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Correlation Between Hippocampal Neuronal Damage and Spatial Learning Deficit Due to Global Ischemia

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BLOCK, F. AND M. SCHWARZ. *Correlation between hippocampal neuronal damage and spatial learning deficit due to global ischemia.* PHARMACOL BIOCHEM BEHAV **56**(4) 755–761, 1997.—Global cerebral ischemia leads to selective neuronal damage in the CA1 sector of the hippocampus and in the striatum. This ischemia leads to a deficit in spatial learning and memory in the water maze. The results of earlier studies that have examined the relationship between neuronal damage and the deficit in the water maze were not clear-cut. It has been observed that neuroprotection reduces both the deficit in the water maze and the neuronal damage. The present study therefore approached the relationship between the neuronal damage and the deficit in the water maze by pharmacological means. Global cerebral ischemia was induced in male Wistar rats by four-vessel occlusion for 20 min. Ischemic rats were then treated with the noncompetitive non-NMDA receptor antagonist GYKI 52466 (30 mg/kg), the radical scavenger LY 231617 (20 mg/kg), the inhibitor of protein kinase C staurosporine (0.1 mg/kg), or solvent. Treatment with GYKI 52466 or LY 231617 reduced the deficit in spatial learning by limiting the increase in swim distance due to ischemia. In addition, LY 231617 reduced the deficit in spatial memory as demonstrated by minimizing the ischemia-induced reduction in time spent in the quadrant of the former platform position during the probe trial. Staurosporine had no influence on the ischemia-induced behavioural changes. Histological examination revealed neuronal damage in the hippocampus and in the striatum in all of the ischemic rats. However, treatment with GYKI 52466 or LY 231617 reduced the hippocampal damage. Correlation analysis demonstrated a correlation between hippocampal damage and total swim distance ($r = 0.88, p < 0.001$). No correlation was found between hippocampal damage and quadrant time of the probe trial ($r = -0.24, p > 0.1$). No correlation was observed between striatal damage and either total swim distance of the escape trials ($r = 0.28, p > 0.1$) or quadrant time of the probe trial ($r = -0.08, p > 0.6$). It is concluded that a correlation exists between hippocampal damage and the deficit in spatial learning following global cerebral ischemia. © 1997 Elsevier Science Inc.

Spatial learning Spatial memory Cerebral ischemia Rat

FINDINGS from clinical studies have provided strong evidence that the hippocampus is critical for memory function in humans (10,39). Hippocampal lesion studies in nonhuman primates have offered further support for the involvement of the hippocampus in memory function (38). In rats, hippocampal lesions lead to an impairment of spatial learning (18,19). Moreover, pharmacological blockade of those processes within the hippocampus that are thought to represent the electrophysiological basis of learning induces a deficit in spatial learning (17).

Pyramidal neurons of the CA1 sector of the hippocampus display a selective vulnerability to transient global cerebral ischemia. This can be observed in humans who have suffered from global cerebral ischemia due to cardiac arrest (10,26). In experimental animals, this global cerebral ischemia may be induced by permanent occlusion of both vertebral arteries and transient occlusion of both carotid arteries (in rats) or by transient occlusion of both carotid arteries (in gerbils), which in both cases leads to selective cell death of the CA1 pyramidal hippocampal neurons (14,27,28). Depending on the duration of this ischemia, selective neuronal cell death may also occur in the dorsolateral striatum and in the cortex (27). From experimental studies, it has been suggested that a massive release of glutamate, intracellular overload with calcium, and enhanced production of free radicals are three major pathophysiological processes that contribute to selective neuronal damage (2,30,31). Furthermore, activation of the enzyme protein kinase C due to ischemia has been suggested as

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another factor contributing to neuronal damage after ischemia (24).

