.

dominated radial transport from the part of the anulus farthest from the catheter tip. The progressively smaller difference between the measured concentrations at  $r = 1$  mm and  $r = 2$  mm as the tip location is approached from the posterior side (Fig. 7) indicates a shoulder on the radial concentration profile and the presence of a convective component, as would be expected from the higher anulus pressure near the tip. Model prediction may also be compared to experimental findings on the anterior side of the catheter tip. Muscimol distribution in this region is consistent with fluid flow across a small hemispheric surface capping the end of the catheter. As above, local pressure and area normal to the flux can be used to estimate that only approximately 7% ( $0.1 \mu\text{l}/\text{minute}$ ) of the total  $1.4 \mu\text{l}/\text{minute}$  inflow volume crosses this hemispheric surface. The concentration profile in this region may thus be predicted by the steady state solution of the convection-diffusion-permeation-reaction equation in spherical coordinates ( $0 = \phi D_c \nabla^2 c - \nabla \times v c - k c$ , where  $v(r)$  is the radial fluid velocity) coupled with an effective  $0.1 \mu\text{l}/\text{minute}$  hemispheric flow rate, and the  $\phi D_c$  and optimized  $k$  values previously used in the analysis of the

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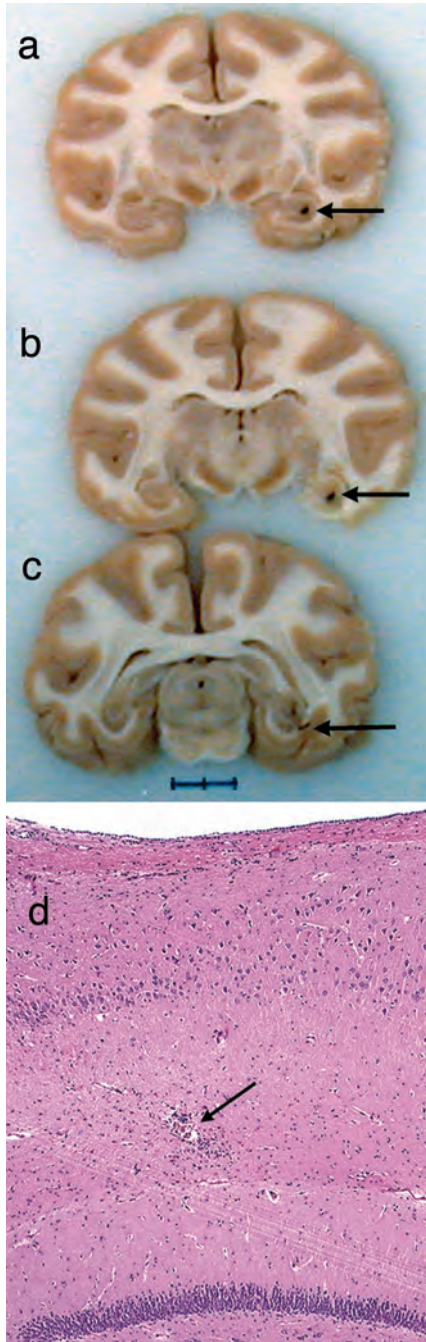


FIG. 5. Photographs of brain sections demonstrating where the infusion catheter (arrows) had been left in place, passing to the hippocampus (a and b) from the posterior temporal lobe white matter (c). Photomicrograph (d) of the region where the infusion catheter had been removed demonstrating a narrow zone of tissue necrosis and gliosis around the tip of the catheter electrode (arrow). Identical changes were observed in animals without muscimol infusion. H & E, original magnification  $\times 10$  (d).

distal axial region. Numerical solution yielded the radial concentration profile exhibited as the line in Fig. 7. The computed radial values (expressed in a spherical coordinate system centered on the catheter tip) may be compared with the experimental values measured in each transverse plane

