Characteristics of Transient Cerebral Ischemia-Induced Deficits on Various Learning and Memory Tasks in Male Mongolian Gerbils

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ABSTRACT—We examined the characteristics of 5-min cerebral ischemia-induced behavioral deficits in spontaneous locomotor activity and their effects on the performance of habituation (HAB), passive avoidance (PA) and 8-arm radial maze (RM) tasks in Mongolian gerbils. Performances in HAB, PA and RM were impaired within 2 days after occlusion, and gerbils showed hyperlocomotion during this period. Ten days after ischemia, the hyperlocomotion disappeared and performance in the HAB and PA was the same as that in the sham-operated group. Retention in the RM was impaired at that period, but this impairment was overcome, and retention recovered easily to the sham-operated level with a few additional trials. When the acquisition trial in the RM began at 11 days after occlusion, severe learning impairment was found. Destruction of hippocampal CA1 neurons appears from 2-3 days after ischemic insult, with most CA1 neurons having disappeared by day 7. These findings suggest that the impairment of performance in the HAB and PA within 2 days after occlusion may be related to an early phase of CA1 neuronal death and to hyperlocomotion, although the impairment of spatial learning and memory was clearly associated with CA1 injury 10 days after ischemia.

Keywords: Mongolian gerbil, Ischemia, Hyperlocomotion, Learning and memory, Neuronal death (CA1)

Neurons in the central nervous system are vulnerable to ischemia. During cerebral ischemia, abrupt loss of neurological function occurs, which, if serious, leads to death. This neurological dysfunction is naturally reflected in the deterioration of behavioral performance and memory function. In fact, cerebral ischemia induced by an injection of microspheres results in poor retention of the active avoidance response in rats (1, 2). Pulsinelli and Brierley (3) have introduced a method for producing cerebral ischemia in unanesthetized rats by temporarily occluding the common carotid arteries and permanently interrupting the vertebral arteries. Although this method requires a delicate and time-consuming operation, at present it is generally employed as an animal model of learning and memory impairment (4-6).

Mongolian gerbils, which are characterized by frequent anomalies in the circle of Willis (7-10), have been used as an experimental model of cerebral ischemia (11-14), since it is very easy to produce cerebral ischemia in these animals by occluding only their bilateral carotid arteries. It could be expected, therefore, that gerbils would be used in behavioral studies of learning and memory tasks as an animal model of cerebral ischemia. However, they have some habits that make them unsuitable for behavioral studies, in that they exhibit excessive general activity and excessive reactivity to novel external stimuli. To date, there have been few behavioral studies of these animals (15-17). We carried out the present study with Mongolian gerbils to elucidate the characteristics of cerebral ischemia-induced deficits in several learning and memory tasks in these animals and to determine whether these gerbils would be an appropriate behavioral model of cerebral ischemia. The cerebral ischemia was produced by transient bilateral common carotid artery occlusion.

MATERIALS AND METHODS

Animals

Eight-week-old male Mongolian gerbils, supplied by Seiwa Jikken Dobutsu Lab. (Fukuoka), were housed in an air-conditioned room controlled for temperature (23±0.5℃), humidity (60±0.5%), and light (9:00-21:00).

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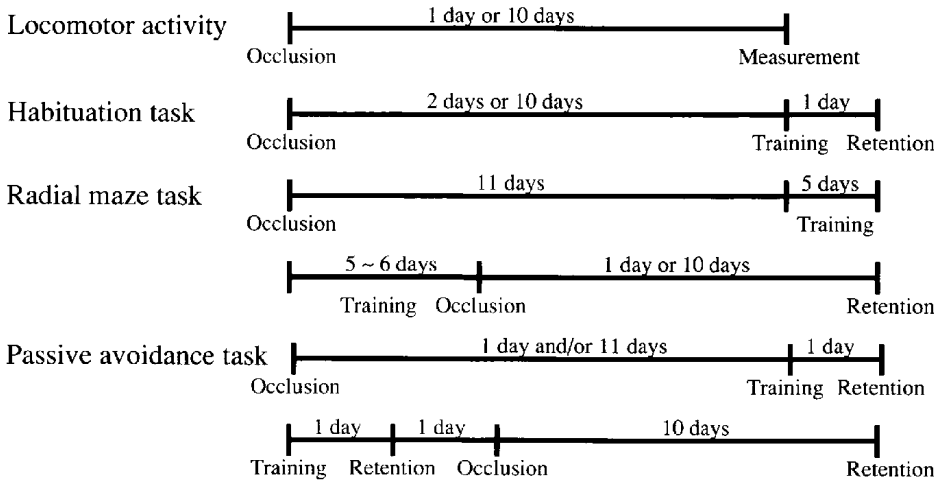

Fig. 1. Experimental schedule for the measurement of locomotor activity and performance in the habituation, radial maze, and passive avoidance tasks. Details of these experiments are described in the Methods and Results sections.