Following transient global cerebral ischemia, rodents exhibit deficits in spatial learning and memory. Using the Morris water maze, several groups have demonstrated that ischemia results in an increase in the time to find the hidden platform (1,13,21,23,36). Further analysis has revealed that this increase in escape latency was due to an increase in swim distance and not a result of motor impairment, as the swimming speed was not altered by ischemia (6,23). Two studies have previously addressed the question of whether the deficit in spatial learning is due to hippocampal neuronal damage. The results so far obtained are not clear-cut. One study found no correlation (21), whereas another found only a weak correlation between hippocampal damage and the spatial learning deficit (23). Pharmacological studies that have examined the neuroprotective effects of substances interacting with the pathophysiological processes leading to ischemic neuronal damage have shown that both hippocampal neuronal damage and deficits in spatial learning can be reduced (3,5–7,34,37). These findings suggest that there might be a correlation between hippocampal neuronal damage and deficits in spatial learning, and the present study was designed to examine this correlation further. For this purpose, rats that had undergone four-vessel occlusion (4VO) were treated with either GYKI 52466 (a noncompetitive AMPA receptor antagonist), LY 231617 (a radical scavenger), staurosporine (an inhibitor of protein kinase C), or solvent. A correlation analysis was then performed based on the results of the behavioural testing in the water maze and on the quantification of neuronal damage in the hippocampus and striatum. The data for LY 231617 have been partly reported elsewhere (6).

METHODS

The procedures of the experiments presented herein were in accordance with institutional guidelines.

Cerebral ischemia was induced by 4VO in male Wistar rats (250–280 g). Both vertebral arteries were occluded by electrocauterization under pentobarbital anesthesia (60 mg/kg IP). The animals were allowed to recover for 24 h, with free access to water but not to food. On the next day, the carotid arteries were exposed under 2% halothane in 30% oxygen/70% nitrous oxide anesthesia and were occluded for 20 min using microvascular clamps. Subsequently, both clamps were removed and both arteries were inspected for immediate reperfusion. During the operation and over the following 6 h, the head temperature (monitored by a thermoprobe located in the temporal muscle) and rectal temperature were kept at 37 ± 0.5°C with a heating lamp. As a control, both vertebral arteries of sham-operated animals were cauterized under pentobarbital anesthesia and the following day the common carotid arteries were exposed but not clamped under 2% halothane in 30% oxygen/70% nitrous oxide anesthesia. Five groups of animals were used: one group of sham-operated animals (n = 11); one group of 4VO rats that received an intraperitoneal injection of solvent (n = 10); and three groups of 4VO rats that were injected with GYKI 52466, 30 mg/kg (n = 9; kindly supplied by Dr. Tarnawa, Drug Research Institute, Budapest, Hungary); LY 231617, 20 mg/kg (n = 11; kindly supplied by Dr. Panetta, Lilly Research Laboratories, Indianapolis, IN, USA); or staurosporine, 0.1 mg/kg (n = 9; Sigma Chemicals, St. Louis, MO, USA). GYKI 52466 was administered 20 min before induction of ischemia; LY 231617 and staurosporine were applied immediately after occlusion of both common carotid arteries. The doses of the substances were chosen according to the results of other studies that demonstrated protective effects of the substances (6,15,22).

One week after surgery, spatial learning was tested in a water maze (18), a black, circular pool (diameter 100 cm, height 40 cm) filled to a depth of 22 cm with water (26°C). Six days after occlusion, the rats received a habituation trial (1 min) in which there was no platform present. Over three subsequent days, each rat performed 20 escape trials (five blocks of four trials). During a particular trial, the rats were able to escape from the water only by climbing onto an invisible, submerged platform (diameter 6 cm). For each rat, the location of the hidden platform remained unchanged during the whole experiment. A trial was terminated as soon as the rat found the platform; if it did not succeed within 120 s, it was placed on the platform by hand. The animal was allowed to stay on the platform for 20 s before the next trial started. Immediately after the final escape trial, each rat was subjected to a probe trial (60 s) in which there was no platform present. The time spent in the quadrant of the former platform position was obtained as a measure of spatial bias. All trials were recorded on video tape for analysis of escape latency, swim distance, and swim speed during the escape trials and for analysis of time spent in the quadrant of the former platform location during the probe trial, using a computer-assisted image analyzer (Watermaze Software, Edinburgh, Scotland).

