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Characteristics of Memory Impairment in Cerebral Embolized Rats at the Chronic Stage

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KIYOTA, Y., M. MIYAMOTO AND A. NAGAOKA. *Characteristics of memory impairment in cerebral embolized rats at the chronic stage*. PHARMACOL BIOCHEM BEHAV **28**(2) 243–249, 1987.—The effect of cerebral embolization on learning and memory in rats was studied at the chronic stage (8 weeks or more after embolization). At the chronic stage, embolized rats showed no significant change in emotional behavior, but exhibited an increase in ambulation in the open-field test. Rats with cerebral embolization exhibited marked impairment of the passive avoidance response at the early stage of cerebral infarction, however this gradually diminished and no impairment of the response was observed at the chronic stage. In a two-way active avoidance task, embolized rats showed accelerated acquisition of the avoidance response in comparison with a sham-operated control group. However, at the chronic stage, embolized rats exhibited marked impairment of light-dark discrimination learning. Spatial memory impairment was also observed in embolized rats, as demonstrated by a significant decrease in initial correct responses and an increase in total errors in the radial maze task. Upon microscopic examination, multi-focal necroses were detected in several brain regions, being particularly obvious in the hippocampus and internal capsule of the embolized hemisphere. These results demonstrate that embolized rats show definite impairment of memory and learning at the chronic stage, and suggest that the impairment may be qualitatively different from that observed at the early stage.

Cerebral embolization Passive and active avoidance tasks Discrimination task Radial maze task
Memory impairment Animal model for dementia

IT is generally agreed that senile dementia will soon become a major medical and social issue as a result of the rapid increase in the population of aged people. Dementia can be pathologically classified into two main types, dementia associated with multiple infarction and senile dementia of the Alzheimer type. It is necessary to establish animal models of human dementia for the development of suitable therapeutic drugs. We have previously demonstrated that rats with cerebral embolism, produced by injection of microspheres into the left carotid artery, showed significant impairment of memory and learning in several learning tasks. We proposed that such cerebral embolized rats might be useful as an animal model of vascular-type dementia [11,12]. These impairments were detected at the early stage of cerebral infarction (within 2 weeks after embolization), whereas the impairment of acquisition of the passive avoidance response gradually diminished and had disappeared by the chronic stage (more than 8 weeks after embolization). However, the effects of cerebral embolization on the function of the central nervous system did not seem to have completely disappeared at the chronic stage, as slight cerebral edema and marked decreases in the activity of choline acetyltransferase (CAT) were still observed in the left cerebral hemisphere [16]. In the present study, we attempted to clarify the learning behavior of embolized rats at the chronic stage using different tasks from those used in the previous studies [12]. The results were compared with those obtained at the early stage.

METHOD

Subjects

The subjects were male JCL/Wistar rats weighing 250–300 g, aged 8–10 weeks at the start of the experiments. They were housed five to a cage in stainless steel cages (35×25×20 cm) with free access to food and water. The rats were maintained on a 12-hr light/dark cycle (lights on at 7:00 a.m.) in a temperature- and humidity-controlled room (25±1°C and 55±5%, respectively).

Cerebral Embolization

The procedure used for cerebral embolization has been described in detail in the previous reports [11,12]. Briefly, rats were anesthetized with ethyl ether and the left carotid artery bifurcation was exposed. After ligation of the pterygo-palatine artery, a PE-50 polyethylene tube filled with saline was inserted through the external carotid artery into the common carotid artery. Two thousand carbonized microspheres (35±5 μm in diameter) suspended in 50 μl saline containing 20% dextran were injected into the internal carotid artery through the tube. Sham-operated rats were

injected with the same volume of 20% dextran solution. Behavioral testing was started at 8 weeks after the operation except where otherwise described.