The animals were housed in groups of five or six per cage and were given food and water ad lib. They were maintained at approximately 85% of their initial free feeding weight (50–60 g) during the 8-arm radial maze experiment. All the animals had become thoroughly familiar with being handled and were allowed at least 5 days following adaptation to laboratory conditions before the experiments began.

Procedure

The experimental procedure is shown schematically in Fig. 1. The same animals were tested in sequential order of experiments using different kinds of tasks. Only the animals used in the passive avoidance task were not used in other tasks.

Ischemic treatment

The gerbils were anesthetized with 4% (introduction) or 2% (maintenance) halothane and placed in the supine position. A midline incision was made in the ventral neck, and the carotid arteries were carefully separated from the adjacent vein and sympathetic nerves. Following the cessation of anesthesia, the animals showed palinesthesia, at which time the exposed arteries were occluded with aneurysm clips for 5 min, after which blood flow was restored. Both occlusion and reflow were visually confirmed. Sham-operated animals were treated in an identical manner, except that the carotid arteries were not clamped. Rectal temperature was monitored throughout the procedure, and it was maintained between 36 and 37℃ with a heating pad and an incandescent lamp. Operated animals were kept heated in the cage until the righting reflex was recovered. After all tests were completed, histopathological examination of tissues was performed. The animals were anesthetized with 4% halothane, and the brains were perfused with 10% buffered formalin solution through the left cardiac ventricle and removed from the skulls. The hippocampal region was cut coronally into 3- to 4-mm thick slices, which were embedded in paraffin and processed by the step section technique. The slices were stained with cresyl violet. Animals in which no ischemic neuronal damage was observed in the hippocampal CA1 neurons were excluded from the behavioral data: One out of 59 animals has been eliminated from the behavioral analysis.

Locomotor activity

Locomotor activity in a novel environment was measured in a photocell activity cage (30 × 30 × 30 cm), using photobeams placed at 1-inch intervals along two sides of the cage (Columbus Instruments, Columbus, OH, USA). The measurements were made for 15 min, at 3-min intervals after the gerbil was placed in the cage.

Habituation task

The habituation of locomotor activity was investigated as an experimental memory process model (18). The locomotor activity of the gerbils was measured for two 15-min sessions in the photocell activity cage. The locomotor activity test described above, carried out on the first day, served as an acquisition session; and the retention test was done in the same way on the second day. A decrease in activity from the first to the second day served as an index of retention. Data from the first 3 min of each session were used for analysis of the habituation task. Both sessions were conducted at the same time of day to exclude any effects due to circadian rhythm.

Eight-arm radial maze task

Apparatus: We used an 8-arm transparent Plexiglas radial maze, described previously (19–21), with minor modifications. The apparatus was used as a corridor radial maze. The central platform (30-cm diameter) was octagonal in shape and contained a starting box; the arms were 50-cm-long and 10-cm-wide, with 50-cm-high side walls. A small circular food cup was placed at the end of each arm; one food pellet (about 20 mg; O'Hara & Co., Ltd., Tokyo) was placed in each cup during the training and retention tests. The entire maze was elevated 3 cm above the floor. Behavioral testing was conducted in a well-lit room, with a variety of navigation cues surrounding the maze. Animals were gradually deprived of food, until they were approximately 85% of their expected free-feeding weight, beginning 3 days prior to this test.

Procedure: The animals were habituated to the apparatus and food pellet 1 day before training began. Five or six animals were placed in the apparatus until they ate 5–7 pellets placed in each food cup (about 20 min). This habituation procedure was repeated 3 times in a day. The habituation interval was more than 1 hr. For each training trial, a gerbil was placed randomly in the starting box facing one of the eight arms. The trial ended when all the arms had been entered and all the pellets had been eaten or after 10 min had elapsed. Two or three training trials were given per day. The animals were trained until they had made more than 7 out of 8 correct arm entries and less than 1 out of 8 total error entries (i.e., entering arms not previously chosen on that particular trial) on 3 consecutive training trials.

