Age-Dependent Increase in Infarct Volume Following Photochemically Induced Cerebral Infarction: Putative Role of Astroglia

Alexander Kharlamov, Elena Kharlamov, and David M. Armstrong

This study demonstrates that the photochemically induced model of stroke is an extremely viable method of inducing cerebral infarction in old animals. The lesions are reproducible both in terms of location and size and compatible with long-term survival of the animal. With this model we demonstrated, one week following surgery, a significantly larger infarct in rats 20 and 24 months of age compared to 4-month-old rats. The older rats also sustained greater neurologic deficits as assessed on a rotarod task. Older rats also were characterized by a glial response that was far less intense than in young animals. While the precise relationship between glia activation and cerebral damage remains to be determined, it would appear that a better understanding of those factors that contribute to the astrocytic response in the aged rat may be of particular benefit in designing therapeutic strategies aimed at reducing the pathologic consequences of cerebral infarction in elderly humans.

IN the aged brain, neurons often display an increased vulnerability following injury. Although the precise mechanism underlying this increase remains unclear, one hypothesis suggests a failed plasticity in the aged brain. Notably, this failure is exacerbated during an extreme condition such as ischemic stroke, which requires the mobilization of all neuroplastic (i.e., compensatory) reserves. Following ischemia, the affected zone undergoes a cascade of events resulting in cell injury and/or death. For example, the depletion of energy reserves, accumulation of lactic acid, release and accumulation of excitatory amino acids, increased concentrations of intracellular Ca^{2+}, and production of oxygen-free radicals all play a role in the resultant cerebral ischemic injury. Although the extent to which these events uniquely affect the aged brain is not known, a number of investigators have demonstrated a larger infarct in the brains of aged rodents compared to young (1–3). However, the correlation between brain damage and age is not linear, in part reflecting the region of the brain affected and variability between rodent strains (3,4).

In order to adequately test various therapeutic interventions in aged animals, it is necessary to utilize a model of stroke that is both reproducible in terms of size and location and compatible with the survival of the animal. The photothrombotic model of inducing cerebral infarctions is ideally suited for aged rats because it is relatively noninvasive and results in a very low rate of morbidity and mortality. In addition, the model results in a highly reproducible infarct which, when positioned within the sensory-motor area of the neocortex, results in specific behavioral deficits. In the present study, we produced photochemically induced focal ischemia damage in the brains of rats 4, 20, and 27 months of age in order to investigate the age-dependent alterations in infarct volume. In addition, as an index of the brain’s compensatory ability, we examined the response of reactive astrocytes in the peri-infarcted zone and measured the performance of the rat on a rotarod.

METHODS

Animals.—Fischer 344 (F344) retired breeder rats ages 4, 20, and 27 months were obtained from NIH-NIA aging colonies (Harlan Sprague-Dawley, Indianapolis, IN). Animals were housed individually for at least 7 days prior to the study and maintained on a 12/12 hour light/dark cycle. Food and water were provided ad libitum. Studies were approved by the AUHS Institutional Animal Welfare Act and the Public Health Services policy on the humane care and use of laboratory animals.

Photothrombotic cortical brain ischemia.—Throughout these studies we employed a model of photochemical thrombotic lesion (5) with some minor modifications (6,7). Rats were deeply anesthetized with Equithesin® (ip .06–.01 ml/100 g body weight) and placed in a stereotaxic apparatus. A photosensitive dye, rose bengal (disodium 4,5,6,7-tetraiodofluorescein; Sigma, St. Louis, MO) was dissolved in saline (0.9% NaCl) and injected into the tail vein (80 mg/kg). Animals were randomly assigned into operated and nonoperated (sham) groups. Sham-operated animals received only saline injections (i.e., vehicle). The skin was incised and the left side of the skull was exposed for 10 min to cold white light (Olympus Denmark A/S, Glostrup, Denmark), corresponding to a region 1.8 mm posterior to bregma and 2.8 mm left of the midline. The underlying brain area corresponded to the parietal sensorimotor neocortex. The distance between the skull surface and light source was 4 inches. Following light irradiation, the skin incision was sutured and the rats were left to recover from an-
esthesia. Body temperature was monitored and maintained at 37° ± 0.2°C using a thermoregulated pad. Animals received a subcutaneous injection (5 ml) of lactated Ringer’s solution immediately following surgery as well as 1 and 2 days postoperative. Rats were allowed free access to laboratory chow and water.