Two weeks after surgery, the animals were deeply anesthetized with pentobarbital and intracardially perfused with Ringer's solution and then with formaldehyde. The entire brain was embedded, and frontal sections (5 μm) were prepared according to a stereotaxic atlas (25) and stained with cresyl violet. Histological sections were examined by one of us (M.S.) without knowledge of the experimental protocol. Measures were taken at two levels in the striatum (+0.2 mm, −0.3 mm from the bregma) and in the hippocampus (−3.8 mm, −4.3 mm from the bregma) from both hemispheres. The area of the whole striatum and the area of neuronal damage within the striatum were traced using an image-processing system (Image-Pro-Plus, Silver Spring, MD, USA); the area of neuronal damage was expressed as percentage of the whole striatum. Within the CA1 sector of the hippocampus, the intact neurons were counted. The percentage of neuronal cell loss was calculated by subtracting the counted number from the known neuronal counts of naive rats (166 ± 9; mean ± SEM) (33). The values obtained from the striatum and the CA1 sector of the hippocampus were then expressed by means of a 6-point scale for neuronal cell loss: 0 = 0–10%, 1 = 11–30%, 2 = 31–50%, 3 = 51–70%, 4 = 71–90%, 5 = 91–100%.

Group differences in escape latency, swim speed, and swim distance were determined by an analysis of variance with repeated measures. Probe trial data and histological scores were compared by Mann–Whitney U-test. A Spearman rank order correlation analysis was performed between swim distance and probe trial versus hippocampal damage and striatal damage, respectively.

RESULTS

All animals were able to swim normally during the habituation trial. As illustrated in Fig. 1A, all rats were able to locate the hidden platform during the escape trials, although 4VO rats required significantly more time than sham-operated controls. Analysis of escape latency revealed a significant effect between groups [$F(1, 19) = 19.98$, $p < 0.001$]. The swim distance of 4VO rats during the escape trials was significantly

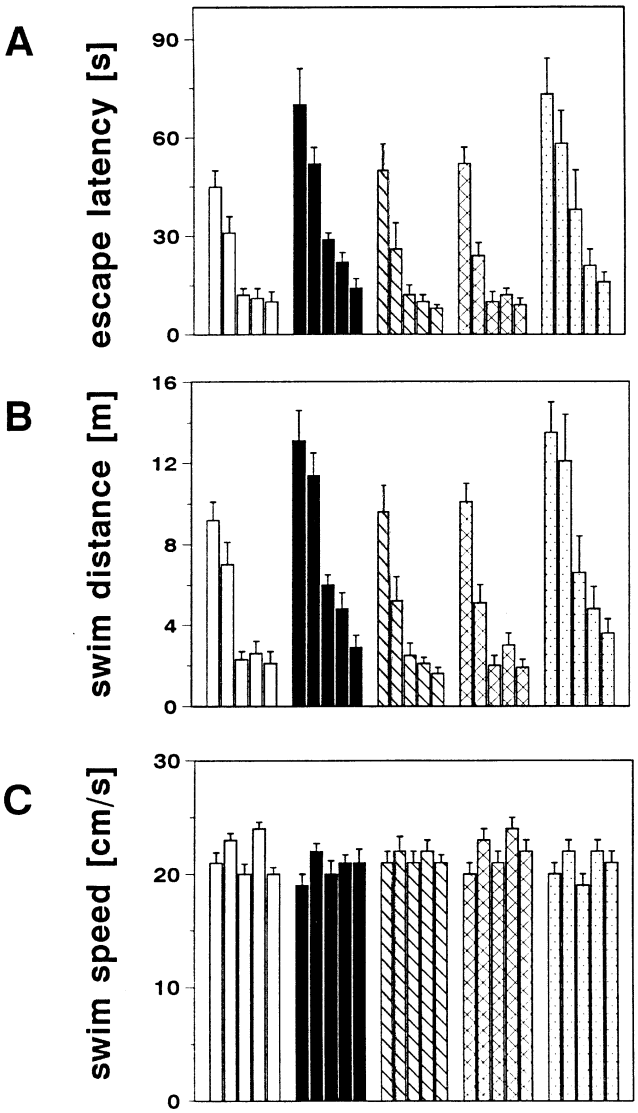

FIG. 1. Escape latency (A), swim distance (B), and swim speed (C) for sham-operated controls (n = 11, white bars), 4VO rats that received solvent (n = 10; black bars), and 4VO rats treated with GYKI 52466, 30 mg/kg (n = 9; hatched bars), with LY 231617, 20 mg/kg (n = 11; crossed bars), or with staurosporine, 0.1 mg/kg (n = 9; dotted bars), for each block of four escape trials throughout the experiment. Values are mean + SEM.

longer than that of sham-operated controls [$F(1, 19) = 20.22$, $p < 0.001$] (Fig. 1B). The swim speed of 4VO rats did not differ from that of sham-operated rats in any of the escape trials [$F(1, 19) = 1.13$, $p > 0.1$]. Analysis of the swimming performance during the probe trial revealed that 4VO rats spent significantly less time in the quadrant of the former platform position than did sham-operated controls (Fig. 2).