Passive avoidance task

The passive avoidance task was performed according to the method described by Karasawa et al. (15) with minor modifications. The gerbils were trained in a conventional step-down type passive avoidance apparatus, divided into safe and grid parts. The experimental chamber ($30 \times 30 \times 30$ cm), made of acrylfiber, was set in a semi-soundproof wooden box with a small electric lamp (15 W). The floor had a grid of stainless rods connected to a 4-mA scramble shock generator (BRS/LVE, SGS-004; Laurel, MD, USA). The safe part ($10 \times 30 \times 3$ cm), made of wood, was at one side of the chamber. Each animal was initially placed on the grid and received a foot shock. When the animal stepped up onto the platform, it was taken out of the chamber. Any animal that showed an abnormal response to the electric stimulation was removed from this experiment. In the second part of the experiment, the gerbil was placed on the safety platform, but it stepped up and down repeatedly and received foot shocks when it stepped down. Finally, it eventually remained on the platform. This acquisition trial lasted for 300 sec. The step-down latency in Mongolian gerbils did not increase with only one foot shock; this result is completely different from that seen in mice and rats. However, there was a marked prolongation of the latency in the passive avoidance task in animals that received repeated foot shocks within 5 min. In the retention test, performed 24 hr or 12 days after the acquisition trial, the gerbil was again placed on the safety platform; and the latency to step down to the grid floor, the number of step-down actions, and the total time spent on the grid were measured. If the gerbil did not step down to the grid floor within 180 sec, a ceiling score of 180 sec was assigned.

Statistical analyses

The results were analyzed by the two-tailed Mann-Whitney *U*-test. The criterion for statistical significance was $P < 0.05$ in all statistical evaluations.

RESULTS

Locomotor activity at 1 or 10 days after occlusion

The effects of 5-min occlusion on spontaneous locomotor activity are shown in Fig. 2. One day after occlusion, locomotor activity in a novel cage was 300 percent more than that in the sham-operated group. However, this activity decreased to the baseline level at 10 days after occlusion.

Habituation task at 2 or 10 days after occlusion

The effects of 5-min occlusion on the habituation task are shown in Fig. 3. Habituation under our conditions was defined as the decrease in spontaneous locomotor activity observed when gerbils were exposed for a second

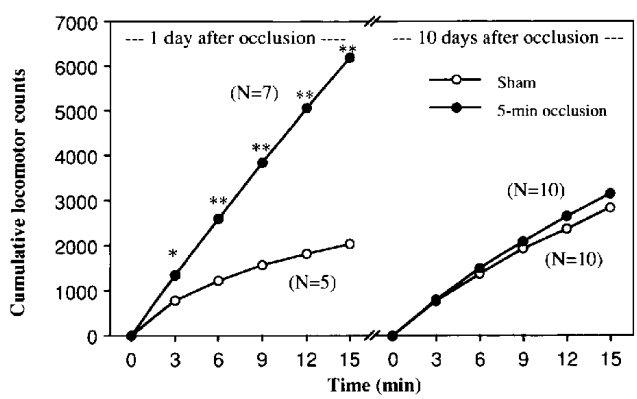

Fig. 2. Effects of 5-min occlusion on spontaneous locomotor activity in Mongolian gerbils. Locomotor activity at 1 day after occlusion (left) and 10 days after occlusion (right). Numbers in parentheses represent the numbers of gerbils tested. Each point represents the mean. $^{*}P < 0.05$, $^{**}P < 0.01$, compared to the corresponding sham-operated animals.

