In vitro Simulation of Neural Trauma by Laser

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Abstract. A serious lack of knowledge about central nervous system trauma is encountered on the cellular level, where the inability to create precise experimental lesions of known magnitude has limited our understanding of the reactions of single cells to injury. We used a laser cell surgery technique developed in this laboratory to manipulate neurons in a controlled environment, in order to observe pathologic reactions during and immediately after the injury. This technique is especially suited for axonal and dendritic amputations close to the perikaryon. The laser provided three different physical modes of injury to neurites: (a) direct vaporization of cytoplasm, (b) pressure wave damage from external vaporization of substrate material, and (c) photochemically-induced localized cytoskeletal destruction leading to the slow pinching of processes followed by transection. Our data indicated a greater similarity between laser impact damage and the cellular damage produced by physical trauma to the central nervous system.

Key Words: Amputation, neurite; Cells, cultured; Laser surgery; Trauma, central nervous system.

INTRODUCTION

The traditional designation of non-fatal head injury as "contusion" or "concussion" is usually based on the surmise, supplemented by laboratory tests, that actual tissue damage has, or has not, occurred (1). This concept is being displaced by experimental evidence (2-5) that injuries to nerve fibers and blood vessels conform to a continuum of effects of acceleration of the head from the blow and the duration of the blow. One clinically significant manifestation of this deeper understanding has been delineation of the diffuse axonal injury syndrome (6). This advance depends upon earlier work from human pathological material which clearly established that damage to nerve fibers is a component of head injury (7, 8).

The next step will be to understand central nervous system (CNS) trauma on the cellular level. An example is important new evidence that cholinergic sites in the pons are activated by concussion (9). It will be essential that major efforts be made to determine the types of neuronal and glial responses to various types of physical injury such as osmotic shock, pressure waves, membrane stretching, distortion of the cytoskeleton, and process transection. It is necessary to ask at what point a neuron or its processes becomes injured and how that injury is functionally manifest.

Limitations of existing techniques have forced investigators to focus on damage at the tissue level (10-16). With the advent of reliable culture methods, the observation of ultrastructural changes accompanying trauma to isolated, single neurons became possible (17-19). Further investigations have been hampered by the inability of mechanical methods of transection to create experimental lesions in single cells with sufficient precision. Involvement of neighboring structures and especially target

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process distortion and tearing invariably accompany such manipulations. This paper describes a new approach to the generation of precise point lesions at any locus in a cultured cell. The method uses a pulsed ultraviolet (UV) laser firing through a microscope and yields minimum lesion diameters of 1 μm with energy densities of sufficient magnitude to produce a variety of lesions ranging from vaporization of cytoplasm to external shock wave damage. It is our purpose to describe the ultrastructural disruptions caused by different types of lesions in order to demonstrate the great similarities between physical and laser impact trauma. In view of this similarity it seems likely that the precision and control offered by laser cell surgery will substantially accelerate investigations of neuronal trauma and recovery on the single cell level.

METHODS

Aspects of the laboratory practices and equipment used in this study have been published in detail (20–22). Here brief descriptions are provided for convenience.

Laser Microbeam System

The work laser was a pulsed nitrogen instrument operating at the fixed wavelength of 337 nm and pulse duration of 12 ns (Laser Service Inc., Rochester, N.Y.). Maximum power output was 14 kW, or approximately 168 μJ of radiant energy per pulse. However, this is reduced 60% by loss in the optical system, thus the maximum energy available at the specimen was about 67 μJ per pulse. The laser output was constantly monitored by a UV-sensitive photodiode before the beam entered the microscope. Energy output of the laser varied in the single shot mode by 5% near the maximum energy, 10% at half maximum, and by as much as 30% at low energy settings. Thus, we reduced the beam intensity with filters whenever possible. Frequency of pulse production could be varied from 4 to 120 Hz. The work laser was supplemented by a continuous helium-neon target laser projected collinearly with the nitrogen laser into the microscope to target the lesion site. Since the position of the laser beam in the optical system is fixed, the position of cellular constituents was varied by moving the microscope stage.