**Tissue preparation.**—Following postoperative periods of 12 hours, 24 hours, 3 days, and 7 days, the animals were anesthetized with chloral hydrate and killed by cardiac perfusion with 0.9% saline in 0.1 M phosphate buffer (PB, pH 7.4) followed by 4% buffered paraformaldehyde (250–300 cc). The brains were rapidly removed and post-fixed overnight in the same solution at 4°C and then cryoprotected in 30% sucrose solution in PB for 2–3 days. Care was taken to preserve portions of the skull illuminated by the light for subsequent determination of its thickness and measurements of light penetrance. Using a sliding freezing microtome, the brains were sectioned at 40 μm in a coronal plane throughout the rostrocaudal extent of the lesion, collected in 24-well culture dishes and stored in cryoprotectant solution consisting of ethylene glycol/glycerol/phosphate buffer.

**Measurement of the thickness of the skull and intensity of the light under the skull.**—For each animal the thickness of the skull was determined with a micrometer. In addition, the intensity of light as a function of thickness of the skull was determined using an INS Lux meter (Fluke 87, True RMS multimeter and photosensor). First, a photocell was placed directly under the light, and the intensity of the light spot on the surface was measured, thus reflecting the intensity of light on the surface of the skull. Subsequently, the photocell was covered with a corresponding piece of skull bone and illuminated. The latter provided a measurement of the intensity of the light beneath the skull (i.e., equivalent to the pial surface).

**Immunohistochemistry.**—The immunohistochemical procedure was adapted from the avidin-biotin peroxidase method of Hsu and colleagues (8) and has been described in detail by Armstrong and coworkers (9). Briefly, free-floating tissue sections were removed from the cryoprotectant solution and rinsed in 0.1 M PB followed by a 30 min incubation in 0.3% hydrogen peroxide in PB. After several rinses in 0.1 M Tris-buffered saline (TBS, pH 7.4) the tissue was incubated for 30 min in TBS containing 3% bovine serum albumin (BSA) and 0.25% Triton X-100 (TX-100). Tissue sections were incubated overnight at 4°C with primary antibody against microtubule associated protein 2 (MAP2; Sigma, St. Louis, MO), vimentin (Boehringer Mannheim), or gliarial fibrillary acidic protein (GFAP; Boehringer Mannheim) diluted 1:1500, 1:100, or 1:500, respectively, in TBS containing 1% BSA and 0.25% TX-100. After several rinses, the tissue sections were incubated for 1 hour at room temperature in biotinylated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) diluted 1:500 in TBS containing 1% BSA, followed by a 1 hour incubation in avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA). The bound peroxidase was treated with imidazole acetate buffer (pH 9.6) containing 0.05% diaminobenzidine, 2.5% nickel ammonium sulfate, and 0.005% H2O2. The immunolabeled tissue sections were mounted onto gelatin-coated glass slides, dehydrated through graded alcohols, and covered with plastic coverslips. As a control for nonspecific staining, preimmune serum was substituted for the primary antibody, and the tissue was processed as described. In no instance did we observe any evidence of immunolabeling when the tissue was processed using the aforementioned procedure.

**Measurement of the infarcted area.**—Using a 1:6 series throughout the frontoparietal cortex, the infarcted zone was measured using a computer-assisted image analysis system. The infarcted area was readily recognized in tissue sections stained for MAP2. In previous studies we and others have demonstrated that this protein is conspicuously absent from the infarcted region (10). For each tissue section the infarcted zone was outlined on the computer screen, and the volume (mm³) was determined by integrating the appropriate area with the section interval thickness.