Open-Field Behavior

General behavior of the rats was observed using the open-field test as described by Hall [8]. The apparatus consisted of a circular floor 60 cm in diameter enclosed with a wall 50 cm in height. The floor was divided into 19 equivalent sectors with black lines. The open field was illuminated by a 100-W bulb placed 80 cm above the center of the floor. A rat was introduced into the center of the floor, and the total number of sectors crossed by the rat and its frequency of rearing were counted for 3 min as "ambulation" and "rearing," respectively.

Flinch-Jump Threshold

Nociceptive thresholds were measured as described in the previous report [12]. A rat was placed in the test box (30×30×30 cm) and after a 1-min habituation period, eight shocks of different AC current (0.10–1.0 mA) were applied in ascending order, each for 1.0 sec, at 30-sec intervals through the grid floor. The minimum shock intensity at which the rat exhibited each form of response (flinch, jump and vocalization) was taken as the threshold.

Passive Avoidance Learning

Rats were tested using a step-through-type passive avoidance task [1,12]. The experimental apparatus consisted of two compartments, one (25×10×25 cm) illuminated and the other (30×30×30 cm) dark. In the acquisition trial, a rat was placed in the illuminated compartment and allowed to enter the dark one. As soon as the rat entered the dark compartment, an unescapable footshock (2.5 mA, 3 sec) was delivered through the grid floor. In the retention test, the rat was again placed in the illuminated compartment and the latency to enter the dark compartment was measured. If the rat avoided entering for longer than 300 sec, a ceiling score of 300 sec was assigned. The acquisition trial was carried out 3, 7, 14, 28 or 56 days after embolization, followed by the retention test performed 24 hr after each acquisition trial. Different groups of rats (N=9–13) were used in each test.

Active Avoidance Learning

Rats were trained using a shuttle box-type active avoidance apparatus [3,12], which consisted of two compartments (24×21×22 cm) separated by a stainless steel plate with an opening (9 cm in diameter), through which rats were able to move from one compartment to the other. A buzzer and a lamp were placed on the ceiling of the apparatus as the source of the conditioned stimulus (CS). A rat was placed in one compartment and, after a 5-min habituation period, the CS was presented for a maximum of 5 sec until the rat crossed to the opposite side. If the rat did not cross to the opposite compartment during the presentation of the CS, an unconditioned footshock stimulus (0.8 mA) was delivered through a grid floor for a maximum of 5 sec until the rat crossed to the opposite side. Rats were given one daily session of 30 trials with a 30-sec intertrial interval for 6 consecutive days. Training was started at 8 weeks after the operation. Three parameters were recorded: "pre-training activity," "intertrial response" and "avoidance response" being expressed as the number of crossings occurring during the 5-min habituation period, during the intertrial interval and during the presentation of the CS, respectively.

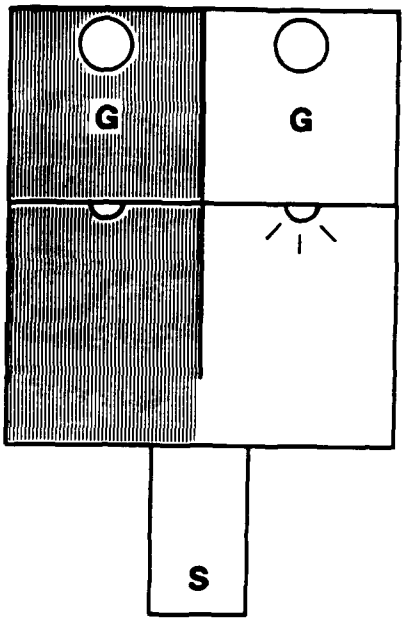

FIG. 1. Schema of apparatus used in the discrimination learning task. The apparatus consisted of a start box (S) and two goal boxes (G) with an entrance (7×7 cm) between them. Pilot lamps were fitted above the entrances of the goal boxes.

