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Selective Vulnerability in the Gerbil Hippocampus Following Transient Ischemia

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Summary. Following brief ischemia, the Mongolian gerbil is reported to develop unusual hippocampal cell injury (Brain Res 239:57–69, 1982). To further clarify this hippocampal vulnerability, gerbils were subjected to ischemia for 3, 5, 10, 20, and 30 min by bilateral occlusion of the common carotid arteries. They were perfusion-fixed after varying intervals of survival time ranging from 3 h up to 7 days. Following brief ischemia (5–10 min), about 90% of the animals developed typical hippocampal damage. The lesion was present throughout the extent of the dorsal hippocampus, whereas damage outside the hippocampus was not observed. Each sector of the hippocampus showed different types of cell reaction to ischemia. Ischemic cell change was seen in scattered CA4 neurons, and reactive change was found in CA2, whereas CA1 pyramidal cells developed a strikingly slow cell death process. Ischemia for 3 min did not produce hippocampal lesion in most cases. Following prolonged ischemia (20–30 min), brain injury had a wide variety in its extent and distribution. These results revealed that the gerbil brief ischemia model can serve as an excellent, reliable model to study the long-known hippocampal selective vulnerability to ischemia. Delayed neuronal death in CA1 pyramidal cells was confirmed after varying degrees of ischemic insult. These findings demonstrated that the pathology of neuronal injury following brief ischemia was by no means uniform nor simple.

Key words: Cerebral ischemia – Selective vulnerability – Hippocampus – Cell death – Gerbils

Introduction

The hippocampus has long been known to be one of the most vulnerable regions in the brain to ischemia (Brierley 1976). This vulnerability differs throughout the hippocampus. The CA1 (Sommer's sector) and CA4 (endfolium) subfields are the most susceptible to anoxia-ischemia, whereas CA3 and the dentate gyrus tolerate a relatively severe ischemic insult.

Recently, a novel change in the gerbil CA1 subfield has been reported (Kirino 1982). Following a brief bilateral cerebral ischemia, extensive loss of the CA1 pyramidal cells was observed. This change in CA1, however, differed from traditionally described ischemic injury of neurons in that it developed very slowly and involved massive growth of the endoplasmic reticulum (ER) instead of rapid decomposition of neurons.

This slowly developing cell death in the hippocampus may afford some clues for further research in ischemic brain injury and its treatment. Delayed neuronal death in CA1 following ischemia is not a rapid destruction of neurons. There is a latent period for almost a day before any overt process of neuronal death is initiated. This means that we will have more opportunities to protect neurons from death of this type after brief ischemia provided that enough knowledge of this phenomenon is available. This possibility might be of considerable clinical significance. Moreover, the hippocampus is the area where anatomic, physiologic and behavioral investigations have been copiously documented (Isaacson and Pribram 1975; Isaacson 1982). Since delayed ischemic neuronal death in the hippocampus seems to be related to the anatomic and physiologic characteristics of this region, it may be advantageous to conduct further research using the available knowledge of this structure.

The Mongolian gerbil has been used as an experimental model for cerebral ischemia (Levine and Payan 1966; Kahn 1972; Ito et al. 1975; Levy et al. 1975). Although it has several beneficial features, the model has a disadvantage. The animal develops a "seizure-like" abnormality especially during and after relatively prolonged ischemia (Levy et al. 1975), which is sometimes so severe that it may jeopardize experimental data

Table 1

Duration of Ischemia	Survival time						
	3 h	6 h	12 h	1 d	2 d	4 d	7 d
3 min	–	–	–	–	–	–	5
5 min	–	–	–	4	5	4	10
10 min	–	–	4	4	4	4	4
20 min	–	–	5	4	5	–	4
30 min	4	4	4	–	–	–	–

The number of gerbil brains observed as plastic sections. Following ischemia longer than 5 min, five failed to develop definite hippocampal lesion and were not included in this table. Animals which died before fixation were also excluded. Four normal gerbils were used as controls

(Pulsinelli et al. 1979). Brief bilateral ischemia in the gerbil, however, does not cause this drawback (Kirino 1982). At least 90 % of the animals developed consistent and similar changes in the hippocampus, and cell injury was absent or very rare, if any, outside the hippocampus. In other words, brief bilateral ischemia in the gerbil offers an appropriate model to study the changes in the hippocampus. In addition, this procedure can almost exclusively eliminate the bilateral CA1 pyramidal cells. This fact could be noteworthy because gerbils are also used in the behavioral sciences (Walters et al. 1963), including studies on the effect of hippocampal lesions (Glickman et al. 1970).