**Vimentin-positive cell counting.**—The number of vimentin-positive cells was determined by two independent investigators blind to the experimental condition. For each brain, a 1:6 series throughout the frontoparietal cortex was immunolabeled using antibodies against vimentin. From these sections three were pseudorandomly selected for cell counting. For each immunolabeled tissue section, four regions within the peri-infarcted zone were selected for cell counting. Using a computerized image analysis system, the regions undergoing analysis were standardized to a size 300 × 300 μm. Within each field all vimentin-positive profiles were counted. In addition to their staining pattern, glial elements were also identified according to morphologic criteria.

**Rotarod testing.**—An automated 4-lane rotarod unit was used to assess motor coordination. The rotarod consisted of a rotating spindle (diameter 7.3 cm) and individual compartments (lane) for each rat. The unit allowed for preprogramming of protocols with varying rotational speeds ranging from 0–80 rpm. Infrared beams were used to detect when a rat had fallen onto the grids beneath the rotarod. Pretest training consisted initially of placing the rat on the rotarod at 0 rpm for 3 × 3 min for 2 consecutive days. The rat was placed perpendicular to the rotating axis with its head opposite to the direction of the rotation, thus requiring the rat to move forward in order to stay on the rod. Further testing consisted of placing the rat on the rod at an initial rotating speed of 5 rpm for an additional 3 days. The time on the rotarod was recorded up to a maximum time of 3 min. Following an intertrial interval of 30 min, the rat was retested. All testing was conducted in the afternoon. Following the lesion, testing commenced on the second postoperative day and continued for 7 days.

**Statistical analysis.**—A one-way analysis of variance (ANOVA) was performed to analyze skull thickness in rats of different ages, age-related changes in light intensity under the skull, and lesion-induced vimentin response in rats from different age groups. A two-way ANOVA was used to
analyze infarct volume in different age groups following multiple post-stroke survival times (3×4 ANOVA) and to analyze presurgical behavioral performance on a rotarod (3×6 ANOVA). Three-way ANOVA was utilized for postsurgical analysis of performance on a rotarod (3×5×2 ANOVA). Duncan multiple range with post-hoc analyses were used to determine any differences between groups. Differences were considered significant at the p < .05 or p < .01 level of confidence. All statistical analyses were performed using PharmCalc Statistic computer program.

**Results**

**General observations.**—Initial experiments with photochemically induced lesions in aged animals indicated the necessity to make some changes in our anesthetic protocol and rehabilitation strategies for long-term survivals. Previously, young rats were routinely anesthetized by ip injection of 360 mg/kg of chloral hydrate. This procedure, however, proved to be unacceptable in older rats, as it resulted in a >25% mortality rate. Subsequent experiments demonstrated a near-zero mortality rate when Equithesin® was used as the anesthetic. Our initial experiments also demonstrated that old rats recovered from the surgery more slowly than young rats and at times had difficulty feeding and drinking within the first 24 hours postsurgery. For this reason, young and old rats received subcutaneous injections of lactated Ringer’s solution during the first 48 hours following the lesion.

**Skull thickness and measurement of the intensity of light under the skull.**—Table 1 illustrates the thickness of the skull from rats 4, 20, and 27 months of age. The skull of the 27-month-old rats was nearly two times thicker than the skull of 4-month-old animals. The difference in skull thickness between middle-aged and old rats was not significant. As a result of the thicker skull, the intensity of the light under the skull of 27- and 20-month-old rats was reduced circa 22% and 16%, respectively, compared to 4-month-old rats. The difference in light intensity between old and middle-aged rats was not significant (Table 1). Age-related differences in the thickness of the skull also raised the possibility that the temperature of the underlying brain may vary as a function of age. Although the narrow spectral band of the light source reduces the likelihood of any temperature elevation, we nevertheless monitored brain temperature during 10 min of light exposure using a small thermosensitive probe. In all animals the cortical brain temperature in the area of illumination remained at 37°C (data not shown).