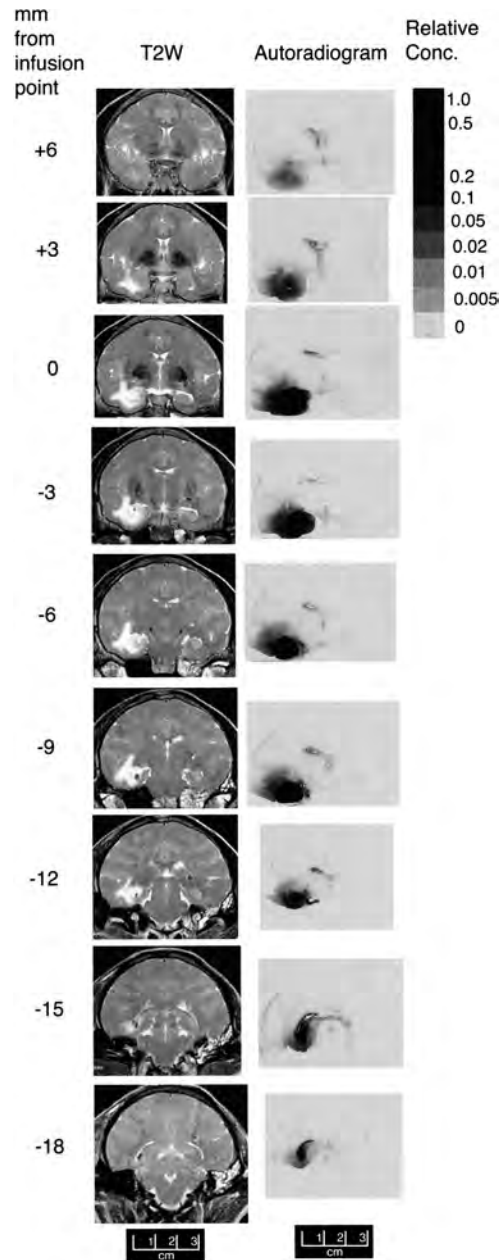


FIG. 6. Several T<sub>2</sub>-weighted MR images and brain autoradiographs comparing coronal tissue sections. The part of the temporal lobe with increased fluid signal (white area) on the T<sub>2</sub>-weighted MR images corresponds to the part with the highest concentration of muscimol (dark gray area) on the autoradiographs.

along the anterior extension of the catheter axis, here approximated by each of the reported values at  $r = 1$ . The predicted line is consistent with these experimental points and accurately defines the leading edge of the anterior spread. The computed profile slightly underestimates the ( $r = 1$ ) concentrations near the catheter tip and overestimates concentrations at the AP positions 5 and 6, observations suggesting respectively that the effective volumetric rate of inflow into this region might be slightly more than  $0.1 \mu\text{l}/\text{minute}$  and that the actual permeation rate  $k$  lies toward the upper bound value yielded by our posterior axial analysis.