The purpose of this experiment is, therefore, to further clarify the changes in the hippocampus following varying degrees of ischemic insult ranging from 3 min up to 30 min. Observations by light microscopy was performed on the gerbil forebrain and special attention was focused on the changes in each sector of the hippocampal formation. A similar, slowly developing cell death after forebrain ischemia has also been described in a rat model recently (Pulsinelli et al. 1982a).

Materials and Methods

Male adult Mongolian gerbils (60–80 g) were anesthetized with halothane. The bilateral carotid arteries at the neck were exposed, freed from surrounding connective tissue, and occluded with aneurysm clips. This procedure is known to cause severe reduction of cerebral blood flow, which is close to zero, in the gerbil forebrain (Crockard et al. 1980). Anesthesia was discontinued as soon as the clips were placed. Five groups of animals were prepared according to the duration of carotid occlusions; i.e., 3 min, 5 min, 10 min, 20 min, and 30 min. The gerbils were kept in a warm cage (27–30° C) until they started moving. After varying intervals, as indicated in Table 1, they were fixed by transcardiac perfusion. A total of 99 gerbils including four normal controls were used in this experiment.

The fixative consisted of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.3). After perfusion, the brains were kept in the same fixative at 4° C overnight. They were then cut into coronal sections which contained the hippocampal area 0.5–1.0 mm posterior to the most rostral tip of the hippocampus or 1.4–1.9 mm posterior to the bregma (Loskota et al. 1974). These specimens were postfixed with 1 % OsO_4 in cacodylate buffer, mordanted in uranyl acetate solution, dehydrated with graded ethanol, and then embedded in Araldite CY-212. One-micrometer sections were cut on an ultramicrotome and stained with toluidine blue. Observation by light microscopy was performed on each subfield of the hippocampus and the overlying cerebral cortex.

In the animals fixed 7 days following ischemia, vibratome sections (25 μm thick) were prepared. In five gerbils of the 5-min group, serial vibratome specimens were cut every 200–300 μm of the forebrain. The planes of these sections were coronal, horizontal, or perpendicular to the long axis of the hippocampus. These sections, stained with cresyl fast violet, were used to examine the extent of the hippocampal damage and the change outside the hippocampus.

Results

"Seizure" Activities and Survival

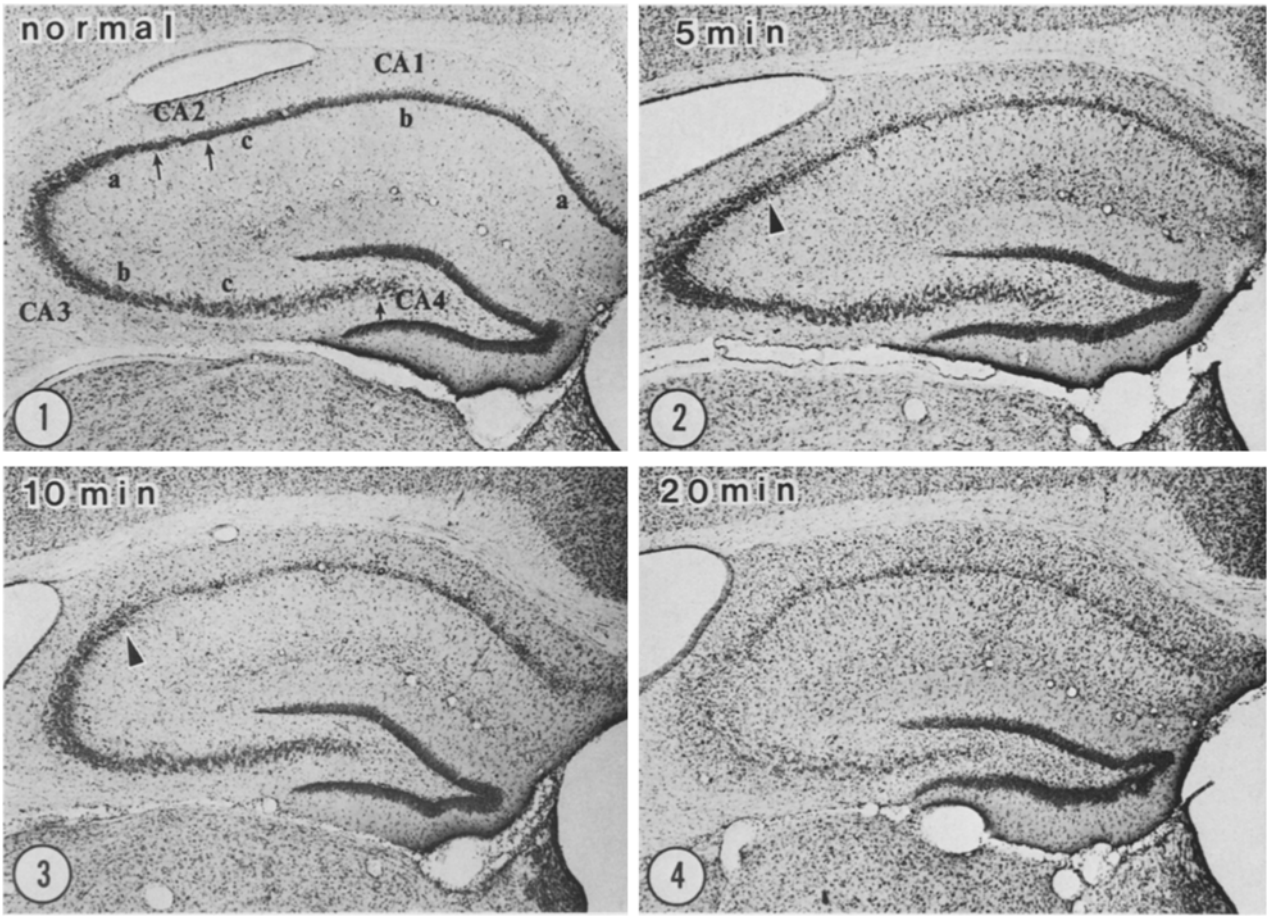
Behavior of the gerbils during and after 3- or 5-min occlusion did not differ from previous work (Kirino 1982). During bilateral carotid occlusion, abnormal "epileptic" motor movement (Brown et al. 1979) was not observed until occlusion lasted longer than 7 min. Such "seizure-like" motor behavior was observed progressively more often as ischemia was elongated. Severe "seizure" activity was seen in 50 % of the animals in the 10- and 20-min groups and in all of the 30-min group during and after the ischemic insult. Death occurred in three animals (14.3 %) of the 20-min group and in nine (42.9 %) of the 30-min group.