**The size of the infarcted core.**—As described previously (6,7), the lesion was restricted to the gray matter of the parietal cortex. In young animals the infarcted area steadily increased, reaching near-maximum size 24 hours postlesion with no further increases in volume observed 3 and 7 days postlesion (Figure 1). In the 20- and 27-month-old rats, the infarct too underwent the majority of its growth within the first 24 hours postlesion (Figure 1). In fact, at 24 hours postlesion no difference in infarct size was noted when comparing any of the three age groups. However, in contrast to the younger rats, the infarcted area within the older rats continued to increase in size up to 7 days. While there was no difference in the size of the infarction between 20- and 27-month-old rats at 3 or 7 days postlesion, the infarcted area in both groups was significantly larger (23% increase) than in young (4-month-old) animals 7 days postlesion.

![Image of Table 1](https://example.com/table1.png)

**Table 1. Thickening of Skull With Increasing Age and Decrease of Intensity of Light Penetration**

<table>
<thead>
<tr>
<th>Age (n)</th>
<th>Skull Thickness (mm) (Mean ± SD)</th>
<th>Skull Thickness as Percentage to Thickness of Skull of 4-month-old Rats</th>
<th>Light Intensity Under the Skull (Lux × 100) (Mean ± SD)</th>
<th>Light Intensity as Percentage to Intensity of the Light Under the Skull of 4-month-old Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 month (12)</td>
<td>0.56 ± 0.03</td>
<td>100%</td>
<td>120.1 ± 1.6</td>
<td>100%</td>
</tr>
<tr>
<td>20 month (10)</td>
<td>0.91 ± 0.01*</td>
<td>163%</td>
<td>100.4 ± 1.7*</td>
<td>84%</td>
</tr>
<tr>
<td>27 month (10)</td>
<td>1.07 ± 0.03*</td>
<td>191%</td>
<td>93.5 ± 1.7*</td>
<td>78%</td>
</tr>
</tbody>
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*Notes: Two one-way ANOVAs with post-hoc Duncan multiple range test revealed significant differences (p < .01) in skull thickness between young (i.e., 4-month-old) and old rats (i.e., 20- and 27-month-old rats) (F = 7.32, df = 2/29). (*) indicates significant differences in rats 20 and 27 months of age compared to rats 4 months old. Likewise, the light intensity below the skull of older rats was significantly different (*) compared to young rats (F = 5.21, df = 2/29, p < .05).

![Image of Figure 1](https://example.com/figure1.png)

**Figure 1.** 24 hours postlesion the infarct obtains the vast majority of its size and does not differ between rats 4, 20, and 27 months of age. Thereafter, infarct size remains relatively constant in the 4-month-old rats. In contrast, infarct size continues to increase in the 20- and 27-month-old rats and 7 days postlesion is larger in the older rats compared to 4-month-old rats. Two-way A N O V A with post-hoc Duncan multiple range test revealed significant difference (*) in infarct volume between old rats (20 and 27 months of age) and young rats (4 months old) at 7 days postlesion (F = 4.23, df = 2/17, p < .05).
Glial response.—Reactive astrocytes were observed immunohistochemically using antibodies against vimentin and GFAP. In nonlesioned animals as well as on the contralateral side, vimentin-positive astrocytes were relatively scant whereas GFAP-labeled profiles were comparatively abundant. Because of the pronounced difference in baseline levels of vimentin versus GFAP, we opted to focus our quantitative efforts on those tissue sections labeled with vimentin. Modest increases in reactive astrocytes could be readily detected in these latter tissue sections.

