Experimental cerebral concussion

Part 1: An electron microscopic study

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Cerebral concussion was produced in rats by an iron pendulum hitting the external
occipital protuberance. This resulted in loss of consciousness lasting from 3 to 10
minutes with prompt recovery and no focal neurological signs. The energy absorbed
by the head at the impact was calculated to be about 1450 gm/cm. Light microscopic
survey showed only minor pathological changes. However, electron microscopic
observation revealed considerable alteration which began at 30 minutes, reached a
peak at 1 hour, and disappeared at 24 hours after concussion. The salient changes in-
cluded severe swelling of the neuronal mitochondria at the point of impact (occipital
cortex), and extracellular edema at the site of contre coup (frontal lobe). Topographically,
the most severe alteration was seen in structures at the craniospinal junction (medulla oblongata and upper cervical cord), consisting of both mito-
ochondrial and edematous changes. Although there was no visible opening of the
capillary interendothelial junctions, extravasated ferritin particles were accumulated in
the edematous regions, indicating a transient increase in the permeability of the
blood-brain barrier.

KEY WORDS • cerebral concussion • cerebral edema •
mitochondria • electron microscope

Cerebral concussion has been defined
as a clinical entity characterized
by a reversible loss of consciousness of
short duration without localizing neuro-
logical features. There has not been any
anatomical substrate associated with con-
cussion in the form of detectable pathology, and nor has there been any evidence of physical
damage to the brain. However, the
permeability of the blood capillaries in the
concussed brain is increased for a short du-
ration. The lack of anatomical changes in the
brain became a conditio sine qua non, a
matter of great definitional importance.
Upon this condition rests the differentiation
between concussion and contusion. Yet all the
cardinal statements of the past referring to
this point were based on examination by light
microscopy, a method that reveals little about
finer changes of the nervous system. We
decided to study the matter by electron
microscopy. Animals were used as ex-
perimental subjects because human brains
from autopsies with known and graded times
from the onset of cerebral concussion are vir-
tually nonexistent.

Material and Methods

The experiments were carried out in 12
young adult rats of Sprague-Dawley strain,
weighing 250 to 350 gm. Litter mates were
used as controls. The experimental animals were subjected to cerebral concussion by a specially designed iron pendulum (Fig. 1). The device provided a known angle for the pendulum to hit the head and a constant position for the rat to rest its head without being held in position by any means. The head moved under the impact and consequently was a true replica of the human head receiving a blow.

Under light ether anesthesia, it was placed on the horizontal wooden board with its head resting on an adjustable end piece at 45° to the horizontal board (Fig. 1). The head and nape were shaved to minimize the loss of concussion impact. As soon as the rat showed a slight sign of waking up, the iron pendulum was released, and hit the head from a position 90° to the vertical line of gravity. At the time of impact, the head moved slightly forward, and the pendulum swung further for a distance of 35° from the vertical line. The lower end of the pendulum was marked with colored chalk and its point of impact could be identified in relation to the external occipital protuberance. Rats not hit at the protuberance were not included in this report, and those that showed signs of hemorrhagic contusion were discarded. The control animals were left on the wooden board to wake up without being struck by the pendulum.

The dynamic force of the impact was determined by trial and error in our pilot experiments. There was no loss of consciousness or any other evidence of concussion in the rats when hit by the pendulum dropped from a position 45° to the vertical plane. When the pendulum was dropped from a horizontal position of 90° to the line of gravity, the rats showed transitory unconsciousness for 3 to 10 minutes. Thus, the pendulum swung from 90° to the vertical plane was selected for the experiments on 12 rats, and the energy absorbed by the head was calculated.

The energy absorbed by the rat’s head was determined from the difference in potential energy corresponding to the initial and final positions of the pendulum. If the initial angle and the angle after impact are \( A_0 \) and \( A_f \), respectively, the difference in potential energy \( (E_0 - E_f) \) can be calculated as:

\[
E_0 - E_f = W \cdot R \cdot (\cos A_f - \cos A_0),
\]

where \( W \) is the weight of the pendulum and \( R \) is the distance from the center of gravity to the support along the pendulum (Fig. 2).

Friction energy loss of the testing system was determined by measuring the difference between the initial and final angles in a “free-swing” situation. This angle due to friction loss was subtracted from the initial angle in computing the energy absorption of the animal.

In the experiments conducted, the initial angle was always set at 90°. This had an angle of friction loss of 5° in the system. Therefore an initial angle of 85° was used. Other values used in the computation were: \( W = 1505 \) gm and \( R = 13.1 \) cm. The energy absorption curve is given in Fig. 2. The dotted line shows that for a 35° angle after impact, the energy absorbed is approximately 1450 gm/cm.