Extent of Ischemic Neuronal Damage

Following 3 min of occlusion, one of five animals developed scattered destruction of CA1 pyramidal cells, whereas the brain of the other gerbils looked normal. After ischemia for longer than 5 min, most of the brains showed damage in the hippocampus (Figs. 2–4). The frequency of brain damage following an ischemic insult longer than 5 min was 93.9 % in this experiment.

In serial sections of gerbil brains fixed 7 days following 5 min of ischemia, no neuronal damage was observed outside the hippocampus. Specimens cut coronally or obliquely (perpendicularly to the long axis of the hippocampus) demonstrated that hippocampal damage was present throughout the dorsal hippocampus which had a length of 2.0 mm along the long axis. At the most posterior pole of the hippocampus where it bends ventrally, as examined by horizontal sections, tissue damage was slight or absent.

Seven days following 10 min of ischemia (Fig. 3), the extent of hippocampal lesion was similar to the 5-min group (Fig. 2). In the 20-min group, cell damage extended to the CA3 subfield, and the whole hippocam-



Figs. 1–4. Dorsal hippocampus. Vibratome section, cresyl fast violet. × 26

Fig. 1. Normal. Subfields of the hippocampus (*CA1–4)* and their subdivisions (*a, b,* etc.)

Fig. 2. One week following 5-min ischemia. CA1 and a part of CA2 have been destroyed and replaced with glial cells. *Arrow* indicated the border of the change

Fig. 3. One week following 10-min ischemia. The area of neuronal destruction is similar to that in Fig. 2

Fig. 4. One week following 20 min ischemia. The whole hippocampal area has been lost. The dentate gyrus looks unchanged

