

# CHANGES IN GFAP IMMUNOREACTIVITY FOLLOWING IMMUNOTOXIC LESIONS IN THE RAT BRAIN

We used immunocytochemistry against glial fibrillary acidic protein (GFAP), a specific marker for astrocytes, to study the role of these cells in neuroprotection. If, in fact, astrocytes are involved in the neuroprotective process, then we expect to see an increase in GFAP immunoreactivity following a lesion in the rat brain. To explore this issue, we stained brain sections containing the region of the basal forebrain cholinergic system with an antibody against GFAP and quantified the levels of expression of this protein. Our results indicate that in animals deprived of estrogen, astrocytes in the lesioned side of the brain are not activated because the levels of expression of GFAP are above the levels seen in the intact side of the brain. These results are preliminary, but they are compatible with the suggestion that estrogen contribute to astrocytic responses to injury.

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## INTRODUCTION

Alzheimer's disease (AD) is a complex neurodegenerative disorder that is characterized by a progressive, irreversible decline in a wide range of cognitive abilities, including short and long-term memory, abstract thinking, and personality and behavioral changes. The brains of patients that die with AD show a number of morphologic changes, such as neuritic plaques, neurofibrillary tangles, and widespread neuronal degeneration. Earlier studies have suggested that estrogen can protect neurons against a toxic insult.<sup>1</sup> Astrocytes are believed to protect neurons in the event of damage. Astrocytes contain glial fibrillary acidic protein (GFAP), and because no other cellular element in the brain contains this protein, it serves as a specific marker to identify them.

## METHODS

We used 16 female Fisher 344 rats, 8 ovariectomized, 8 nonovariectomized. They all received a lesion with the immunotoxin 192 IgG-saporin. These lesions were made in the horizontal limb of the diagonal band of Broca in the right side of the brain with stereotaxic procedures. The animals were allowed to recover, and then half of each group received estrogen and the other half received placebo pellets for 60 days. At the end of the treatment, the rats were sacrificed and perfused. The brains were cut on a microtome and stored for histologic analysis, as described elsewhere.<sup>2</sup>

One series of sections from all these brains was processed with immunocy-

tochemical techniques, using an antibody against GFAP. This staining technique consisted of a two-day process and a long series of steps. First, a 30-minute wash in 25% PBT and 30% hydrogen peroxide, two 5-minute rinses in phosphate-buffered saline buffer, a 60-minute incubation in blocking solution (3% milk), and an overnight incubation in the primary antibody, a polyclonal anti-GFAP made in rabbit (1:25,000 dilution). On day 2, after three 10-minute rinses in phosphate-buffered saline, the sections were incubated for 60 minutes in a solution containing the secondary antibody, a biotinylated anti-rabbit IgG made in goat (1:1,000 dilution), then rinsed three times for 10 minutes, incubated for 60 minutes in ABC complex (dilution 1:500), rinsed again three times for 10 minutes in phosphate-buffered saline, incubated in DAB for 15 minutes, and rinsed again; sections were then mounted onto glass slides.

## DATA ANALYSIS

By using a digital camera mounted onto a microscope and connected to the computer, the sections were photographed with the 10x/.25 objective. The capture parameters were fixed, so the camera always took the photographs with the same magnification. The images were then transferred to Photo-shop, converted to black and white, and a grayscale threshold was applied. The images were inverted, and the computer calculated the number of pixels occupied by staining. This measurement was used as an indirect estimate of GFAP abundance. Three sections from each

brain were measured. For each section, the percentage change in GFAP staining was calculated in the lesioned side (L) with respect to the contralateral (C), intact side as follows:  $L/C \times 100$ . Then the three percentages were averaged to obtain a single value per animal.

## RESULTS

Results were plotted according to four groups of animals: ovariectomized + estrogen, ovariectomized + placebo, nonovariectomized + estrogen, and non-ovariectomized + placebo. In nonovariectomized animals, the lesion induced a small, not statistically significant increase in GFAP, compared to the intact side. The levels of GFAP in ovariectomized animals treated

with estrogen were similar for both lesioned and control sides of the brain. By contrast, in ovariectomized animals treated with placebo (and therefore completely devoid of estrogen), the levels of GFAP in the lesioned side were higher than in the control side.

## DISCUSSION

Although these results are preliminary because the number of subjects in each group was not sufficient to establish statistical significance, we speculate that lack of estrogen may have a negative effect on the capacity of the brain to respond to a lesion. We need to repeat these measurements in more animals to verify that this is the case. In addition,

future work will seek to correlate our GFAP data with an analysis of neuronal survival to ensure that in all these cases the lesions were of comparable size. Otherwise, we could have a superimposed effect of the lesion.

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## REFERENCES

1. Saenz C, Dominguez R, de Lacalle S. Estrogen contributes to structural recovery after a lesion *Neuroscience Letters*. 2006;392:198–201.
2. Hartonian I, Mufson EJ, de Lacalle S. Long-term plastic changes in galanin innervation in the rat basal forebrain. *Neuroscience*. 2002; 115(3):787–795.