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. 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. 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. 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. 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.

Therefore, we aimed to evaluate whether the photothrombotic stroke model is suitable for the investigation of specific cognitive impairments after stroke. (Stroke. 2014;45:00-00.)

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

A brief report

Cortical Photothrombotic Infarcts Impair the Recall of Previously Acquired Memories but Spare the Formation of New Ones

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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 photothermbotic strokes are well suited for the investigation of specific cognitive impairments after stroke.

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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 intravenously. 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). To examine long-term memory retention, we performed probe trials on day 7, 14, and 28 after ischemia.

The tasks of experiment 1 were performed on a total number of 18 animals subdivided randomly into 2 experimental groups (ischemia group n=9; sham group n=9). Twenty animals were included in experiment 2, subdivided randomly into the experimental groups (ischemia group n=10; sham group n=10). Twenty animals were used in experiment 3, subdivided randomly into the experimental groups (ischemia group n=11; sham group n=9).

Behavioral Assessment

Water Maze

The water maze task was performed according to a modified protocol described by Morris. Briefly, animals learned to use spatial cues in the room to navigate to the escape platform positioned at a fixed location below the water surface. On each of the acquisition sessions, each animal performed 4 trials. The latency to reach the platform and the total distance moved were recorded. The probe trials were performed with the platform removed from the pool. Each trial lasted 60 seconds. 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. A cued version of the water maze test was performed 1 day before the acquisition period to test for possible confounding sensory and motor deficits.

Sensorimotor Tests

The adhesive tape removal test, the cylinder test, and the Rotarod test were performed to assess sensorimotor function.

For detailed Materials and Methods see the online-only Data Supplement.

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

![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.](image-url)
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

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).
Experiment 2

A  Memory retrieval following photothrombotic stroke

B  Acquisition of new memory content following photothrombotic stroke

Experiment 3

C  Long-term memory retention

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 photothrombotic stroke (latency to reach platform area, time spend 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 $12.92\pm1.72$ mm$^3$. 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, photothrombotic 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 photothrombotic 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.

Several previously published articles have described the occurrence of cognitive impairment after experimental stroke. However, in studies using the most common model of stroke, MCAO, deficits in water maze tasks have often been confounded by disturbed sensorimotor functions. 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. Photothermal infarcts, by contrast, cause only minor sensorimotor deficits, thus allowing a more reliable, unbiased detection of cognitive deficits. Moreover, our results show that photothrombotic 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 photothrombotic cortical infarcts are not affected by co-occurring general motor impairments and can be reliably assessed in the Morris water maze. The photothrombotic 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.

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