

Ultrastructural Changes of Macroglial Cells in the Rat Brain Following an Exposure to a Non-penetrative Blast

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Abstract

The present study examined the ultrastructural changes in astrocytes and oligodendrocytes in rats following an exposure to a non-penetrative blast. At 1 and 7 days after the blast, the astrocytes in the cerebral and cerebellar cortex were hypertrophied; their end-feet associated with the blood vessels were also swollen, bearing sparsely distributed organelles. The above changes were not observed in experimental rats when the survival interval was prolonged. It is concluded from this study that the blast could have disrupted the integrity of the blood-brain barrier resulting in possible abnormal entry of serum-derived substances thereby leading to astrocytic hypertrophy. The reversible nature of the changes is evidenced by the seemingly normal appearance of astrocytes in rats killed at 14, 21 and 28 days after the blast. Oligodendrocytes remained unaffected at various time intervals after the blast.

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Introduction

Exposure to detonation of explosives is known to produce injuries to various organs in the body, e.g. perforations of the tympanic membrane¹ and injuries to the lungs,^{2,3} middle ear and gastrointestinal tract.⁴ Detonation of explosives in the air produces a region of instantaneous rise in pressure generally termed a blast wave² or a shock wave³ which transverses the body and causes injury to living tissues without external trauma.³ Recently,⁵ we reported signs of neuronal lesions in rats exposed to a non-penetrative blast.⁵ Accompanying the neuronal lesions was microglial activation in the cerebral and cerebellar cortices as manifested by their upregulation of surface antigens detected immunohistochemically as well as showing ultrastructural changes.⁵ The aim of the present study was to find out whether the macroglial cells in the brain, namely astrocytes and oligodendrocytes,⁶ would also respond to the blast.

Materials and Methods

Six adult male Wistar rats (weighing about 250 g) were exposed to a blast in an underground concrete

chamber. They were kept in laboratory cages (North Kent, England) which were fastened to a wooden plank that was further secured to the floor of the underground concrete chamber. Details of the design of the chamber have been reported previously.⁵ The explosive (110 kg TNT equivalent) was then detonated on the ground surface at a distance of 12.5 metres from the underground chamber in which the animals were kept. One rat each was sacrificed at 1, 4, 7, 14, 21 and 28 days after the blast. For controls, 2 normal rats of equivalent body weight were sacrificed.

The rats were perfused with Ringer's solution following deep anaesthesia with 7% chloral hydrate until the lungs and liver were cleared of blood. This was followed by a mixed aldehyde solution composed of 2% paraformaldehyde and 3% glutaraldehyde in 0.1M phosphate buffer. The brains were removed and further fixed in the same fixative overnight at 4°C. Coronal vibratome sections (100 µm in thickness) of the brains were cut. Blocks of cerebral (somatosensory region) and cerebellar cortices from the above sections were removed and postfixed in 1% osmium tetroxide in 0.1M phosphate buffer, pH 7.4 for 1 hour. Following dehydration in a

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graded series of alcohol, the tissue blocks were embedded in Araldite mixture. Ultrathin sections stained in lead citrate were viewed in a Philips CM120 microscope.

Results

No external trauma was observed in the rats following an exposure to the blast. At 1 and 7 days after the blast, the astrocytes and their processes associated with the blood vessels were considerably swollen (Fig. 1). The astrocytic cytoplasm appeared extremely watery bearing a few cisternae of rough endoplasmic reticulum and mitochondria (Fig. 1). Activated microglia showing ruffling of their plasma membrane were often observed in close approximation to the swollen astrocytic end-feet on the blood vessels (Fig. 2). Besides the blood vessels, the astrocytic processes around the dendrites, closely applied to the neuronal soma as well as those forming the glia limitans were also distended. In the cortical tissues of control rats processed with those from the experimental rats, the astrocytes and their end-feet around the blood vessels presented typical ultrastructural features (Fig. 3) described previously.⁶ At 14, 21 and 28 days after the blast, the ultrastructural features of astrocytes and their processes did not show any noticeable difference from the control rats. Neuronal somata of the cortical neurons did not exhibit striking structural alterations at 7 and 14 days after the blast, although darkened dendrites were ubiquitous in the

neuropil. Darkened dendrites had never been encountered in control rats as well as at 21 and 28 days after the blast. Oligodendrocytes, majority of them being the dark type, appeared structurally unaltered at the various time intervals after the blast (Fig. 4).

