Behavioral Effects of Transient Cerebral Ischemia

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CA1 neurons in the hippocampus, a brain structure involved in learning and memory, are selectively vulnerable to ischemic effects. In this study, the authors examined if duration of ischemia is directly related to extent of CA1 damage and degree of spatial learning deficit. Adult female Wistar rats received either 5-min or 10-min ischemia or sham surgery. Following recovery, rats were tested in the Morris water maze. Histological analysis showed moderate cell loss in CA1 (31%) and CA3 (12%) and minimal cell loss in CA2 (4%) with 5-min ischemia. Increased cell loss was seen in CA1 (68%), CA2 (16%), and CA3 (23%) with 10-min ischemia. Behavioral testing revealed that animals with 10-min ischemia have greater spatial learning deficits and they remain impaired across the test days compared to the 5-min ischemic group. Furthermore, degree of CA1 cell loss accounted for approximately 45% of the variance in spatial learning deficits in the ischemic group. The authors conclude that cell loss is largely confined to CA1 region in rats who received 5 and 10 min of ischemia and that increased ischemic duration results in persistent learning deficits in female rats; also, the degree of behavioral impairment is related to extent of CA1 cell loss.

Key words: *Hippocampus, cerebral ischemia, learning and memory, female rats*

The normal adult human brain uses 15% of cardiac output for energy to sustain electrical and metabolic activities and other functions (Meyer 1993). Because the brain has decreased glucose and oxygen stores and uses aerobic metabolism exclusively, it is highly vulnerable to fluctuations in blood flow. Cerebral ischemia induced by severe transient reductions of cerebral blood flow (CBF) results in selective damage to vulnerable groups of neurons, namely, the CA1 pyramidal neurons of the hippocampus (Martone and others 1999; Olsen and others 1994; Tabuchi and others 1995), a structure believed to be involved in learning and memory. Clinical conditions such as cardiac arrest and traumatic brain injury can cause transient cerebral ischemia. Duration of ischemia in the clinical setting may be time limited because of increasing progress in the resuscitation of cardiac arrest and head-injured victims; thus, it is particularly important to determine the "shortest" duration of ischemia that would result in persistent behavioral impairment. Additionally, it is important to know if the functional consequences of cerebral ischemia seen in male animal models are the same as in females.

Clinically, the link between ischemic hippocampal damage and learning and memory impairment was established after a thorough postmortem histological

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examination of the patient R. B. This patient developed anterograde amnesia following massive blood loss (5000 ml) during coronary artery bypass procedure (Zola-Morgan and others 1986). Subsequent clinical studies have confirmed that global cerebral ischemia results in selective memory loss and difficulties in learning, in the absence of intellectual impairment (Auer and others 1989; Nunn and others 1994; Volpe, Pulsinelli, and others 1992); this clinical landmark finding prompted the development of a number of animal models of global cerebral ischemia. Here we report the results of the behavioral consequences and histological analysis of 2 time periods of transient global cerebral ischemia.

Literature Review

Cognitive impairments in ischemic animals have been found largely to conform to patterns of deficits shown by animals with electrolytic hippocampal lesions (Davis and Volpe 1990). This is hardly surprising since the hippocampus is the brain area of predilection for ischemic damage, and within the hippocampus, CA1 pyramidal neurons are selectively vulnerable to reductions in blood flow (Martone and others 1999; Meldrum 1990; Olsen and others 1994). This pattern of "selective vulnerability" holds across almost all species, including humans. Numerous reports have shown that damage to the hippocampus following cerebral ischemia is associated with deficits in spatial learning when animals must use allocentric (focuses on the spatial relationships of elements in the environment to each other) cues for efficient solution of navigational task. It has been demonstrated that the CA1 region of the hippocampus is the major area of damage seen in ischemic rats subjected to either 15-20 min of 2-vessel (2-VO) or 4-vessel occlusion (4-VO) and that these animals show impairment in the acquisition of water maze place task (Hagan and Beaughard 1990; Jaspers and others 1990; Nunn and others 1994), T-maze (Volpe, Davis, and others 1992), and the 8-arm radial maze (Kiyota and others 1991; Volpe, Pulsinelli, and others 1992) task. These studies provide converging evidence that the hippocampal CA1 region is the most consistent area that is damaged in animals demonstrating behavioral impairment following cerebral ischemia. Thus, it is likely that the behavioral deficits seen in rats subjected to transient global cerebral ischemia are primarily a function of damage to the hippocampal CA1 region.

Attempts to establish quantitative relationships between extent of CA1 cell loss and degree of spatial learning impairment reported inconsistent results. There are few reports of positive correlations between performance deficit in the water maze (Rod and others 1992), 8-arm radial arm maze (Kiyota and others 1991), and T-maze (Volpe, Davis, and others 1992) and extent of CA1 cell loss. On the other hand, others found no correlations between extent of CA1 damage and degree of behavioral impairment in the 8-arm radial maze (Lyeth and others 1990) and water maze (Netto and others 1993; Nunn and others 1994). The conflicting findings reported in these studies may be attributed to differences in the complexity of the behavioral task (some investigators used a battery of spatial learning tests while others used only place learning tasks) and the statistical procedures (some reports included sham animals in the correlational analysis while others did not) used.

