

CAVERNOUS SINUS TUMOR MODEL IN THE CANINE: A SIMULATION MODEL FOR CAVERNOUS SINUS TUMOR SURGERY

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OBJECTIVE: The recent limitations of working hours for neurosurgical trainees carry the risk of decreasing the amount of microsurgical experience. In the absence of enough surgical exposure to some pathological states, an alternative option of a more continuous source of tactile and visual experience that simulates the real-life state is needed. To help with this problem, we established a cavernous sinus tumor model in the canine.

METHODS: A gliosarcoma cell line that was harvested from a tumor model in nude mice was implanted in six mongrel dogs. In the first group (two dogs), the cell line was implanted in the dural leaflets of the cavernous sinus. (Immunosuppression was used in one dog.) In the second group (four dogs), the cell line was implanted in the region of the gasserian ganglion. (Immunosuppression was used in all four dogs.) The condition of each dog was followed through neurological examinations and serial magnetic resonance imaging. The cavernous sinus region later was explored, after which the dogs were later killed and histopathological evaluations of the cavernous sinus region was carried out.

RESULTS: The initial cell line implanted within the dural leaflets of the cavernous sinus showed no evidence of tumor growth. The tumor grew in all four dogs that had the gliosarcoma cell line implanted in the region of the gasserian ganglion. The clinical and radiological features as well as the experience of the surgical dissection of these tumors simulated cavernous sinus tumors in humans.

CONCLUSION: We established the first cavernous sinus tumor model in the canine. This model simulates the real-life pathological state, and it can be used as an alternative source of surgical experience to advance surgical skills.

KEY WORDS: Cavernous sinus tumor, Cranial base tumor, Neurosurgical training

The recent introduction of regulations regarding limited working hours for neurosurgical trainees carries the risk of compromising the amount of microsurgical training achieved during the period of residency and/or fellowship. This becomes more relevant in pathological entities that are of higher complexity and/or lesions that are not encountered on routine weekly basis. Tumors involving the cranial base, especially the cavernous sinus, are such an example (1, 3, 4, 12). The anatomic complexity of the cranial base is now better defined and understood (5, 6). This anatomy, however, is significantly changed in the presence of pathological processes.

In the absence of enough surgical exposure to the pathological state, an alternative option of a more continuous source of tactile and visual experience that simulates the real-life state is needed. Although there are ongoing attempts at achieving this goal using computer technology simulation, when it comes to neurosurgery, this field is still in its infancy.

To help with the above-mentioned problems, we established a cavernous sinus tumor model in the canine. This model is to be used for both the better understanding of the pathological state and as an alternative source for surgical skills. To our knowledge, this is the first time a model of a cranial base tumor is reported.

MATERIALS AND METHODS

The following protocols met all the guidelines of and were approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences.

Growth of Tumor Cells in the Nude Mouse

Canine gliosarcoma cells, which had been induced chemically in a dog, were obtained from Dr. John Hilton (Johns Hopkins Oncology Center). The frozen tumor stock solution was thawed rapidly before its use, and 50 μ l of the stock solution (approximately 2.4×10^5 cells/ μ l) was injected subcutaneously in each flank of an adult nude mouse (nu/nu, 4–6 wk, 20–25 g; Harlan Sprague-Dawley, Inc., Indianapolis, IN). A drop of the tumor cell suspension was plated with antibiotic free media and incubated overnight at 37°C with 5% CO₂ to test for the presence of bacterial contamination.

Within 1 week, a tumor mass was visible in the skin. The tumor was allowed to grow for an additional 2 to 3 weeks and then was harvested at the time of surgical implantation into the canine. Before tumors were transplanted into the dog, the nude mouse was anesthetized with 0.05 mg/g of sodium pentobarbital and kept alive while the tumor was harvested and implanted into the canine. This was carried out to ensure adequate perfusion of the tumor mass and the survival of the tumor cells. The tumor samples, typically several centimeters in diameter, were minced, and whole pieces were injected with a large-bore needle.

The unused tumor fragments were minced further in media and were spun down at 5000 revolutions per minute for 10 minutes. The supernatant was removed, and the pellet was suspended again in fetal bovine serum and 10% dimethyl sulfoxide. A drop of the tumor cell suspension was plated with antibiotic free media and incubated overnight at 37°C with 5% CO₂ to test for the presence of bacterial contamination. The cell suspension was made into aliquots, slowly frozen overnight, and then stored in a liquid nitrogen tank. These cells later were implanted into nude mice and then transplanted into dogs.