GYKI 52466 and LY 231617 reduced the prolongation of escape latency induced by 4VO [$F(1, 17) = 12.6$, $p < 0.01$; $F(1, 19) = 23.5$, $p < 0.001$], whereas staurosporine had no effect on this latency [$F(1, 17) = 0.25$, $p > 0.6$] (Fig. 1A). Treatment with either GYKI 52466 or LY 231617 resulted in a decrease in the exaggeration of swim distance induced by 4VO [$F(1, 17) = 17.9$, $p < 0.001$; $F(1, 19) = 28.4$, $p < 0.001$],

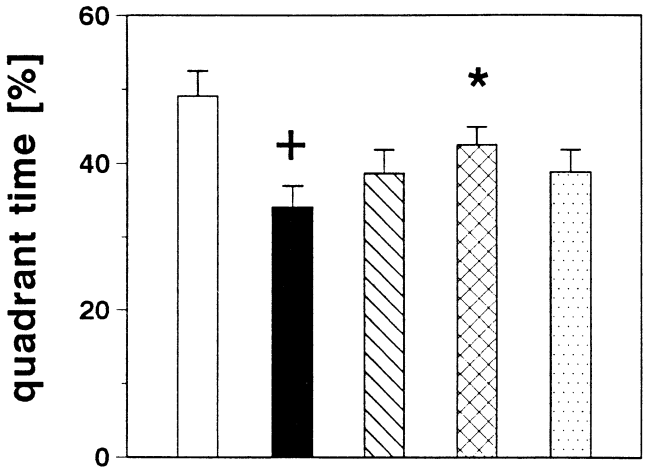

FIG. 2. Time spent in the quadrant of the former platform position (as percentage of total time) during the probe trial for sham-operated controls (n = 11; white bar), 4VO rats that received solvent (n = 10; black bar), and 4VO rats treated with GYKI 52466, 30 mg/kg (n = 9; hatched bar), with LY 231617, 20 mg/kg (n = 11; crossed bar), or with staurosporine, 0.1 mg/kg (n = 9; dotted bar). Values are mean + SEM. $+p < 0.05$ vs. Sham; $*p < 0.05$ vs. 4VO; Mann–Whitney U-test.

whereas staurosporine did not affect this measure [$F(1, 17) = 0.13$, $p > 0.7$] (Fig. 1B). The swim speed during the escape trials was influenced by neither GYKI 52466 [$F(1, 17) = 0.66$, $p > 0.4$], LY 231617 [$F(1, 19) = 0.78$, $p > 0.3$], nor staurosporine [$F(1, 17) = 0.001$, $p > 0.9$] (Fig. 1C). The reduction in time spent in the quadrant of the former platform position induced by 4VO was attenuated by treatment with LY 231617 ($p < 0.05$) but not with GYKI 52466 ($p > 0.2$) or staurosporine ($p > 0.4$) (Fig. 2).

Histological examination of the brains of sham-operated animals revealed no cell damage at all. Twenty minutes of 4VO led to necrosis of pyramidal cells of the CA1 region of the hippocampus and neuronal damage in the dorsolateral striatum (Fig. 3). Treatment with GYKI 52466 or LY 231617 resulted in a significant decrease in neuronal cell damage in the hippocampal CA1 region. In addition, LY 231617 slightly but not significantly reduced striatal neuronal damage. Treatment with staurosporine did not protect against ischemic cell damage in either the hippocampus or the striatum. The brains of all ischemic animals treated with GYKI 52466, LY 231617, staurosporine, or solvent displayed no obvious neuronal damage in other brain regions.