Discussion

The present study showed that although no external trauma was produced in rats following an exposure to a non-penetrative blast, the brain (in particular the cerebrum and cerebellum) showed some ultrastructural alterations, a notable feature being the astrocytic swelling at 1 and 7 days after the blast. Astrocytic hypertrophy in response to brain injury has been well documented in different experimental models.^{7,8} Landis⁷ described that astrocytic swelling in brain injury might be attributed to disordered potassium homeostasis, altered neuronal or neurotransmitter metabolism. It is possible that such may also be the case in the brain following the blast as darkened dendrites and axon terminals indicative of neuronal degeneration^{5,9} were a common feature. The astrocytic swelling around the blood vessels at 1 and 7 days after the blast may be indicative of some functional disturbances at the blood-brain barrier which may have allowed the entry of some serum-derived substances into the neuropil. This could have led to the activation of microglial cells as has been reported previously.⁵

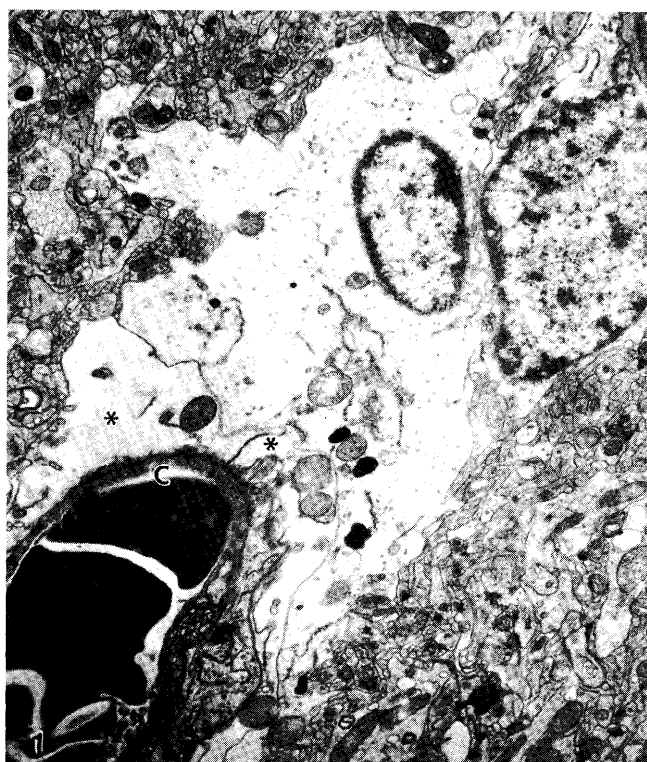


Fig. 1. A hypertrophied astrocyte in the cerebral cortex of a rat at 4 days after the blast, showing its watery cytoplasm which contains a few cisternae of rough endoplasmic reticulum and mitochondria. Note that the astrocytic end-feet (asterisks) resting on the capillary (C) wall are extremely dilated ($\times 7000$).

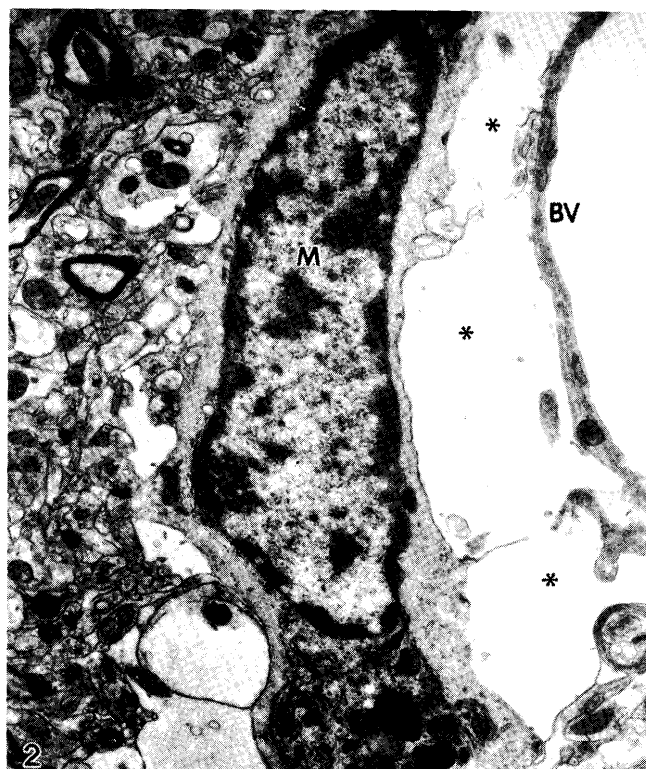


Fig. 2. Profiles of swollen astrocytic end-feet (asterisks) on a blood vessel (BV) in the cerebral cortex at 7 days after the blast. A microglial cell (M) with ruffled plasma membrane is lying in close apposition to the swollen astrocytic processes ($\times 7820$).