A Leitz Orthoplan microscope was used equipped with Zeiss Ultraplan quartz objectives of ×10, ×32, and ×100 magnification. Minimum focus diameters of the laser beam were 4.5, 2.3, and 0.7 μm respectively. A phase condenser allowed sufficient contrast to visualize the unstained living cells before, during, and after laser irradiation. The culture dishes were maintained at 37°С by a copper DC heating plate on the microscope stage. A humidified stream of 10% CO2 in air was directed over the dish to maintain pH. Because the objectives are heat sensitive and may be damaged above 30°C a ventilated plastic sleeve containing a matched quartz coverslip was used to protect the lenses of the 32 and 100 power objectives.

Cell Culture

NB41A3 mouse neuroblastoma cells were purchased from the American Type Culture Collection, Rockville, Md. Dissociated spinal cord cells from 13–14 day mouse embryos were cultured according to a modified method of Ransom (23). Cells were grown in Falcon or Lux Permanox dishes or on Lux Thermopan or glass coverslips at 37°C under 10% CO2 in Eagle's minimum essential medium supplemented with 10% fetal bovine serum. Spinal cord cells grown in culture for 12 to 45 days were used for experiments. These cultures produced a variety of glial and neuronal cells which were interconnected by single and grouped cell processes. The cells were usually attached to an underlying "carpet" of fibroblasts and astrocytes. Distinction of the different cell types was carried out on the basis of light and electron microscopic characteristics. The cells were photographed through the trinocular head of the microscope on Polaroid film.

TABLE 1

<table>
<thead>
<tr>
<th>Lesion method</th>
<th>Site</th>
<th>Neuroblastoma</th>
<th>Glia</th>
<th>Neuron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaporization of cytoplasm (direct</td>
<td>Soma</td>
<td>43</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>irradiation)</td>
<td>Process</td>
<td>72</td>
<td>10</td>
<td>92</td>
</tr>
<tr>
<td>Pressure waves from vaporized</td>
<td>Soma</td>
<td>50</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>substrate (&quot;near miss&quot;)</td>
<td>Process</td>
<td>20</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>Subthreshold multishot</td>
<td>Soma</td>
<td>340</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>(direct irradiation)</td>
<td>Process</td>
<td>40</td>
<td>0</td>
<td>240</td>
</tr>
</tbody>
</table>

Electron Microscopy

Identification of specific cells for subsequent electron microscopy (EM) was facilitated by using the nitrogen laser to burn a series of 2–4 μm craters in the plastic substrate, giving a "cross hair" effect. Fixation was achieved by adding 3% glutaraldehyde in phosphate buffer to the culture medium to a final concentration of 1 percent. After five minutes (min) the dilute fixative was replaced by 3% fixative for one hour (h), followed by post-osmication (1%) and dehydration in ethanol. For scanning EM, the cells were critical point-dried, coated with gold, and examined in an AMR 1200 scanning electron microscope. For transmission EM, after dehydration propylene oxide was added and the cells still attached to the dishes were polymerized in an Epon–Araldite mixture. Disks containing the cells of interest were cut and glued to plastic rods. Thin sections were mounted on Formvar-coated grids, stained with uranyl acetate and lead citrate, and examined with a Siemens 101 transmission electron microscope.

Injury Techniques

Thus far, we have devised three methods for "surgery" of cells and their processes in tissue culture. The first method is direct irradiation of the cell by the laser beam at relatively high energy density (about 10 μJ/μm²) to produce vaporization of cytoplasm. This occurs because of nonlinear absorption and subsequent rapid (10 ns) heating in the focus of the laser, and is inevitably associated with intracellular pressure or shock waves.

The second method of inducing cell injury is to direct the laser beam into the plastic substrate below or near the cell or its process. This "near miss" produces a miniature pressure wave in the adjacent medium of sufficient magnitude to disrupt cells and processes in the immediate vicinity. Plastic culture substrates have low damage thresholds and can be cratered at specific energy densities ranging from 0.2 to 3 μJ/μm² at 337 nm. This method may have special relevance for the topic of this paper because some of the forces involved in human head injuries are similarly projected through the brain substance.