Taken together, these studies provide evidence that a general relationship exists between CA1 cell loss following cerebral ischemia and behavioral deficits. However, the prolonged duration of ischemia used in these studies does not take into account the possibility of early resuscitation, although these ischemic animal models were designed to simulate cardiac arrest. That is, ischemic duration in a clinical context may be time limited because of increasing progress in resuscitation efforts in cardiac arrest and traumatic head-injury victims. Thus, it is important to determine the "shortest" duration of ischemia that would result in persistent behavioral impairment, which may be a more representative model of the human circumstance that nurses encounter clinically. Additionally, results regarding learning and memory impairments seen in these prolonged ischemia models must be interpreted with caution since these animals have extrahippocampal damage. Furthermore, even though evidence exists that female animals are more impaired in the spatial learning tasks in the water maze compared to their male counterparts following electrolytic hippocampal lesions (Therrien 1982), no studies have been done on cerebral ischemia using female animal models. Thus, it is not known whether gender differentially affects the behavioral impairments seen following cerebral ischemic damage. In this study, we were interested in determining (1) the duration of ischemia in female rats that would result in confined damage to the hippocampus, and (2) if the extent of CA1 damage and degree of spatial learning deficits are related to the duration of cerebral ischemia.

Materials and Methods

Sample

Twenty-one adult female Wistar rats weighing 230-275 g at the time of surgery were included in the study. All animals were housed individually in a temperature-controlled environment (25 °C) with free access to standard rodent food and water. The animals were handled daily and maintained on a reverse light-dark cycle (lights on at 6 PM and off at 6 AM). Behavioral testing took place during the animals' active period, which is between 11 AM and 1 PM.

Surgery

Animals were randomly assigned to receive either ischemic lesions ($n = 13$) or sham surgery ($n = 8$). In addition, animals in the ischemic lesion group were further randomized to receive either 5 min ($n = 6$) or 10 min ($n = 7$) cerebral ischemia. From a clinical perspective, it is important to know the "shortest" duration of ischemia that would result in persistent behavioral deficit; thus, these ischemic periods were used in this study. Existing global ischemia models show that ischemic periods greater than 10 min result in damage not only in the hippocampus but also in extra-hippocampal areas; thus, it would be difficult to make conclusions regarding behavioral effects. On the other hand, ischemic periods of less than 5 min do not produce persistent behavioral impairment (Nunn and others 1994).

Using an adaptation of the 4-VO technique (Pulsinelli and Brierly 1979), surgery to induce transient global cerebral ischemia was performed across 2 consecutive days. On the 1st day, once animals were anesthetized, the neck was shaved using a razor and the skin disinfected with Betadine followed by 70% alcohol using appropriate scrubbing in a circular pattern. In addition, Lacrilube was put in the animals' eyes to prevent drying. After preparing the animals, they were placed supine and both upper teeth hooked to an attachment in the stereotaxic table to keep the airway open. A specialized cone was used to provide continuous general gas anesthesia (2.5% isofluorane and 30% oxygen). If additional anesthetic was needed during surgery, isofluorane was increased to 3%. Temperature was monitored and maintained at 37 °C throughout surgery and during the recovery period with the use of a heating blanket. Using a stereomicroscope, both common carotid arteries were isolated via a ventral, midline cervical incision. A silastic ligature was then loosely placed around each common carotid artery without interrupting blood flow, then the incision was closed using silk sutures with the ligatures projecting through the skin. Following isolation of both common carotid arteries, animals were then placed in a prone position. An incision, approximately 1 cm in length, was made behind the occipital bone directly overlying the first two cervical vertebrae. Through this incision, the paraspinal muscles were separated from the midline and again with the use of the stereomicroscope, the left and right alar foramina of the first cervical vertebrae were exposed. Because the rat's vertebral arteries travel within the cervical canal and pass beneath the alar foramen before entering into the posterior fossa, a 0.05 mm diameter electrocautery needle was inserted through each alar foramen and both vertebral arteries were electrocauterized and permanently occluded. Following cauterization, layers of muscle and skin were closed with silk sutures and the animals kept warm with a heating pad until fully awake. On the second day, animals were physically restrained (immobilized by holding them firmly in the back of the neck) while awake and the silastic ligatures were tightened one after the other to occlude both common carotid arteries. The timing of carotid occlusion was done using a stopwatch and was started once both ligatures were tightened. Following carotid occlusion, the animals were monitored for loss of righting reflex indicating a decrease in cerebral blood flow by 10 ml / 100 g / min. Once the predetermined period of occlusion (5 or 10 min) was achieved, the silastic ligature was gently removed to immediately restore cerebral blood flow. The animals were then kept warm using heating pads. Animals were continuously monitored for return of the righting reflex and until fully awake. Once ambulatory, animals were returned to their respective cages and assessed daily for postoperative complications. A total of 26 animals were used, and 5 were excluded from the study because they (1) did not lose righting reflex (indicating incomplete ischemia) during carotid occlusion ($n = 2$), (2) developed seizures following carotid occlusion ($n = 1$), (3) had excessive weight loss ($n = 1$) following carotid occlusion (< 20% of preoperative weight), and (4) developed paralysis ($n = 1$). The control animals were subjected to sham surgery, which consisted of a neck incision without manipulation of the carotid arteries and an incision behind the occipital bone without electrocauterization of vertebral arteries.

