Photochemically induced graded spinal cord infarction

Behavioral, electrophysiological, and morphological correlates

RICARDO PRADO, M.D., W. DALTON DIETRICH, PH.D., BRANT D. WATSON, PH.D., MYRON D. GINSBERG, M.D., AND BARTH A. GREEN, M.D.

Departments of Neurological Surgery, Neurology, Anatomy and Cell Biology, and the Cerebral Vascular Disease Research Center, University of Miami School of Medicine, Miami, Florida

Neurological and morphological outcome was evaluated in a rat model of graded spinal cord infarction initiated by a photochemical reaction. In this model, light-dye interactions induce primary microvascular stasis, resulting in consistent patterns of tissue necrosis. Four groups of rats underwent photoinduction times ranging from 30 seconds to 10 minutes. Neurological and electrophysiological functions were assessed starting 1 week after irradiation and continuing for 8 weeks. A functional neurological score was obtained by combining results from sensory and motor tasks, and electrophysiological function was evaluated from the somatosensory evoked potential recordings. In rats irradiated for short periods (30 seconds and 1 minute) mild behavioral deficits were documented. In contrast, electrical conduction was suppressed acutely in both groups; this recovered by 8 weeks to baseline or near baseline in the 30-second group but not in the 1-minute group. In rats irradiated for longer periods (5 and 10 minutes), severe behavioral and conduction abnormalities were detected at both the subacute and chronic testing periods. Although no significant difference in behavior was documented between the 5- and 10-minute groups acutely, the rats with 5-minute photoinduction time demonstrated a significant improvement in behavior over time whereas the group with 10-minute photoinduction time showed no improvement. A severe conduction block was present in both animal groups during the course of the study. Histopathological examination combined with morphometric measurements of the lesion area in cross section revealed four different degrees of spinal cord necrosis which correlated significantly with photoinduction times and neurological scores at 8 weeks. Reproducible degrees of ischemic damage to spinal cord parenchyma following primary microvascular occlusion result in a predictable sequence of behavioral and functional abnormalities, which in some cases recover with time.

KEY WORDS • spinal cord injury • photochemical injury • infarction • rat

TRAUMATIC injury to the spinal cord is associated with a complex series of pathological events, including both primary and secondary components. Immediately following spinal cord injury, paraplegia, loss of electrical conduction, and shifts in electrolytes have been documented. These early alterations most likely are related to trauma-induced membrane dysfunction, but secondary damage to the microvasculature has also been suggested as an important component of the injury process. In several experimental studies, early endothelial damage has been shown to occur following spinal cord injury, with endothelial alterations appearing as early as 90 seconds after impact. Additionally, platelet aggregation and edema formation also appear to be early consequences of impact injury. It is clear that trauma-induced microcirculatory failure and edema formation could result in irreversible damage to the spinal cord.

The present study was undertaken to determine if reproducible graded spinal cord infarction could be produced in rats by a recently developed photochemical method. This insult results in primary microvascular dysfunction with subsequent infarction within predetermined spinal cord areas. Specific aspects of interest include: 1) the subacute and chronic electrophysiological and behavioral consequences of four grades of injury; 2) the relationship of these functional consequences to the final pathological lesion size; and 3) the nature of the vascular alterations associated with the early stages of spinal cord infarction.

Materials and Methods

Animal Preparation

Male Wistar rats, each weighing 230 to 260 gm, were anesthetized with 4.5% halothane in a mixture of O2 and N2O (30:70) and were maintained with 1.3%
halothane, through a closely fitting Mylar face mask. The tail vein was exposed and catheterized for the administration of the photosensitizing dye, rose bengal. The back was closely shaved and sterilized with Betadine solution. A midline longitudinal skin incision was made, followed by paramedian incisions into the paraspinal muscles to expose the underlying vertebrae (T6–10). The T-8 vertebra was chosen for photoradiation. The animals were then positioned beneath the irradiation apparatus, which has previously been described in detail.25,27