The third method is to use multishot irradiation of neurites at very low energy densities (0.5–2 μJ/μm²). This exposure does not vaporize cytoplasm or the usual culture substrates. It does not produce any obvious, measurable reactions in the cytoplasm until the energy from several hundred shots has accumulated (22). At that point a gradual process pinching is seen which is followed by total transection of the neurite. The pinching is reversible if irradiation is stopped during the initial stage of cytoplasmic contraction. It has been hypothesized (22) that absorption at 337 nm by NADPH and NADH causes disruption of mitochondria and a concomitant release of calcium ions. This in turn leads to a local destabilization of the cytoskeleton and possibly to local contractile events followed by catastrophic permeability changes. Together these events lead to local cytoskeletal dissolution and cell process transection. The types and numbers of cells studied in the various lesion categories are shown in Table 1. The illustrations are drawn from representative examples of effects at different times after the lesion was induced.
RESULTS

A typical pressure wave transection of a multifiber cable is shown in Figure 1. The single shot energy density of 3 μJ/μm² focused on the plastic (polystyrene) substrate generated a large pressure wave which transected the target cable as well as an adjacent cable 20 μm distant. A residual bubble of vaporized substrate material and water can still be seen in Figure 1B near the arrow, 3 s after the 10 ns laser pulse. Although the magnitude of this pressure wave has not yet been measured or accurately calculated, it is clear that pressure wave lesions can be generated reproducibly for quantitative studies of trauma on the cellular level. The intensity of the pressure wave depends on the substrate material, and can be varied by changing the energy density in the laser focus with filters or laser operating voltage controls. Smaller, more localized lesions are shown in Figures 2 and 3. The scanning electron micrograph (Fig. 2A) shows a small crater at the arrow, retraction of the proximal neurite segment and a narrowing of the distal segment near the lesion. The conical appearance of the proximal segment seems to reflect a local collapse of the cytoskeleton which must have occurred to allow the 8 μm retraction from the crater.

The highly localized nature of small pressure wave lesions is demonstrated in Figure 3A and B. In this case a cell process was transected 5 μm from the edge of the perikaryon and only 5 μm from a smaller process of another cell that passed underneath the perikaryon. The latter process was undamaged despite a total transection at the target site. Such localization of damage has been repeatedly observed also on the EM level. However, the cell body of the targeted process does show cytoplasmic disruption beyond the laser impact area. In fact mitochondrial vacuolation can be clearly distinguished in regions of the perikaryon adjacent to the lesion (Fig. 3C, D). Therefore, the tearing of cellular structures by external pressure waves was accompanied by a spread of damage beyond the immediate site of the lesion. Similar cellular reactions appear to be associated with the generation of cytoplasmic pressure waves due to single shot irradiation of the cytoplasm (22, Fig. 3). However, the relatively low absorption of cytoplasm at 337 nm required high energy densities for the generation of such lesions. Those energy densities which lie above 6 μJ/μm² are at the upper limit of our present laser microbeam system and cannot be attained.
Fig. 2. A. Large neuronal process one min after indirect pressure wave transection (note crater at arrow). The proximal process has retracted 8 μm from the laser impact site. Both proximal and distal processes show tapering indicative of cytoskeletal collapse. No major cytoplasmic spillage from the severed processes can be seen. Energy density: 2 μJ/μm². Substrate: Lux Thermaflex. Scanning electron micrograph. ×2,280. B. The HeNe target laser can be seen in the light micrograph B at arrow before transection. Phase microscopy. ×400.

reliably. A more dramatic depiction of the cytoplasmic disruption is shown in Figure 4 representing a mouse spinal neuron three and a half min after a laser shot (3 μJ/μm²) into the substrate below the process. Dense material within the cytosol and cup-like vacuoles were present in the cytoplasm; mitochondria had small vacuoles, and the cytoskeleton was lost.

The third and least traumatic method of imparting damage has already been reported (22). It is being developed as a means of effecting morphological changes in neurons and neuronal networks during electrophysiological analysis in culture. In this case the neurite is subjected to energy densities far below the damage thresholds of substrates or cytoplasm. However, the cumulative effect of many shots eventually triggers a slow, gentle cytoplasmic pinching in the laser focus. Depending on the irradiation parameters, the cytoplasmic pinching may reverse or it may lead to a total transection. A morphological recovery from such a laser-induced process-pinching is shown in Figure 5. Whether the recovery shown in Figure 5F is complete also on the ultrastructural level has not yet been investigated.