Behavioral Testing

The behavioral procedure for this study tested the animals' learning ability and was initiated following 4 days of recovery from surgery to allow for the significant evolution of ischemic damage (Colbourne and others 1999; Hu and others 1998; Martone and others 1999; Yamamoto and others 1990). This procedure was adopted from the study on place learning and disorientation (Therrien 1982). In this test, an animal swims in a large tub to locate a platform submerged beneath the water's surface that it can neither see, smell, nor hear. This adaptation of the Morris water maze is an excellent apparatus for testing large-scale spatial learning ability, allowing for complete control of even the most subtle intramaze cues while offering any number of directional choices at any given point in the tank. The testing apparatus, a large circular tub made of galvanized steel, measures 1.52 meters in diameter, and the interior surface is painted white. During testing, the tub was filled with tepid water (20 ± 2 °C) and made opaque by the addition of powdered milk. An inverted white flower pot, submerged 2 cm beneath the water's surface, served as the goal/platform and allowed the animals the means for escape. Extramaze cues such as overhead lighting, windows, and room noise were held constant during testing; furthermore, the animal cages, principal investigator, and a second investigator served as fixed distal cues.

Before behavioral testing was begun, a single swim trial was conducted wherein the animals were allowed to swim for 10 s and then placed on the hidden platform located away from any of the assigned locations used for testing. Rats swim well but do not like water. This pretrial swim was done so that all animals learned that a means of escape from the water is available in the tub. All animals received 4 trials per day for a total of 6 days to establish a learning curve. For each trial, rats were placed in different randomly chosen starting points (north, east, west, and center of the maze) while the platform remained in the same location throughout the test days. Each trial lasted either until the rat had found the platform or for a maximum of 180 s (to prevent fatigue). All rats were allowed to rest on the platform for 30 s. A minimum of 2 min was used between trials to provide a rest period for the animals and avoid "practice effect." In addition, all animals were placed in a covered holding cage between trials. Swim latency (time to locate the platform) in 10ths of seconds was recorded by the investigator. First, one of the investigators placed the animal in the water with its nose facing the side of the tub, then used a stop watch to record the time from release of the animal until both its front paws touched the hidden platform. Then, the second investigator manually recorded the paths the animals took to reach the goal on a schematic drawing of the tub during swim time, noting the initial direction taken (directional heading) once the animal straightened its body and moved forward for a distance of 1 body length. From the directional heading, directional heading error (a measure of divergence from the direct path to the goal) was computed by measuring the angle, in degrees, between the heading and a straight line drawn between the goal and the starting point. Thus, a score of zero degrees indicates that the path taken by the animal would lead directly to the goal. This manual recording procedure has been successfully documented to provide a reliable representation of swimming routes (Sutherland and others 1983; Therrien 1982).

Histology

Following completion of testing, all animals were overdosed with sodium pentobarbital and perfused through the heart with isotonic saline solution followed by 10% neutral phosphate buffered formalin (pH = 7.4). The brains were then removed and stored in 10% neutral phosphate buffered formalin and cryoprotectant (30% sucrose solution) for approximately 1 week. Using standard embedding technique, the brains were dehydrated with alcohol and embedded in paraffin. The brains were then cut into 5 μm coronal sections. Every 30th brain section was mounted on a gelatinized slide, which resulted in 5 sections of quantifiable levels of cerebral cortex, hippocampal formation, and striatum. The cerebral cortex and hippocampal formation were quantified at 5 levels (Paxinos 1997)—7.2, 6.2, 5.7, 4.2, and 3.2 mm from the interaural line—and at 1 level in striatum (5.7 mm from the interaural line).

The brain sections were then stained with hematoxylin and eosin and neuronal cell loss was quantified using the Leica Q500 MC image analysis program (Cambridge, UK) with an Olympus microscope at a magnification of 40x. Mean neuronal cell loss was calculated by determining the ratio of degenerated to viable neurons. A viable neuron was defined as a neuron with a clear and distinct nucleus, and neurons with shrunken cell bodies were defined as degenerated. Using the image analysis program, tissue sections were placed under the microscope and projected on a monitor. Once the brain area for analysis was projected in the monitor, measurements were performed on the region contained within the *measure frame* (rectangular box overlaid on the displayed image); the measure frame was calibrated at 100 pixels (1 pixel = 1 μm). The measuring frame was moved over the entire region of the structure being examined for each target tissue section (right and left and 5 coordinates for each animal). An average of 45 to 50 measuring frames were analyzed for each animal, with control and ischemic animals having comparable numbers of measuring frames. Means for all the variables were calculated for each rat from the values obtained from the 5 coronal sections.