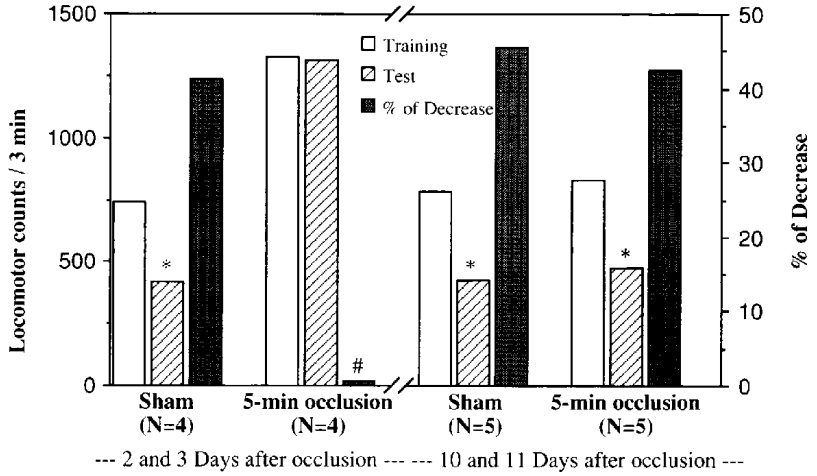

Fig. 3. Effects of 5-min occlusion on the performance of a habituation task by Mongolian gerbils. Left, locomotor activity for 3 min at 2 days (training) and 3 days (test) after occlusion; right, locomotor activity at 10 days (training) and 11 days (test) after occlusion. % of decrease = [(locomotor activity at training; A) − (locomotor activity at retention test)]/A × 100. Numbers in parentheses represent the numbers of gerbils tested. Each value represents the mean. $^{*}P<0.05$, compared to corresponding locomotor activity at training; $^{\#}P<0.05$, compared to the corresponding sham-operated animals.

time to a simple exploratory situation in a photocell activity cage. An acquisition session was carried at 2 or 10 days after occlusion, and the retention session was carried out at 24 hr after each acquisition session. The sham-operated group showed a marked decrease in locomotor activity, but poor retention was observed in animals which had been subjected to ischemia for 5 min. The 5-min ischemia produced hyperlocomotion at both the acquisition and retention sessions. At 10 days after occlusion, retention was the same as that of the sham-operated animals.

Eight-arm radial maze task

Task acquisition begun at 11 days after occlusion: The sham-operated and ischemic groups gradually acquired the 8-arm radial maze task. Averaged choice times of the sham group were 19.1, 15.4 and 14.5 sec and those of the occluded group were 11.3, 14.5 and 13.6 sec at the first, second and third trial of the 11-day training, respectively. There was no difference in the averaged choice time between the sham and occluded groups. The running trespass of these animals was different in every trial. In the radial maze task, sometimes we observed that the animals stopped and stared around at the turning points of the 8 arms. This behavior looks like the animals were searching for navigation cues surrounding the maze and/or selected the arm. However, the ischemic group had a higher total

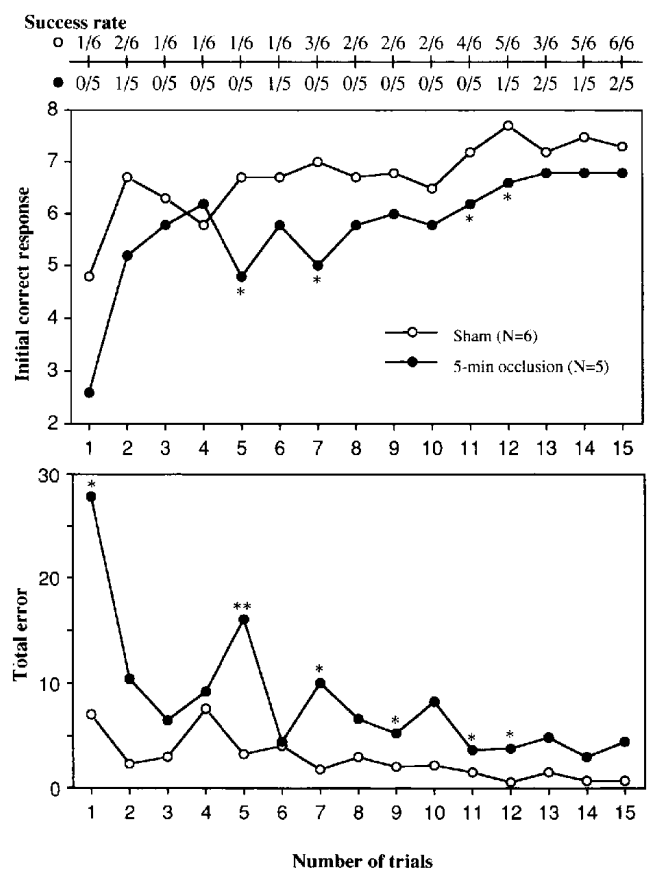

Fig. 4. Effects of 5-min occlusion before training on acquisition of the radial maze task in Mongolian gerbils. Training began at 11 days after occlusion. Three training trials were given per day. The success rate shows the number of animals that had more than 7 out of 8 correct arm entries and less than 1 out of 8 total error entries/number of gerbils tested at each training trial. Upper and lower figures show the initial correct response and total error, respectively. Numbers in parentheses represent numbers of gerbils tested. Each point represents the mean. $^{*}P<0.05$, $^{**}P<0.01$, compared to the corresponding sham-operated animals.

error (TE) score and lower initial correct response (ICR) score overall than the sham-operated group. In the sham-operated group, the number of animals reaching the criterion increased with repeated trials; however, in the ischemic group, this number did not increase after the 12 trials, and the animals that reached the criterion were different in each trial (Fig. 4).