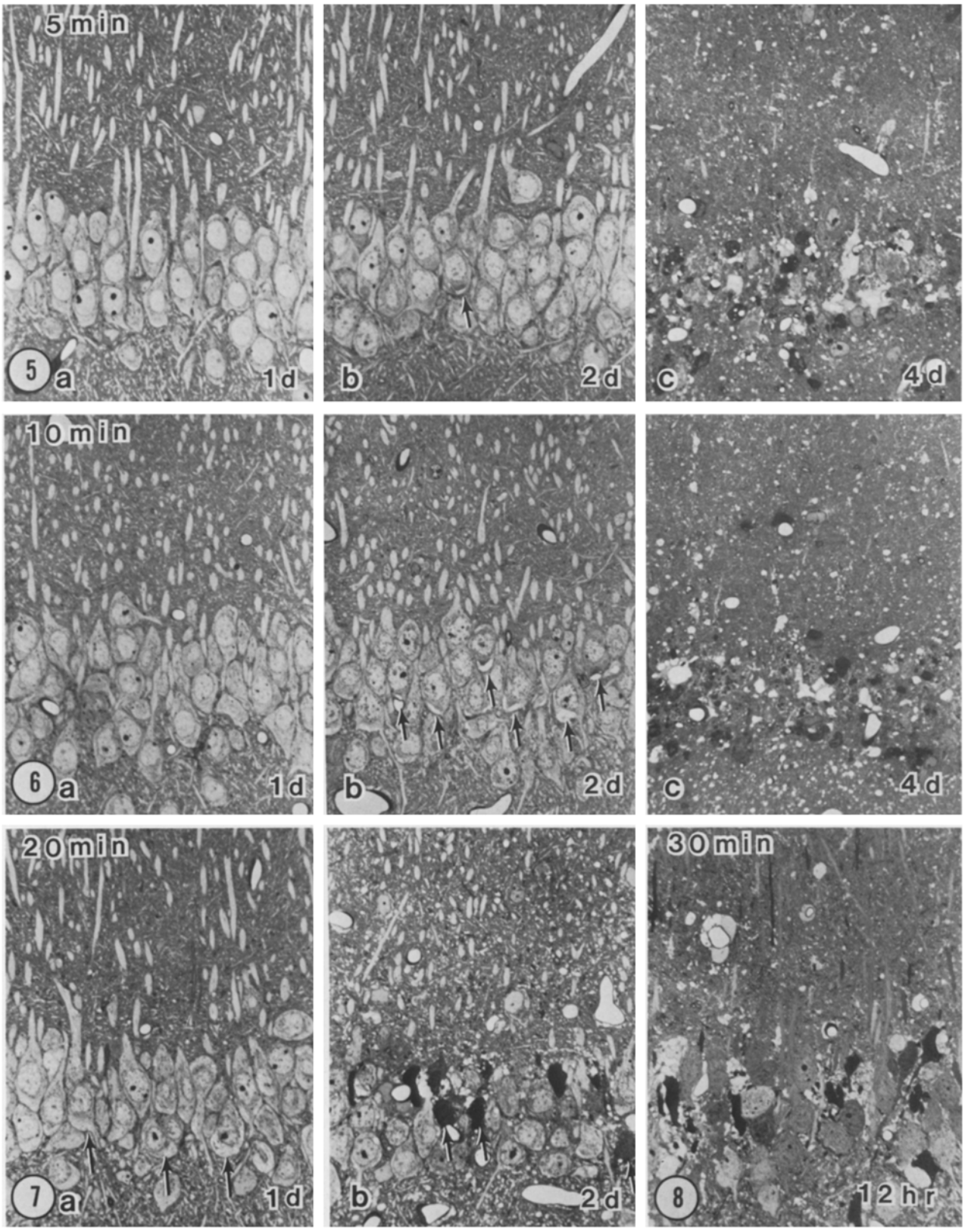
pus was lost, while the dentate gyrus looked intact (Fig. 4).

In the rest of the animals, only the dorsal hippocampal formation and overlying cerebral cortex were examined as plastic-embedded specimens. The results are described according to each area of the structure. The definition of each subfield in shown in Fig. 1.

CA1 (Sommer's Sector)

The change in CA1 was slow. One day following brief ischemia (5–10 min), definite alteration was hardly seen except that the cell nucleus occasionally looked more inhomogeneous than normal (Fig. 6a). On day 2, pyramidal cells showed slight clumping of the nuclear chromatin and slits in the basal side of the cytoplasm (Figs. 5b, 6b). These initial signs of alteration were followed by extensive destruction of almost all pyramidal cells observed on the 4th day (Figs. 5c, 6c). In the later stage of CA1 cell change, some pyramidal cells were darkly stained whereas others were swollen and stained only slightly (Figs. 5c, 6c). No uniform type of cell decomposition was detected at this stage.