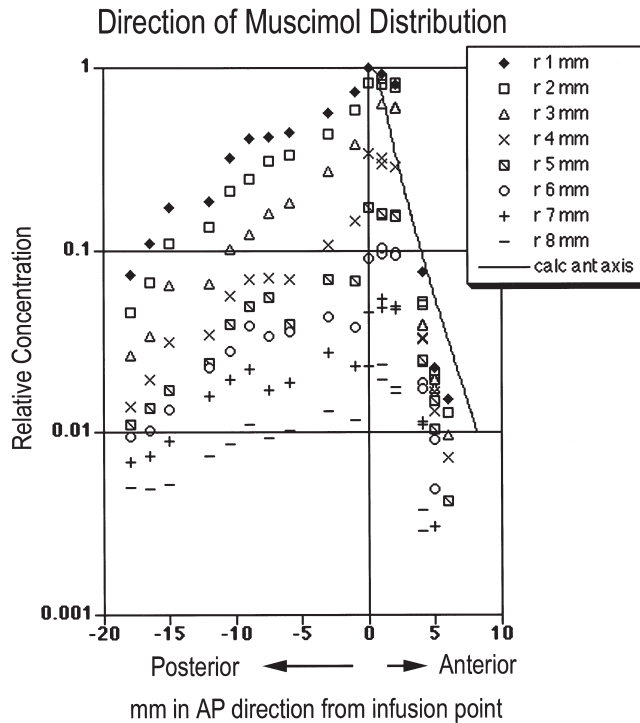


FIG. 7. Graph depicting the distribution of muscimol around the catheter (discrete data points). Concentration is expressed relative to its tissue value immediately adjacent to the tip and shown as a function of the AP distance from the catheter tip. The  $r$  values refer to radii within a given transverse plane. The *solid curve* is the radial concentration profile computed from the convection-diffusion-permeation-reaction equation in spherical coordinates (with an effective hemispheric inflow rate of  $0.1 \mu\text{l}/\text{minute}$ ) and applied to the region anterior to the catheter tip. It corresponds to concentration data points lying along the anterior extension of the catheter axis and approximates the experimental values at  $r = 1 \text{ mm}$ . Calc ant = calculated anterior.

If applied to an infusate concentration of  $0.125 \text{ mM}$ , the anterior profile is consistent with a drug concentration that decreases to the minimum effective concentration of  $150 \text{ nM}$  at approximately  $10.5 \text{ mm}$ . A decrease in measured concentrations (at a given  $r$ ) with an increasing posterior distance from the catheter tip is behavior consistent with diffusional extraction of an agent from fluid moving through an anulus.<sup>4,7</sup>

### Discussion

Convection-enhanced delivery of muscimol resulted in high concentrations of the agent within the entire ipsilateral hippocampus and medial temporal lobe. The sylvian and choroidal fissures acted as barriers to the direct intraparenchymal spread of muscimol from the temporal lobe to the adjacent frontal and parietal lobes and brainstem. The infusion contained muscimol at  $14$  to  $114 \mu\text{g}/\text{ml}$  artificial CSF ( $0.125$ – $1 \text{ mM}$ ). This concentration was  $1.4$  to  $11.4\%$  of the concentration ( $8.8 \text{ mM}$ ) previously used in direct injection of small boluses in humans to inhibit activity in the GPI and thalamus.<sup>36,38</sup> Results of this investigation have shown that it is possible to deliver muscimol throughout the hippocampus and amygdala via infusion through a catheter elec-

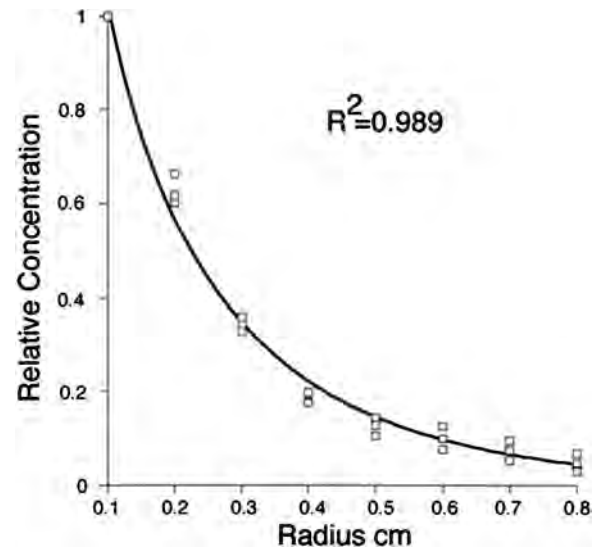


FIG. 8. Graph demonstrating the radial concentration profile of muscimol in transverse sections most posterior to the catheter tip ( $\text{AP} = -18$  to  $-15 \text{ mm}$ ), with concentration expressed relative to its value at  $r = 1 \text{ mm}$  from the catheter (discrete data points). The *solid curve* is the corresponding concentration computed from the diffusion-permeation-reaction equation in cylindrical coordinates and applied to the transverse plane (Equation 2).