The gradual nature of the process-pinching allows ultrastructural analysis of the underlying cytoskeletal changes. These changes are seen after the photobiological events have occurred, and are therefore not necessarily directly linked to interactions with photons at the wavelength of 337 nm. In Figure 6, a neuroblastoma cell process has been fixed during the first stages of cytoplasmic pinching. The electron micrographs reveal loss of microtubules, reduction of neurofilaments, an accumulation of organelles on either side of the lesion and damaged mitochondria. So far our data

Fig. 3.  A. Pressure wave lesion 5 μm from perikaryon. Note target laser at arrow. Phase microscopy. ×200. Intact neighboring process 5μm distant despite complete lesion at arrow. Phase microscopy. ×200. C and D. Vacuolation in mitochondria of the affected cell is greater than normal, especially near the traumatized region. Note electron dense residual bodies in C. Energy density: 2.5 μJ/μm². Substrate: Lux Permanox. Fixation: Three min after shot. C, ×3,150. D, ×19,200.
Fig. 4. Typical cytoplasmic disruption resulting from pressure wave lesion. A and B. Mouse spinal neuron before and three min after single laser shot 3 μJ/μm² into substrate below neurite at arrow. Phase microscopy. ×400. C and D. Neuron and process segment near lesion three and a half min after shot. Note extensive vesiculation in addition to mitochondrial vacuolation. Substrate: Lux Permanox. C, ×2,400. D, ×14,000.
Fig. 5. Process pinching and recovery in response to a cumulative energy density (energy density/pulse x number of pulses) below the threshold for complete process transection. A and B. Target cell and process before irradiation with HeNe target beam at arrow in B, C, and D. Target area five and 30 s after laser injury. E and F. Recovery of process three and eight min after injury. Irradiation parameters: 1 μJ/μm², 20 Hz, 1.5 s. Phase microscopy. A, x150. B, x300. C–F, x500.

show that disruption of mitochondria and loss of microtubules are always associated with process-transection or with process-pinching. The two neuronal cell processes in Figure 6 demonstrate this ultrastructural change. Neurofilaments were reduced in number or disappeared entirely. However, this phenomenon was not obvious,
Fig. 6. Early stages of pinching of neuroblastoma process after multishot laser irradiation. Vacuolated mitochondria are present above and below laser impact area in D (site of laser focus within circle). The mitochondria have probably migrated from the irradiation site during the 30 s from onset of radiation to fixation. Regions adjacent to the laser focus (E and G) show intact microtubules whereas no microtubules are present at the laser focus (F). Light micrograph inserts A and B show the 25 μm perikaryon before and five s after termination.
developed gradually, and was linked to complete transection. Stumps of microtubules ending in a clear area in the distal process are seen in Figure 7E.

A total transection of a neurite fixed 42 s after initiation of laser irradiation is shown in Figure 8. The 2.3 μm laser focus diameter has been depicted as a circle at the transection site. The electron micrographs show an unusually clean separation of proximal and distal segments and suggest a high degree of membrane reorganization. The brief time needed for resealing of the severed stumps was somewhat faster than was measured by membrane potential in another system (24).

Figure 9 shows a doubly damaged neuron which survived after a pressure wave lesion and a multiple-shot subthreshold lesion. Typical mitochondrial swelling, vacuolation of cytoplasm, and loss of microtubules are seen in the stump of the gradually irradiated process (Fig. 9E). Despite this extensive damage to neurites, the neuron survived and was fixed one h after irradiation.

The detailed parameters that ensure cell survival after surgery are still not completely understood. However, survival is a direct function of lesion distance from the perikaryon and the somal volume and an inverse function of the target process diameter and of the actual size of the lesion. If a large process is amputated too close to the cell body, the deterioration of the cell is often very rapid. This is shown in Figure 10 where a spinal glial cell had a 6 μm diameter process transected 30 μm from the soma. Within eight min the entire cell revealed severe signs of degeneration. For this to occur, a spread of the damage of at least 0.6 μm/s can be estimated.

**DISCUSSION**

Use of the laser for these experimental manipulations requires attention to the unique properties of this form of electromagnetic irradiation. At high levels of laser output, the burst of photons can produce nonspecific absorption, generating heat of several thousand degrees, which vaporizes cytoplasm, substrate, or medium (25, 26). In comparing our results to others in the literature, it is imperative to stress the differences in wave length and pulse duration between our instruments and others in use clinically and experimentally. Our laser operates in the ultraviolet while most other instruments operate at visible wavelengths. Our laser has about a 1000-fold shorter pulse duration than such instruments. Nevertheless, heating of the cytoplasm is a major variable to be considered in our technique.