Light-Dark Discrimination Learning

The experimental apparatus consisted of a start box (28×13×19 cm), two goal boxes (30×30×25 cm) and a choice area (40×60×25 cm) as shown in Fig. 1. Rats were able to move freely in the appartus through openings (7×7 cm) between the compartments. A food cup was placed in each goal box and a pilot lamp was installed above the opening of each goal box. One of the lamps was lit during each trial, the illuminated side being chosen randomly. The rats were adapted to 80% of initial body weight by a food deprivation regime for 2 weeks before the start of training. A rat was placed in the start box and the guillotine door (7×7 cm) of the start box was raised 5 sec after placement. When the rat chose either goal box, the trial was terminated. Half of the rats were able to obtain a food pellet (45 mg) in the illuminated goal box; the others were able to obtain a pellet in the dark box. Each rat was given a block of 10 trials daily for 8 days. Correct response was expressed in terms of the percentage of correct choices per 10 trials. In addition, the response latency in choosing either goal box after opening the guillotine door was recorded.

Radial-Arm Maze Learning

The apparatus was a radial 8-arm maze set 33 cm above the floor [18]. The arms (10×80 cm) with 2.5-cm-high walls projected from an octagonal center platform (36 cm across). The rats were adapted to 80% of initial body weight by a food deprivation regime for 2 weeks before the start of training. In each test, one pellet of food (45 mg) was placed in a food cup located at the end of each arm. A rat was placed in the center of the apparatus and allowed to choose freely among all the arms. Throughout the training period, the positions of

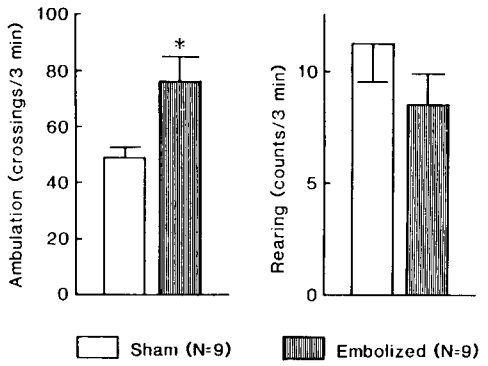

FIG. 2. Ambulation and rearing in the open-field test. Behavioral testing was conducted at 8 weeks after the operation. The values represent the frequencies of each form of behavior, and are shown as mean±S.E. *$p<0.05$, compared with sham-operated rats.

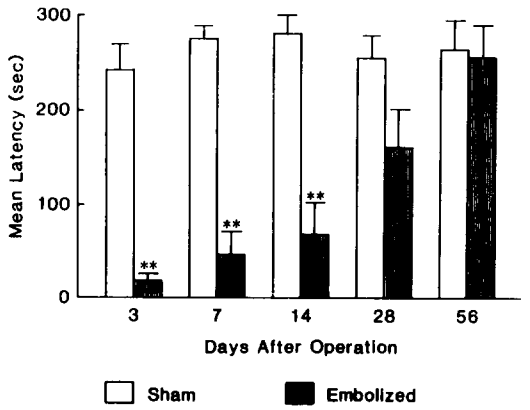

FIG. 3. Acquisition of passive avoidance response at various times after cerebral embolization in rats. Different groups of rats were used in each retention test. The retention test was performed 24 hr after the acquisition trial. **$p<0.01$, compared with sham-operated rats.

extra-maze cues (e.g., table, chair, rack, door, lamp, observer, etc.), were held constant. Each rat was given one daily trial for 18 days and the 18 trials were divided to 6 training blocks. We measured two parameters: "initial correct response" expressed as the number of initial consecutive choices without error, and "number of errors" expressed as the number of errors made until the rat got all the pellets of food.

Histology

Rats were anesthetized with pentobarbital and were perfused with 10% formalin through the left cardiac ventricle at one or 8 weeks after embolization. The brains were removed and kept in 10% formalin for at least 7 days. Brains were frozen and sectioned coronally at 40 μm with a frozen-stage microtome. Sections taken every 200 μm were stained with cresyl violet.

Statistics

Student's t-test, Mann-Whitney U-test (two-tailed) and analysis of variance (ANOVA) followed by Newman-Keuls test were used for statistical analysis.