Following longer ischemia (20–30 min), the changes appeared faster. One day after 20 min of ischemia, the tendency of slit formation was accentuated, and cell nuclei were more inhomogeneous than after brief ischemia (Fig. 7a). On day 2, the majority of the pyramidal cells showed increased staining with toluidine blue (Fig. 7b). Some had a dark cytoplasm with small vacuoles (Fig. 7b). Twelve hours following 30 min of occlusion, some CA1 pyramidal cells were at



Figs. 5–8. Hippocampal CA1 subfield. The changes appear faster when ischemia lasts longer. Plastic section, toluidine blue; ×410

Fig. 5. One day following 5-min ischemia (**a**) pyramidal cells appear normal. On day 2 (**b**), a slit-like change is seen (*arrow*) on the basal side of the perikarya. The nucleus looks slightly clumped. No normal CA1 pyramidal cells are present on day 4 (**c**)

the stage of overt cell disintegration. They had darkly stained, shrunken cell bodies with surrounding empty spaces (Fig. 8). These findings in CA1 pyramidal cells following the longer ischemia resembled classical ischemic cell change of neurons in their light-microscopic appearance. Even the changes following 20 min of ischemia, however, took place during days 1 and 2 and were by no means fast cell deterioration.

After longer ischemic insults (20–30 min) in the most medial part of CA1 (CA1a or paramedian region according to Pulsinelli 1982a), cell death appeared faster. When the rest of the CA1 subfield showed slit formation and slight change in cell nuclei, CA1a neurons were in the stage of overt cell destruction. The CA1a pyramidal cells, however, were less vulnerable to brief ischemia because following 3–5 min of ischemia, CA1a neurons were preserved in most cases. In rare cases, pyramidal cells were preserved as clusters even in CA1b and CA1c. Except for these survivors, most of the CA1 pyramidal cells were lost (Figs. 2, 3).

CA2 and CA3

Alteration in the CA2 and CA3 subfields took place within a day. Following brief ischemia (5–10 min), pyramidal cells in the CA2 sector became swollen (Figs. 9, 10). Numerous dark granules were seen in the enlarged cytoplasm, and the cell nuclei were displaced to the periphery and had a crescent shape. These are characteristic of the change which has been called reactive change or selective chromatolysis. This cell alteration culminated on days 1 and 2; then it resulted in cell death in most parts. In some cases, there was no definite CA2 cell injury in the presence of damages in CA1 or CA4 regions when observed later than day 7 following ischemia. After longer ischemic insult, reactive change appeared faster and more severely (Fig. 11). No change in CA3 was noticed after brief ischemia (Fig. 12), whereas following more protracted ischemia (20–30 min), giant pyramidal cells in CA3 showed widespread reactive change (Fig. 13). This was most conspicuous in the CA3a area which is contiguous to CA2. This cell alteration resulted in almost total loss of the pyramidal cells of the hippocampus (Fig. 4).

CA4 (Endfolium) and Dentate Gyrus

The CA4 pyramidal cells showed a fast change. They became darkly stained with shrunken cell bodies and empty spaces surrounding them (Figs. 14, 15). Ocassionally, injured cells contained numerous vacuoles, but they were so intensely stained that the cell's internal structures could hardly be identified by light microscopy. These darkly stained cells were scattered in the CA4 subfield among seemingly unaffected pyramidal cells (Fig. 14). Although the number of cells injured increased as ischemic insult was prolonged, there was no clue as to the type of cell which had been selectively injured.

The dentate gyrus was the most tolerant to ischemia among the structures examined here (Fig. 14). A longer ischemic insult (20–30 min) caused swollen cytoplasm in some dentate granule cells. In one gerbil following 20-min ischemia, there was 1/3 loss of the dentate granule cells. Otherwise, the dentate gyrus looked unchanged. One week after 20-min ischemia, there were numerous dark granules in the inner part of its molecular layer. This change was identical to what has been described as degenerating terminal boutons in this area in gerbils (Kirino and Sano 1980).