The 12 rats were killed at the following times after concussion: two at 30 minutes, four at 1 hour, two at 2 hours, two at 6 hours,
Experimental cerebral concussion

and two at 24 hours. The rats were anesthetized by an intraperitoneal injection of a 3.5% chloral hydrate solution (1 ml/100 gm body weight). Cadmium-free ferritin in the amount of 0.3 to 0.5 ml (4.5 mg/ml) was injected into the tail vein, 5 to 15 minutes before death. The animals were killed by perfusion of 20 to 30 ml of warm phosphate buffer (0.1 M at 37° C) through the ascending aorta, followed by 100 to 120 ml of cold 4% glutaraldehyde (in 0.1 M phosphate buffer at 4° C). Immediately after the perfusion-fixture the cranium was opened, and the brain and upper cervical spinal cord removed. The following specimens were collected: the right and left frontal cortex and subcortical white matter, the right and left occipital cortex and subcortical white matter, the cerebellar cortex and white matter, both superior colliculi, the tegmentum of the medulla oblongata, and the gray and white matter of the first two segments of the cervical cord. All specimens were postfixed in cold osmium tetroxide for 90 minutes, dehydrated in graded ethanol, and embedded in Maraglas mixture.

Thick (1 μm) and thin (60 nm) sections were cut from each specimen on a Porter-Blum MT-2 ultramicrotome.* The thick sections were stained with 0.1% toluidine blue for light microscopy. The thin sections were stained with uranyl acetate and lead citrate for examination by a JEOLCO 100B electron microscope.†

Results

All the rats were unconscious for 3 to 10 minutes after the concussion. During this time the corneal and light reflexes were absent and the animals did not respond to painful stimulation. After they awakened, they remained sluggish for 15 to 20 minutes. Thereafter they behaved and fed normally, and showed no evidence of focal or generalized neurological abnormality.

On gross examination the brains of the concussed rats were normal. In particular there was no evidence of swelling or hemorrhages.

*Porter-Blum MT-2 ultramicrotome manufactured by Ivan Sorvall, Inc., Norwalk, Connecticut.
†Electron microscope supplied by Jeol, Inc., 477 Riverside Avenue, Medford, Massachusetts.

Light microscopy revealed slight edema in the frontal white matter, the medulla, and upper cervical cord. A few neurons in the occipital cortex and a considerable number of neurons in the cervical cord appeared slightly swollen but, in contradiction to previous findings,* there was no evidence of chromatolysis in the nerve cells. In general, the central nervous system specimens showed only minor changes under the light microscope.

Electron microscopy demonstrated significant changes in the brain ultrastructure of the concussed rats; the abnormalities showed great regional variation.

Fig. 2. Upper: Diagram showing the initial and final positions of the iron rod with reference to the line of gravity where the rat's head received the impact. Lower: Graph representing the variation in energy absorption as a function of the angle after impact, for an initial angle of 90°. For a postimpact angle of 35°, the energy absorbed is 1450 gm/cm. The curve has been adjusted for friction energy loss of the testing system.
The brains of control animals revealed a well-known ultrastructural picture of intact mitochondria and narrow extracellular spaces (Fig. 3 left). By comparison, in the occipital cortex of the concussed rats, the point of impact, many mitochondria of the neurons were swollen (Fig. 3 right). The mitochondrial cristae were pushed to the periphery by excess fluid. The mitochondria of glial cells remained normal. The nucleus, Nissl substance, and Golgi complex of the neurons were not altered, nor was there any evidence of chromatolysis. There was no edema in the occipital cortex or white matter. Changes in the cerebellar cortex were similar to those found in the occipital lobe. The superior colliculi remained entirely intact.

The frontal cortex, the site of the contre coup, revealed minor changes in terms of slight swelling of the perivascular astrocytic processes and mild enlargement of the extracellular space (Fig. 5 left). Although the "tight junction" of the capillary endothelium was not widened, numerous ferritin particles were present in the basal lamina of the capillaries and venules. A few ferritin particles were even seen in the extracellular space, indicating increased permeability of the blood-brain barrier. There was occasional

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**Fig. 3.** *Left:* Occipital cortex of control rat revealing intact neuronal mitochondria and narrow extracellular spaces. $\times$ 15,000. *Right:* Occipital cortex in an experimental rat 1 hour after concussion. Many neuronal mitochondria (m) are moderately swollen, and their cristae are pushed to the periphery. The ribosomes (R), the endoplasmic reticulum (Er) (Nissl substance), the Golgi complexes (G), and the nucleus (N) are normal. $\times$ 5000.
Experimental cerebral concussion

**FIG. 4.** Left: Neuropil of the gray matter of the upper cervical cord 1 hour after concussion. Numerous neuronal mitochondria (m) are swollen, and most of their cristae can be seen only over part of the periphery. Other subcellular organelles and synapses (s) appear intact. The extracellular space (e) is slightly enlarged. × 3000. Right: White matter of the upper cervical cord 1 hour after concussion. The extracellular space (e) is greatly enlarged. The myelin sheaths (Ms) of some nerve fibers show slight rufflings. The axons (Ax) appear somewhat overhydrated. A great concentration of gliofilaments (Gf) is evident in the processes of fibrous astrocytes. × 8500.