Statistical Treatment

Independent *t* test was used to analyze the extent of cell loss following ischemic damage. Analysis of variance with repeated measures was used to examine behavioral performance in the water maze, and post hoc comparisons were done using the Scheffé test. To quantify the relationship between CA1 cell loss and behavioral deficits, Pearson product-moment correlations were performed on the percentage of CA1 cell loss and behavioral performance as measured by swim latency and directional heading error on consecutive test days. Finally, to determine if extent of CA1 cell loss can predict the degree of spatial learning impairment, regression analysis was performed.

Table 1. Histological Comparison of Neuronal Cell Loss in All Groups

Area Damaged	Shams (n = 8)	5 Min (n = 6)	10 Min (n = 7)
Hippocampus	None	16% ± 0.50%	36% ± 2.52%
Cortex	None	None	None
Striatum	None	None	None

NOTE: Mean ± *SEM* (standard error of the mean).

Results

Histological Analysis

Sham-operated animals showed no cell loss in any brain region examined, while ischemic rats demonstrated cell loss confined to the hippocampal area (Table 1).

Rats exposed to 5-min ischemia showed cell loss in the dorsal region of the hippocampus ranging from 15% to 20%, while those exposed to 10-min ischemia had cell loss ranging from 29% to 44%. Extent of cell loss in the hippocampal area in the 2 ischemic groups was significantly different (t_{12} = –4.78, $P < 0.05$) in that animals who received 10 min of 4-VO ischemia had increased cell loss compared to rats who received 5 min 4-VO ischemia. These results showed that extent of total hippocampal damage more than doubled as the duration of ischemia was increased (mean of 16% ± 0.50% for 5 min vs. mean of 36% ± 2.52% for 10 min).

Comparison of damage to hippocampal subregions in the 5- and 10-min ischemic groups revealed varying extent of cell loss in the different regions as shown in Figures 1 and 2. More specifically, 10-min ischemia produced significantly increased (t_{12} = –7.08, $P < 0.01$) damage to viable neurons in the CA1 region compared to 5-min ischemia (68% ± 3.01% vs. 31% ± 3.00%, respectively).

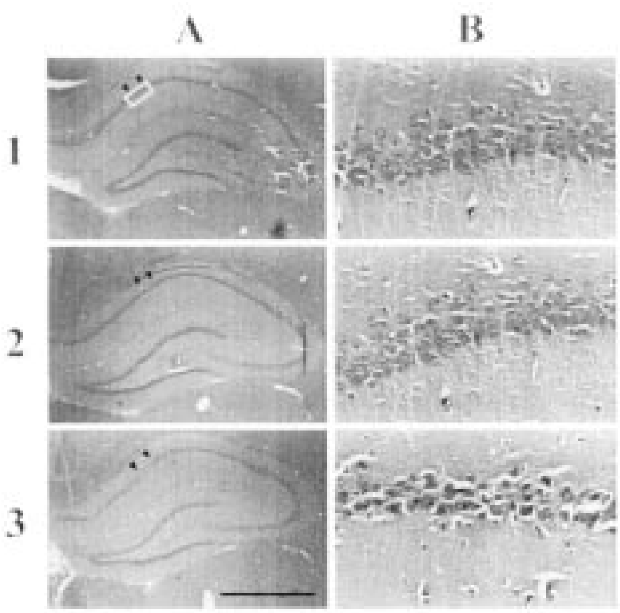

Figure 1. Photomicrographs of representative coronal sections (6.2 mm from interaural line) of dorsal hippocampus in sham-operated (1), 5-min (2), and 10-min (3) ischemic rats. (Column A: 4x objective; Column B: 40x objective). In column A, note the decreased cell density in the CA1 region (arrowheads) of the 10-min ischemic rats, whereas no notable difference can be seen in the 5-min ischemic rats compared to shams. Column B is a magnification of the area within the rectangular frame illustrating viable CA1 neurons with a clear and distinct nucleus in the sham and 5-min ischemic animals. In contrast, there are fewer viable CA1 neurons seen in the 10-min ischemic rats. Scale bar = 100 μm.