TABLE 1

NOCICEPTIVE THRESHOLDS IN EMBOLIZED RATS AT THE CHRONIC STAGE

	Flinch	Jump	Vocalization
Sham	0.147 ± 0.01	0.229 ± 0.02	0.239 ± 0.01
Embolized	0.148 ± 0.01	0.234 ± 0.01	0.244 ± 0.01

Nociceptive thresholds were measured 8 weeks after cerebral embolization. Each value is the mean ± S.E. (mA) for 9 rats.

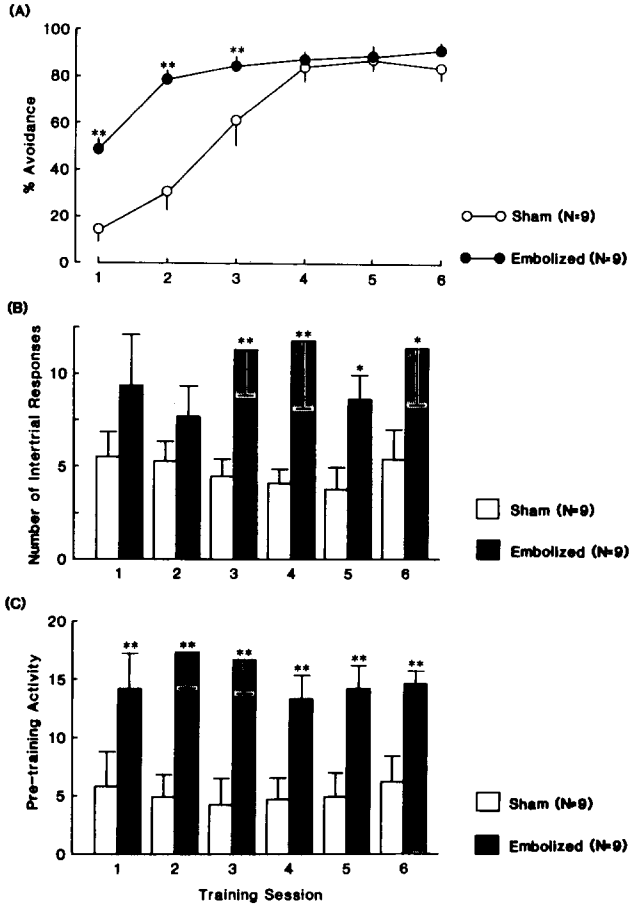

FIG. 4. Effects of cerebral embolization on active avoidance response in rats. Training was started at 8 weeks after the operation. The values represent the percentage of avoidance response (A), the number of intertrial responses (B), and activity during the pretraining habituation period (C), as means±S.E. *$p<0.05$, **$p<0.01$, compared with sham-operated rats.

RESULTS

General Behavior

The numbers of crossings and rearings in the open-field test are shown in Fig. 2. The embolized rats showed a significant increase in ambulation ($t=2.60$, $p<0.05$) but not in rearing, compared with the sham-operated controls. There were