The following paradigms were chosen for correlation analysis: with respect to performance in the water maze, the total distance of all 20 escape trials and the quadrant time during the probe trial; and with regard to morphology, the neuronal damage in the CA1 sector of the hippocampus and in the striatum. A correlation was found between the total swim distance and hippocampal damage ($r = 0.88$, $p < 0.001$) (Fig. 4). No correlation existed between the quadrant time during the probe trial and hippocampal damage ($r = -0.24$, $p > 0.1$) (Fig. 5). No correlation was found between behavioural data and striatal damage ($r = 0.28$, $p > 0.1$ for total swim distance; $r = -0.08$, $p > 0.6$ for quadrant time during the probe trial) (Figs. 4, 5).

DISCUSSION

A consistent deficit in spatial learning following global cerebral ischemia has been demonstrated using the water maze

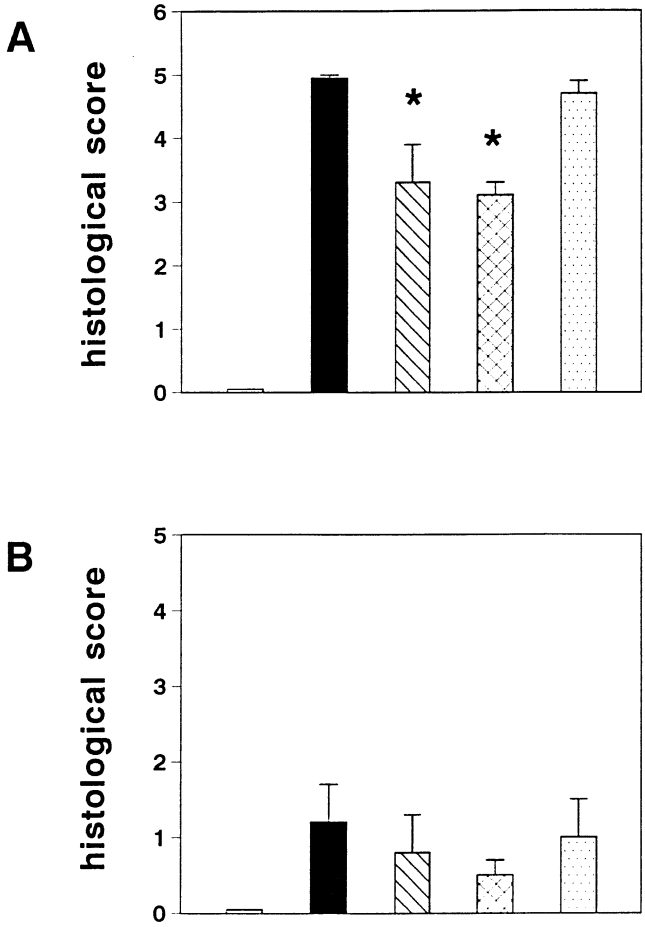

FIG. 3. Histological scores in the CA1 sector of the hippocampus (A) and in the striatum (B) of sham-operated controls ($n = 11$; white bars), 4VO rats that received solvent ($n = 10$; black bars), 4VO rats treated with GYKI 52466, 30 mg/kg ($n = 9$; hatched bars), with LY 231617, 20 mg/kg ($n = 11$; crossed bars), or with staurosporine, 0.1 mg/kg ($n = 9$; dotted bars). Values are mean + SEM. $*p < 0.05$ vs. 4VO; Mann–Whitney *U*-test.