Transplantation of Tumor Cells into the Canine Cavernous Sinus

Six adult female mongrel dogs (Martin Creek Kennels, Wiliford, AR) weighing approximately 27 to 31 kg were used in this study. Five had their immune systems suppressed; one did not. For immunosuppression, the dogs were treated for 1 to 4 weeks with cyclosporine (100 mg orally twice daily), azathioprine (50 mg orally twice daily), and prednisone (10 mg orally twice daily) mixed into their food. (This regimen was previously used in an intraparenchymal tumor model in canines [14].) The dogs were kept on the immunosuppressive regimen for the duration of the study.

The dogs were anesthetized with intravenous injections of 22 mg/kg of tiletamine and zolazepam (Telazol; Parke-Davis, Morris Plains, NJ). They were then intubated and placed on a

ventilator. Anesthesia was maintained during surgery with isoflurane at concentrations ranging from 0.5 to 3%, depending on the vital signs, which were monitored throughout the procedure. The scalp was shaved, scrubbed, and draped in a sterile manner and a scalp incision was made over the frontoparietal region. The temporalis muscle was split with cautery and retracted. The underlying cranium was exposed, and a temporal craniectomy, which was 1.5 to 2 cm in diameter, was performed (Fig. 1). The base of the cranium was drilled in the subtemporal region to provide better access to the cavernous sinus. The dural layer was dissected from the base of the skull to the lateral wall of the cavernous sinus. There, the dural leaflets forming the lateral wall of the cavernous sinus was split from the dura propria covering the temporal lobe and exposing the gasserian ganglion in the posterior cavernous sinus wall. The tumor was harvested from the nude mouse host and immediately was transplanted into the dog.

In the initial phase of the study, the tumors were implanted in the dural leaflet forming the roof of the cavernous sinus, which was split. This method was used in two dogs, one whose immune system was suppressed and one whose immune system was normal. No evidence of tumor growth appeared in either dog, which was confirmed by magnetic resonance imaging (MRI) and histopathological studies.

The remaining four dogs were immunosuppressed, and the tumor specimens were implanted into the region of the gasserian ganglion. After the tumor was harvested from the mice, small pieces were minced and then injected with a large-bore needle into the region of the gasserian ganglion. The overlying muscle and skin were then sutured in routine fashion.

White blood cell counts were measured routinely in the animals to detect occult infection or signs of sepsis from the

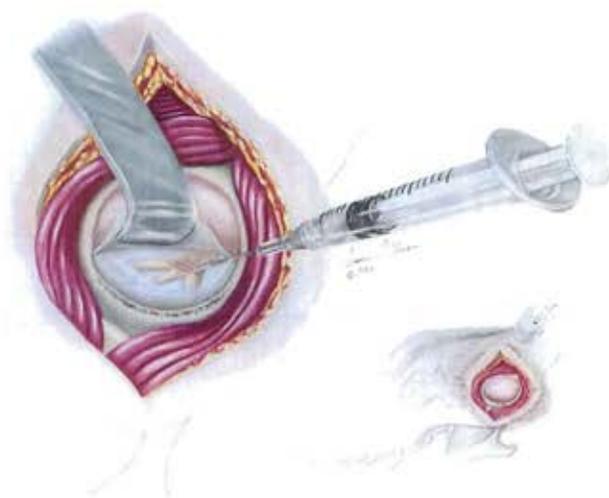


FIGURE 1. Lower right, illustration of the craniectomy site (the skin incision, the split temporalis muscle, and a low temporal craniectomy). Left and upper right, illustration of the temporal lobe retracted upward with the outer dural leaflet of the roof of the cavernous sinus; the tumor cells are injected into the region of the gasserian ganglion.

immunosuppressive regimen. Moderate elevations of the number of white blood cells were attributed to the steroid therapy. Superficial cellulitis at the surgical site occurred in two dogs and was treated adequately with intramuscular antibiotics.

The procedure used in the third dog was repeated in the remaining three dogs. The growth of the tumor was predictable and to the same extent.