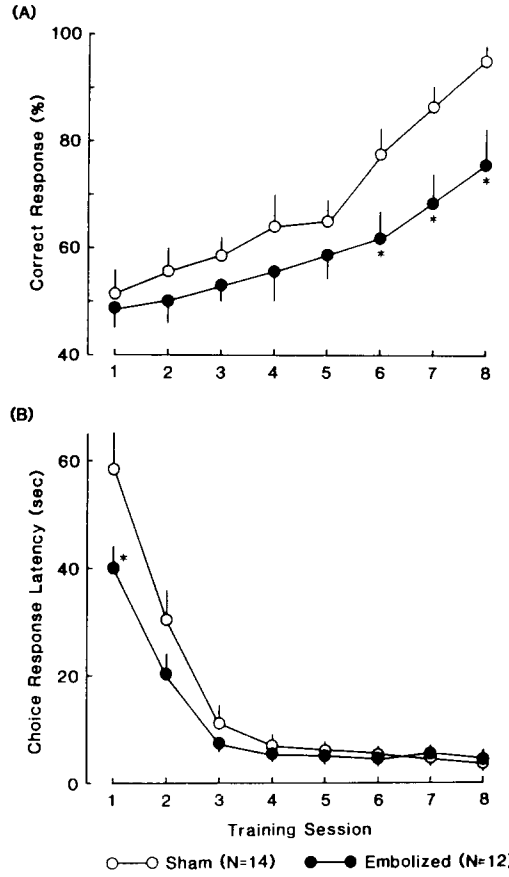

FIG. 5. Effects of cerebral embolization on discrimination learning in rats. Training was started at 8 weeks after the operation. The values represent the percentage of correct responses (A), and the choice response latency (B), as means±S.E. *$p<0.05$, compared with sham-operated rats.

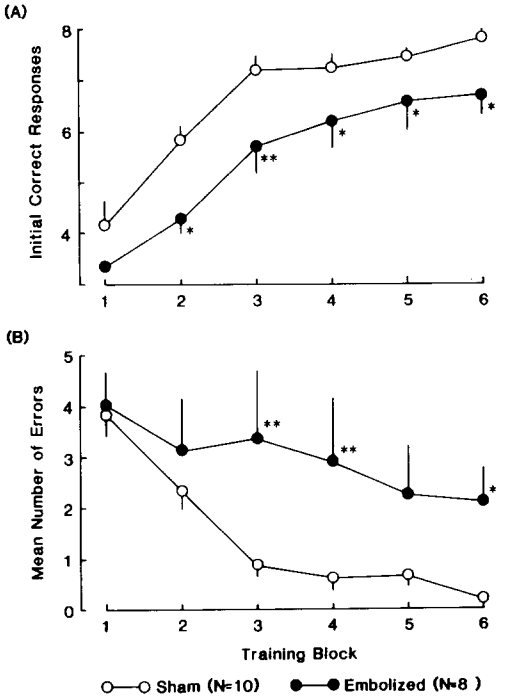

FIG. 6. Effect of cerebral embolization on radial maze performance in rats. Training was started at 8 weeks after the operation. The values represent the number of initial correct responses (A), and the number of total errors (B), as means±S.E.. *$p<0.01$, compared with sham-operated rats.

no differences between the two groups with regard to nociceptive threshold (Table 1).

Passive Avoidance Learning

Sham-operated control rats showed long response latencies on each test day. In contrast, the response latencies in embolized rats were significantly shorter than those of the controls when tested 3 ($U=1$, $p<0.01$), 7 ($U=7.5$, $p<0.01$) and 14 days ($U=10$, $p<0.01$) after embolization. However, the shortened response latencies gradually increased to the level of the control rats, and a normal avoidance response was seen 56 days after embolization (Fig. 3).

Active Avoidance Learning

As shown in Fig. 4, the percentage of avoidance response increased with training in both the sham-operated and embolized rats as shown by a significant effect of the trial blocks, $F(5,80)=6.17$, $p<0.01$. However, the percentage of avoidance response in embolized rats was abnormally high for the first 3 training sessions. Significant differences between the two groups were observed, $F(1,16)=11.2$, $p<0.01$. Follow-up Newman-Keuls comparisons revealed that the embolized group had significantly greater avoidance responses compared with the control group for sessions 1 through 3 inclusive, $p<0.01$. As shown by a significant interaction between group and the trial sessions, $F(5,80)=10.5$, $p<0.01$, significant differences between the two groups were observed only in the early phase of training (Fig. 4A). Embolized rats also showed significant increases in both intertrial responses, $F(1,16)=8.07$, $p<0.05$, and pre-training activity, $F(1,16)=13.8$, $p<0.01$, as shown in Fig. 4B and 4C, respectively.