The multiple shot, low energy density cytoplasmic transection method is the most gentle of the three transection techniques. We believe it to be triggered by mitochondrial heating due to absorption of the 337 nm radiation by NADH and NADPH. Even under these conditions, the mitochondria do not disintegrate. The heat produced is therefore relatively small; certainly it does not approach the boiling point of water. Olson et al (27) calculated a rise of only 6°C above ambient temperature in mitochondria subjected to 540 nm radiation at 0.19 μJ/μm² and 1 μs irradiation. Hence the heating expected from a 12 ns pulse might be even less despite differences in chromophore absorption and energy density.

Our pressure wave transection method requires vaporization of substrate to produce the pressure wave. Vaporization of substrate is caused by heating of the material in the laser focus. However, the heat is trapped in the concomitant cavity and

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of irradiation (B) at arrow. Fixation: 18 s after termination of irradiation. Irradiation parameters; 0.2 μJ/μm², 115 Hz, 12 s. Substrate: Lux Permanox. A and B, phase microscopy. ×400. C, ×840. D, ×15,000. E, F, and G, ×37,000.

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Fig. 7. Two lesions in separate neurons irradiated near the threshold for neurite transection. 
A and B. Mouse spinal neurons before and two min after laser injury at 2 μJ/μm² (160 shots). 
Note pinching, loss of microtubules and deterioration of organelles. Phase microscopy, × 300. 
C, D, and E. Neuron before and three and a half min after irradiation at 2 μJ/μm² (220 shots). 
This amount of energy resulted in a transection with the usual organelle deterioration in 
Fig. 8. Large neuronal process after complete transection achieved with the multiple shot, low-energy method of laser cell surgery. A. Cell before laser irradiation. Arrow indicates visible HeNe beam over target area. Phase microscopy. ×400. B. Cell with severely pinched target region two s after termination of laser irradiation. Phase microscopy. ×400. C. Appearance of target area 42 s after irradiation. The circle represents the diameter of the laser focus (2.3 μm) with the ×32 objective. Note possible membrane reformation and (reassembled?) microtubules near severed ends. Irradiation parameters: 2 μJ/μm², 20 Hz, 11 s. Substrate: Lux Permanox. ×18,000.
Fig. 9. Cell survival despite transection and pressure wave trauma to two cell processes at distances less than 15 \( \mu \text{m} \) from the edge of the cell body. A. A neuron before laser injury. Arrow points to target region. Laser was focused on the inner edge of the right cell process. Phase microscopy. \( \times 400 \). B. Cell process after pressure wave trauma induced by firing into the substrate (Lux Permanox). The crater of vaporized plastic is visible to the left of the arrow. Note process distortion. Energy density: 3 \( \mu \text{J} / \mu \text{m}^2 \). Phase microscopy. \( \times 400 \). C. Multiple shot
subsequent bubble formation. The cavitation, in turn, is a function of the energy density and absorbing material used. In our experiments the bubble diameter rarely exceeded 5 μm. Damage beyond this diameter (as in the secondary process in Fig. 1B) is due primarily to pressure wave effects. However, if substrate vaporization is produced directly below a neurite, a combination of heat and pressure wave damage must be expected. This also requires some of the laser photons to pass through the process above the substrate, and consequently some heating of mitochondria cannot be avoided. The heat from vaporization is thought to be localized since the hot gases rise away from the impact site and dissipate in the medium. Under these conditions, this specific transection method introduces some protein denaturation and free radicals at the lesion which may not be present in the usual kinetic brain injuries. However, at our present level of analysis, no differences can be detected between "near miss" and "below the process" pressure wave lesions. Furthermore, even the non-pressure wave (sub-threshold multishot) process transections which presumably result from local collapse of the cytoskeleton (22) show similar ultrastructural changes. The only reproducible difference is a less extensive cytoplasmic vacuolation obtained by the latter technique.