Findings on MRI

MRI scans were carried out as soon as neurological symptoms developed in a dog or just before sacrifice if no neurological symptoms developed (Days 41 and 52 for Dogs 1 and 2). These images were made with a 1.5 Tesla General Electric machine (General Electric, Milwaukee, WI). The dogs were sedated with intravenous injections of 22 mg/kg of tiletamine and zolazepam (Telazol), which was repeated every 15 to 20 minutes as needed. When the animals were fully sedated, they were laid prone and MRI scans (3–5 mm thickness) of the head were obtained with and without contrast enhancement with gadolinium-diethylenetriamine penta-acetic acid. Approximately 5 ml of contrast material was used for each dog.

Harvesting and Histopathological Studies of Transplanted Tumors

When neurological deficits developed in the dogs (usually cranial neuropathy), the tumors were large enough to fill the cavernous sinus. After obtaining the MRI images and confirming the presence of tumor growth, the dogs were anesthetized for exploration and dissection, and they then were killed with intravenous injections of 0.22 ml/kg sodium pentobarbital (6 g/ml).

The previous incision was reopened, and the bone flap from the first procedure was removed. The tumor site was inspected, and the intracavernous neural and vascular structures were dissected carefully using microsurgical techniques that are routinely applied to the region of the cavernous sinus. Specimens were harvested, fixed in paraffin, embedded, and sectioned at 5 μ m. The paraffin was removed from the slides, and the specimens were stained with routine hematoxylin and eosin for histopathological studies.

RESULTS

MRI

The immune system in the first dog was not suppressed, and the tumor cells were implanted in the dural leaflets of the cavernous sinus. No evidence of tumor growth appeared at either the time the MRI scan was performed or at the time of sacrifice (6 wk after implantation). The immune system of the second dog was suppressed, and the tumor cells were implanted in the same anatomic location, but no evidence of tumor growth appeared on either the MRI scan or after sacrifice (>7 wk after implantation). The immune system of the third dog was suppressed for 8 weeks before the tumor cells

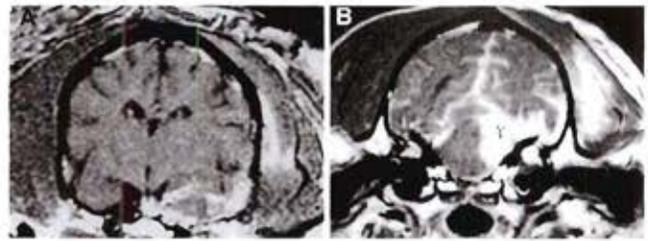


FIGURE 2. Coronal enhanced MRI scans of (A) Dog 3 showing the tumor (*) filling the left cavernous sinus and pushing the temporal lobe superiorly and (B) Dog 4 with tumor extending back to the level of the brainstem.

were implanted. Twelve days after implantation, neurological deficits developed in the dog, including gait difficulty and extraocular dysfunction. MRI scans taken 2 days later showed the tumor filling the cavernous sinus (Fig. 2). The dog was killed 1 day later; histopathological studies confirmed the presence of the gliosarcoma (Fig. 3). The immune system of the fourth, fifth, and sixth dogs were suppressed for only 12 days before implantation. Neurological deficits occurred at approximately the third week after implantation in the form of abnormal extraocular movement. Two days after the occurrence of symptoms, MRI scans of the head were obtained and revealed the tumor filling the cavernous sinus region (Fig. 2). The dogs were explored and the cavernous sinus region was dissected using microsurgical techniques normally used in the resection of human cavernous sinus lesions. The tumor spread and its dissection was very similar in its visual and tactile experience to human benign cavernous sinus tumors, such as meningiomas, and malignant cavernous sinus tumors, such as adenoid cystic carcinomas (Fig. 4). In two of the dogs, the tumor spread extended posteriorly through the region of Meckel's cave and had to be dissected off the brainstem.

Histopathological Studies

Hematoxylin and eosin stains of the tumor specimens harvested from the cavernous sinus showed the tumor invading the overlying tentorial dura (Fig. 3). The tumor was hypercel-

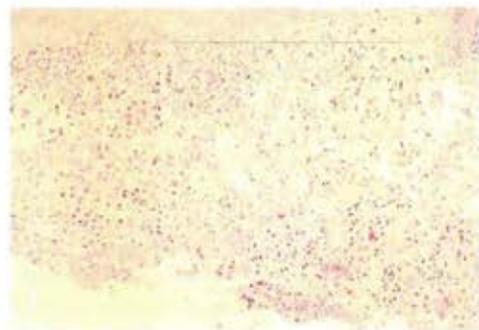


FIGURE 3. Light microscopic examination of the harvested tumor showing gliosarcoma cells with hypercellularity, extremely pleomorphic nuclei, and marked atypia (hematoxylin and eosin).