For the present experiments, the apparatus previously used to induce spinal cord injury photochemically in rats27 was slightly modified to produce a train of high-powered pulses by electromechanically gating a 300-W xenon lamp, run at its maximum output.* This modification significantly decreased the length of irradiation periods necessary to produce the desired insult. The transmitted beam was focused by a 75-mm focal-length fused silica lens. An interference filter,† mounted near the aperture of an electromechanical shutter, spectrally modified the incident visible beam to produce an exit beam centered at 560 nm (the absorption maximum of rose bengal) with a bandwidth of 60 nm. The beam shutter‡ was mounted in a housing to which was attached a manual optical aperture, 1 in. in diameter. In the present studies the beam was gated on/off at 350/600 msec (37% duty cycle). The (peak) beam intensity during the open-shutter cycle was 2.2 W/sq cm, and the average intensity was 0.92 W/sq cm. A dual fan system was operated during irradiation in order to maintain physiological temperature in the irradiated spinal cord segments. The animal was covered up to the incision with a heating blanket, which helped to maintain the body temperature at 37° to 37.5°C. Following irradiation, the incisions were closed in layers and the rats allowed to recover in their cages. In all rats, an antibiotic agent (cefazolin, 40 mg) was given intraperitoneally preoperatively and postoperatively for all rats, an antibiotic agent (cefazolin, 40 mg) was given immediately following the injection, animal groups were irradiated for various periods depending on the desired injury grade: four

Experimental Design

To produce the photochemical insult, a solution of rose bengal (30 mg/ml in 0.9% saline) was injected into the tail vein over a 5-minute period to yield a body concentration of 40 mg/kg. Immediately following the injection, animal groups were irradiated for various periods depending on the desired injury grade: four

were irradiated for 30 seconds; four for 1 minute; six for 5 minutes; and five for 10 minutes. A control group of five animals was prepared identically to those described above, injected with saline, and irradiated for 10 minutes. A group of animals injected with rose bengal but not irradiated has been previously described.27 Baseline somatosensory evoked potentials (SEP's) were recorded 24 hours before the induction of spinal cord injury under pentobarbital anesthesia (35 mg/kg). Light anesthesia was maintained with supplemental doses as required. Subsequent to the induction of spinal cord injury, SEP's were measured on a biweekly basis for the duration of the experiment. Animals undergoing behavioral, electrophysiological, and histopathological analysis were allowed to survive for 8 weeks.

Neurological Evaluation

Neurological testing of the 19 rose bengal-injected irradiated animals and the control group of five rats was initiated 1 week after injury and continued each week thereafter for 8 weeks. Neurological function was evaluated by a scoring system similar to that described by Gale, et al.,10 which combines the indices described below to yield a functional neurological score (or percent deficit) ranging from 0% for a normal rat to 100% for a completely paralyzed rat. The functional score is derived from the sum of the points allotted for each test (Table 1). Motor function was evaluated by a modified Tarlov grading system, and consisted of observing the rat on an open field and grading spontaneous hindlimb movement (Table 1). A water-bath test was used to evaluate locomotor function. Rats were

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>motor function</td>
<td>0 loss of voluntary motor function (flaccid paralysis)</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>1 barely perceptible movement of hind limb, no weight-bearing</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>2 alternate movement of hind limbs, without weight-bearing</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3 walking &amp; weight-bearing, with a deficit</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>4 normal walking</td>
<td>0</td>
</tr>
<tr>
<td>locomotor function (water-bath test)</td>
<td>0 no movement</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1 barely perceptible movement</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>2 weak random flexion &amp; extension movements of hind limbs</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>3 vigorous rhythmic movements of hind limbs</td>
<td>0</td>
</tr>
<tr>
<td>inclined-plane stability</td>
<td>25° to 30° angle</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>35° to 40° angle</td>
<td>10</td>
</tr>
<tr>
<td>withdrawal reflex to painful stimuli</td>
<td>45° angle</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0 no reaction to pinch (flaccid paralysis)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>1 hyperactive flexion &amp; extension of lower limbs</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2 normal flexion withdrawal</td>
<td>0</td>
</tr>
</tbody>
</table>

* Xenon lamp manufactured by ILC Technology, Sunnyvale, California.
† Interference filter manufactured by Corion Corp., Holliston, Massachusetts.

