ABSENCE OF GLIAL FIBRILLARY ACIDIC PROTEIN RESPONSE TO CRYOGENIC INJURY IN SKATE (RAJA ERINACEA)

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Glia, since their discovery by Virchow in 1846, have been thought of as supportive cells, which nurture more important activities of neurons. As such glia represent an essential cellular component of normal brain. Therefore, in disease states of brain, glia can be expected to either influence or be influenced by neuropathic processes. Hence, by monitoring the patterns and mechanisms by which glia modulate their function in response to injury, one may gain insight to the pathogenesis of various brain diseases. For example, edema is a virtually universal response of brain to injury which commonly involves alterations in glial volume regulation. Such edema is absent from the elasmobranch brain after it is injured from excessive cold (Klatzo et al. In: Brain Edema, I. Klatzo & F. Seitelberger, eds., Springer-Verlag, N.Y., pp. 554-563, 1967). This latter result suggests the elasmobranch brain or its glial cells may be fundamentally different from their counterparts in mammals. Accordingly, we examined another common response of glia to injury: The transformation of certain astrocytes into reactive species after cold injury as a first step toward providing further evidence of a difference between glia of elasmobranchs and those of other vertebrates.

Seven skates of either sex (Raja erinacea) (1-2 kg) were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and respirated by passing fresh, running sea water (16^{o}C) through their spiracles at a rate of 1-2 l/min. A craniotomy was made to expose the tectum and anterior cerebellum. Next the dura was retracted from the right side of the exposed brain. A small piece (approximately 5 mm in diameter) of dry ice was applied to the brain at this latter site for 30-60 sec (n=4). Finally, a thin rubber patch was used to close the craniotomy site by securing it to the surrounding skull area with cyanoacrylate. Glue was applied to the skull so as to preclude its entry into the brain case. Animals were first returned to a recovery pond where artificial ventilation was continued (approximately 24 hours) until they began to spontaneously swim and breath. Subsequently animals were placed in a common pond. During this latter period their feeding and swimming behavior appeared normal. Seven days after cryogenic injury animals were re-anesthetized and sacrificed by intracardiac perfusion (>300 ml) with 4% paraformaldehyde in skate Ringer (288 mM NaCl, 4 mM KCl, 5 mM $CaCl_2$, 3 mM $MgCl_2$, 350 mM urea, and 100 mM phosphate buffer at 7.2 pH). Brains were removed and post fixed for one hour before being stored in skate Ringer at 4 ^{o}C. Other brains (n=3) were processed as controls from unoperated animals.

Fixed brains were either 1) embedded in parafin, sectioned, and stained with hematoxylin and eosin; or 2) frozen with dry ice, sectioned, and processed for immunohistochemical visualization of glial fibrillary acidic protein (GFAP).

Dry ice applied to the tectum and cerebellum produced evidence of coagulation necrosis. Neurons and glia within lesioned areas had shrunken pyknotic nuclei. Vacuolated areas and some liquefaction was evident at the base of necrotic zones. In addition inflammatory cells were present throughout lesioned areas. No edema was evident. Although GFAP staining was evident in astrocytes and radial glia of all animals, no increased staining was seen in

cold-injured brain (Fig. 1). This is in contrast to the rat where increased GFAP staining is evident as early as 30 minutes after cold injury (Ammaducci et al., Neurosci. Letters 27:27-32, 1981).

These results, though preliminary, suggest that elasmobranch glia do not increase their GFAP content after a necrotizing lesion. Furthermore, if increased GFAP content is a neccessary concomitant of the transformation of glia into reactive species, these results imply such altered cells are absent in the elasmobranch after injury.