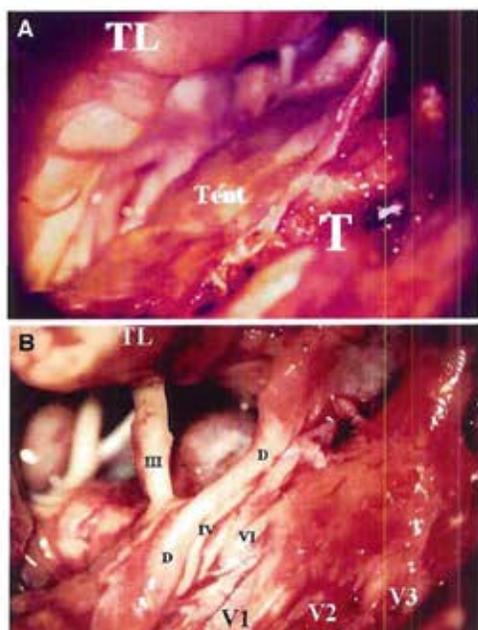


FIGURE 4. Intraoperative photos taken during tumor harvest in Dog 4. A, the temporal lobe (TL) elevated and the tumor (T) filling the cavernous sinus and elevating the overlying tentorial dura (Tent). B, the cavernous sinus after the tumor has been harvested. All anatomic structures have been preserved. III, oculomotor nerve; IV, trochlear nerve; VI, abducens nerve; V1, ophthalmic branch; V2, maxillary branch; V3, mandibular branch of the trigeminal nerve; TL, temporal lobe; D, dura of the roof of the cavernous sinus.

lular, with marked pleomorphic cells consistent with a gliosarcoma.

DISCUSSION

Reproducible tumor models in different species of animals have been available for many years and are used currently and frequently in tumor research. The earliest reproducible animal models of intracranial neoplasms were made to promote a better understanding of the behavior of tumors and the pathophysiological changes caused by the tumors. The use of *in vivo* tumor models also can help to develop new therapeutic methods that later can be used in clinical trials to treat similar tumors in humans (7).

Intracranial tumor models were first developed in rodents through either chemical induction or direct viral inoculation. For example, nitrosourea and the avian sarcoma virus have been used successfully to induce brain tumors in rats (2, 13). But intracranial tumor models in large animals are necessary because of the advantages of studying tumors and their pathological effects using the radiological studies routinely used in clinical practice. Rabotti et al. (9, 10) were among the first to induce brain tumors in rabbits and dogs using the Rous sarcoma virus. Early attempts to transplant tumors into the

brains of large animals such as dogs, rabbits, and guinea pigs were reported in 1967 by O'Malley and O'Doherty (8). These investigators were unsuccessful in transplanting all homologous and heterologous tumors, except for an infectious venereal lymphosarcoma implanted into a dog's brain. This implantation was a subdural placement of solid tumor pieces harvested from another dog with a subcutaneous tumor. Although the tumor did not originate from a nervous system cell line, this successful attempt was significant for the later development of brain tumor models.

In 1982, Salzman et al. (11) modified the transplantable canine glioma model developed previously. They successfully passed brei from the original tumor from dog to dog, crossing over different strains, until an intracerebral injection in a weanling successfully and reproducibly induced fatal brain tumors that had similar features to human glioblastoma. The refinement of this model allowed subsequent collection of data on the pathophysiology of brain tumors in large animals. These data eventually were translated into clinical trials. Whelan et al. (14) used a canine glioma model to study the differences in computed tomographic and MRI scans of brain tumors and the potential use of light therapy to treat posterior fossa tumors. These studies were performed with intracerebral injections of canine glioma cells. But no attempts to develop and study cranial base tumor models have been reported.