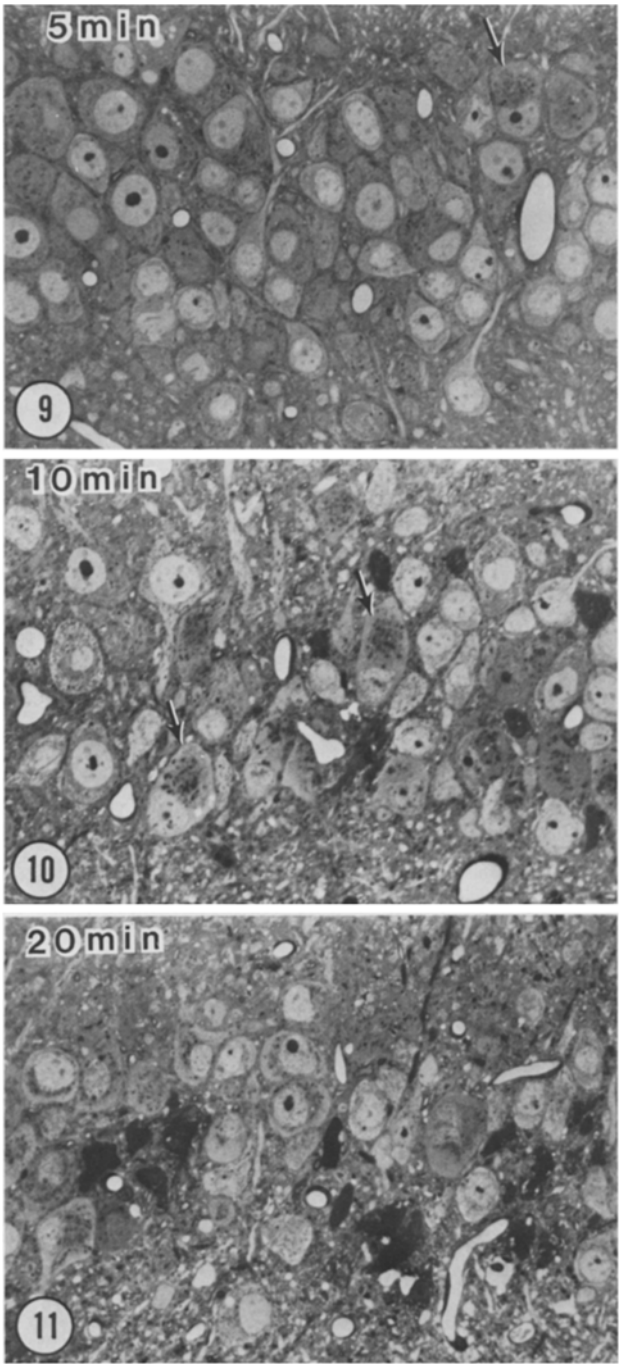
Discussion

This experiment has shown that brief bilateral carotid occlusion in the Mongolian gerbil offers an excellent, reliable model to solve the long-known problem of hippocampal selective vulnerability (Scholz 1959). Ischemia lasting 5 min produced the typical hippocampal lesion in about 90% of animals. The hipocampal damage was seen throughout the dorsal hippocampus, whereas brain injury outside the hippocampus was not observed. Brief ischemia for 5 min seemed to produce pure CA1 pyramidal cell deprivation except for scattered CA2 and CA4 neurons that were damaged. The change following 10 min of ischemia was similar to that of the 5 min group. Following longer ischemia (20–30 min), similar damage were seen in more rapid and severe forms. This is a clear example of "maturation phenomenon" (Ito et al. 1975). The brains, however, showed varying degrees of injury after longer ischemia. In some animals lesion was restricted to the hippocampus, whereas others had widespread neuronal changes outside the hippocampus. Ischemic insult for 3 min failed to cause reproducible changes in the hippocampus.

Fig. 6. One day following 10-min ischemia (**a**) the cell nucleus is slightly inhomogeneous. Two days after the insult (**b**) numerous slit-like changes appear (*arrows*). By day 4 (**c**) almost all of CA1 has been destroyed

Fig. 7. Twenty-minute ischemia causes multiple slit-like structures in the perikarya (*arrows*) 1 day after the insult (**a**). The following day (**b**), some pyramidal cells are darkly stained and look shrunken (*arrows*). There are small vacuoles in the cytoplasm

Fig. 8. Twelve hours following 30-min ischemia the changes appear faster. Some neurons are intensely dark and shrunken. They are surrounded by empty spaces. Some cells show swollen cytoplasm



Figs. 9–11. CA2 subfield. One day following ischemia. Plastic section, toluidine blue; ×430

Fig. 9. Five-minute ischemia. There are few swollen pyramidal cells with increased intracytoplasmic granules (*arrow*)

Fig. 10. Ten-minute ischemia. Cells displaying typical reactive change increase (*arrows*). Some neurons are darkly stained