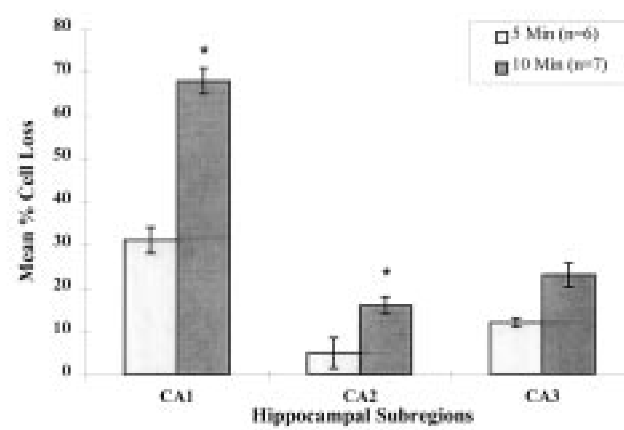

Figure 2. Mean percentage of cell loss in the hippocampal subregions for rats subjected to 5- and 10-min 4-VO ischemia. *$P < 0.05$.

Table 2. Hemisphere Comparison of Mean Percentage of Cell Loss in the Hippocampus of Ischemic Animals

	Hemisphere	5 Min (Cell Loss)	10 Min (Cell Loss)
CA1	Left	35 ± 4.41	70 ± 3.26
	Right	34 ± 3.79	68 ± 2.84
CA2	Left	5 ± 2.52	15 ± 2.24
	Right	6 ± 2.40	15 ± 2.40
CA3	Left	13 ± 0.67	22 ± 2.69
	Right	11 ± 1.20	24 ± 2.66

Furthermore, comparison of damage in the CA2 region also showed significant differences ($t_{12} = -3.17$, $P < 0.05$) between the two ischemic groups in that 10-min ischemia produced 3 times as much cell loss compared to the 5-min ischemic rats (16% ± 2.03% vs. 5% ± 3.5%, respectively). Although appreciable damage to the CA3 region was seen under both 5- and 10-min ischemic conditions (12% ± 1.00% vs. 23% ± 2.71%, respectively), no difference was found in the extent of cell loss between the 2 ischemic groups. Although both 5- and 10-min ischemic durations produced damage to the hippocampus proper (CA regions), limited cell loss was seen with shorter duration of ischemia. None of the ischemic rats exhibited unilateral neuronal damage, and as can be seen in Table 2, very little left-right hemisphere variability was seen in terms of hippocampal cell loss.

Behavioral Effects of Cerebral Ischemia

Swim latency. The swim latency profiles of all groups are parallel ($F_{10/28} = 1.78$, $P = 0.11$), suggesting no interaction effect between test days and ischemia effect. Ischemic rats have significant increase in mean swim latency ($F_{2,18} = 4.65$, $P < 0.05$) compared to sham-operated animals, as shown in Figure 3A, suggesting that it took them more time to locate the goal. A change over time was also evident ($F_{5,14} = 7.21$, $P < 0.05$), which suggests that all groups demonstrated a learning curve. A significant amount of learning took place on day 1 ($F_{2,18} = 15.99$, $P < 0.001$) and day 2 ($F_{2,18} = 8.38$, $P < 0.05$), with sham animals reaching asymptotic performance a day earlier compared to ischemic rats.

Post hoc analysis revealed that rats who received 10 min of carotid occlusion took significantly longer in locating the platform on day 1 (25 ± 4.74 s vs. 59 ± 15.39 s, respectively, $P < 0.05$) compared to sham-operated animals and the 5-min ischemic group. Interestingly, no difference was seen between shams and the 5-min (25 ± 4.74 s vs. 30 ± 4.51 s, respectively, $P <$ 0.05) ischemic group. Comparison of the 2 ischemic groups showed significant differences on day 5 (5 ± 0.48 s for 5 min vs. 10 ± 1.38 s for 10 min, $P < 0.05$) and day 6 (6 ± 0.84 s for 5 min vs. 15 ± 4.29 s for 10 min, $P < 0.05$) in that the 5-min ischemic group performed as well as controls on the last 2 test days.

Directional heading error. Directional heading error profiles of all groups are parallel ($F_{10,28} = 1.44$, $P <$ 0.21), suggesting no interaction between test days and ischemia effect. The sham-operated animals made significantly less heading error ($F_{2,18} = 6.31$, $P < 0.05$) compared to animals with ischemic damage, as shown in Figure 3B, which suggests that healthy animals took more direct routes and were able to reach the goal quickly compared to ischemic rats. Although animals with ischemic damage showed impairment in directional heading, they demonstrated a learning curve as did sham rats as shown in a significant change over time in their performance ($F_{5,14} = 12.24$, $P < 0.001$).