Retention test at 1 or 10 days after occlusion: Animals were trained in the radial maze task, after which they were occluded. The effects of 5-min occlusion on the retention test are shown in Figs. 5 and 6. Ischemic gerbils exhibited peculiar behavior at 1 day after occlusion, running in the radial maze apparatus with a running trespass of 135° or 180°. They thus had lower ICR values, and higher TE values than the sham-operated animals (Fig. 5). On the retention test at 10 days after occlusion, the TE value recovered to the level of the sham-operated group, but the ICR value did not (Figs. 5 and 6). However, following repeated re-training, the decreased ICR value recovered to the control level (Fig. 6).

Passive avoidance task

Acquisition trial at 1 or 11 days after occlusion: In the first experiment, the acquisition and retention tests for the

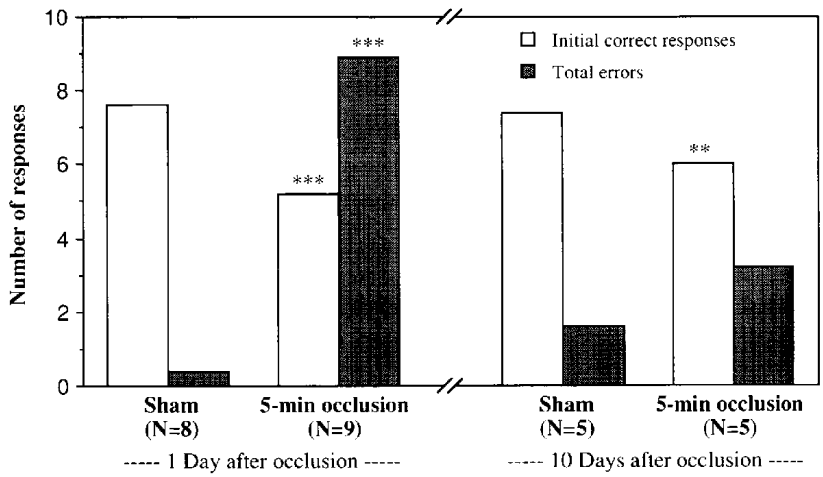

Fig. 5. Effects of 5-min occlusion on retention of radial maze task in Mongolian gerbils. The animals were trained for the radial maze task and were afterwards occluded for 5 min. Left, number of responses at 1 day after occlusion; right, number of responses at 10 days after occlusion. Numbers in parentheses represent numbers of gerbils tested. Each value represents the mean. $^{**}P<0.01$, $^{***}P<0.001$, compared to the corresponding sham-operated animals.

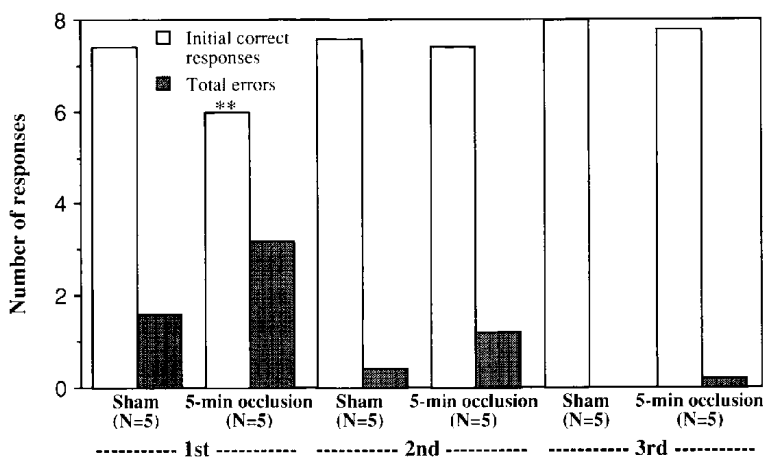

Fig. 6. Effects of re-training on retention of the radial maze task in Mongolian gerbils at 10 days after 5-min occlusion. Three repeated re-trainings were given at 10 days after occlusion, at intervals of about 1 hr. Data shown for the 1st re-training in this figure are the same as the data shown on the right in Fig. 5. Numbers in parentheses represent numbers of gerbils tested. Each value represents the mean. $^{**}P<0.01$, compared to the corresponding sham-operated animals.

