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Leksell gamma knife lesioning of the rat hippocampus: the relationship between radiation dose and functional and structural damage

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Object. The goals of the study were to determine at what dosage and after what interval impairment of hippocampal function occurs after Leksell gamma knife radiosurgery (GKS) of the rat hippocampus and to assess the associated structural changes.

Methods. Long–Evans rats were irradiated with maximum doses of 25, 50, 75, 100, and 150 Gy, and four 4-mm isocenters were used to cover the hippocampus bilaterally. The impairment of hippocampal function, which is associated with a loss of memory, was measured by testing the impairment of the rats' orientation in a Morris water maze. Changes in the irradiated tissue were measured using magnetic resonance imaging (Bruker 4.7/20 experimental spectrometer). The data were compared with histologically demonstrated changes.

Significantly higher incidences of edema, necrosis, and behavioral changes were observed following administration of doses higher than 50 Gy. No edema, necrosis, or behavioral changes were observed when doses were 25 Gy.

Conclusions. It would seem that rats can be used for experiments involving the induction of complex brain lesions by using four 4-mm isocenters. Testing retention memory for behavioral changes after bilateral GKS of the whole hippocampus proved insensitive; acquisition memory should be tested to assess functional changes of hippocampus. Significantly higher incidences of edema, necrosis, and behavioral changes were observed for doses higher than 50 Gy. There seems to be a therapeutic window during which doses may affect epilepsy without impairing the memory of the rat.

KEY WORDS • radiosurgery • gamma knife • rat • memory • radiation necrosis • edema

DESPITE limited experience with this procedure, radiosurgery for epilepsy represents a promising treatment.[1,3,8,20,21] An experimental basis for a rational application of this method, however, must be established in detail. Previous experimental radiosurgical studies in animals have been focused on histopathological changes of normal or neoplastic tissue.[2,10–12,14] Experimental studies on the anticonvulsive effect of radiosurgery applied in a rat epilepsy model, however, have been published during the last 2 years.[6,13,15] Radiosurgery performed using subnecrotic doses has been shown to control epilepsy in a rat model without causing subsequent behavioral impairment.[13] Only unilateral lesioning of the dorsal hippocampus has been studied. The aim of the present study was to clarify at what dosage level and after what latency interval impairment of hippocampal function occurs following radiosurgery of the rat hippocampus. The dose was delivered using the Leksell gamma knife, and associated structural changes were also studied.

Abbreviations used in this paper: GKS = gamma knife radiosurgery; MR = magnetic resonance.

Materials and Methods

The experiment was performed with the approval of the ethical committee of the Na Homolce Hospital and the Animal Care Committee of the 2nd Medical Faculty of Charles University, Prague.

Radiosurgical Lesioning of the Hippocampus

Bilateral irradiation of the hippocampus was performed in 64 Long–Evans rats. Their age at the time of irradiation was 3 months, and their weight ranged from 320 to 380 g. Intraperitoneal thiopenthal (40 mg/kg) was used for anesthesia. Rats were fixed to a specially designed MR imaging–compatible stereotactic apparatus (Fig. 1). Stereotactic MR images were then obtained using a Siemens Expert 1-tesla imager in which a T_1-weighted three-dimensional mode was used. The reconstructed slice thickness was 0.8 mm and the matrix was 154 × 256. The images were exported to the GammaPlan treatment planning workstation, and radiosurgical lesions were made.

A pilot group of six rats underwent bilateral irradiation. The dose was delivered to the region of the dorsal hippocampus alone. Two 4-mm collimator isocenters were used. The six rats were divided into two groups of three, which received a maximum dose of 100 and 150 Gy, respectively. In all the remaining rats the entire hippocampus was covered bilaterally with the 70% isodose by using four 4-mm isocenters.

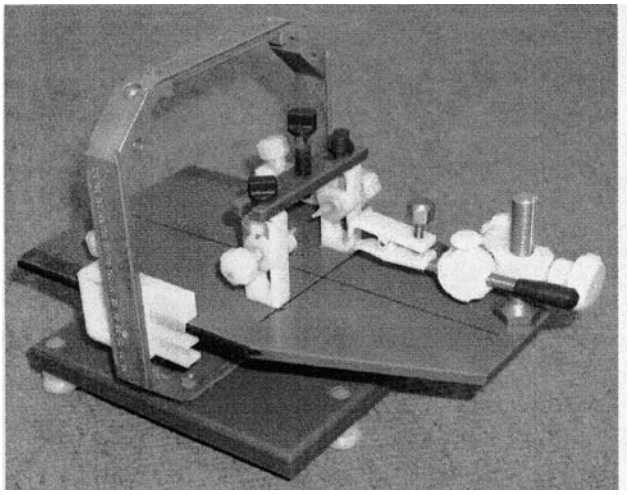

FIG. 1. Photograph showing the stereotactic MR imaging–compatible apparatus for rat fixation.