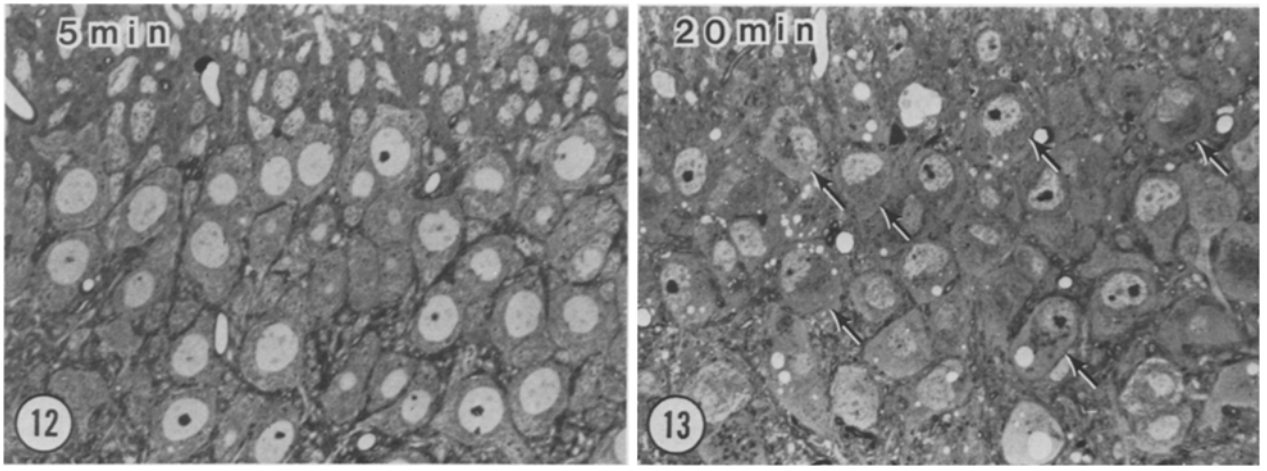
Fig. 11. Twenty-minute ischemia. Most of the CA2 neurons are affected. The number of cells showing dark cytoplasm has increased

The change in CA1 pyramidal cells after brief ischemia differed from what has been known as ischemic neuronal alteration (McGee-Russell et al. 1970; Brown and Brierley 1972). It was a slowly progressing pathologic change. It took 2 days before the cell change became apparent by light microscopy. The change was not a rapid destruction nor decomposition of neurons. Suzuki et al. (1983) showed that the gerbil's hippocampal CA1 sustained electric activity on day 1, while it disappears althogether on day 2 following 5 min of ischemia. It seemed that CA1 pyramidal cells retained their viability for 1 or 2 days and then deteriorated. Research into this delayed neuronal death will be of special clinical significance since neurons are not subject to rapid cell destruction in this type of ischemic cell injury. It may allow great possibility to protect or shift neurons during the initial latent period from the process of cell death. However, the period when neurons become irreversibly damaged, or the "point of no return" (Trump 1980), is yet to be clarified.

The exact mechanism of delayed neuronal death is not known. Since gerbils have been believed to demonstrate unusual tissue reaction following ischemia (Brown et al. 1979), it is possible that delayed neuronal death in CA1 is a process inherent only to the gerbil model. This is unlikely, however, as Pulsinelli et al. (1982a) have reported slowly progressing neuronal death after ischemia in the rat. They showed a delay in the onset of ischemic damage which was also noticed by increasing the duration of ischemia. Our observation in the gerbil is in accordance with that in the rat by Pulsinelli et al. (1982a).

Events following reestablishment of recirculation after ischemia are thought to be primarily important in the process of selective vulnerability (Jenkins et al. 1981). Since a state of hypermetabolism has been reported to occur following recirculation (Diemer and Siemkowicz 1980), imbalance of energy metabolism between supply and demand (Levey et al. 1979) could be the cause of delayed neuronal death. However, Arai et al. (1982) examined the CA1 subfield in the same gerbil model and confirmed that there was no decrease of ATP during the period of cell deterioration. Also, Pulsinelli and Duffy (1982) noticed a similar change in energy metabolism in their rat ischemia model. They found that, although transient hypermetabolism appeared to be related to the onset or early progression of the neuronal damage following ischemia, imbalance of regional blood flow and glucose metabolism did not correlate to the pattern of neuronal injury (Pulsinelli et al. 1982b). Therefore, cell death due to impairment of energy metabolism does not seem to be the main cause.