passive avoidance task were carried out 1 and 2 days, respectively, after occlusion. As shown in the left panel of Fig. 7, the step-down latency in the retention test was significantly prolonged in the sham-operated group. The mean number of step down actions in the sham-operated animals was 7 and 1 in the training and test sessions, respectively. Furthermore, the mean total time spent on the grid of the sham-operated animals in the test session was 10 sec, which was 1/18 the duration of the test session period (180 sec). In fact, in the retention test, gerbils stayed on the platform, and they hesitated to step down to the grid floor. The step-down latency in the retention test was significantly decreased in the ischemic group compared to that in the sham-operated group. In contrast, the number of step-down movements and the total time spent on the grid in the ischemic group was significantly increased (data not shown). Most sham-operated gerbils did not step down within 180 sec (Fig. 7).

In the second experiment, these animals were trained again at 11 days after the 5-min occlusion. When the retention test was carried out at 24 hr after the second training,

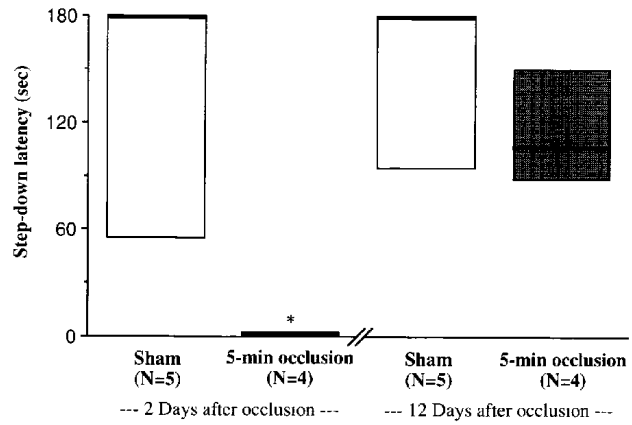

Fig. 7. Effects of 5-min occlusion before training on retention of the passive avoidance task in Mongolian gerbils. Training for the passive avoidance task was carried out 1 day and 11 days after occlusion. Step-down latency in the retention test at 24 hr after the first training (left) and at the 24 hr after the second training (right). Numbers in parentheses represent numbers of gerbils tested. Each value represents the median (horizontal bar) and interquartile range (column). *$P<0.05$, compared to the corresponding sham-operated animals.