A second pilot group of six animals (maximum of 100 Gy in three and 150 Gy in three) underwent irradiation, with the 70% isodose corresponding to the medial border of the hippocampus (the coordinates of the isocenters covering the dorsal part of the hippocampus were 1 mm above the center of hippocampus). The experimental groups of rats were irradiated in such a way that the coordinates of the isocenters covering the dorsal part of the hippocampus were centered right in the middle of hippocampus (Fig. 2). The maximum dose was 150 Gy in three rats, 100 Gy in nine, 75 Gy in 12, 50 Gy in 16, and 25 Gy in 12. The rats were divided, together with nonirradiated control rats, into groups for testing at 1, 3, 6, and 12 months after radiosurgery. A group of four rats irradiated with 50 Gy, together with two control rats, were tested repeatedly for hippocampal function. Magnetic resonance imaging was also performed for a 14-month period after irradiation to track the lesion evolution over time.

Doses reported in this paper are those prescribed in the GammaPlan treatment planning system. Additional study, which focused on the evaluation of inaccuracies in the stereotactic irradiation of the rat brain, demonstrated that it is necessary to apply a correction factor for the absolute dose. This means the exact values of applied doses may be obtained by multiplying the calculated doses by a factor of 1.078. Detailed findings of the study, which determined this factor, will be published elsewhere.

Testing of Hippocampal Function

Behavioral testing of learning was performed in the Morris water maze.[16] The Morris water maze is a circular pool, 196 cm in diameter, filled with water at a temperature between 20 and 23˚C. Escape is possible on a hidden platform with a diameter of 10 cm, which is submerged 2 cm below the water surface so the rat cannot see it. The platform remained at the same position during the 5 consecutive days of testing. Multiple extramaze landmarks on the walls and ceiling of the room provided cues for the spatial relationship of the hidden platform. The testing was performed eight times each day. During the first two trials, the rat swam around the edges of the pool. This is natural behavior because the greatest chance of escape from water in nature is usually to be found at the shore. The rat then started to cruise the pool in different directions and would finally find the platform by chance. If this did not happen within 60 seconds the rat was led to the platform by hand. The rat then was allowed to rest on the platform for 15 seconds while trying to determine its spatial coordinates and to remember them. On the 5th day a normal unimpaired rat would find the island in about 7 seconds if released from any location. A rat with impaired hippocampal function would search for the island for significantly longer or even fail to find it.[9,17,18] From the eight measurements performed during a single day, the result of the first trial was omitted and the sum of the seven remaining latencies was calculated. The theoretical value of the maximum sum of latencies was taken to be 420 seconds for each rat. When the group of rats treated with the same dose was evaluated, the group mean of these sums of latencies was calculated.

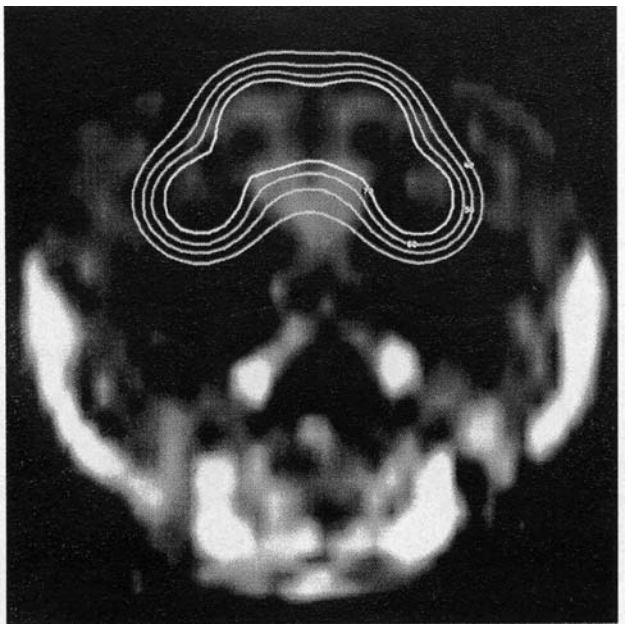

FIG. 2. The whole hippocampus was covered bilaterally by the 70% isodose (70, 60, 50, and 40% isodoses are shown).

Magnetic Resonance Imaging

After the functional behavioral testing was completed, all the experimental rats together with 10 controls underwent imaging in a Bruker Biospec 47/20 spectrometer (4.7-tesla/200 MHz) with a 200 mT/m gradient system and equipped with a surface coil made in-house. During the examination, rats received inhalational isofluran anesthesia (Forane; Abbott, Czech Republic). The anesthesia was induced by a 3% concentration of the anesthetic in air and maintained at a concentration of 1.5% during the whole examination. A set of T_2-weighted images was acquired using a standard turbo–spin echo sequence (TR = 2000 msec, TE = 35.1 msec, FOV = 4 cm, matrix 256 × 256, slice thickness/distance = 1:1 mm). The volume of any edema was evaluated by manual segmentation using an MaZda program. The T_2 relaxation times were evaluated from a set of T_2-weighted MR images obtained using a CPMG sequence with 30 echoes (TE = 8.63 msec, TR = 2500 msec).