Cranial Base Tumor Model

A large variety of benign and malignant brain tumors frequently involve the cranial base. These lesions previously were considered impossible to operate on and difficult to treat. Their outcome used to be associated with a high mortality and/or significant morbidity. The recent improvements in cranial base surgical techniques have had a positive impact on the outcome of patients with these tumors, especially those with benign lesions (1, 3, 4, 12). These results were achieved by the hands of few and are not easy to replicate. The trend of decreasing working hours is going to make it very difficult for neurosurgical trainees even to be present while the surgery progresses. This is why finding alternatives to the classic sources of surgical experience becomes important. Recent efforts to establish computer-generated simulations are still faced with a significant challenge of replicating the tactile and visual experience of the real-life state. Our model was able to replicate such an experience. Its value was best proven in establishing a dissection plan that will help to identify the course of the intracavernous cranial nerves and the internal carotid artery before tumor resection is started. This can help trainees in their microdissection plan, which will improve the safety of the procedure. This model also was helpful in learning techniques to control cavernous sinus bleeding with minimal blood loss. The dissections of the tumor off the intracavernous cranial nerves, without injury, was a very valuable exercise (Fig. 4).

CONCLUSION

To our knowledge, this is the first reported cranial base and cavernous sinus tumor model. This model simulates the real-life pathological state. It can be used as an alternative source of surgical experience to advance surgical skills. In addition to its potential use for studying different treatments, this model has significant potential for studies that can help surgeons to understand better the pathophysiological and hemodynamic changes occurring in the cavernous sinus when it is invaded by a tumor.

REFERENCES

1. Al-Mefty O: *Surgery of the Cranial Base*. Boston, Kluwer Academic Publishers, 1989.
2. Copeland DD, Vogel FS, Bigner DD: The induction of intracranial neoplasms by the inoculation of avian sarcoma virus in perinatal and adult rats. *J Neuropathol Exp Neurol* 34:340-358, 1975.
3. Dolenc VV: Direct microsurgical repair of intracavernous lesions. *J Neurosurg* 58:824-831, 1983.
4. Dolenc VV, Kregar T, Ferluga M, Fettich M, Morina A: Treatment of tumors invading the cavernous sinus, in Dolenc VV (ed): *The Cavernous Sinus*. New York, Springer-Verlag, 1987, pp 377-391.
5. Harris FS, Rhoton AL Jr: Anatomy of the cavernous sinus. *J Neurosurg* 45:169-180, 1976.
6. Krishit A, Barnett DW, Barrow DL, Bonner G: The blood supply of the intracavernous cranial nerves: An anatomic study. *Neurosurgery* 34:275-279, 1994.
7. Merker PC, Wodinsky I, Geran RI: Review of selected experimental brain tumor models used in chemotherapy experiments. *Cancer Chemother Rep* 59:729-736, 1975.
8. O'Malley WE, O'Doherty D: Growth of a transplanted tumor in the dog's brain. *Trans Am Neurol Assoc* 92:277-278, 1967.
9. Rabotti GF, Grove AS Jr, Sellers RL, Anderson WR: Induction of multiple brain tumours (gliomata and leptomeningeal sarcomata) in dogs by Rous sarcoma virus. *Nature* 209:884-886, 1966.
10. Rabotti GF, Sellers RL, Anderson WA: Leptomeningeal sarcomata and gliomata induced in rabbits by Rous sarcoma virus. *Nature* 209:524-526, 1966.
11. Sakman M, Scott EW, Schepp RS, Krupp HC, Broadwell RD: Transplantable canine glioma model for use in experimental neuro-oncology. *Neurosurgery* 11:372-381, 1982.
12. Sekhar LN, Jannetta PJ: Petroclival and medial tentorial meningiomas, in Sekhar LN, Schramm VL Jr (eds): *Tumors of the Cranial Base: Diagnosis and Treatment*. Mount Kisco, Futura, 1987.
13. Swenberg JA, Koestner A, Wechsler W: The induction of tumors of the nervous system in rats with intravenous methylnitrosourea (MNU). *J Neuropathol Exp Neurol* 30:122, 1971.
14. Whelan HT, Clanton JA, Wilson RE, Tulipan NB: Comparison of CT and MRI brain tumor imaging using a canine glioma model. *Pediatr Neurol* 4:279-283, 1988.
15. Whelan HT, Schmidt MH, Segura AD, McAuliffe TL, Bajic DM, Murray KJ, Moulder JE, Strother DR, Thomas JP, Meyer GA: The role of photodynamic therapy in posterior fossa brain tumors: A preclinical study in a canine glioma model. *J Neurosurg* 79:562-568, 1993.

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topathological studies. This study was supported by Grant R118922108 from the National Science Foundation.