746 J. Neurosurg. / Volume 67 / November, 1987
placed in a glass water tank 37.5 cm deep, 60 cm long, and 30 cm wide. The water temperature was maintained at 37°C. Spontaneous hind limb movement was observed and graded as shown in Table 1. Animals were also subjected to testing by the inclined plane method of Rivlin and Tator. The maximum angle at which the animal could maintain a stable position for 5 seconds on the inclined plane was recorded and graded (Table 1). Sensory function was tested by observing the hind-limb response of animals subjected to painful stimuli by pinching the skin on the dorsal foot pads with a hemostat. The observed withdrawal reflex was graded as shown in Table 1.

Somatosensory Evoked Potentials

Summed responses to sciatic nerve stimulation were recorded from small stainless steel epidural electrodes overlying the corresponding hind-limb projection area of the somatosensory cortex. A bipolar stimulating electrode was placed on the sciatic nerve just rostral to its bifurcation. The stimulus had a duration of 0.1 msec with a repetition rate of 1.1 Hz. The stimulus intensity was increased gradually until it was 1.5 to 2 times motor threshold. The recording system had a frequency response with 3-dB cutoff points at 100 Hz and 3 KHz. The computer averager was triggered by the synchronized pulse from the stimulator, and 128 responses were routinely summed over an analysis time of 52 msec. For a permanent record, averages were photographed on Polaroid film from the screen of the oscilloscope.

The cortical evoked responses analyzed consisted of the two positive peaks (P1 and P2), each followed by corresponding negative peaks (N1 and N2). Latencies were measured for each component wave, and waveforms were analyzed by interpeak amplitudes of the component waves. The cortical SEP recordings allowed us to evaluate the integrity of spinal cord afferent pathways.

Morphological Methods

Routine light microscopic analysis was carried out on animals 8 weeks following photoactivation. At this time, animals were reanesthetized with halothane and perfused transcardially at a pressure of 100 mm Hg with FAM (formaldehyde:glacial acetic acid:methanol, 1:1:8) for 20 minutes following a brief saline wash. Spinal cords were left in situ overnight at 4°C before removal. The cords were blocked in cross section and processed for paraffin embedding. Paraffin sections were then stained for histopathological analysis with hematoxylin and eosin and/or Luxol fast blue.

The lesion epicenter (area of maximum necrosis) was determined by computer-assisted planimetry of serial sections from the spinal cord. The histological sections were projected onto paper by a camera lucida attached to a Nikon light microscope. Intact tissue, including normal staining myelinated axons and gray matter were then traced and subjected to planimetric analysis using a digitizing table interfaced to a PDP 11/44 microcomputer.

Blood-Spinal Cord Barrier Assessment

To document early blood-spinal cord barrier disruption, a 2% saline solution of the protein tracer, Evans blue, was injected intravenously into the rats immediately following 10 minutes of irradiation. Thirty minutes later the rats were perfused transcardially with saline for 2 minutes. The spinal cords were then removed from the spinal column with the aid of a dissecting microscope, and placed in chilled buffered formaldehyde.