Histological and Cytochemical Examinations

After functional testing and MR imaging, the rats were killed by exsanguination in deep whole-body anesthesia (intraperitoneal injection of 5% narkamon, 0.2 ml/100 g body weight). The brains were transcardially perfused with 0.9% NaCl and flushed with 4% buffered formaldehyde. For basic histological examination either the whole brains or only the right halves were postfixed in 4% buffered formaldehyde or Carnoy solution (6:3:1) and embedded in Paraplast-Plus (Polysciences Inc., Warrington, PA). The 7-μm-thick coronal sections were stained with toluidine blue. Cytochemical analysis was performed on the coronal cryostat sections, prepared from the remaining unfixed or prefixed left hemispheres. It included immunocytochemical detection of glial fibrillary acidic protein (Mabs GF-01, 1:25; Exbio, Prague, Czech Republic), synaptophysin, and syntaxin, (Mabs Clone SPV-38 and HPC-1; Sigma, St. Louis, MO) revealed by biotinylated goat anti–mouse immunoglobulin G (Fab fragment 1:200) and extravidin–peroxidase conjugate (1:100, both Sigma) and was terminated by a conventional diaminobenzidine reaction. Morphometric evaluations, including determination of the width of the neocortex and the hippocampus, the population density of pyramidal hippocampal neu-

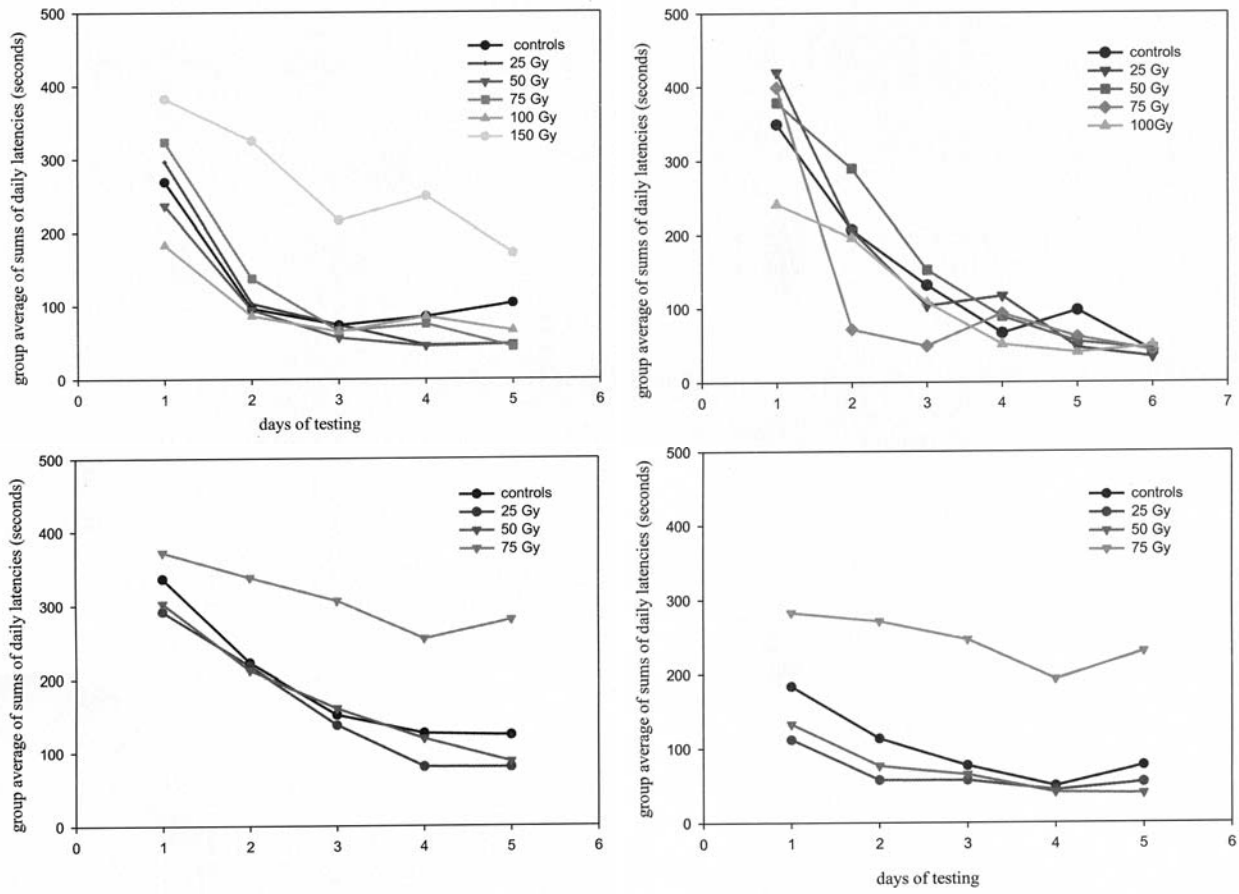

FIG. 3. Graphs showing acquisition curves in Morris water maze–tested rats. *Upper Left:* One month after radiosurgery. *Upper Right:* Three months after radiosurgery. *Lower Left:* Six months after radiosurgery. *Lower Right:* Twelve months after radiosurgery.

rons, and the concentration of the immunocytochemical staining product were performed using an eye-piece micrometer and an ocular grid (20 × 20) or the Advanced Image Data Analyzer Program (AIDA 2.11; Raytest Isotopengeraete GmbH, Germany) applied to digitalized microscopic pictures (Olympus Provis).

