Background and Purpose—Despite a high incidence of poststroke dementia, there is no specific treatment for this condition. Because the evaluation of poststroke cognitive deficits in animal models of stroke is exceedingly challenging, the preclinical evaluation of candidate drugs is limited. We aimed to explore the impact of small cortical photothrombotic strokes on poststroke cognition, thereby assessing the suitability of this experimental stroke model for the investigation of cognitive impairment after stroke.

Methods—Photothrombotic cortical infarcts were induced in 19 adult male Wistar rats. Nineteen sham-operated animals served as controls. Using the Morris water maze, we analyzed the impact of photothrombotic stroke on both the acquisition of new memories and the recall of previously acquired memories. The cylinder test, the adhesive tape removal test, and the rotarod test were performed to investigate sensorimotor deficits.

Results—Photothrombotic stroke significantly impaired the recall of previously acquired memories (P < 0.05), whereas the acquisition of new memories remained largely intact. The analysis of the animals’ swimming speed in the water maze and the rotarod test showed no confounding motor impairments after photothrombotic stroke. The adhesive tape removal test and the cylinder test revealed mild sensorimotor deficits in lesioned animals (P < 0.05).

Conclusions—Photothrombotic cortical infarcts impair the recall of memories acquired before stroke, whereas the formation of new memories remains unimpaired. The observed deficits in the water maze are not confounded by disturbed motor functions. Overall, experimental photothrombotic strokes are well suited for the investigation of specific cognitive impairments after stroke. (Stroke. 2014;45:00-00.)

Key Words: brain ischemia ■ mild cognitive impairment ■ animal model ■ stroke

Among patients with stroke, ≈10% of them develop a new dementia after a first stroke, and after recurrent stroke, ≈30% of the patients have dementia.1 The risk of developing poststroke dementia is more closely related to the number of strokes than to pre-existing vascular risk factors, which emphasizes the causal role of stroke itself.1 Despite the high disease burden, there is no specific treatment for poststroke dementia. The reason for this is that no suitable animal models are available that reliably allow for the investigation of mechanisms of poststroke cognitive deficits and for the preclinical evaluation of candidate drugs.2–4 Middle cerebral artery occlusion (MCAO) in rodents is the widely used method for the induction of cerebral ischemia in experimental stroke research. However, animals that underwent MCAO exhibit severe sensorimotor deficits, which confound the evaluation of cognitive functions.2–4 The frequently applied Morris water maze is sensitive for cortical and striatal damage but has an increased susceptibility to hippocampal dysfunctions. Because MCAO irregularly causes hippocampal damage, the evaluation of cognitive deficits in the Morris water maze may be imprecise.2–4

Therefore, we aimed to evaluate whether the photothrombotic stroke model is suitable for the investigation of cognitive impairment after stroke. To examine whether photothrombotic cortical infarcts differently affect the acquisition of new memories and the retrieval of remote memories, we performed 3 different experiments. Experiment 1 was performed to detect an impaired acquisition of new memories; experiment 2 was designed to uncover impairments in the recall of memories acquired before stroke; and experiment 3 was performed to investigate potential deficits in long-term memory retention.
Materials and Methods
All experiments complied with animal welfare regulations, and experimental protocols were approved by the local ethics committee and were performed in a fully randomized and blinded fashion.

Photothrombotic Ischemia Model
Photothrombotic infarcts were induced according to a previously published protocol. Briefly, animals were anesthetized with an intraperitoneal injection of ketamine hydrochloride (100 mg/kg body weight; Ketanest) and xylazine hydrochloride (8 mg/kg body weight). The left femoral vein was cannulated with a PE-50 tube for Bengal Rose infusion. The rectal temperature was maintained at 37°C by a thermostat-controlled heating pad (Föhr Medical Instruments). Photothrombotic ischemia was induced in the right frontal cortex. For illumination, a laser spot of 8 mm in diameter (G Laser Technologies) was placed stereotaxically onto the skull 0.5 mm anterior to the bregma and 3.5 mm lateral from the midline. The skull was illuminated for 20 minutes. During the first 2 minutes of illumination, the dye Bengal Rose (0.133 mL/kg body weight, 10 mg/mL saline) was injected intravascularly. Sham-operated animals underwent the same procedure including Bengal Rose injection but without illumination of the skull.