**FIG. 5.** Left: Frontal cortex 1 hour after concussion. The perivascular astrocytic processes (A) are moderately swollen. The extracellular space (e) in the neuropil is slightly enlarged. A few mitochondria (m) appear partially hydrated. The “tight junction” (T) of the endothelial cells is not widened. Numerous ferritin particles can be seen in the basal lamina (B) of a capillary, and some in the swollen astrocytic processes and the enlarged extracellular space (arrows). L = lumen of the capillary. × 4000. Right: Subcortical white matter of the frontal lobe 1 hour after concussion. The extracellular space (e) is enlarged. The axons (Ax) and their mitochondria (m) appear intact. The myelin sheaths of some nerve fibers show rufflings (Ms). × 3250.
mild swelling of the neuronal and glial mitochondria. In the frontal white matter, there was a definite extracellular matter edema as evidenced by the enlarged extracellular spaces (Fig. 5 right). Some ruffling of the laminae of the myelin sheaths was also observed.

These alterations were already observed 30 minutes after the concussion but in a lesser degree of severity. They reached their peak at 1 hour, declined afterward, and virtually disappeared by 24 hours. The severe changes of the mitochondria were completely reversible within 24 hours, while a slight enlargement of the extracellular space of the white matter in the frontal lobes and upper cervical cord remained. The same changes were observed in the brains of rats sacrificed in the same period of time after concussion.

Discussion

Previous studies of experimental brain concussion were performed on animals without a known force of the impact on the skull or with a calculated force but after exposure of the brain. More accurate data were obtained from the experiments of Ommaya, et al. We believe that the simple adjustable pendulum used in our experiments, exerting its force on the intact skull, comes closest to the situation prevailing in the average head injury in man that results in cerebral concussion, because the transient unconsciousness is followed by complete recovery without any focal neurological signs. It is still amenable to the calculation of the force of impact in dynamic terms, readily providing us with the actual energy absorbed by the head at impact.

Our observations confirm the statement made by Brodersen that the severity of clinical symptoms is proportional to the energy absorbed by the head on impact. Our findings also indicate that the major damage occurs in the frontal region and at the craniospinal junction, while the mesencephalon and the caudal diencephalon are the least vulnerable. This is comparable with the findings by Ommaya and Gentile. From a mechanical point of view, the changes are caused by contre coup as far as the frontal lobes are concerned and stretch-shearing effects at the craniospinal junction.

In summarizing the effect of concussion at the ultrastructural level, mitochondrial changes occur in the neurons of the occipital cortex, the region of the impact (Fig. 3 right). The changes in the structure of the neuronal mitochondria are even more severe in the medulla and upper cervical cord, areas somewhat remote from the impact but subject to traumatic stretch and shearing (Fig. 4). However, it should be pointed out that swelling of neuronal mitochondria is not specific to brain concussion. It was observed in a variety of neurological diseases including acute and chronic hypoxia and radiation damage, to name a few.

Interestingly enough, edema does not develop in the impact region. It occurs in areas farther away, such as the frontal lobes and the craniospinal junction. The fact that the mesencephalon, as represented by the superior colliculi, remains intact can be explained by the basic mechanical principles involved. The skull and its contents can be viewed as an oval globe, round in cross section and oval in horizontal section. Any force hitting the skull at one point can be resolved in two component forces that meet at the farthest opposite point, giving rise to a contre-coup effect and to a shearing action at the craniospinal junction. Since the superior colliculi are close to the center of globe, they receive zero force from any impact to the periphery of the ovoid mass.

The localized cerebral edema caused by concussion is of the vasogenic type and reflects the transient increase in the permeability of the blood-brain barrier as shown by the extravasation of ferritin. Similar findings were reported earlier with the use of methylene blue, fluorescein, Evans blue-albumin, and P. In most experiments utilizing vital dyes, increased permeability was restricted to the first 24 hours.

Previous experiments by Bakay, et al., using nuclear magnetic resonance technique revealed that the edema fluid in trauma consists of two components. One is motionally restricted, “bound” water, the other motionally unrestricted, “bulk” water. The rapid development, shift, and disappearance of excess water in concussion must be attributed to the fast moving water component.

The sudden onset of mitochondrial swelling and its prompt disappearance in 24 hours is probably not caused by a complete disappearance and reappearace of mitochondrial enzymes. A more likely explana-
Experimental cerebral concussion

tion is a sudden increase in the permeability of the mitochondrial outer membrane. The inward flow of excess fluid results in a swelling of the organelle, which pushes the cristae to the periphery and dilutes the mitochondrial enzymes. Hamberger and Rinder found a brief increase in the succinoxidase activity and concluded that concussion activated the existent enzyme in the neuronal mitochondria rather than increasing the synthesis of the enzyme. Detailed histochemical studies now in progress in our laboratory might shed light on this aspect of cerebral concussion.

These morphological investigations do not by themselves explain the loss of consciousness in concussion. However, the edematous and mitochondrial changes of the medulla and upper cervical cord may explain such clinical findings as respiratory distress, cardiovascular alterations, and slowing of cerebral circulation.3,7,10

References

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