Statistical Analysis

To evaluate the changes in the rat brain over time postirradiation three events were studied as a function of the maximum dose delivered: MR imaging–documented edema, necrotic changes verified by the histological examination, and behavioral changes in the Morris water maze. The log-rank test was used to determine the level of the peripheral dose causing a significantly higher incidence of the three aforementioned clinical events. Results were considered to be significant if log-rank probability values were less than 0.05. All statistical analyses in this study were performed with the SPSS statistical software (version 10.0).

Results

Behavioral Testing of Memory Retention

Initially, the two pilot groups of rats were tested for retention of memory. The rats in these groups were trained to find the hidden platform in the Morris water maze and the GKS was performed thereafter. The rats were tested twice a week postirradiation until memory impairment was detected. The first pilot groups consisted of six rats in which only the dorsal part of the hippocampus was irradiated using two 4-mm isocenters. The maximum dose was 100 Gy in three rats and 150 Gy in another three rats. Rats irradiated with 100 Gy showed no sign of memory impairment until the 78th day after GKS, and the latency of seven daily measurements did not exceed 70 seconds. Two rats irradiated with a maximum 150-Gy dose exhibited memory impairment on the 35th day postirradiation and one after the 50th day, when latency exceeded 70 seconds.

The second pilot group consisted of six rats and the whole hippocampus was irradiated using four 4-mm isocenters. The maximum dose was again 100 Gy in three rats and 150 Gy in the other three. The rats irradiated with 100 Gy had no sign of memory impairment until the 50th day postradiosurgery and the latency of seven daily measurements did not exceed 70 seconds. The rats irradiated with a maximum dose of 150 Gy showed memory impairment on the 32nd day postirradiation (one rat) and no impairment was shown even 36 days postirradiation in the other two rats.

Behavioral Testing of Memory Acquisition

Tests for memory retention induced by preirradiation training proved to be insensitive for the purpose of this experiment. Consequently, the remaining rats were tested for acquisition of spatial memory. Irradiation of the hip-

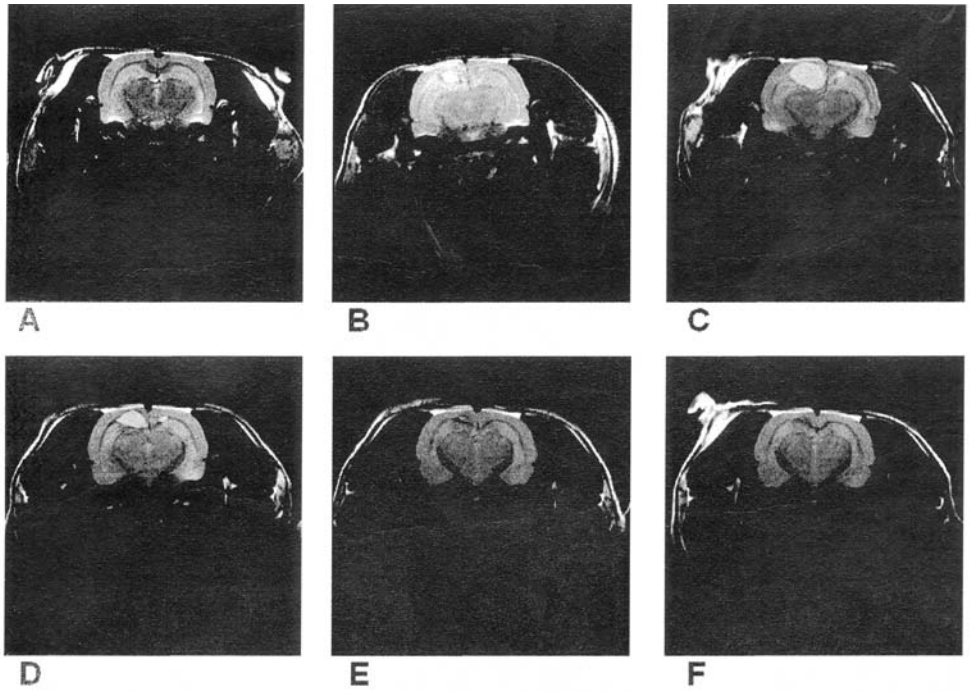

FIG. 4. Evolution of the lesion in the hippocampus of a rat irradiated with a dose of 50 Gy at 6 months (A), 8 months (B), 9 months (C), 10 months (D), 12 months (E), and 14 months (F).

pocampus was performed in untrained rats and the whole hippocampus was irradiated using four isocenters (4-mm collimator). The sensitivity of this functional testing was verified in the group of three rats irradiated with the maximum dose of 150 Gy. This group was tested 1 month postirradiation, and their latencies in finding the hidden platform were two- to threefold worse compared with the control group (Fig. 3 *upper left*).