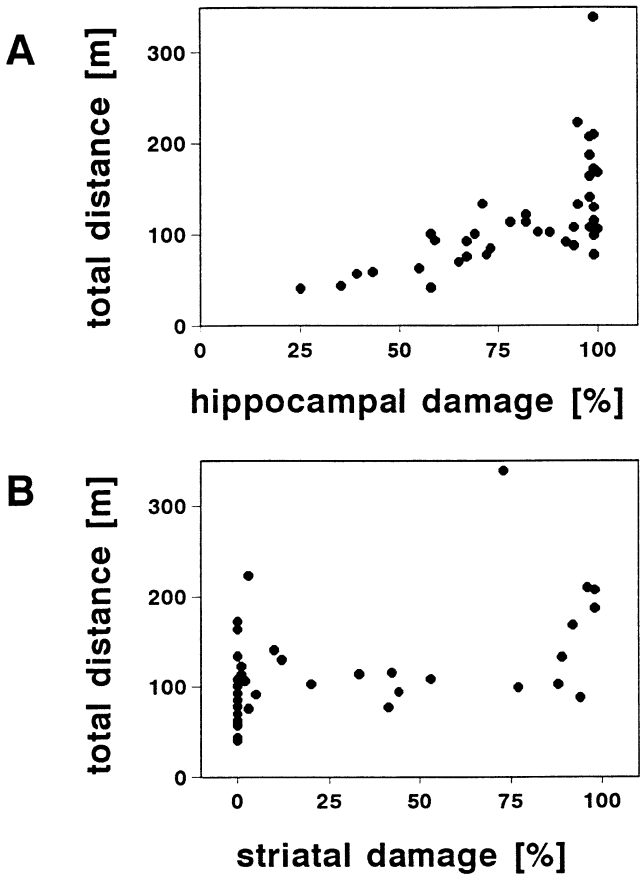

FIG. 4. Spearman rank order correlation analysis (r and p values) for hippocampal (A) or striatal damage (B) vs. cumulative swim distance for all 20 escape trials for all ischemic animals. (A) $r = 0.88$, $p < 0.001$; (B) $r = 0.28$, $p > 0.1$.

(1,13,21,23). In the present study, 4VO rats also displayed an increased escape latency in comparison to sham-operated controls. It has been shown that several substances that interact with the pathophysiological processes leading to ischemic cell death attenuated the deficit in spatial learning induced by ischemia (3,5–7,34,37). In the present experiments, we examined the effects of the noncompetitive non-NMDA receptor antagonist GYKI 52466, the radical scavenger LY 231617, or the protein kinase C inhibitor staurosporine on the deficit in spatial learning and memory and the neuronal damage produced by 4VO. The ischemia-induced increase in escape latency was reduced by treatment with GYKI 52466 or LY 231617. Because the escape latency is determined by swim speed and distance, analysis of these two parameters is helpful in evaluating the underlying mechanisms. Swim speed, which was not altered by 4VO, was also unaffected by GYKI 52466 or LY 231617. These results suggest that motor performance during behavioural testing in the water maze was not influenced by treatment with GYKI 52466 or LY 231617. Treatment with GYKI 52466 or LY 231616 counteracted the increase in swim distance induced by 4VO, suggesting that treatment with GYKI 52466 or LY 231617 reduced the deficit in spatial learning. The reduction in time spent in the quadrant of the former platform position during the probe trial evoked by 4VO was significantly attenuated by treatment with LY 231617. This means that the deficit in spatial memory induced by global ischemia was reduced by LY 231617. Treatment with staurosporine, however, did not affect the deficit in spatial learning and memory caused by global cerebral ischemia. Previous studies had demonstrated that staurosporine at 0.1 mg/kg, the same dose as used in the present study, attenuated deficits in learning and memory following global cerebral ischemia or ibotenic acid lesions of the basal forebrain (20,22). Some differences in the design of the studies might be responsible for the different results. In the other study on global ischemia, behavioural testing was performed 24 h after ischemia, whereas in the present study behavioural testing was done between days 7 and 10 after ischemia (22). In the study with excitotoxic basal forebrain lesions, staurosporine was given each time before behavioural testing, whereas in the present experiments it was applied once 7 days before behavioural testing was started (20).

Treatment with GYKI 52466 or LY 231617 resulted in a significant reduction of the hippocampal neuronal damage normally induced by 4VO. The finding that GYKI 52466 attenuates ischemic hippocampal damage is somewhat contradic-