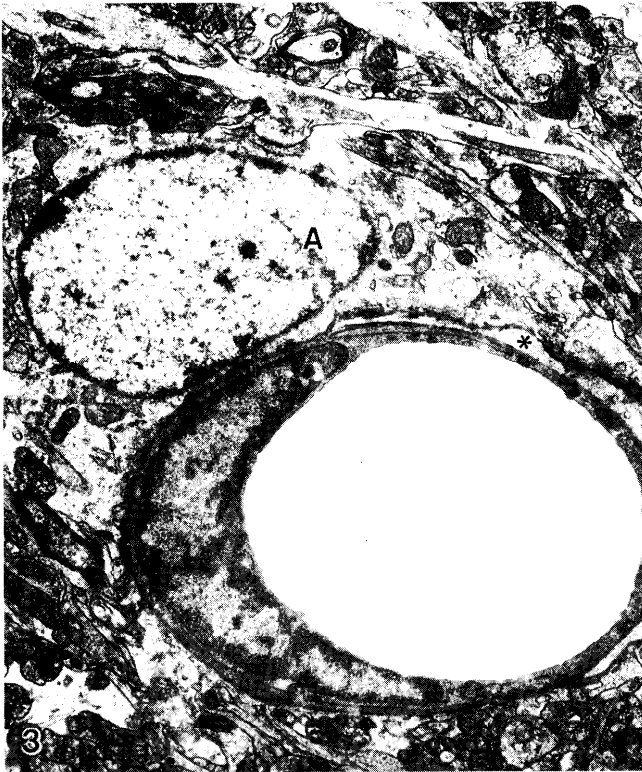


Fig. 3. A capillary in the cerebellar cortex of a control rat is partially enclosed by an astrocyte (A) (x7000).

Astrocytic hypertrophy was not observed at 14, 21 and 28 days after the blast although the incidence of darkened dendrites was sustained at least until 14 days. It is possible that astrocytic hypertrophy is an early sign of glial response to brain injury helping to limit the neuronal cell death by secreting 'some neurotrophic factors. Indeed, a recent study has shown that glia-derived neurotrophic factors have been described to be important in limiting neuronal cell death following axon transection.⁷ Reactive astrocytes and microglial cells are known to secrete neurotrophic factors which may provide a microenvironment conducive to neuronal sprouting following injury to the central nervous system.¹⁰

In the present study, oligodendrocytes did not show any alteration at any time interval following the blast. This is confirmed by the lack of change in the myelin sheaths associated with the numerous axons.

Conclusion

It is concluded from this study that an exposure to a single non-penetrative blast would result in hypertrophy of astrocytes and their end-feet associated with blood vessels. One possible contributing factor leading to this change is disruption of the blood-brain barrier. The induced ultrastructural changes, however, are reversible, suggesting the recovery of the astrocytic function in longer surviving animals.

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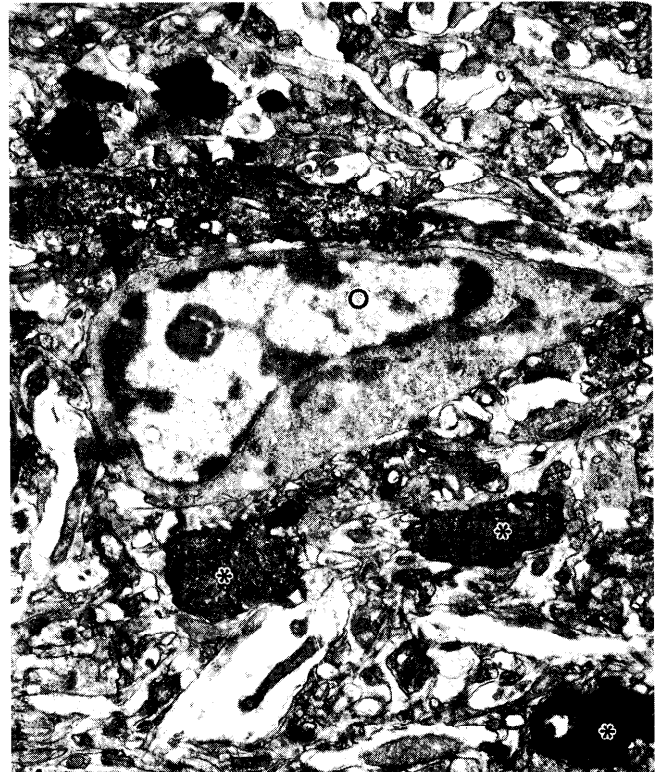


Fig. 4. A dark oligodendrocyte (O) in the cerebellar cortex at 14 days after the blast. The cell shows a nucleus with dense chromatin clumps. The moderate amount of cytoplasm contains mitochondria and cisternae of rough endoplasmic reticulum. Darkened dendrites (asterisks) are widely distributed in the neuropil (x7700).

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