The first group of 12 untrained rats was irradiated with maximum doses of 25, 50, 75, and 100 Gy (three animals for each dose) and tested 1 month after the radiosurgery, when no differences were observed in comparison with the control group (Fig. 3 *upper left*). The second group of untrained rats was irradiated with maximum doses of 25, 50, 75, and 100 Gy (three animals for each dose), tested 3 months after the radiosurgery, and no differences compared with the control group were observed (Fig. 3 *upper right*). The third group of 12 untrained rats was irradiated with 25, 50, 75, and 100 Gy to the maximum (three animals for each dose). Rats irradiated with the 100-Gy dose died between the 4th and 5th month after radiosurgery and the rest were tested 6 months after radiosurgery. No difference was observed between rats irradiated with 25 and 50 Gy and those in the control group. Rats irradiated with 75 Gy were significantly worse (Fig. 3 *lower left*). The fourth group of nine untrained rats was irradiated with maximum doses of 25, 50, and 75 Gy (three animals for each dose). The 100-Gy dose was omitted because rats receiving this dose had died within the 6 previous months. Rats in this group were tested 12 months after radiosurgery. No differences compared with the control group were observed in rats irradiated with the 25- and 50-Gy doses. Rats irradiated with 75 Gy were significantly worse (Fig. 3 *lower right*).

Lesion Evolution Observed on Magnetic Resonance Imaging

A pilot study performed in rats irradiated with doses of 100 and 150 Gy and two isocenters revealed massive lesioning with edema in the hippocampi and adjacent tissue 3 months after irradiation. No structural changes were observed on the T_2-weighted images of hippocampi during the first 6 months in rats irradiated with doses up to 75 Gy. Hippocampal T_2-weighted values were the same in the control rats (mean 65.3 msec). The rats irradiated with 25 Gy had no visible lesions by 12 months postirradiation. In the case of the rats irradiated with higher doses (50 and 75 Gy) response to the treatment was not uniform.

All four rats receiving 50 Gy suffered edema, which developed approximately 8 months postirradiation. Its maximum volume was observed after 9 months. In two cases the edema slowly disappeared, with only a small necrotic scar remaining after 14 months. In the other two edema persisted. Its volume did not change, although it was filled with a connective tissue with a similar T_2 relaxation time to that found in the surrounding tissue as shown in Fig. 4. A lesion was observed in one rat 6 months after GKS with 50 Gy and its edema volume was 13.8 mm^3. No edema was observed in the group of rats evaluated 12 months after irradiation, although a hypointense signal was demonstrated in the hippocampus of one rat, probably indicating necrotic tissue after edema absorption.

In two of five rats irradiated with 75 Gy a large asymmetrical lesion (edema volume 345.9 $\pm$ 176.3 mm^3) covering both hippocampi and a considerable part of the cortex was revealed 6 months after irradiation (Fig. 5). The two rats with these lesions were examined once again 14 months after irradiation, and both were found to have a massive edema of up to 624.9 $mm.^3$

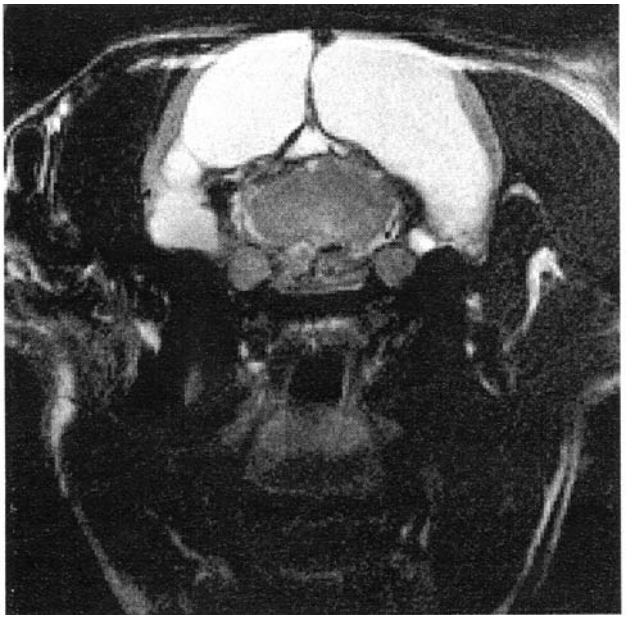

FIG. 5. Edema and necrotic lesion in the hippocampus of a rat irradiated with a dose of 75 Gy 6 months after radiosurgery.