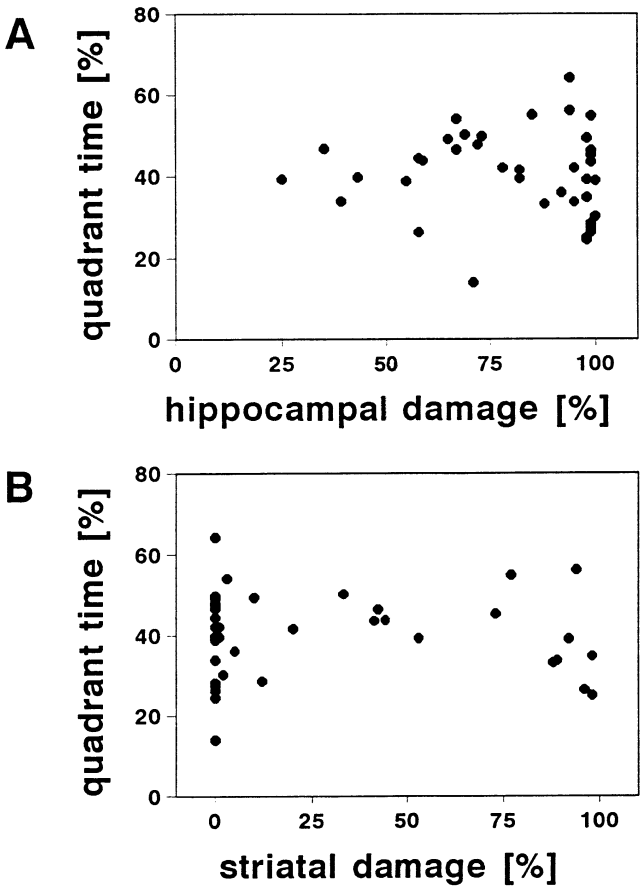

FIG. 5. Spearman rank order correlation analysis (r and p values) for hippocampal (A) or striatal damage (B) vs. time spent in the quadrant of the former platform position during the probe trial for all ischemic animals. (A) $r = -0.24, p > 0.1$; (B) $r = -0.08, p > 0.6$.

tory to the results of another study that demonstrated no protective effect of GYKI 52466 on hippocampal damage following global ischemia (15). In this study, GYKI 52466 was applied after the induction of ischemia, whereas in the present study it was given 20 min before ischemia. Thus, the different time points of application of GYKI 52466 with respect to ischemia might be the reason for the different effects observed. Consistent protective effects of the competitive non-NMDA receptor antagonist NBQX have been reported (16,29). The protective effect of LY 231617 in the present experiments is in accordance with other studies that also showed a reduction in hippocampal damage after treatment with LY 231617 (8,37). It has also been shown for other radical scavengers that they are able to reduce hippocampal neuronal damage following global ischemia (8,32). Treatment with staurosporine did not lead to a reduction in neuronal cell damage within the hippocampus. This result is in contrast to another study that demonstrated a protective effect of staurosporine on ischemic hippocampal neuronal damage (12). In that study, however, staurosporine was administered intraventricularly and not systemically as in the present study. This difference in route of application may be the reason for the difference in efficacy.

The present results show that functional improvement and reduction of ischemic cell damage appear in parallel: treatment with GYKI 52466 or LY 231617 reduces both the deficit in spatial learning and the neuronal damage, whereas treatment with staurosporine affects neither the functional nor the morphological changes induced by ischemia. This finding raises the question of whether the parallel appearance of functional and morphological improvement is causally related. Is the reduction of neuronal damage in the hippocampus and/or the striatum the basis for the reduction of the increase in swim distance? Analysis of the present study revealed a correlation between hippocampal damage and the total distance of all 20 escape trials. Earlier studies have previously examined whether the histological damage in the CA1 sector of the hippocampus relates to the deficit in spatial learning in the water maze. Olsen et al. found that a negative correlation existed between a reduction in escape distance and an increasing number of intact CA1 hippocampal neurons (23). For their correlation analysis, they included sham-operated controls and three groups of 4VO rats that were subjected to global ischemia of either 6, 9, or 12 min duration. For the histological examination, they counted intact CA1 neurons within the whole hippocampus. The coefficients for the correlation between reduction in escape distance and increase in number of intact CA1 neurons were rather poor. This may be due to the fact that the ventral hippocampus was included in the histological examination. This region is less vulnerable to ischemic insults than the dorsal hippocampus: the CA1 sector of the ventral hippocampus displays a necrosis of 10–20% of the neurons, whereas in the dorsal hippocampus 90–100% are damaged (1,23). In another study, no correlation between hippocampal damage and deficit in spatial learning was found (21); for their study on four groups of ischemic animals with duration of ischemia of 5, 10, 15, or 30 min, Nunn et al. chose the mean escape latency of trials 17–20 and 21–24 as a behavioural parameter and neuronal cell loss using a 6-point scale as a morphological parameter. In comparison to the present study, they used behavioural data from rather late trial blocks, for which they could demonstrate a significant difference between the groups with regard to escape latency. Several studies, including the present one, have found that no difference between the groups with respect to escape latency or swim distance exists in these late trial blocks (3,5–7, 11,13,23). Some methodological differences concerning the behavioural testing in the water maze between the study of Nunn et al. (21) and the present one exist, in particular the size of the pool, maximum time allowed to locate the platform, and the duration of the stay on the platform. These methodological differences might partially explain the different results regarding the course of escape latency and swim distance among the trials. Nevertheless, these differences seem also to reflect a different type of learning. In the case of Nunn et al., all animals started off at almost the same level with respect to escape latency, and with increasing numbers of trials the difference between the groups enlarged (21). In contrast, in other studies, including the present one, the difference between the groups was most pronounced at the beginning of behavioural testing in the water maze, and with increasing number of trials this difference declined (3,5–7,11,13,23). Thus, the somewhat contradictory findings of the study of Nunn et al. (21) and the present one regarding the correlation between hippocampal damage and escape latency or swim distance may be due to the fact that a different type of learning was evaluated.