trode with appropriate matching of the catheter diameter and volumetric inflow rate ( $1.3 \text{ mm}$  diameter and  $1.4 \mu\text{l}/\text{minute}$  inflow rate in the present study). The associated distribution of the agent is an elongated tapered cylinder or teardrop shape consistent with the establishment of a back-flow anulus around the catheter by elastic expansion of the surrounding tissue (Fig. 7).<sup>32</sup> The increased surface area of this teardrop shape for fluid flux, relative to the small surface of the catheter tip, is associated with lowered hydrodynamic pressure and thus lowered convective velocities across this surface. As a consequence, significant convective delivery of the solute to tissue occurred only within the anulus and  $1$  to  $2 \text{ mm}$  from the catheter tip. At axial distances far from the catheter tip ( $\sim 18 \text{ mm}$ ), diffusion rather than convection equations describe steady-state concentration profiles in planes transverse to the catheter axis. The shape and extent of these concentration profiles are largely determined by the balance between diffusive flux and local permeation loss. The small magnitude of the permeation-reaction rate constant is consistent with low rates of microvascular efflux<sup>39</sup> and with the slow approach to a steady-state concentration, during as many as  $10$  to  $24$  hours depending on the rate of muscimol entry into cells. Data and simulations show that tissue muscimol concentrations decrease more than two orders of magnitude within  $8 \text{ mm}$  of the catheter surface. Because muscimol has an  $\text{IC}_{50}$  of  $30 \text{ nM}$  for the GABA-A receptor *in vitro*<sup>15</sup> and a dissociation constant ( $K_d$ ) of  $5.4$  to  $16 \text{ nM}$ ,<sup>13</sup> a free muscimol tissue concentration greater than  $12$  to  $50 \text{ nM}$  ( $0.01$ – $0.04\%$  of the  $0.125 \text{ mM}$  infusate) should affect GABA-A receptors. Data in our study indicate that these levels can be achieved throughout the ipsilateral hippocampus (Figs. 7 and 8).

During the infusion, the animals were evaluated for changes in neurological function or EEG recordings that

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would indicate suppression of neuronal activity. Higher concentrations (that is, 0.5 and 1 mM) of infused muscimol consistently exceeded the sedation threshold, 0.25 mM sedated one of two animals, but a lower concentration (0.125 mM) was well tolerated. Although facilitated spike discharges have been recorded by other investigators after stopping chronic infusions of GABA into the cerebral cortex of baboons or rats,<sup>6,29</sup> in our study the discontinuation of muscimol infusion reversed sedation and did not result in seizures or epileptiform activity. The total dose of muscimol administered, 28 to 230  $\mu\text{g}$  (3.5–28  $\mu\text{g}/\text{kg}$ ), was far below the oral dose of 5 to 10 mg/day ( $\sim 100 \mu\text{g}/\text{kg}$ ) that was free of systemic toxicity in a previous clinical trial.<sup>43</sup>

Convection-enhanced delivery, a method of infusing pharmacological agents into the extracellular space of the brain, has been used to treat a variety of brain diseases.<sup>5,8,21,22,24,26,32,33,35</sup> Convection utilizes the pressure of a syringe infusion pump transmitted to the catheter tip to move the fluid that contains the therapeutic agent throughout the extracellular space of the brain in the perfused region from which it can exchange with brain cells. This delivery technique was safe when used to deliver an immunotoxin to brain tumors in humans<sup>22</sup> and has been used successfully to deliver *N*-methyl-D-aspartate-receptor excitotoxins to selectively eliminate the neuronal population of the GPI in primates with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.<sup>23,25</sup>

Muscimol has not been used to treat epilepsy in humans, although it has been used clinically as a test injection during stereotactic surgery. Injection of muscimol (2.5  $\mu\text{g}$  in 2.5  $\mu\text{l}$  normal saline, 8.8 mM) into the GPI in a patient with Parkinson disease temporarily relieved rigidity and bradykinesia in the contralateral hand and induced no side effect.<sup>38</sup> Test injections of muscimol into the thalamus transiently relieved essential tremor in six patients and predicted the effect of radiofrequency lesioning or chronic electrical stimulation.<sup>36</sup> A similar strategy for the treatment of complex partial seizures involves the injection of muscimol into the epileptic focus to provide targeted suppression of postsynaptic neurons there and to predict the effect of surgical removal or selective elimination of neurons in this region.<sup>9</sup>