Post hoc comparisons showed that 5-min ischemic rats have greater directional heading error compared to sham-operated animals on day 2 (55 ± 9.29 degrees vs. 27 ± 2.89 degrees, respectively, $P < 0.05$) and day 3 (47 ± 11.19 degrees vs. 22 ± 2.18 degrees, respectively, $P <$ 0.05). However, directional heading error of these 5-min ischemic groups approximated those of shams on day 5 (18 ± 3.38 degrees vs. 22 ± 3.98 degrees, respectively) and day 6 (12 ± 2.95 degrees vs. 17 ± 3.96 degrees, respectively). Comparison of performance in the ischemic groups revealed that although both groups were impaired in heading error on the first 3 days of behavioral testing, rats who received 10 min of carotid occlusion continued to have significant increase in directional heading error compared to animals that have 5 min of ischemic damage on day 5 (33 ± 6.31 degrees vs. 18 ± 3.38 degrees, respectively, $P <$ 0.05) and day 6 (30 ± 5.71 degrees vs. 12 ± 2.95 degrees, respectively, $P < 0.05$). Thus, even though overall performance of all the groups showed improvement across the test days suggesting that ischemic animals can learn, the 10-min ischemic animals did not reach the same level of efficiency as did the 5-min ischemic group and controls.

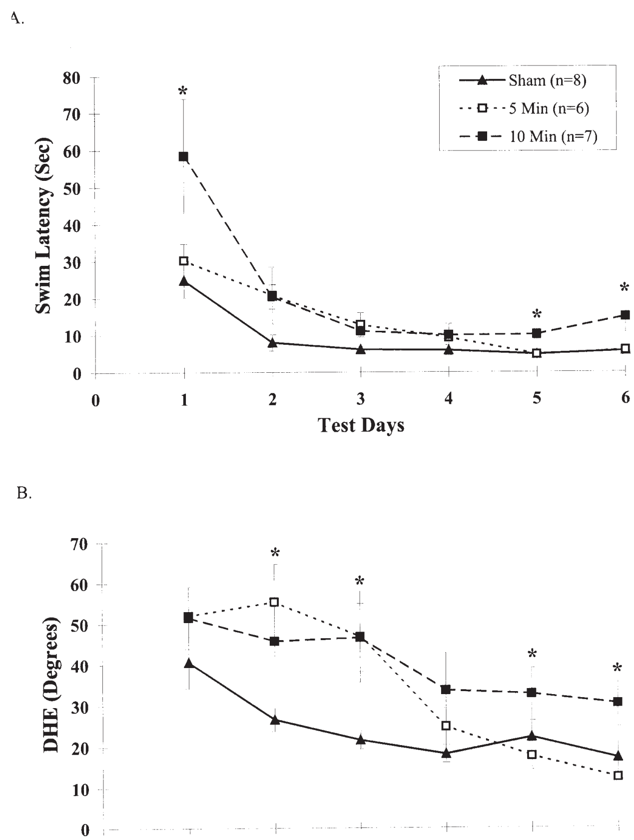

Figure 3. Comparison of mean swim latencies (A) and directional heading error (B) for control and ischemic rats. Note that all groups demonstrated some degree of learning and that the 5-min ischemic group was able to achieve the same efficiency in performing the tasks as sham animals in the last 2 testing days; however, the 10-min ischemic group showed persistent learning impairment.
**P* < 0.05 for 5-min and 10-min ischemic animals.

CA1 Cell Loss and Behavioral Performance

As previously noted, under both 5- and 10-min ischemic conditions, rats showed impairment in spatial learning performance compared to sham animals. Although a general relationship between CA1 cell loss and behavioral deficits is evident, an important issue in this study is whether extent of CA1 cell loss can quantifiably be related to degree of spatial learning impairment.

Correlation results of mean swim latency on consecutive test days and percentage of CA1 cell loss showed that performance on day 1 ($r = 0.53$, $P < 0.05$), day 3 ($r = 0.65$, $P < 0.05$), and day 5 ($r = 0.64$, $P < 0.05$) are significantly related in that increases in the percentage of CA1 cell loss brought about increases in mean swim latency. Although the relationship between percentage of CA1 cell loss and swim latency performance was not significant on test day 2 ($r = 0.50$, $P = 0.07$) and day 6 ($r = 0.43$, $P = 0.13$), there was a trend toward an increase in mean swim latency on these days related to extent of CA1 damage.

When directional heading error on consecutive test days was correlated with percentage of CA1 cell loss, no relationship was found between performance on the 1st day of testing and amount of CA1 damage. However, a significant relationship was found in directional heading error performance and percentage of CA1 cell loss on day 2 ($r = 0.59$, $P < 0.05$), day 3 ($r = 0.73$, $P < 0.005$), and again on day 4 ($r = 0.53$, $P < 0.05$), suggesting that on these test days as CA1 cell loss increased, heading error impairment also increased. Although directional heading error performance on test day 6 was not significantly related to percentage of CA1 cell loss ($r = 0.39$, $P = 0.16$), there was a trend toward an increase in heading error on this day in rats with greater CA1 damage. However, these results must be interpreted with caution because when correlational analysis was done excluding the sham animals, the only significant relationship seen was on swim latency performance on test day 5 ($r = 0.75$, $P < 0.05$). This discrepancy in correlation analysis results can be due to several factors and will be addressed in the discussion section.