In all experimental animals the number of vimentin-positive astrocytes was relatively few in the peri-infarcted area 24 hours postlesion (data not shown). Dramatic increases in vimentin immunolabeled astrocytes in the peri-infarcted zone were observed in young rats 3 and 7 days postsurgery (Table 2; Figure 2A,B). In 20- and 27-month-old rats the number of vimentin-positive elements was relatively scant 24 hours postlesion (data not shown). While the number of vimentin-positive elements rose substantially 3 and 7 days postlesion in older rats, the response was significantly less than that observed in 4-month-old rats (Table 2; Figure 2C–F). Although no attempt was made to count the number of GFAP-labeled astrocytes, we too observed an increase in GFAP-labeled elements in the lesioned cortex of young rats compared to the contralateral side (data not shown). Labeled astrocytes in the peri-infarcted region showed evidence of hypertrophy and hyperplasia. In addition, the number of GFAP positive astrocytes in the peri-infarcted zone of 20- and 27-month-old rats was less pronounced than in young rats (data not shown).

Rotarod testing.—During the pretest period, old rats displayed marked impairment with this task compared to young rats (Figure 3A). Differences were most pronounced during the first couple days of testing. However, after 4–5 days of training all animals performed equally well. Following surgery, the performance of sham-operated rats did not differ from presurgical levels. These later data were true regardless of the age of the rat (Figure 3B). In contrast, the performance of lesioned rats was significantly affected (Figure 3C). Specifically, in 4- and 20-month-old rats marked deficits in performance were observed during the first and second days of testing. However, by the third day of testing (i.e., 4 days after ischemia), performance was not significantly different from pretest levels. Twenty-seven-month-old rats also performed poorly during the first couple days of testing. Yet, unlike younger rats, they failed to improve and continued to perform at substandard levels throughout the duration of the testing period.

Table 2. Decrease in Vimentin-Positive Cells in Relation to Age

<table>
<thead>
<tr>
<th>Age (n)</th>
<th>Vimentin-Positive Cells (Mean ± SD)</th>
</tr>
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<tbody>
<tr>
<td>4 month (8)</td>
<td>103.2 ± 3.3</td>
</tr>
<tr>
<td>20 month (8)</td>
<td>68.8 ± 5.5*</td>
</tr>
<tr>
<td>27 month (6)</td>
<td>50.0 ± 11.1*</td>
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Note: In the area penumbra (i.e., that region immediately adjacent to the infarcted core), vimentin-positive profiles are significantly (*) fewer 7 days postlesion in rats 20 and 27 months of age compared to 4-month-old rats ($F = 4.65, df = 2/19, p < .05$).
Figure 3. Performance on a rotarod task. (A) Prior to surgery, 20- and 27-month-old rats displayed greater difficulty in acquiring this task than did rats 4 months of age. However, by the sixth day animals of all ages were performing at similar levels. Two-way (3×6) ANOVA with post-hoc Duncan multiple range test demonstrates significant differences in rotarod performance between old (20- and 27-month-old rats) and 4-month-old rats within the same trial (F = 3.60, df = 2/21, p < .05). (B) Following surgery, sham-treated animals, regardless of age, performed comparable to preoperative levels. (C) In contrast, all infarcted rats demonstrated marked deficits in performance during the first two days of testing. While performance improved dramatically in the 4- and 20-month-old rats, the 27-month-old animals displayed only modest improvement throughout the entire test period. Postsurgical performance was analyzed by three-way (3×5×2) ANOVA with Duncan multiple range test. (*) indicates significant differences in rotarod performance between different age groups within the same trial (F = 3.48, df = 2/31, p < .05). (#) indicates significant differences in behavioral performance between sham and lesioned animals within the same age group (F = 4.53, df = 1/31, p < .05).

DISCUSSION

This study demonstrates that the photochemically induced model of stroke is a highly viable method of inducing cerebral infarction in old animals. The lesions are reproducible both in terms of location and size and compatible with long-term survival, making studies of recovery and plasticity of the aged brain possible. The study also underscores the importance of recalculating the dose for anesthetic agents and for changing rehabilitation strategies for middle-aged and aged animals. Using this model of stroke, we demonstrated a significant increase in infarct size in 20- and 27-month-old rats compared to 4-month-old rats. Our data support the results of previous investigations demonstrating a significantly greater extent of brain damage in old animals after middle cerebral artery occlusion (1,2,3). In interpreting our data it is important to note that the calvarium of old rats is nearly twice the thickness of 4-month-old rats, thus reducing the amount of light penetrating the skull (i.e., 25% reduction) and interacting with the photosensitive dye, rose bengal. Had we elected to adjust for this variable and increase the intensity of light for aged rats, we might have predicted an even greater age-related difference in infarct size than observed in the present study. Notably, our study supports biological rather than technical and/or mechanical factors contributing to the age-related increases in the size of the lesion.