Light-Dark Discrimination Learning

The percentage of correct responses increased gradually with training in the two groups, $F(7,168)=26.3$, $p<0.01$. Embolized rats showed lower choice accuracy than did the sham-operated control rats at each session and a significant difference between the two groups was found, $F(1,24)=4.48$, $p<0.05$ by ANOVA. Follow-up comparisons showed significant differences between the two groups for the last 3 sessions, $p<0.05$ by Newman-Keuls test (Fig. 5A). By the 8th session, most of the control rats showed perfect discrimination, whereas half of embolized rats showed a chance level of correct responses. However, there was no difference between the two groups in the choice response latency, $F(1,24)=1.99$, $p>0.05$ (Fig. 5B).

Radial-Arm Maze Learning

The number of initial correct responses gradually increased with training in the two groups, $F(5,80)=38.4$, $p<0.01$, but in all training blocks embolized rats showed lower values than did the sham-operated rats, a significant difference was found between the two groups, $F(1,16)=5.30$,

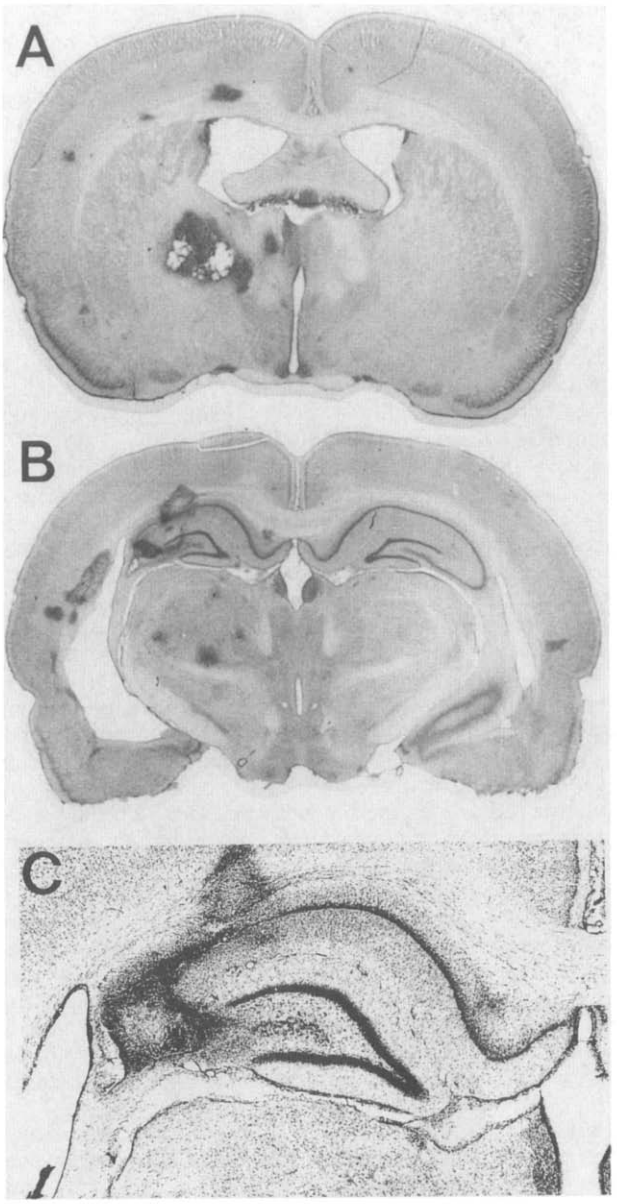

FIG. 7. Photographs of coronal sections of rat brain taken 1 week after cerebral embolization (A and B). Photomicrograph (C): a coronal section of the left hippocampus of a cerebral embolized rat. The sections were stained with cresyl violet after fixation with 10% formalin.