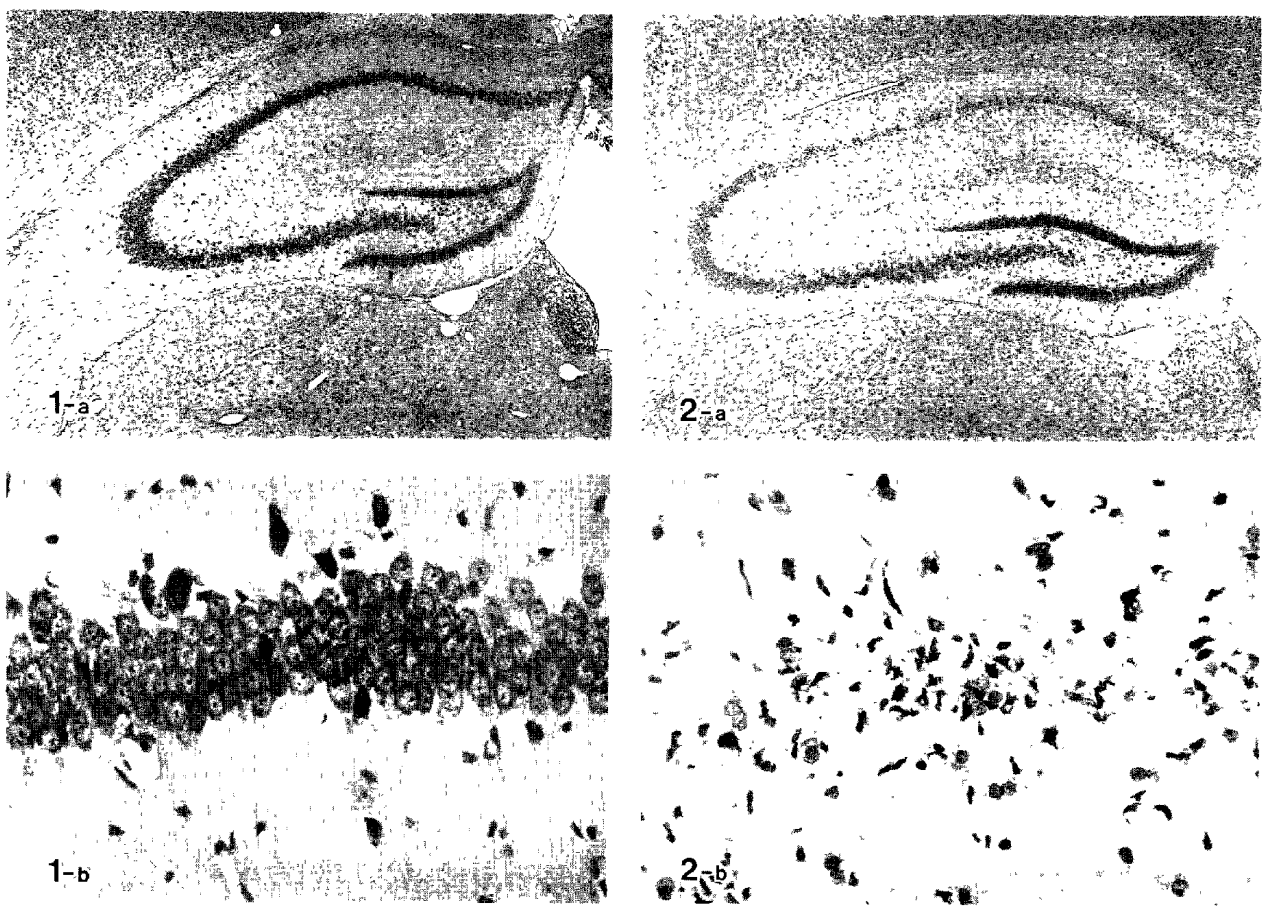

Fig. 8. Typical micrographs of the hippocampus of sham-operated (1-a, 1-b) and ischemic gerbils (2-a, 2-b). 1-a, 2-a: ×30, 1-b, 2-b: ×300.

ischemic effects did not occur, and all groups showed a similar step-down latency (Fig. 7).

Retention test at 10 days after occlusion: The acquisition trial and the first retention test were carried out at 48 hr and 24 hr, respectively, before the occlusion. When a gerbil stepped down onto the grid floor, during the first retention test, it received a foot shock. A second acquisition trial was therefore necessary for some animals. All of the animals stayed on the platform for 180 sec after the first retention test. Although these animals were occluded after training and after the first retention test, they showed the same and maximum (180 sec) step-down latency in the second retention test for the passive avoidance task at 10 days after occlusion (data not shown). Clearly, they had retained the task.

Histological changes in CA1 neurons

Figure 8 shows typical micrographs of the hippocampus of sham-operated (1-a, 1-b) and ischemic gerbils (2-a, 2-b) at 10 days after occlusion. In the sham-operated animals, the entire population of CA1 neurons in the hippocampus was easily observed. At 10 days of recirculation after 5-min occlusion, the CA1 neurons in the hippocampus appeared to be destroyed and were not evident by light microscopic examination.

DISCUSSION

Mongolian gerbils are characterized by frequent anomalies in the circle of Willis (7–10). It is very easy to produce cerebral ischemia in these animals by occluding only their bilateral carotid arteries. Interruption of the blood flow to the forebrain in these animals may be accomplished by common carotid occlusion, while flow to the basal brain via the vertebral arteries remains unimpaired (22). The continuation of basal brainstem function during transient forebrain ischemia insures that spontaneous respiration and ingestive behavior will be maintained. Thus, any behavioral or biochemical alterations observed following ischemia can be attributed to forebrain effects, and not to basal brain or systemic effects (17). Transient forebrain ischemia induces neuronal death in a variety of brain areas in neonates and adults (23–25), the CA1 region of the hippocampus being particularly vulnerable to ischemic damage (26–29). The hippocampus is the brain region responsible for controlling learning and memory behaviors (19–21, 30–33). Therefore, amnesia may be induced by the neuronal damage produced by ischemia in the hippocampal CA1 subfield in gerbils. It could thus be of interest to study the effects of ischemia on learning and memory in the Mongolian gerbil.