In considering these biological factors, it is important to acknowledge the putative age-related changes in the microcirculation. For example, previous studies have reported decreases in the diameter of the lumen of capillaries as well as reductions in capillary endothelial cells (11). With advancing age the microvascular also has been shown to undergo a number of morphologic alterations including increased tortuosity and deformity (12). In the aged brain, intramural collagen also has been demonstrated to increase, resulting in vascular thickening (13). These structural changes in the microvascular may provide an explanation for the age-related impairments in cerebral autoregulation as well as declines in cerebral blood flow [CBF; (14,15)]. Although the extent, if any, that vascular alterations contribute to our observations is unclear, it is reasonable to consider that any condition that compromises CBF, and hence reduces circulating concentrations of rose bengal, would tend to minimize rather than exaggerate differences between young and old rats following photochemically induced cerebral infarction.

When investigating the various mechanisms of neuronal damage following stroke, it is also reasonable to consider the role of glutamate. In this regard, it is important to bear in mind that following stroke there are a number of reports supporting a massive rise in extracellular glutamate levels (16,17). Thus, one may hypothesize that the aged brain, with greater sensitivity to glutamate and less efficient means of glutamate disposal, may be at greater risk to the pathologic consequences of glutamate receptor activation. In support of this hypothesis are a number of studies reporting an age-related increase in the sensitivity to kainate (KA) neurotoxicity, suggesting that kainate and N-methyl-D-aspartate (NMDA) receptor activation could be important mediators for excitotoxic damage in the aged brain (18–20). In contrast, work by Kesslak and colleagues (21) demonstrated a decreased toxicity to KA with age. Although the reason for these discrepant findings remains to be determined, it is important to note that this study utilized intrahippocampal injections of KA, while in earlier investigations excitotoxic damage was induced following systemic or subcortaneous injections of KA (19,20) or in the case of humans following accidental ingestion of domoic acid (18). Furthermore, age-related variations in the permeability of the blood-brain barrier or changes in drug metabolism may result in alterations in the availability of KA to the brain and thus account for differences between studies. Aged rats also have been reported to have higher basal levels of glutamate (22). Potential contributors to these latter observations include reduced affinity of...
the high energy glutamate reuptake mechanisms (23,24). Whether glutamate uptake mechanisms are further impaired following stroke and thus contribute to the age-related increase in infarct size remains to be determined. Although a controversial subject, age-related decreases have been reported in the number of KA, NMDA, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors (25). Although the numbers of these receptors may decrease with age, it too has been reported that the functional sensitivity of NMDA receptors is enhanced with age (26). Moreover, recent work demonstrates that following stroke there is the downregulation of specific glutamate receptor subunits [i.e., GluR2; (27)]. These alterations likely have marked effects on the responsiveness of the receptor following glutamate activation including the conductance of calcium through the ion channel. Whether these stroke-induced alterations in glutamate receptor subunits occur to a similar extent in the aged brain remains the subject of further investigations. Studies have also demonstrated that the ischemia-induced release of taurine, one of the inhibitory amino acids, is significantly diminished in aged spontaneously hypertensive rats (SHRs) compared to adult SHRs (28). In contrast, glutamate levels remained similar between aged and adult SHRs. These data raise the possibility that the age-related vulnerability of neurons to ischemic insult is more likely to be attributable to impairment of an inhibitory mechanism rather than to excessive excitotoxin release. Comparable mechanisms may too be contributing to the observations of the present study.