Regression analysis results showed that, indeed, percentage of CA1 cell loss is a predictor of overall mean swim latency ($F_{1,19} = 10.70$, $P < 0.05$) and directional heading error ($F_{1,13} = 9.19$, $P < 0.05$) performance in the water maze and that CA1 cell loss accounts for 47% and 43%, respectively, of the variance seen in these behavioral measures. Scatterplots depicting the relationship between extent of CA1 cell loss and degree of spatial learning impairment in the water maze as measured by overall swim latency and directional heading error are shown in Figures 4A and 4B.

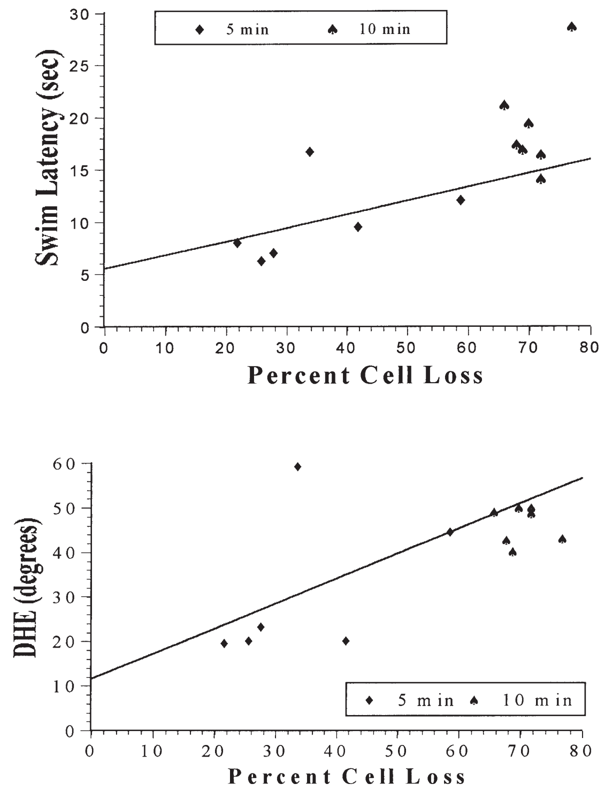

Figure 4. Scatterplots of overall mean swim latency (A) and directional heading error (B) versus mean percentage of CA1 cell loss.

Discussion

The present study supports the hypothesis that duration of ischemia is related to extent of dorsal hippocampal damage. Within the hippocampus, the greatest damage as a result of ischemia was seen in the CA1 region, although some degree of cell loss was also observed in the CA2 and CA3 regions. Prolonging the duration of ischemia from 5 to 10 min dramatically increased the extent of cell loss in the CA1 region while considerable cell loss was also seen in the CA3 region. These results underscore the fact that the CA1 region of the hippocampus is selectively vulnerable to changes in cerebral blood flow and that with increased ischemic duration the CA3 region is more vulnerable than the CA2 region of the hippocampus. The selective vulnerability of CA1 pyramidal cells is partly due to its morphology. That is, CA1 pyramidal cells express a high density of glutamate receptors (Monaghan and Cotman 1985; Auer and others 1989; Mattson 1998; Rusakov and Kullmann 1998), primarily N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA). Excessive levels of extracellular glutamate seen following ischemic damage then results in increased receptor activation and increased calcium influx, which is not only mediated by NMDA activation but also triggered secondarily by sodium influx through AMPA receptor-gated channels eventually leading to excitotoxicity (Lee and others 1999; Nunn and others 1994).

The evidence of a linear function between time of carotid occlusion and degree of damage to the hippocampus is basically in line with findings from other studies (Kiyota and others 1991; Nunn and others 1994; Volpe, Pulsinelli, and others 1992) that used male animal models, suggesting that the increased hippocampal cell loss as a consequence of prolonging the duration of ischemia seen in female rats is similar to that seen in male animal models. Furthermore, the evidence of confined damage to the dorsal hippocampus in both 5- and 10-min ischemic conditions suggests that the ventral hippocampal area may be less vulnerable to the effects of transient forebrain ischemia. Future studies are needed to fully understand if there is a difference in “selective” vulnerability between the ventral and dorsal hippocampus and the contribution of each area in learning and memory.

Behavioral testing results showed that ischemic animals were impaired in spatial learning performance in the water maze compared to shams as evidenced by increased swim latency and directional heading error. Although both ischemic groups demonstrated spatial learning deficits, the behavioral effects seen in the 5-min ischemic group were of brief duration whereas persistent impairment was seen in rats who received 10 min of ischemia. Although the possibility exists that the longer mean swim latency of the ischemic animals is influenced by lack of motor coordination, a complication of ischemic damage, this factor alone could not account for the behavioral impairment seen in the 10-min ischemic rats. That is, given that the 5-min ischemic animals were eventually able to perform similar to controls, the persistent increase in mean swim latency in the 10-min ischemic animals is most likely due to cognitive impairment rather than deficits in motor function. This persistent behavioral deficit in the 10-min ischemic group may be due to progressive cell loss during the evolution of ischemic damage. This line of reasoning is supported by the fact that the 5-min ischemic animals eventually achieved the same efficiency as shams in accomplishing the spatial learning task. The behavioral effects seen in ischemic animals in this study parallel those of others (Hagan and Beaughard 1990; Jaspers and others 1990; Nunn and others 1994) with one notable difference. That is, the profile of behavioral deficits seen in these female animals who received 10 min of ischemia is similar to that reported by others in male animals who received 15 min of ischemia (Nunn and others 1994; Volpe, Davis, and others 1992), suggesting the possibility that females are more sensitive to the effects of reductions in cerebral blood flow.