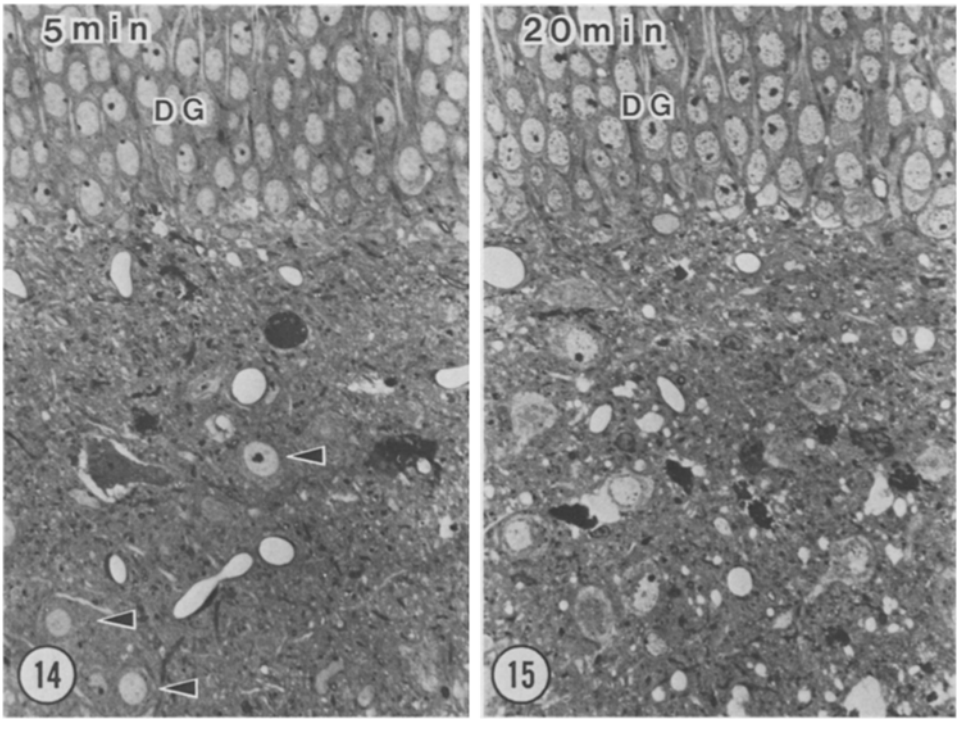
The hippocampus can be divided into two major parts; CA1 and CA3 (Shepherd 1979). The CA3 subfield connects with mossy fiber terminals from the



Figs. 12, 13. CA3 subfield. One day following ischemia. Plastic section, toluidine blue; × 430

Fig. 12. Five-minute ischemia does not produce any change in the CA3 subfield (cf. Fig. 2)

Fig. 13. Most of the CA3 neurons show reactive change (*arrows*) following 20-min ischemia. Later on, this results in total cell death (cf. Fig. 4)



Figs. 14, 15. CA4 subfield and dentate gyrus. One day following ischemia. Plastic section, toluidine blue, × 440

Fig. 14. Five-minute ischemia. Scattered CA4 neurons are affected, darkly stained, and shrunken. Other pyramidal cells (*arrowheads*) look unchanged. The dentate granule cells (*DG*) appear similar to normal

Fig. 15. Twenty-minute ischemia. Change in the CA4 neurons increases. Also, the neurophil looks spongy. The dentate granule cells (*DG*) do not show definite alteration

dentate granule cell and sends off Shaeffer collaterals to CA1 pyramidal cells. The CA1 sector in turn receives fibers from CA3 and have connections with the subiculum. Interestingly, each subdivision of the hippocampus showed discrete forms of cell change following brief ischemia in this experiment. Therefore, it is more understandable that this phenomenon depends on the position and interrelation of neurons in the total circuitry within the hippocampus.

One controversial cell alteration has been reactive change in CA2 (Bubis et al. 1976). Brown et al. (1979) attributed this change to "epileptic" movement in gerbilis. Gerbilis subjected to 5-min ischemia, however, usually did not show overt "epileptic" movement during and after ischemia, yet did develop reactive change in the hippocampus. Moreover, the gerbil brains were reported to be electrically silent during ischemia and consequently "epileptic" movement, if

any, did not arise from the ischemic brain itself during ischemia (Cohn 1979). These observations may open the question of reactive change. One intriguing observation is that when the hippocampus was lost altogether, CA2 and CA3 pyramidal cells had been destroyed by reactive change.

The change in CA4 was rather similar to tranditional ischemic cell change (Brown and Brierley 1972). Why this cell alteration took place only in CA4 is obscure. We wish to emphasize that reaction of the brain tissue to brief ischemia is by no means a uniform one.

When gerbils were subjected to longer ischemia, CA1 pyramidal cells in some specimens developed change which was rather similar to well-defined ischemic cell change (Brown and Brierley 1972). The cell body became darkly stained and shrunken. A question has arisen whether there was a jump in cell change from "delayed neuronal death" type to "ischemic cell change" type following a certain amount of ischemic insult, or if there was a qualitative continuity in the cell pathology. This problem awaits further study using electron microscopy (Kirino and Sano 1983).

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