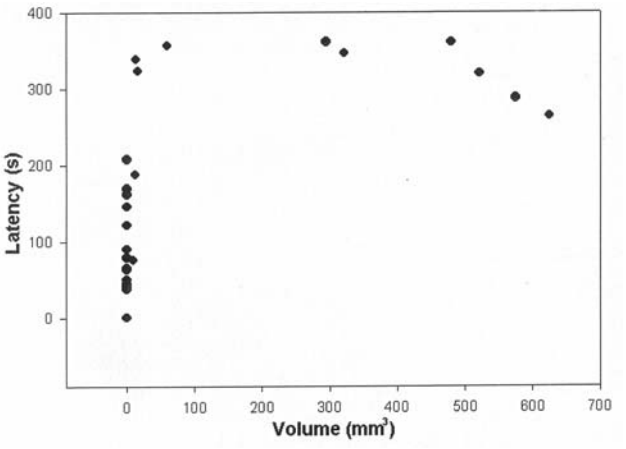

FIG. 6. The Morris water maze test results (latency) correlated to the edema size. These data were acquired 6 and 12 months after irradiation.

Another group of three rats evaluated 12 months after irradiation had small lesions of 16.3 mm^3 or less. Edema was always accompanied by a considerable change of T_2 relaxation time. The T_2 values in the edema increased up to 148 msec, whereas those in the necrotic tissue revealed after the disappearance of the edema were low at 29.8 msec.

Results of the Morris water maze test, assessed by latency value, were correlated with the volume of edema at 6 and 12 months after irradiation. A significant functional impairment was found in the rats suffering from edema. Figure 6 shows the dependence of functional impairment on the edema volume.

Morphology and Cytochemistry

Severe necrotic lesions were found 3 months after GKS in the hippocampus as well as in the adjacent dorsooccipital cortex and corpus callosum in all rats irradiated with 100 and 150 Gy applied with two isocenters. At that time point, irradiation with a dose of 100 Gy, applied with four isocenters, induced only mild reduction in the thickness of the neocortex (−30%) above the hippocampus, a slight reduction in the number of pyramidal cells (−16.3%), dilation of capillaries of the lateral ventricles, and a diffuse astrogliosis. After the 50-Gy dose, the choroid plexus of the lateral ventricles appeared slightly hypertrophic in one of two rats.

After a 6-month postirradiation interval, the dose of 50 Gy applied with four isocenters produced small focal necrotic lesions in one of three rats in the medial parts of the dorsooccipital cortex and the corpus callosum above the hippocampus. The lateral ventricles were also dilated in some segments. After administration of a 75-Gy dose, postnecrotic lesions and/or cavities were more extensive and most evident in the dorsooccipital cortex. Moreover, partial ablation occurred in some segments of the hippocampus. The layer of the pyramidal cells of the hippocampus was slightly disfigured, and there was a mild astroglial reaction, which was independent of the dose. Apart from a mild astrocytic reaction, no changes were found after 25 Gy. The same result remained true of the 25-Gy group 1 year after radiosurgery.

At 12 to 14 months after 50-Gy irradiation, small, solitary foci of necrosis occurred in one of four rats. After 75-Gy irradiation, we found necroses and/or postnecrotic cavities. In all the rats, the caudal parts of the hippocampus were more affected than the rostral segments. The size of the hippocampus was reduced in both the 50- and 75-Gy groups (−18 to −30%) and the pyramidal neurons were hyperchromic. In some rats, there was dilation of capillaries, a pericapillary edema, and distension and deformation of the lateral ventricles. An astrocytic reaction, although not entirely proportional to the radiation dose, was apparent in all these rats. No significant changes in the expression of synapsin and syntaxin, markers of the pre- and postsynaptic vesicles, were found except for a less diffuse distribution of the immunohistochemical-staining product. Representative histological findings are shown in Fig. 7.

Significantly higher incidences of edema ($p < 0.001$), necrosis ($p < 0.001$), and behavioral changes ($p = 0.031$) were observed when maximum doses were in excess of 50 Gy. There was no edema, necrosis, or behavioral change observed when the dose was 25 Gy.

Discussion

The current surgical treatment of epilepsy consists of the resection of the epileptogenic focus. Radiosurgery has been studied as a noninvasive alternative for the microsurgical removal of an epileptogenic focus. The first clinical studies were performed in patients with temporal lobe epilepsy and mesiotemporal sclerosis,[20] and experimental studies were performed in animals with focal epilepsy.[6,7,15] The results of these animal experiments indicated that a decreased frequency of epileptic seizures can be achieved with subnecrotic doses. In a rat model of epilepsy, the delivery of 20 Gy to the ventral part of the hippocampus led to a decrease in the frequency of epileptic seizures within 2 months after GKS, and this effect was even more

pronounced 4 to 6 months after radiosurgery. The dose of 40 Gy led to a decrease in epileptic seizures within 1 month, producing a maximum effect during the 2nd month after irradiation. The dose of 20 to 40 Gy applied to the ventral hippocampus using two 4-mm isocenters did not cause necrosis 10 months after the radiosurgery.[6] In another study the positive effect of a 20-Gy GKS dose in a rat with focal epilepsy was observed 2 weeks after radiosurgery.[15] Although the behavioral testing in the Morris water maze was also performed,[13] only one half of the hippocampus unilaterally was irradiated using one 4-mm isocenter. These experiments[6,13,15] would appear to show that a positive antiepileptic effect can be achieved using a subnecrotic, low dose of 20 Gy. This is in agreement with the results of Barcia-Salorio and Barcia[3] who observed the antiepileptic effect of a 20-Gy dose in patients with temporal lobe epilepsy. Experience with the radiosurgical treatment of brain tumors and arteriovenous malformations with secondary epilepsy shows that the effect on secondary epilepsy can also be achieved with relatively low doses of radiation.