Electron Microscopic Analysis

For ultrastructural studies, rats were perfused transcardially immediately following a 5-minute irradiation period. These rats were initially perfused with 0.9% sodium chloride solution followed by 2% paraformaldehyde and 2.5% glutaraldehyde in a 0.1-M sodium phosphate buffer. Perfusion pressure was monitored throughout the procedure and was maintained at a pressure of 100 mm Hg. Following fixation, the cords were removed from the spinal column and placed in fresh chilled fixative (4°C) for 2 hours. The cords were then sectioned into coronal blocks containing the irradiated areas. The blocks were returned to chilled 0.1-M sodium phosphate buffer (4°C) for 2 hours. Specimens were dehydrated in graded ethanol preparations and propylene oxide and embedded in Poly-Bed 812. Sections 1 mm thick were cut with glass knives on a Sorvall ultramicrotome, then stained with toluidine blue and examined with a light microscope. Once the area of interest was selected, tissue blocks were trimmed and sectioned with a diamond knife on an ultramicrotome. These thin sections were then collected on 200-mesh copper grids, stained with uranyl acetate and lead citrate, and examined and photographed in a Zeiss EM-10C electron microscope.

Statistical Analysis

All data were analyzed using one-way analysis of variance with p < 0.05 being considered significant. Values are given as means ± standard error of the means. The relationships between the lesion area at the epicenter, irradiation time, and the final neurological deficit (%) were analyzed using linear regression. Cortical evoked responses were analyzed with regard to latency and amplitude, and are described as a percentage of baseline. Analysis of variance was performed on the absolute values of the individual waveforms for latency (msec) and amplitude (mV).

Results

Control Group Findings

The five control rats (irradiated for 10 minutes following saline injection) demonstrated no behavioral or electrophysiological abnormalities during the course of
Fig. 1. Neurological deficits obtained by combining various behavioral tasks (see Table 1) as a function of time. Values are means ± standard error of the means obtained from 1 week to 8 weeks. No behavioral deficit was seen in the control or 30-second irradiation groups.

Fig. 2. Representative examples of the alterations in somatosensory evoked potentials seen in the five experimental groups over time following photochemically induced spinal cord injury. C = control group.

the investigation. The functional neurological score for this group of animals was 0% at all time periods (Fig. 1) and SEP's demonstrated no change from baseline (Fig. 2). The histopathological appearance of the spinal cord was unremarkable at 8 weeks following irradiation (Fig. 3a). The white-matter tracts appeared intact, and the neuronal cell bodies appeared normal. Planimetric analysis of spinal cord cross sections taken at the level of irradiation demonstrated a mean total cross-sectional area of 2.99 ± 0.05 sq mm. Additionally, the blood-spinal cord barrier was shown to be intact by Evans blue infusion in our control series. Thus, irradiation of the spinal cord parenchyma with 560 nm light at 2.2 W/sq cm (0.82 W/sq cm average intensity) was without structural or functional consequences.

Experimental Group Findings

30-Second Irradiation Group. The rats that underwent 30 seconds of irradiation were only mildly injured and showed no functional neurological abnormalities during the course of the investigation (Fig. 1). Behavioral testing over the course of 8 weeks resulted in 0% neurological deficit as assessed by the battery of tasks described in the Materials and Methods section (Table 1).

Electrophysiological function was impaired at 2 weeks following irradiation (Fig. 2 and Table 2). This abnormality was associated with decreased amplitudes of all the wave components with a difference from the control levels of 75% for P1−N1 (p < 0.05), 82% for N2–P2 (p < 0.05), and 92% for P2–N2 (p < 0.01). Increased latency of N2 was also seen at 2 weeks, with a difference of 72% from the control level (p < 0.01). One of the four animals in this group recovered to baseline; however, two of the four animals had isoelectric SEP's at 2 weeks, which in one animal recovered to baseline by 6 weeks only to deteriorate slightly at 8 weeks. In the other animal, there was some recovery of the evoked potential, but not to baseline.

Morphological lesion assessment at 8 weeks following irradiation revealed necrosis of the dorsal columns up to but not including the level of the corticospinal tracts, and to the posterior section of the dorsolateral funiculi (Fig. 3b). There was also partial damage to the dorsal gray horns at the level of irradiation. Regions of necrosis included areas of gliosis with macrophage infiltration. White-matter tracts not appearing damaged displayed normal staining characteristics except for occasional vacuolation. Morphometric analysis of the lesion area at the level of irradiation revealed the total cross-sectional area of remaining normal tissue to be 2.19 ± 0.20 sq mm. This value was significantly less than the control level (p < 0.01).