We determined that following stroke, aged rats were characterized by a glial response that was far less intense than in young animals. Astrocytic activation is of central importance following brain injury and can either promote or deter recovery of central nervous system (CNS) function (29). With increasing age, the astrocytic population undergoes a number of changes including increases in basal levels of GFAP mRNA and protein (30–32). Likewise age-associated increases in GFAP in the human brain have been reported, and considerably larger increases have been observed in brains of patients with Alzheimer’s disease (33). It is not clear, however, the extent (if any) to which aging affects the ability of CNS astrocytes to respond to injury. For example, the work of Morgan and colleagues (34–36) supports an exaggerated astrocytic response, albeit sometimes delayed, in aged rodents following a variety of injury paradigms. In contrast, the studies of Scheff and colleagues (37) and Kane and associates (38) suggest a less robust astrocytic response in brain and spinal cord of older animals. In interpreting the latter findings, Scheff and colleagues (37) suggest that one factor affecting the astrocytic response in old animals is an impaired or suppressed response to injury. In this regard, it is interesting to consider the relationship between astrocytes and macrophages and the fact that macrophage activation may increase at the injury site well before increases in astrocytic proliferation (39,40). Macrophages are known to secrete cytokines, including interleukin 1 (IL-1), which in turn are reported to stimulate astrocytes (41). In aged rats, macrophage activity within cerebral infarcts has been reported to be markedly reduced (1). Notably, a reduced macrophage response and associated decreases in IL-1 production may contribute significantly to the extent of astrocytic hypertrophy following injury. In support of this hypothesis is the work of Schroeter and coworkers (42), who suggest that the persistent astrocytic response in the zone immediately surrounding the photothrombotic infarct might be induced by leukocyte-derived cytokines. In considering the mechanism underlying the astrocytic activation, it is important to understand that following photothrombotic insult, reactive astrocytes are present both in the peri-infarcted zone (i.e., adjacent to the lesion) as well as throughout the entire ipsilateral cortex [(42) and unpublished observations]. Notably, the mechanism underlying the activation of these two populations of astrocytes may differ. For example, Schroeter and colleagues (42) demonstrated that when lesioned rats were treated with MK801, a noncompetitive NMDA receptor antagonist, the glial response in the border zone of the lesion was unaffected, whereas the response at more distal cortical sites was completely abolished. These latter data suggest that functionally the glial response immediately adjacent to the infarct might be mediated via leukocyte-derived cytokines, while NMDA-receptor activation may mediate remote responses.

Our findings—that with age there are greater deficits on the rotarod task—are consistent with previous studies demonstrating the influences of age and selective brain injury or traumatic brain injury on sensorimotor performance (43). While photothrombotic infarction resulted in initial sensorimotor deficits in animals of all age groups, the older animals displayed a significantly longer recovery period. Although a number of factors may contribute to this delayed recovery, one must consider that the size of the lesion undoubtedly plays a significant role in the initial onset of deficits and in the ability of the animal to recover from them. Notably, our studies demonstrate the utility of the rotarod test for evaluating basic motor abilities and changes induced by experimental manipulations. In the future, this task should prove highly useful in evaluating the efficacy of various therapeutic interventions aimed at ameliorating neurologic deficits following cerebral infarction.

In summary, our studies demonstrate that the photochemical model of stroke represents a reproducible model of cerebral infarction that is compatible with short- and long-term survival in aged rats. With this model, we demonstrated one week postoperative a significantly larger infarct in aged rats compared with young animals. The larger infarct also resulted in sustained neurologic impairment. While the mechanism underlying these latter observations is unclear, it is highly tempting to correlate increased infarct volume with decreased astrocytic proliferation. A further understanding of those factors which contribute to the astrocytic response in the aged rat may be of particular benefit in designing novel therapeutic strategies aimed at reducing the pathologic consequences of cerebral infarction in elderly humans.

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Address correspondence to Dr. David M. Armstrong, Lankenau Medical Research Center, Thomas Jefferson University, 100 Lancaster Avenue, Wynnewood, PA 19096. E-mail: ArmstrongD@mlhs.org
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