At low levels of laser output, energy absorption is linear, and is delivered to specific chromophores which must, of course, have absorption spectra compatible with the wavelength of laser output. At our fixed wavelength of 337 nm major chromophores are probably the reduced nucleotides NADH and NADPH which are concentrated in mitochondria. Mitochondrial vacuolation is a prominent, early, and localized phenomenon in our material. We recognize that for many years mitochondrial changes of this type have been considered artifactual, due to tardy fixation. However, fixation in our material is acceptable in other areas remote from the laser focus. Other possible causes would be interaction between lysosomes and mitochondria (28) or a calcium activated protease (29). We will explore these possibilities in future experiments. Only minimal mitochondrial vacuolation was evident in other trauma studies (5, 30) but tubulovesicular changes were present, similar to the cytoplasmic vacuoles we observe. One difference between those studies and ours is that our briefest intervals of fixation were probably faster than were achieved in those whole animal experiments. The study by Olson et al (27) using lasers in the visible spectrum did show prominent effects on mitochondria, including vacuolation. We have developed a hypothesis (22) that energy absorption by reduced nucleotides leads to the early vacuolation of the mitochondria, and is probably linked to release of calcium ions from the mitochondria. Release of calcium could, in turn, trigger microtubule dissolution (31) leading to cytoskeletal collapse and local pinching of the neurite. This hypothesis will be tested in future experiments. Once the cell membrane has been breached by injury, there is strong evidence, for example from experiments with horseradish peroxidase (32), that exogenous material is taken into the damaged cell. This would include calcium from the extracellular space, which would contribute to further deterioration of the cytoskeleton.

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transsection of left process. Irradiation parameters: 2.0 μJ/μm², 8 Hz, 20 s. Right process has almost recovered its previous diameter. Phase microscopy. ×400. D. Electron micrograph showing both target regions (circles). Fixation: 30 min after lasing. ×1,050. E. Higher magnification revealing ultrastructure of proximal stump of transected (left) neurite. Note swelling, mitochondrial disruption, vacuolation and absence of microtubules near the end of the retracted proximal process. ×18,000.

Fig. 10. Irreversible cell deterioration eight min after laser amputation of large (6 μm) cell process within 30 μm of the cell body. The perikaryon becomes swollen and the other processes are beaded, shortly after irradiation. Rapid deterioration was often seen when surgery was performed close to the cell body. A. Cell before irradiation. B, C, and D. Cell 1 min, 3 min, and 8 min after laser exposure. Arrows point to the HeNe target laser over target site. Irradiation parameters: 1.5 μJ/μm², 20 Hz, ten s. Substrate: Glass. Phase microscopy. × 500.

While the direct effects of laser irradiation are of great interest, it is the secondary effects of pressure waves on the nerve processes which make the method highly relevant to trauma research. Head injury is a complex of vascular, nerve fiber and glial damage (4) often complicated by mass effects and hypoxia. These injuries are conveyed by linear and angular accelerations, as well as by the propagation of pressure waves (3). However, pressure waves have been demonstrated experimentally (33, 34) to result from blows to or movements of the head, and clinical evidence points to fiber tract damage from propagated forces as a major aspect of human head injury (6–8). Experimental studies have verified that nerve fiber damage can occur in isolation, or accompanying other injuries (5). A few studies on nerve fibers in vitro have shown effects from stretching (35) and torsion (36) of nerve fibers. Our micro-
scopetic method is limited since it presently attempts to reproduce only one of the actual results of human injury (i.e. cell process transection). Nevertheless, this issue is central in research on injury to the nervous system.

There may be a link between the pressure wave effects and laser energy absorption by mitochondrial chromophores. Although there has been little published work on the effects of pressure on mitochondria, one group (37) found that sucrose entry was facilitated by increased hydrostatic pressure at low temperatures, and that in extreme cases, this would lead to mitochondrial swelling and rupture. Whether this phenomenon is operative here will be the subject of future experiments. The morphological evidence presented in this paper suggests that structural changes resulting from all three methods of laser damage (direct vaporization, indirect pressure waves, and gradual photon absorption) have underlying similarities. These include early swelling and vacuolation of mitochondria, dissolution of microtubules, accumulation of cytoplasmic vesicles, and disruption of neurofilaments. These similar effects could have common causal mechanisms.

Beyond the study of the immediate damage to neurites is the issue of cell survival which depends on the type of cell, types of lesions, and proximity of lesion to cell body (38, 39). Further experiments will allow us to define these parameters statistically. Especially significant is the capability to modify the culture environment in order to evaluate compounds which may enhance recovery, or offer some protection against the type of injury which can be induced by laser microbeam techniques.

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