1-Minute Irradiation Group. In contrast to the 30-second irradiation group, which showed no behavioral abnormalities, two of the four rats in the 1-minute group demonstrated mild behavioral abnormalities at 1 week (Fig. 1). These subtle deficits included hyperactive responses to pain as well as minimal motor impairment. Because these abnormal consequences were not seen in all of the animals, a statistically significant difference between this group of rats and the control group was not demonstrated. At 3 weeks, no behavioral abnormalities were detected in three of the four animals; the
### TABLE 2
Summary of electrophysiological features in the experimental groups*

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Time Post-injury</th>
<th>SEP Latency†</th>
<th>SEP Amplitude‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P₁</td>
<td>N₁</td>
<td>P₂</td>
</tr>
<tr>
<td>30-sec irradiation</td>
<td>2 wks</td>
<td>50 ± 29</td>
<td>51 ± 28</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>0.2 ± 3</td>
<td>0.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>6 wks</td>
<td>4 ± 3</td>
<td>5 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>8 wks</td>
<td>4 ± 3</td>
<td>2 ± 8</td>
</tr>
<tr>
<td>1-min irradiation</td>
<td>2 wks</td>
<td>65 ± 20</td>
<td>60 ± 23</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>47 ± 17</td>
<td>44 ± 19</td>
</tr>
<tr>
<td></td>
<td>6 wks</td>
<td>33 ± 4</td>
<td>29 ± 4</td>
</tr>
<tr>
<td></td>
<td>8 wks</td>
<td>28 ± 5</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>5-min irradiation</td>
<td>2 wks</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>6 wks</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>8 wks</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10-min irradiation</td>
<td>2 wks</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>6 wks</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>8 wks</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means.
† Mean percentage increase from baseline in the latency of the somatosensory evoked potentials (SEP's).
‡ Mean percentage decrease from baseline in the amplitude of the SEP's.

Behavioral abnormality in the one remaining animal consisted of an isolated sensory deficit.

A severe conduction block was present in all animals at 2 weeks postinjury (Fig. 2 and Table 2). This consisted of a reduction in amplitudes of 81% for $P₁-N₁$ ($p < 0.01$), 86% for $N₁-P₂$ ($p < 0.01$), and 95% for $P₂-N₂$ ($p < 0.01$) compared to control levels. Latencies were also altered at 2 weeks, with two of the four animals having isoelectric evoked responses, which recovered by 4 weeks in one animal and by 6 weeks in

---

**FIG. 3.** a-e: Cross sections of the spinal cord in the five experimental groups showing histopathological alterations 8 weeks following the various periods of irradiation. Paraffin sections, H & E and Luxol fast blue, × 12.5. a: Cord section of a control rat that was infused with saline and irradiated for 10 minutes. Note the well-preserved gray- and white-matter structure. b: Spinal cord of a rat after 30 seconds of irradiation. c: Spinal cord of a rat after 1 minute of irradiation. d: Spinal cord of a rat after 5 minutes of irradiation. e: Spinal cord of a rat after 10 minutes of irradiation. f: Photograph of the spinal cord of a rat taken 30 minutes after 10 minutes of irradiation at the level of irradiation demonstrating Evans blue extravasation. There is intense staining in the central gray matter of the spinal cord.
cross-sectional area at the level of irradiation was 1.46
gray horns and areas of intermediate gray matter were
the dorsal columns, including the corticospinal tracts
dominated rats demonstrated a consistent lesion involving
pain, and the rats were impaired as to motor function,
week (Fig. 1). At this period there was no response to
irradiation was severely injured, and
demonstrated complete necrosis of the entire cord
thickens except for a thin area of white matter located
at the level of the ventromedial tracts of the anterior
funiculi (Fig. 3e). In these rats, the cross-sectional area
of normal cord was 0.0901 ± 0.01 sq mm. By one-way
analysis of variance this value was not significantly
different from the 5-minute group.