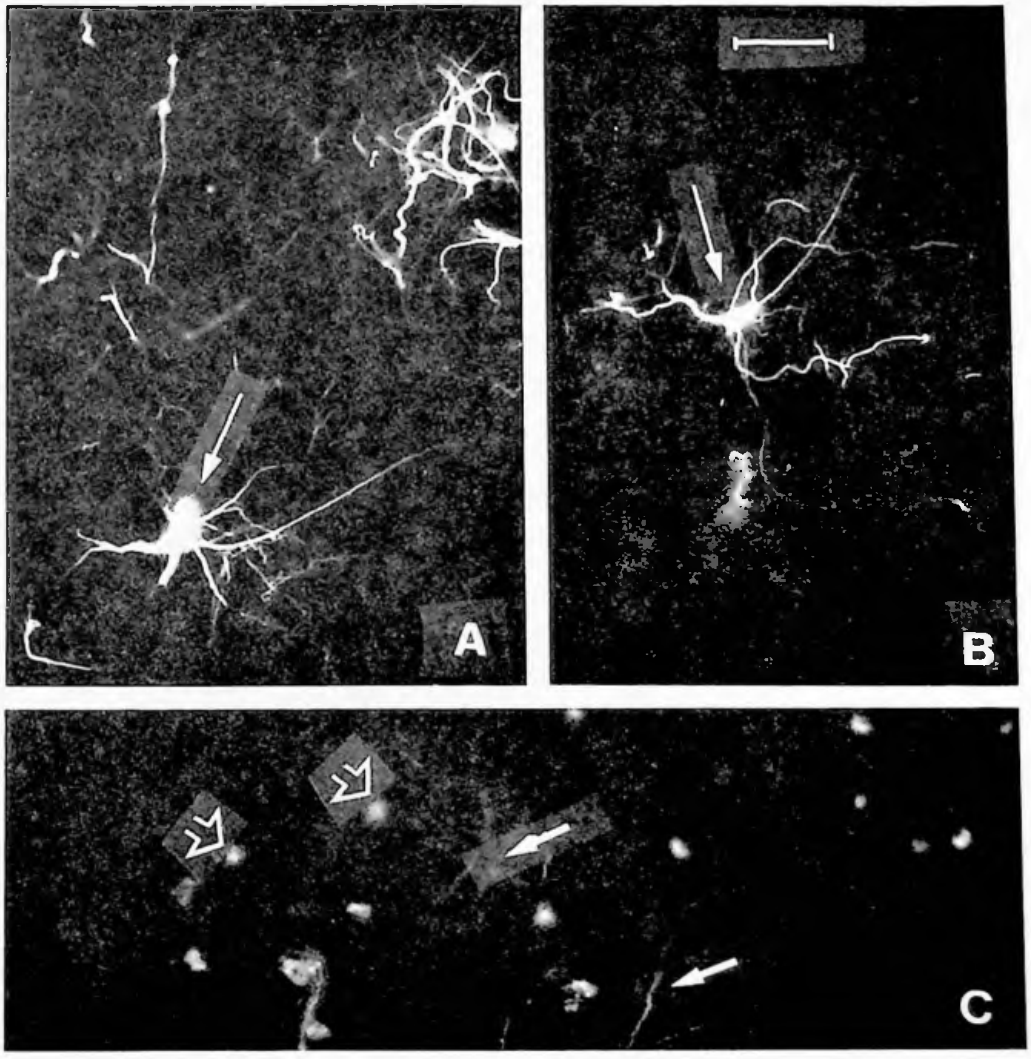

Figure 1. Glial fibrillary acidic protein (GFAP) staining in elasmobranch brain. GFAP positive glia within the tectum are shown from normal (A) and lesioned (B & C) skates. (A) Arrow marks astrocyte in the intermediate tectal layer. (B) Intermediate layer of cold-injured tectum. Note the similar pattern of GFAP staining (arrows) from normal (A) and lesioned (B) brain. (C) Superficial layer of cold-injured tectum. Arrows mark thin GFAP positive fibers. Open arrows point to nonspecific flourescence from lymphocytes which infiltrated the lesioned tectum. All staining was done with anti-GFAP (monoclonal antibody from Boehringer Mannheim; #814369) at a dilution of 1:100. Calibration bar is 25 um.

This work was supported by NS 13742 (CBJ), NS 19108 (RPK) and Teacher Investigator Development Award NS 00767 to RPK.