These animals, however, have some habits which are undesirable in behavioral studies; and to date, few investigators have used them. In the present study, we attempted to elucidate the characteristics of cerebral ischemia-induced deficits in Mongolian gerbils in several learning and memory tasks, namely, habituation, passive avoidance, and radial maze tasks, which are usually employed in experiments with rats and mice.

Mongolian gerbils exhibit excessive general activity; In fact, when the animals first arrived in our laboratory, it was difficult to weigh them because they were so active. However, after they received handling of 5 min/day and were adapted to the laboratory/colony for more than 5 days (usually for 7 days), it was easier to weigh them. We found that gerbils, like rats and mice, accomplished the 8-arm radial maze task. During the acquisition trial for the passive avoidance task, gerbils repeatedly stepped up and down despite receiving foot shocks from the grid when they stepped down from the platform. Their step-down latency did not increase with single foot shocks, and this result is completely different from that in rats and mice. However, they showed a marked prolongation of step-down latency during the retention test when they received repeated foot shocks for 5 min in the acquisition trial. The results of our present studies are summarized in Table 1.

Five-minute ischemia produced hyperlocomotion at 1 and 2 days after occlusion, but at 10 days after occlusion, this behavior had disappeared. Performance in the habituation, passive avoidance, and radial maze tasks was impaired within 2 days after occlusion. Ten days after ischemia, however, the hyperlocomotion had disappeared and performance in the habituation and passive avoidance tasks was the same as that of the sham-operated group. Furthermore, retention of the passive avoidance task at 10 days after ischemia was the same as that of the sham-operated group. Retention of the radial maze task was impaired at this time, but this impairment was easily overcome, and retention recovered to the sham-operated level after a few additional trials. When the acquisition trial

Table 1. Summary of results

Test item	Stage	Time after 5 min-occlusion	
		~2 days	10 days~
Locomotor activity		↑	±
Habituation task	Acquisition	↓	±
Radial maze task	Acquisition	—	↓
	Retention	↓	↘~±
Passive avoidance task	Acquisition	↓	±
	Retention	—	±
CA1 pyramidal cell		Early phase of death	Death

↑: increase, ±: no effect, ↓: impairment, ↘: slight impairment, —: not tested.

for the radial maze task was begun at 11 days after occlusion, severe learning impairment was found. Neurons in the hippocampal CA1 subfield are subject to the phenomenon of delayed death, that is, destruction of CA1 neurons appears from 2–3 days after ischemic insult, and most CA1 neurons have disappeared by day 7 (34) or 4 (35). We found marked destruction and disappearance of CA1 neurons at 10 days after occlusion. These findings suggest that the impairment of performance in the habituation and passive avoidance tasks within 2 days after occlusion may be related to an early phase of CA1 neuronal death and to hyperlocomotion, although the impairment of spatial learning and memory is clearly associated with CA1 injury 10 days after occlusion. In other words, not all kinds of learning and memory tasks may depend on the hippocampus.

When the acquisition trial for the radial maze task was begun at the time when CA1 neurons disappeared, we found that there was severe impairment of spatial learning. It has been shown that injury to the hippocampus produces impairments of working memory (32, 19–21) and that hippocampal ablation causes spatial memory deficits in the Morris water maze task (30, 31). In our experiment, however, occluded animals seem to acquire the radial maze task to some degree when they are repeatedly trained, since the number of ICR tends to increase. It may be possible that the remaining functional neurons compensate for the function of CA1 neurons. As described in the results, in the sham-operated group, the number of animals reaching the criterion increased with repeated trials; however, in the ischemic group, this number did not increase after the 12 trials, and the animals which reached the criterion were different in each trial. Therefore the hippocampus is concerned with the acquisition and/or formation of spatial memory (33).

It is interesting to note that the memory impairment in the radial maze at 10 days after occlusion was easily overcome, and that performance recovered to the normal level after additional training trials. These results indicate the possibility that spatial memory impairment is based on dysfunction at the retrieval stage, and that remaining functional neurons compensate for those which are destroyed. The present results taken together with these other lines of evidence indicate that the hippocampus may play an important role in spatial learning, as a relay station for the acquisition of spatial recognition and the recall stage of spatial memory, the former role being more important.

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