**Blood-Spinal Cord Barrier**

Thirty minutes following irradiation a central area of
vascular stasis, bordered rostrally and caudally by
blue-staining tissue, was apparent. Intense blue staining
on the surface of the cord extended for 1 cm longitudi
ally. Cross sections taken through the irradiated zone
demonstrated that the blue stain was present throughout
the cord thickness. Although both white and gray
structures were stained (Fig. 3f), barrier leakage was
more intense within the central gray matter.

**Ultrastructural Examination**

Transmission electron microscopic examination of
tissue obtained from rats 5 minutes after irradiation
demonstrated a high incidence of luminal platelets (Fig.
4a) and vascular congestion involving both surface and
parenchymal vessels. Vascular alterations at this early
time following irradiation were mainly restricted to the
dorsal columns, dorsolateral fasciculus, and laminae 1
to 5 of the central gray matter. Platelets frequently were
degranulated and had pseudopodia. Endothelial cells
appeared swollen with sites of luminal discontinuity.
There were many pinocytic vesicles lining both the
luminal and abluminal endothelial membranes (Fig.
4b). Perivascular edema with astrocytic swelling was
also a common occurrence. Blood vessels displaying
different degrees of luminal compression were detected
in both gray and white matter. Neuronal cell bodies
appeared relatively normal except for sites of perineu
ronal edema. Some degree of edema was also revealed
within white-matter tracts in the dorsolateral fasciculus.

A significant correlation was demonstrated between
the irradiation time and lesion size at the epicenter
\( r = -0.86; \ p < 0.001; \) Fig. 5a). There was also a
significant correlation with lesion size and the final
behavioral outcome \( r = -0.87, \ p < 0.001; \) Fig. 5b).

**Discussion**

The results of this study demonstrate that a consistent
progression of behavioral, electrophysiological, and his
topathological abnormalities can be documented fol
lowing photochemically induced spinal cord infarction.
This experimental method of injuring the rat spinal
cord is advantageous in that reproducible degrees of
spinal cord infarction can be produced without lami
nectomy. Inasmuch as ischemia may be an important
aspect of traumatic spinal cord injury, the present
infarct model may also be useful in investigating this
component of traumatic spinal cord injury.

The photochemically induced spinal cord lesion may
be reproducibly varied by systematically altering the period of irradiation. The residual tissue area at the epicenter correlated significantly with photoinduction times (p < 0.001, r = -0.86). By the use of such an experimental approach, spinal cord tracts in specific regions can be selectively damaged or left intact. This characteristic of lesion formation therefore allows for the behavioral and electrophysiological consequences to be directly compared to the final neuroanatomical lesion.

Electrophysiological and behavioral testing over the course of the study period revealed changes in functional deficits with time following injury. For example, within the 30-second irradiation group, some recovery of electrical conduction was documented between 2 and 4 weeks after irradiation. Behaviorally, a significant (p < 0.05) recovery trend was seen in the 5-minute irradiation group, in which severe motor deficits at 1 week improved significantly by 2 weeks. In conventional models of the spinal cord injury, most improvement in behavior also appears to take place at fairly early periods following injury. In a study by Gale and
though large degrees of white-matter destruction were
ences in the final neurological outcome were docu-
disrupted by the insult. 
result of undamaged motor tracts taking over functions
by this insult. Therefore, motor recovery might be the
result of undamaged motor tracts taking over functions
disrupted by the insult. At 8 weeks following irradiation, significant differ-
ences in the final neurological outcome were docu-
mented in the 5- and 10-minute irradiation groups
compared to control data. Significant differences be-
tween the neurological scores of the two groups were
also demonstrated. This was especially true of motor
performance as determined by inclined-plane stability
scores. This difference in final behavioral outcome most
likely resulted from histopathological damage to ventral
motor tracts in the 10-minute irradiation group, which
were spared in the 5-minute irradiation group. Al-
though large degrees of white-matter destruction were
associated with both experimental groups, no signifi-
cant difference in lesion area at the epicenter was dem-
Oned.