The hippocampus is a crucial structure responsible for the generation of epileptic seizures in mesiotemporal sclerosis, and radiosurgery of the amygdalohippocampal complex should bring relief to the patient without anatomical ablation of this structure. Despite this promising use of radiosurgery, the effect of radiation on the normal functioning of the hippocampus, particularly in terms of memory, remains unknown. The range of doses that can suppress epileptic activity and not impair the memory function of hippocampus can affect the indication criteria for radiosurgery. Currently, patients with bilateral epileptic foci are disqualified from either epilepsy surgery or radiosurgery. The same applies to patients whose amobarbital and neuropsychological test results indicate that the affected hippocampus may not be sacrificed. Knowledge of the effects of radiosurgery on the normal hippocampus is important in these cases. Investigation of the consequences of a radiosurgical lesion induction on the normal hippocampus is a logical and hitherto absent component of the theoretical basis of GKS for epilepsy.

There has been no previous publication concerning the creation of complex lesions in rat brains, which could be used for the purposes of this experiment. Usually a single 4-mm isocenter has been used to investigate changes in rat brains.[10,12] Because the effect of radiosurgery depends on the irradiated volume, the tolerance of the rat brain to the use of more isocenters (and consequently a higher volume of irradiated brain tissue) has not been investigated. One group of investigators used two isocenters.[6] We have used four 4-mm isocenters for the irradiation of the whole hippocampus bilaterally and found that the rat could be used as an animal model in a wide range of doses creating complex lesions in the brain. This animal could also be tested over at least 1 year.

Damage to the hippocampus from different causes leads to memory impairment. Therefore, testing the memory impairment in the Morris water maze was chosen as a sensitive indicator of hippocampal function. A platform is hidden so that the rat cannot perceive it with any of its senses. The position of platform can be remembered only by correlating it with its surroundings. It is generally assumed that the rat (in common with humans) has a cognitive map of the surrounding world in its memory.[19] The rat can perform operations by using this map, enabling it to find the hidden platform. Navigation in the water maze is strongly impaired after bilateral damage of the hippocampus and interruption of its input. Bilateral and partial unilateral loss of hippocampal function by tetradotoxin significantly impairs the navigation of rats in a Morris water maze.[7] Ibotenic acid lesions of the hippocampus selectively damage the cognitive part of an animal's orientation in a water maze.[17] Damage of the hippocampus after protracted pilocarpine seizures is proportional to the impairment of navigation in a Morris water maze.[4,5]

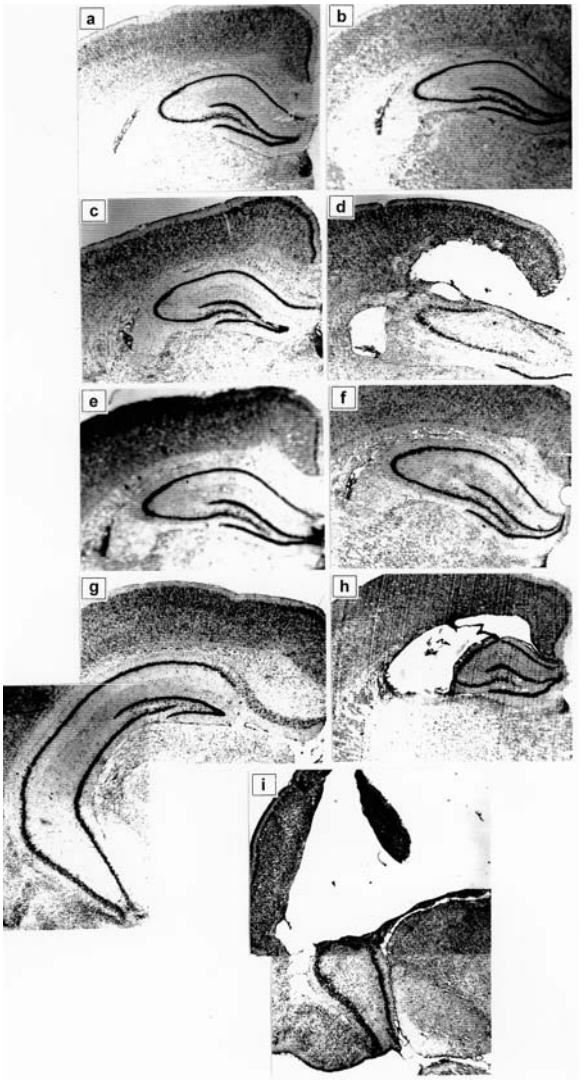

FIG. 7. Morphological features of the dorsal and ventral hippocampi in irradiated rats at 6 and 12 months. a: Dorsal hippocampus control specimen at 6 months. b: Dorsal hippocampus 25-Gy group specimen at 6 months. c: Dorsal hippocampus 50-Gy group specimen at 6 months. d: Dorsal hippocampus 75-Gy group specimen at 6 months. e: Dorsal hippocampus 25-Gy group specimen at 12 months. f: Dorsal hippocampus 50-Gy group specimen at 12 months. g: Ventral hippocampus 50-Gy group specimen at 12 months. h: Dorsal hippocampus 75-Gy group specimen at 12 months. i: Ventral hippocampus 75-Gy group specimen at 12 months. Toluidine blue, original magnification × 25.