Experimental Design
To elucidate the effect of a photothrombotic stroke on the acquisition and retention of spatial memory, we performed 2 different experiments (Figure 1). In experiment 1, we induced photothrombosis on day 0 and performed the water maze trial on days 7 until 11 with an additional probe trial on day 12. Furthermore, a battery of somatosensory and motoric tests was performed on day −1 (baseline) and days 1, 6, and 14. This battery consisted of the cylinder test, the adhesive tape removal test, and the rotarod test. In experiment 2, we performed the water maze trials (day −5 until day −1) before the induction of the photothrombosis (day 0). On day 7 after ischemia, we performed the probe trial. Beginning on day 9 until day 11, an additional acquisition task was executed to examine flexibility of learning. For this, the position of the hidden platform was moved to a different quadrant of the water maze. This relearning task was followed by a probe trial on day 12. In experiment 3, we again performed the water maze trials (day −5 until day −1) before the induction of the photothrombosis (day 0).<ref>
Figure 1. Experimental design. Experiment 1 was designed to detect the impact of photothrombotic stroke on the acquisition of new memory content. Experiment 2 was designed to detect impaired retrieval of memories acquired before the stroke and flexibility of learning. Experiment 3 was performed to investigate long-term retention of memory content acquired before the stroke.
</ref>

Histological Analysis
After behavioral assessment, the animals were perfusion fixed intracardially with buffered formalin. The brains were removed, and serial coronal sections (thickness, 10 μm) were cut, collected at 100 μm intervals, and subsequently stained with toluidine blue (Sigma, St Louis, MO). Infarct volumes were estimated by measurement of the maximum diameter and measurement of the maximum infarct...
area on the slides, as previously described. The extent of the lesions was determined with reference to the stereotaxic atlas by Paxinos and Watson.

Statistical Analysis
The values presented in this study are means±SEM. Statistical analyses were calculated using the Statistical Package of Social Sciences (version 15.0; SPSS Inc, Chicago, IL). Behavioral measurements were analyzed by 2-way repeated-measures analysis of variance followed by the Fisher-protected least significant difference test. Student t test with Bonferroni correction was used to compare data between 2 groups. An error rate of 0.05 was taken as the criterion for significance.

Results
Experiment 1: Acquisition of New Memories
Assessment of Spatial Learning and Memory Function
During the acquisition trials, the distance moved as well as the latency to reach the platform did not differ between lesioned and sham-operated animals (repeated-measures ANOVA; P>0.05; Figure 2A). During the probe trial, there was neither an effect of photothrombotic stroke on the latency to reach the target area (Student t test; P>0.05; Figure 2A) nor on the amount of entries into the target area (Student t test; P>0.05; Figure 2A). Sham-operated animals spent more time in the target quadrant than lesioned animals (Student t test; P<0.05; Figure 2A). The swimming speed did not differ between both groups (P>0.05; Figure 2A). The cued version of the water maze test revealed no between-group differences in escape latency (repeated-measures ANOVA; P>0.05; data not shown).

Assessment of Somatosensory and Motor Function
The cylinder test and the adhesive tape removal test revealed mild somatosensory and motor deficits after photothrombosis (repeated-measures ANOVA; P<0.05; Figure 2B). The rotarod test did not show significant differences between both groups (repeated-measures ANOVA; P>0.05; Figure 2B).

Experiment 2: Recall of Memories
Assessment of Spatial Learning and Memory Function
The probe trial on day 7 after ischemia demonstrated that photothrombosis impaired the retrieval of memories acquired before the induction of ischemia. The latency to reach the target area was significantly longer (Student t test; P<0.05; Figure 3A), and the time spent in the target quadrant was significantly shorter (P<0.05; Student t test; Figure 3A) among lesioned animals compared with sham-operated animals. The analysis of entries into the target area revealed a strong trend toward a reduced number of entries into the target area (Student t test; P=0.06; Figure 3A). These differences were not caused by motor deficits as indicated by comparable swimming speed in both groups (Student t test; P>0.05; data not shown). The relearning task showed an unimpaired acquisition of new memories (repeated-measures ANOVA; P>0.05; Figure 3B). Again, the swimming speed did not differ between both groups (P>0.05; Figure 3B). The probe trial on day 12 revealed a trend toward a reduced ability to retrieve newly acquired memory content after photothrombotic stroke (latency to reach the target area).