Luminal platelet aggregation within the irradiated
zone was an early consequence of the photochemical
insult. This result is consistent with recent findings that
platelet aggregation is an early consequence of photo-
chemically induced cortical infarction. In that study, platelet aggregation was associated with endothelial membrane damage and blood-brain barrier disruption. Rose bengal is the most efficient known gener-
ator of singlet oxygen, which reacts with structural
proteins and lipids to initiate direct peroxidation reac-
tions within endothelial membranes. In the early stages of tissue infarction, occlusive platelet thrombi
within surface and parenchymal vessels of the cord may
directly suppress spinal cord blood flow. Additionally,
because the vascular endothelium is the site of the
blood-spinal cord barrier, photochemically induced en-
the primate. In their study, endothelial alterations in-
cluded separation of endothelial tight junctions and
basal lamina exposure. In a spinal cord injury model in
eats, Kapadia demonstrated the immediate disruption
of endothelial cell junctions and increased pinocytotic
activity within the endothelial cells at the injury site.
Interestingly, blood vessels coursing within white-mat-
ter segments rostral and caudal to the traumatized site
demonstrate similar alterations 2 hours following in-
jury. In other studies, spinal cord edema first occurred
in the gray matter at the site of injury and then spread
rostral and caudal white-matter tracts. Leakage of Evans blue dye in the present study was more pro-
nounced within the central gray region compared to
the surrounding white-matter tracts. This consequence
is likely due to the greater concentration of blood vessels
in gray than in white matter. Interestingly, hemorrhagic
necrosis involving this central gray matter was seen at
7 days in the present model following irradiation. Whether similarities between the histopathological con-
sequences of spinal cord trauma and spinal cord infarc-
tion imply common mechanisms of tissue destruction
merits further consideration.

Conclusions
The present study has demonstrated that graded
spinal cord infarction can be photochemically induced
in rats. Although this method is based on an insult that
results in primary microvascular injury without me-
chanical trauma, the behavioral, electrophysiological,
and histopathological consequences are nonetheless
similar to those reported in conventional trauma
models. Our results demonstrate that, by varying irra-
diation times, selective white-matter tracts may be re-
 producibly damaged and as a consequence show a
consistent pattern of abnormalities. Depending on the
level of injury, functional deficits documented at early
postinjury periods may or may not recover. The pres-
ent experimental model therefore appears to be an
advantageous method for determining mechanisms of
functional recovery following reproducible spinal cord
infarction. Finally, the model may also aid in the as-
essment of the role played by the microcirculation in
spinal cord injury. Therapeutic strategies directed to-
ward injury-induced abnormalities of spinal cord blood
flow, as well as the role of edema formation in lesion
propagation, may also be addressed.

Acknowledgments
We thank Joseph Mora for electrophysiological and behav-
orial testing of the animals, and Mrs. June Thomas and Mrs.
Marcilia Halley for preparation of histological material.

References
1. Balentine JD: Hypotheses in spinal cord trauma research,
in Becker DP, Povlishock JT (eds): Central Nervous Sys-
tem Trauma Status Report 1985. Bethesda: National In-
stitute of Neurological and Communicative Disorders
and Stroke, 1985, pp 455–461
Photochemically induced spinal cord infarction in rats


---

Manuscript received January 21, 1987.
This work was supported by National Institutes of Health Grants NS 05820, NS 23244, and Training Grant NS 07238, by VHA Grant 3263-002, and by the Department of Neurological Surgery, University of Miami. Dr. Dietrich is an Established Investigator of the American Heart Association.
Dr. Ginsberg is the recipient of a Jacob Javits Neuroscience Investigator Award (NS 22603).
Address reprint requests to: Ricardo Prado, M.D., Department of Neurology (D4-5), University of Miami School of Medicine, P.O. Box 016960, Miami, Florida 33101.