Based on previously published results,[9,18] we had assumed that irradiation of the dorsal part of the hippocampus would be sufficient. Therefore, in the first pilot group of rats, radiosurgical lesioning was performed using two 4-mm isocenters only in this dorsal part of the hippocampus. According to published radiobiological studies, with a single 4-mm isocenter 150 Gy produces a necrotic lesion within 1 month and 100 Gy does the same in about half the rats within 3 months after the radiosurgery.[10,12] Rats irradiated with a maximum of 150 Gy to the dorsal part of the hippocampus alone showed no sign of memory impairment within 1 month when tested twice a week. Two animals, however, showed signs of impairment after 35 and 50 days, respectively. Therefore, in the second pilot group the whole hippocampus was irradiated bilaterally using four 4-mm isocenters, with 150- and 100-Gy doses. The 100-Gy dose was used because we did not know if the irradiated volume would cause diffuse postirradiation edema in the rat brain. Although radionecrosis should have developed within 30 days after the 150-Gy dose, these rats showed no impairment in the 36 days following radiosurgery. In these pilot groups, memory retention was tested after the rats had been trained to find the hidden platform and radiosurgery was performed thereafter. No memory impairment was observed in rats irradiated with 150 Gy before the 35th day after radiosurgery and none in rats irradiated with 100 Gy before 70 to 80 days after radiosurgery, when extensive necrotic lesions involving the neocortex were created. We found an explanation for the late impairment in the fact that repeated testing of the retention of memory track in the Morris water maze served as a stimulation of the plasticity processes, and the memory track was probably restored in residual preserved regions in the case where the lesion developed gradually. Therefore, this test could not be viewed as sensitive enough, and the acquisition of the memory track, rather than its retention, was tested in untrained rats for the remainder of the experiments, and irradiation was delivered to the whole hippocampus by using four 4-mm isocenters. When untrained rats were irradiated with a maximum dose of 150 Gy and tested 1 month thereafter, outcome was two- to threefold worse compared with that in the control group, and histology revealed necrotic changes. The untrained rats irradiated with 25 to 100 Gy had no impairment of memory acquisition 1 month after radiosurgery. Likewise no MR imaging–detected changes were observed and the histology showed no necrosis.

Three months after the irradiation of untrained rats with doses of 25 to 100 Gy, there were no changes in the behavioral acquisition test. Magnetic resonance imaging revealed no changes and histology showed no necrosis, although an astrocytic reaction and a deficit of PCNA-positive cells were present in rats irradiated with 25 Gy and with increasing intensity in higher doses.

None of the rats irradiated with the 100-Gy dose survived for 6 months after radiosurgery to be tested. With the exception of a single rat receiving 50 Gy, no untrained rats irradiated with 25- and 50-Gy doses showed any changes in the behavioral acquisition test after 6 months. Magnetic resonance imaging revealed no changes and histology showed no necrosis, except in the case of the one impaired rat in the 50-Gy group. A different situation was observed in the group of four rats irradiated with a maximum of 50 Gy, which underwent MR imaging repeatedly, whereas the rest of the tested animals were tested only once. In this group of four rats the MR imaging changes were more pronounced. These rats differed from the others in that repeated use of anesthesia was performed during the MR imaging examination, which lasted for several hours. We believe that the effect of protracted anesthesia with eventual hypoxia can increase the potential for the development of structural and functional changes in the irradiated tissue, and this hypothesis should be verified in further studies. In all rats irradiated with 75 Gy significant impairment was shown in the behavioral acquisition test, MR imaging revealed brain edema, and the histological examination showed necrotic cavities. Similar results were observed in the groups of animals tested 12 months after irradiation.

Conclusions

Rats can be used for animal experiments in which complex brain lesions are created using four 4-mm isocenters. With maximum doses less than 75 Gy, the rats survive and can be tested for at least 12 months after radiosurgery. Retention memory for the testing of behavioral changes after bilateral radiosurgery of the whole hippocampus was insensitive, and acquisition memory should be tested to track the functional changes of the hippocampus. Significantly higher incidences of edema, necrosis, and behavioral changes were observed in rats receiving doses above 50 Gy, but none was demonstrated when using doses of 25 Gy.

There appears to be a therapeutic window in which the dose affecting epilepsy does not impair memory of the rat. This therapeutic window should be further tested by studying the epileptic rats to verify the sensitivity to memory impairment of a hippocampus harboring an epileptic focus.

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