Muscimol has been studied extensively in animal models. It has been used in nonhuman primates to produce transient focal inactivation of brain regions. Injections have ranged from 0.5<sup>47</sup> to at least 30  $\mu\text{g}$ .<sup>20</sup> In rodents, seizure frequency has been suppressed with bilateral infusions of 0.01  $\mu\text{g}$  (88 pmol)<sup>18</sup> in the mouse and 0.06  $\mu\text{g}$  in the rat.<sup>41</sup> Muscimol is rapidly cleared from the blood of rats following intravenous administration of 8  $\mu\text{mol}/\text{kg}$ .<sup>3</sup> After intracerebroventricular injection of 0.8 nmol (5.3 nmol/kg) muscimol, recovery in the brain was 55% at 30 minutes. In the rat brain, intraparenchymal infusion of 1  $\mu\text{l}$  8.8-mM muscimol over 4 minutes resulted in a radius of detectable drug of 1.7 mm after 2 hours.<sup>27</sup> Factors affecting muscimol distribution include muscimol binding to GABA receptors and other structures, cellular uptake, metabolism in the brain, and capillary permeability. At least some of these processes are likely to be saturable. In an *in vitro* experiment with rat brain preparations, muscimol acted as a strong GABA agonist, with an  $\text{IC}_{50}$  of 0.03  $\mu\text{M}$ , which is approximately 10 times the affinity of GABA itself.<sup>15</sup> The concentration used *in vivo* might have to be adjusted to account properly for binding within the brain extracellular space.

Data from studies in animal models of epilepsy have shown that epileptic activity can be suppressed by direct infusion of reversible neurotransmitter agonists or antagonists, calcium channel blockers, and sodium channel blockers.<sup>6,14,19,28,40,41,44</sup> Results of animal studies have identified neuronal populations, receptors, and ion channels that are most important in seizure onset and propagation. Infusion of ibotenic acid, an excitatory amino acid, which selectively affects the *N*-methyl-D-aspartate-receptor, extinguishes epileptiform activity in a rat model of temporal lobe epilepsy, presumably by eliminating a hyperexcitable focus of neurons.<sup>35</sup> A similar strategy may be pursued in humans for the treatment of focal epilepsy, if preliminary clinical research shows that temporary suppression of the epileptic focus can be achieved using muscimol.

Because depth electrodes modified to include a dialysis probe have been left in place 10 to 18 days in clinical studies without causing infection, we anticipate that an externalized system such as we describe could be used for the same period.<sup>11,12</sup> Short-term infusion of muscimol could be used to evaluate the effect of suppressing neuronal activity in the volume of brain in which muscimol is distributed. For example, infusion of the left hippocampus with muscimol could predict the effect of surgery or selective neurotoxin infusion on verbal memory. In addition, side effects arising from brain structures outside the hippocampus could indicate that muscimol had spread beyond the target. If the effects of the reversible agent were limited to the target structure and seizure frequency, a neurotoxin that was specific for excitatory neurons could be infused into the same region through the external system. Because of the risk of infection with prolonged use of a percutaneous foreign body, however, our external infusion system would be inappropriate for therapy lasting more than a few weeks. To evaluate the safety of chronic infusion of muscimol into the hippocampus would require another animal study using a totally implanted system, which is less likely to become infected than an externalized one.

## Conclusions

To provide a basis in humans for using regional therapy to treat medically intractable epilepsy, we performed a pilot study of muscimol microinfusion into the hippocampus of rhesus monkeys. Muscimol is a GABA receptor-specific agent that can suppress the epileptic focus. Convection-enhanced delivery of muscimol into the brain of a nonhuman primate was safe, allowing high drug levels in the infused hippocampus and low levels in the rest of the brain. The data obtained support clinical studies of short-term muscimol infusion into the seizure focus to predict the effect of ablative surgery or neurotoxin infusion on seizure control and neurological function. The data obtained support clinical studies of short-term muscimol infusion into the seizure focus to predict the effect of ablative surgery or neurotoxin infusion on seizure control and neurological function. It will be necessary to conduct an animal study using a totally implantable system to determine the potential of chronic infusion of muscimol for the treatment of medically intractable epilepsy in patients who are poor candidates for standard surgery because their epileptic focus resides within, or adjacent to, functionally eloquent cortex.

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