COMMENTS

Krishit et al. report a canine tumor model for cavernous sinus surgery. A gliosarcoma cell line was grown in nude mice and harvested for implantation in mongrel canines. The cavernous sinus was accessed via a temporal craniectomy, and minced fresh tumor samples were implanted within the dural leaflet forming the roof of the cavernous sinus or into the region of the gasserian ganglion. Only immunosuppressed dogs with implantation in the gasserian ganglion demonstrated tumor growth. After symptoms developed, a magnetic resonance imaging (MRI) scan was obtained, and the animals were surgically explored by use of microsurgical techniques for the preservation of neurovascular structures. The animals were subsequently killed.

Radiosurgery has become the first-line therapy for many lesions of the cavernous sinus. When undertaken, microsurgery of the cavernous sinus remains technically challenging. Before attempting such surgery, surgeons will benefit from time spent practicing in the cadaver laboratory and using virtual reality simulations. However, these methods lack the added challenges of pathological distortion of normal anatomy and bleeding. The authors have created a canine model that addresses these deficiencies. The main drawback of this model seems to be the resource requirement. Furthermore, a more elegant method of tumor implantation might be percutaneous injection into the region of the gasserian ganglion via the foramen ovale, providing a more virgin territory for subsequent exploration.

Pankaj Gore
Robert F. Spetzler
Phoenix, Arizona

The authors developed a cavernous sinus tumor model in the dog for a better understanding of the pathological state of skull base tumors and as an alternative source of tactile and visual experience for training of surgical skills. They succeeded using injections of whole pieces of canine gliosarcoma tumors into the gasserian ganglion of immunocompromised dogs after the tumors had been grown subcutaneously and harvested from nude mice. The model seems to be effective and reproducible, and thus, other centers may use and profit from this model. It is highly desirable to have an animal model to train such complex surgical skills. We believe that this model has significant potential for studying tumor biology at the skull base. Its use as a source of surgical experience to advance surgical skills might be of benefit for all neurosurgeons or neurosurgical trainees, independent of their working hours.

Volker Seifert
Jürgen Beck
Frankfurt, Germany

Until now, there have been no skull base tumor models in large animals. Krisht et al. describe a new canine tumor model involving the implantation of a gliosarcoma into the gasserian ganglion or dural leaves of the cavernous sinus. The tumor cells were chemically induced in a dog and propagated in nude mice. After implantation in dogs, the tumor grew with many of the features of an invasive tumor and could be imaged with MRI. It will be interesting to see practical applications of this model, including microsurgical training or possible therapeutic investigations such as radiosurgery.

Jeffrey N. Bruce
New York, New York

The authors describe the development of a model designed to mimic intracavernous tumor surgery in humans. The model was developed in dogs such that the size of the tumors and the size of the vessels can simulate what is experienced in human neurosurgical operations. In one set of animals, gliosarcoma cell lines were injected into the leaflets of the dura, and none of the animals produced cavernous sinus or dural tumors. In a second group of animals, the cell line was implanted in the gasserian ganglion, and immunosuppression was used. The animals were followed up neurologically and with MRI studies. The second group of animals all developed tumors in the cavernous sinus region. The authors state that

subsequent dissection of the tumors was similar to that of human tumors.

The premise of the model is that reduction in neurosurgical training hours offers a lower exposure of neurosurgical residents to cavernous sinus tumor surgery. This seems to be a fairly costly model to develop, and one wonders about the actual justification. The indications for invasive cavernous sinus tumor surgery remain controversial. This model could equally be used to examine the chemotherapeutic or endovascular approaches to intracavernous tumors. In addition, radiosurgery could be studied in more detail with this model. In most centers around the United States, combined-modality therapy is used to treat benign tumors of the cavernous sinus. The issue of treating malignant tumors aggressively remains controversial.

The documentation of this model is impressive, including the MRI findings and the intraoperative demonstrations of the tumors themselves. The model does seem to emulate cavernous sinus surgery and gives the surgeon a feel for working around cranial nerves and vascular structures in the cavernous sinus. The final question not addressed in the article is whether or not neurosurgical residents have actually used this model for dissection and how useful this exercise was.

Christopher S. Ogilvy
Boston, Massachusetts

Stem cell-derived dopamine neurons 3 months after transplantation into the brain of a parkinsonian animal. (Courtesy of Lorenz Studer.)