In the present study a correlation between hippocampal damage and deficit in spatial learning was observed. This finding is in line with the results of another study that demon-

strated a correlation between hippocampal damage induced by aspiration lesions and impairment in spatial learning (19).

Striatal damage has also been demonstrated to induce a deficit in spatial learning (4,35). However, the deficit in spatial learning following complete striatal damage is accompanied by a motor deficit, as revealed by a decrease in swim speed (4). Global ischemia for 20 min, as used in the present experiments, results neither in complete striatal damage nor in a reduction of the swim speed. In addition, treatment with any of the substances did not lead to a significant protection of striatal neurons. These findings suggest that the striatal damage does not relate to the deficit in spatial learning. Indeed, analysis of the present results revealed no correlation between striatal damage and swim distance. This finding is in line with the results of Nunn et al. (21), who found no correlation between striatal damage and escape latency during the escape trials.

No correlation existed between hippocampal neuronal damage and the time spent in the quadrant of the former platform position during the probe trial. This is in line with the study of Nunn et al. (21), who also found no correlation between quadrant time during the probe trial and neuronal damage. The analysis of Olsen et al. (23), however, was able to demonstrate such a correlation. As for their other analysis, this group included the data of sham-operated controls. But as the control animals do not display a deficit in spatial memory and do not display neuronal damage, they should not be included in any analysis concerning the correlation between hippocampal neuronal damage and deficits in spatial memory. No correlation between hippocampal neuronal damage and deficit in spatial memory could be demonstrated in the present study.

Striatal neuronal damage induced by excitotoxic lesion induces besides a deficit in spatial learning also a deficit in spatial memory (35). As global ischemia also leads to striatal neuronal damage, it is tempting to examine whether a correlation between striatal damage and the deficit in spatial memory exists. Correlation analysis of the present results clearly rejects this possibility. This finding confirms that of Nunn et al. (21), who also failed to observe a correlation between striatal damage and the deficit in spatial memory. As no correlation between neuronal damage in either hippocampus or striatum and the deficit in spatial memory was found, one may have to look at other regions of neuronal damage to address the question of correlation to the deficit in spatial memory. Neuronal damage occurs also in the cortex and in the thalamus following global cerebral ischemia (27). However, this damage is much less pronounced than that in the hippocampus and striatum, and it appears mainly after 30 min of ischemia (27). In the present study, 20 min of ischemia was used, and no consistent neuronal damage was observed during histological analysis either in the cortex or in the thalamus. The present findings suggest that the deficit in spatial memory following global ischemia would seem to be unrelated to neuronal damage dectable at the level of light microscopy.

In conclusion, the present study demonstrated that a correlation exists between hippocampal damage and the deficit in spatial learning following global cerebral ischemia. No correlation, however, was observed between any neuronal damage resulting from ischemia and the deficit in spatial memory.

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