Experiment 1

**A** Acquisition of spatial memory following photothrombotic stroke

**B** Motor and somatosensory function following photothrombotic stroke

Figure 2. Results of experiment 1. Acquisition of spatial memory after photothrombotic stroke (PT) compared with sham (Sham) in the water maze (A, path length and latency to reach the hidden platform plus velocity/probe trial: latency to reach platform area, amount of platform crossings, and time spent in the target quadrant). Assessment of motor and somatosensory function (B, cylinder test, adhesive tape removal test, and rotarod test). Means±SEM; ANOVA with repeated measures and Student t test with Bonferroni correction, where applicable; *P<0.05, ***P<0.001.
Figure 3. Results of experiments 2 and 3. Retrieval of previously acquired memory content after photothrombotic stroke (PT) compared with sham (Sham) in the water maze (A, latency to reach platform area, time spent in the target quadrant, and amount of platform crossings). Acquisition of new spatial memory content after ischemia (B, path length and latency to reach the hidden platform plus velocity/probe trial: latency to reach platform area, time spent in the target quadrant, and amount of platform crossings). Long-term memory retention (C, probe trials on days 7, 14, and 28 after ischemia: latency to reach platform area, time spent in the target quadrant, and amount of platform crossings). Means±SEM; ANOVA with repeated measures followed by the Fisher-protected least significant difference test and Student t test with Bonferroni correction, where applicable; *P<0.05, **P<0.01.
target area; Student t test; P=0.08; amount of platform crossings; Student t test; P=0.09; Figure 3B). There was no effect on the time spent in the target quadrant (Student t test; P>0.05; Figure 3B). The cued version of the water maze test revealed no between-group differences in escape latency (repeated-measures ANOVA; P>0.05; data not shown).

Experiment 3: Long-Term Memory Retention

Assessment of Spatial Learning and Memory Function

The probe trials performed on days 7, 14, and 28 after ischemia revealed deficits in long-term memory retention after photothermotic stroke (latency to reach platform area, time spent in target quadrant, and amount of platform crossings; repeated-measures ANOVA; P<0.05; Figure 3C). Post hoc tests revealed significant differences between lesioned and sham-operated animals on day 7 (P<0.05) for latency to reach platform area; on days 7 (P<0.01), 14, and 28 (P<0.05) for time spent in target quadrant; and on days 7 (P<0.01), 14, and 28 (P<0.05) for amount of platform crossings.

Histological Analysis

The cortical lesions were maximal in surface area at the cortical surface, and at the midpoint extended in depth to the corpus callosum (Figure 1 in the online-only Data Supplement). Histopathologic analysis revealed a mean total lesion volume of 12.92±1.72 mm³. No evidence of tissue loss was found in the brains of animals from the sham group. The rostral/caudal limits of the lesions ranged from 2.7 mm anterior to the bregma to 2.8 mm posterior to the bregma. There was no evidence of damage in any subcortical area, such as the hippocampus, fornix, caudate, or anterior thalamic nuclei.

Discussion

In the present study, photothermotic cortical infarcts distinctively impair the animals’ ability to recall remote spatial memories, whereas leaving the acquisition of new spatial memories largely unaffected. This impairment of memory recall is still detectable 28 days after the infarct. After photothermotic stroke, the animals exhibit sensorimotor deficits, displayed by the adhesive tape removal test and the cylinder test. However, motor performance on the rotarod and the swimming speed in the Morris water maze were similar in both groups, thus indicating that deficits in the animals’ water maze performance are attributable to an impaired memory function and not a result of confounding motor disturbances. Moreover, we did not observe thigmotactic swimming. Overall, our results pronounce the importance of the cortex for the recall of remote memories. These findings are consistent with common concepts of memory organization, according to which spatial memories are formed in the hippocampus, subsequently integrated by hippocampal-cortical connections and transformed into remote memories in cortical networks.9