Our above findings on the effects of ischemic damage on the behavioral performance in the water maze are at odds with those of others (Kiyota and others 1991) who found no spatial learning deficits in the water maze. This discrepancy in findings may be attributed to the difference in testing apparatus used by others (Kiyota and others 1991), for example, the Styrofoam pieces in the water maze to cover the goal rather than concealing the goal by making the water opaque with the use of powdered milk. Use of Styrofoam pieces may serve as intramaze cues for locating the goal, making the testing paradigm cue learning as opposed to place learning. Indeed, it has been shown that animals with hippocampal damage are able to learn almost as well as controls in the presence of a cue (Holden and Therrien 1993; Therrien 1982; Winocur 1982).

Together, the histological and behavioral findings demonstrate that ischemic damage in the hippocampus, most specifically in the CA1 region, results in spatial learning impairments in the water maze, suggesting that CA1 cells are critical to learning and memory performance. If one considers the unidirectional flow of information through the hippocampus via the trisynaptic circuit (dentate gyrus - CA3 - CA1 - subiculum) or the direct entorhinal - CA1 pathway (bypassing the dentate gyrus and CA3), the major role played by the CA1 region in learning and memory can be clearly understood. However, the possibility that damage to the CA2 and CA3 regions also contributes to the spatial learning deficits seen following cerebral ischemia cannot be discounted, because even with short duration of ischemia, cell loss was not confined to the CA1 region. Furthermore, it can also be speculated that damage to other hippocampal areas outside of the CA1 region may have an additive effect in the impaired behavioral performance of ischemic rats in the water maze.

In the present study, a quantifiable relationship was found between extent of CA1 cell loss and degree of behavioral impairment evidenced in both swim latency and directional heading error when sham animals were included in the analysis, suggesting that as CA1 cell loss increases, behavioral deficits in the water maze will be amplified. Our findings extend those of others (Kiyota and others 1991; Volpe, Pulsinelli, and others 1992) who reported that the extent of CA1 cell loss correlated significantly with performance deficits on the T-maze and the radial arm maze, respectively. Interestingly, when sham animals were excluded in the analysis in the present study, the only significant relationship was found between swim latency on test day 5 and extent of CA1 cell loss, still suggesting an association between CA1 cell loss and behavioral performance although weakened.

Several factors could account for the discrepancies between the 2 correlation analyses. First, while a significant linear relationship between extent of CA1 cell loss and duration of ischemia exists, the relationship between degree of behavioral impairment and extent of CA1 cell loss may not be in a true linear fashion. That is, once extent of CA1 cell loss in ischemic animals reaches a certain threshold, then the behavioral deficits manifested by the animals above this threshold become monotonic. In some ways, the scatterplots on swim latency and directional heading error shown in Figure 5 provide some indication to support this line of reasoning.

Second, the behavioral deficits seen in ischemic animals may not totally depend on extent of CA1 cell loss. It is possible that these behavioral deficits are more a function of the pervasiveness of neuronal dysfunction in the entire hippocampus. For example, deafferentation and changes in neurotransmitter levels, receptor binding, and second messenger systems may not be confined to the CA1 region (Bruckner and others 1999; Colbourne and others 1999; Gennarelli 1997). Thus, although cell loss in the CA2 and CA3 regions of the hippocampus is minimal compared to CA1 damage, it may contribute to the persistent behavioral impairment seen with longer periods of ischemia. Although the present experiment does not address this issue, a strong possibility exists that these factors contribute to the behavioral deficits seen in ischemic animals.

Thus, although mixed results were obtained in determining if a correlation exists between extent of CA1 cell loss and degree of learning and memory, it does not suggest a lack of correlation. In fact, the consistent finding that the CA1 region sustained the most damage in both 5- and 10-min ischemic conditions suggests that the common loss of CA1 cells was responsible for the behavioral deficits seen in ischemic animals. This finding underscores the importance of the CA1 region as the major afferent input from the cortical areas to the hippocampus.

From a clinical perspective, survivors of cerebral ischemic events such as cardiac arrest and traumatic brain injury often lack intact cognitive skills, specifically learning and memory, which increases their dependency on both formal and informal caregivers. Thus, as medical technology becomes increasingly adept in improving mortality rate after cardiac arrest and traumatic brain injury, early recognition of the functional consequences of cerebral ischemia by astute assessment is critical so that appropriate therapies can be developed.

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