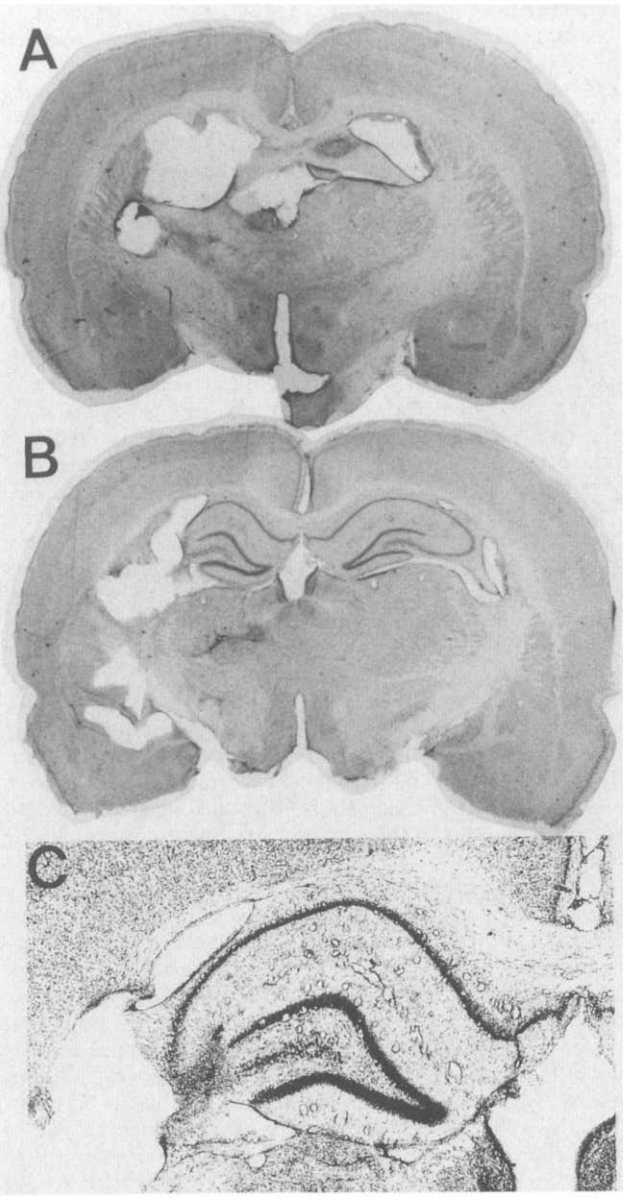

FIG. 8. Photographs of coronal sections of rat brain taken 8 weeks after cerebral embolization (A and B). Photomicrograph (C): a coronal section of the left hippocampus of a cerebral embolized rat. The sections were stained with cresyl violet after fixation with 10% formalin.

$p<0.05$. In addition, follow-up comparisons by Newman-Keuls test showed significant differences between the two groups from block 2 through block 6 ($p<0.05$ or $p<0.01$, Fig. 6A). The number of errors made by embolized rats was significantly greater than that made by sham-operated rats, $F(1,16)=7.89$, $p<0.05$, and Newman-Keuls comparisons showed that embolized rats had significantly increased numbers of errors at blocks 3, 4, and 6 ($p<0.05$ or $p<0.01$, Fig. 6B).

Histology

Abnormally stained spots, indicating necroses induced by embolization, were observed in several regions of the brains of rats embolized 1 week previously. The spots were located mostly in the hippocampus, internal capsule, cortex and their surrounding areas in the embolized left hemisphere (Fig. 7A and 7B). In particular, there was a high frequency of damage to the CA3 subfield of hippocampal pyramidal cells (Fig. 7C). Enlargement of the lateral ventricle indicating atrophy of the hippocampus was also observed in the embolized hemisphere at 8 weeks after embolization (Fig. 8A and 8B).

DISCUSSION

It was demonstrated in our previous study that rats which

had been subjected to cerebral embolization by injection of microspheres into the left internal carotid artery, showed significant memory impairment in several learning tasks; we suggested that such rats might be useful as an animal model of vascular-type dementia [11,12]. With regard to the passive avoidance task, however, the degree of impairment was found to decrease after embolization, although brain damage was considered likely to remain even in the chronic stage. Therefore, in the present study, we attempted to further clarify the changes occurring in the learning ability of cerebral embolized rats at the chronic stage using different learning tasks.