Several previously published articles have described the occurrence of cognitive impairment after experimental stroke.2–4,10–11 However, in studies using the most common model of stroke, MCAO, deficits in water maze tasks have often been confounded by disturbed sensorimotor functions.2–4 After MCAO, animals exhibit severe sensory and motor deficits, which affect the animals’ ability to control their swimming direction, and may also increase the animals’ anxiety, leading to augmented thigmotactic swimming.4 Photothermotic infarcts, by contrast, cause only minor sensorimotor deficits,5 thus allowing a more reliable, unbiased detection of cognitive deficits. Moreover, our results show that photothermotic cortical infarcts persistently impair the recall of remote memories, whereas the formation of new memories remains largely intact. To our knowledge, this is the first study demonstrating that cortical infarcts differently affect the acquisition of new memories and the retrieval of remote memories.

With respect to the high incidence of poststroke dementia and the absence of a specific treatment, the necessity to develop an animal model for the investigation of cognitive impairment after stroke is evident. Cognitive impairments after photothermotic cortical infarcts are not affected by co-occurring general motor impairments and can be reliably assessed in the Morris water maze. The photothermotic stroke model is well suited for the investigation of poststroke dementia and may open up new opportunities for the exploration of specific treatments for this condition.

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Disclosures

None.

References

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Supplemental Material

Supplemental Methods

Behavioral assessment

Water maze

The animals were placed in a circular water pool with a diameter of 1.50m; the water temperature was maintained at 21°C.

Before the training began a habituation trial was performed. During the habituation trial the animals had to swim for 60 s with no platform placed in the pool.

On the day prior to the beginning of each acquisition period, the animals were trained to locate a cued platform presented 2 cm above the surface of the water. Each animal performed four trials; both the cued platform position and the starting position of the animal were randomly varied. On each of the trials, the rat was allowed 90 s to reach the platform and then allowed to remain there briefly before being returned to a holding cage for 60 s before the beginning of the next trial.

For the assessment of spatial memory performance the animals learned to use spatial cues in the room to navigate to the escape platform positioned at a fixed location below the water surface. The rats were released into the pool from randomly varying positions for a maximum trial duration of 90 s. On each of the acquisition sessions each animal performed four trials, with an intertrial interval of 60 s. The latency to reach the platform and the total distance moved were recorded. The probe trials (60 s) were performed with the platform removed from the pool. The time the animal spends swimming in the former platform quadrant, the amount of platform crossings and the latency to reach the platform area were recorded. All trials were recorded with a video camera connected to a computer equipped with the Ethovision XT tracking software (Noldus Information Technology).
Sensorimotor tests

Adhesive tape removal

In all animals, sensorimotor tests were performed 1 day before ischemia (baseline) after a training period of 3 days and at 1, 6, and 14 days after ischemia.

For the adhesive removal test, 2 pieces of adhesive-backed paper dots (113.1 mm²) were used as bilateral tactile stimuli occupying the palmar surface of each forepaw. The time to remove each paper dot from the forelimbs was documented in 3 trials per day for each forepaw. An asymmetry score was calculated as follows: (time to remove ipsilateral dot− time to remove contralateral dot)/(time to remove ipsilateral dot+ time to remove contralateral dot).

For the cylinder test, the rats were placed in a transparent cylinder (16-cm diameter, 21-cm height) and videotaped, spontaneous wall and ground touches of both forelimbs were counted and an asymmetry score was calculated as described above.

For Rotarod tests, rats were placed on an accelerating Rotarod cylinder, and the time the animals remained on the Rotarod was measured. Speed was increased from 4 to 40 rpm within 5 minutes. The trial ended if the animal fell off the rungs or gripped the device and spun around for 2 consecutive revolutions without attempting to walk on the rungs. An arbitrary time limit of 300 seconds was set for rats on the Rotarod cylinder in training and testing procedures. The animals were trained 3 days before ischemia.
Supplemental figure I: Rat brain 14 days after photochemically induced ischemic stroke