In the chronic stage, embolized rats showed a significant increase in locomotor activity with no change in rearing behavior. There was also no difference in shock sensitivity between sham-operated and embolized rats. Facilitation of the active avoidance response was observed in embolized rats associated with an increase in intertrial responses and pretraining activity; this is in contrast to the marked degree of impairment at the early stage [12]. It is known that rats with septal lesions show similar facilitation of performance, which may be mainly due to their hyperactivity [14,20]. Similarly, in embolized rats, hyperactivity may, to some extent, be involved in performance facilitation. Another possibility is that disruption of the behavioral inhibition mechanism in the brain may lead to facilitation of the active avoidance response. The impairment of both active and passive avoidance learning may only be slight in this animal model at the chronic stage. However, significant impairment of light-dark discrimination and radial maze learning was detected in embolized rats at this stage. These results suggest that definite impairment of memory and learning remains at the chronic stage.

Cerebral necroses were observed in several regions of the ipsilateral hemispheres but not on the contralateral side in embolized rats. The changes were located in the hippocampus, internal capsule, deep layer of the cortex, and their surrounding areas. In some cases, the hippocampal damage was observed as a loss of pyramidal cells. It is known that transient cerebral ischemia in man and experimental animals induces selective CA1 pyramidal cell loss [5,19]. In our models, cell loss was observed particularly in the CA3 region. This difference in the regions effected may be due to the distribution of vasculature in the hippocampus.

It is considered that central cholinergic mechanisms are greatly involved in memory and learning, and there is a considerable weight of pharmacological, neurochemical and electrophysiological evidence to support this hypothesis [4, 6, 21]. Major cholinergic inputs to the cerebral cortex and hippocampus originate in the basal forebrain (the nucleus basalis of Meynert in primates) [10,23] and in the medial septum [7,13], respectively. Animals with lesions of these pathways show marked impairment of memory and learning, which also supports the existence of a cholinergic contribution to memory [9,15]. It is interesting that necroses in cerebral embolized rats were located in the hippocampus, one of the major cholinergic terminals, and in the internal capsule involved in the basal forebrain, one of the major cholinergic origins. The memory impairment observed in embolized rats may be due to central cholinergic damage induced by cerebral embolization. However, deterioration of other neural systems might also contribute to the memory impairment in these rats. For example, dysfunction of the striatal dopaminergic system might be at least partially involved in memory impairment, since the nigro-striatal dopaminergic pathway runs through the internal capsule which is preferentially damaged by embolization. The finding that embolized rats showed a low level of dopamine in the striatum (unpublished data) supports this possibility.

The results of the present study suggest that embolized rats have qualitatively different deficits in learning and memory at the early and the chronic stages. At the early stage, passive and active avoidance responses and water maze performance were markedly impaired, but at the chronic stage, there was no impairment in either avoidance task and facilitation was observed in the active avoidance task. However, there were significant deficits in the discrimination task and in the radial maze task at the chronic stage. This qualitative difference could be explained by chronological changes in CAT activity in the forebrain, that is, the activity was markedly decreased in both the cortex and hippocampus at the early stage, but decreased activity was observed only in the hippocampus at the chronic stage [16]. In particular, the impairment shown in the radial maze task, which requires working memory, may be related to the decrease in the hippocampal cholinergic activity, since working memory is markedly disturbed by damage to the septo-hippocampal cholinergic system [2, 17, 22]. Additionally, the impairment of avoidance learning shown at the early stage may be related to the decrease in cortical cholinergic activity. This seems to be supported by evidence that lesioning of the basal forebrain, which projects cholinergic nerve fibers to the cortex, leads to a marked degree of impairment in passive and active avoidance tasks [15].

In conclusion, cerebral embolized rats have been shown to have memory and learning impairment even at the chronic stage. The impairment is qualitatively different from that observed at the early stage suggesting that such embolized rats may be useful as an animal model of vascular-type dementia.

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