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Down-regulation of Glial Fibrillary Acidic Protein (GFAP) during the development of a marine fish (*Dentex dentex* L.)*

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SUMMARY: A band of approximately 52-53 kDa, corresponding to glial fibrillary acidic protein (GFAP) was detected by immunoblot techniques in brain tissue of a marine fish (*Dentex dentex* L). The aim of this study was to quantify the density of GFAP during the life cycle of a marine fish in the wild. The levels of GFAP immunoreactivity were determined at two different stages of development: juvenile (body weight range: 180-360 g) and sexually developing-mature specimens (body weight range: 2329-5550 g) from a natural population in the Western Mediterranean Sea. Our results indicated a negative correlation between the immunoreactivity of GFAP and weight in the sexually developing-mature group of fishes ($r=-0.785$, $p<0.05$, $n=8$). These findings might be of relevance in understanding the role of GFAP in the development of the central nervous system and demonstrate that GFAP down-regulation in *D. dentex* brain tissue during development is a natural physiological phenomenon.

Keywords: *Dentex dentex*, Glia, Glial fibrillary acidic protein, immunoblot, western blot, marine fish development.

INTRODUCTION

GFAP has been considered as the main constituent of intermediate filaments of mammal astrocytes (Eng *et al*., 1971; Eng, 1985) and the expression of GFAP by glia cells is indicative of the differentiation and maturation stage of the cell. The immunological characteristics of this protein are constant in vertebrates from fish to mammals (Mencarelli *et al*., 1993). Few studies have been done on the glial component of the fish central nervous system (CNS) due in part to the difficulty in identifying fish astrocytes by immunohistochemistry (Dahl *et al*. 1985). Monoclonal antibodies to porcine GFAP have been demonstrated to cross react with fish GFAP by immunohistochemistry and immunoblot techniques (Blaugrund *et al*. 1991). Taking advantage of this fact we recently demonstrated in a preliminary report the down-regulation of GFAP expression during the development of *Sparus aurata* L. in captivity (Busquets *et al*., 1996). The common dentex, *Dentex dentex* L. (Pisces: Sparidae), is a littoral species common down to a depth of 50 m, which is also found down to 100 m. It is distributed in the Atlantic from the Bay of Biscay to Cape Blanc and Madeira and it is an important component of the littoral ecosystems of the Mediterranean. This species is relatively abundant on Majorcan waters,

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where all the age classes composing the population are exploited using a combination of different fishing gears. Common dentex is a long living species, reaching 28 years of age, corresponding to weights up to 8000 g. First sexually mature specimens have a mean weight of 443.8 g for females and 691.97 g for males. Fifty percent of the population is mature at 563 g for females and 1960.5 g for males, corresponding to 2-4 years of age (Morales-Nin and Moranta, 1997). The aim of this study was to examine the possible modulation of GFAP levels in the brain tissue during the growth and sexual development of a marine fish in its natural environment. Thus, we have chosen common dentex for the possibility of sampling different size ranges, corresponding to all sexual development stages, and for the knowledge of its biology in Majorca.

MATERIAL AND METHODS

Specimens of the common dentex were sampled in 1995 in the Western Mediterranean Sea around Majorca Island. They were caught with bottom long-lines (pubescent and mature fish with total length between 20 and 80 cm) or trammel nets (juvenile immature fish with total length from 17 to 30 cm). Fishes were immediately kept on ice, the brains were dissected upon arrival at the laboratory and stored at -80°C. Sexual development stage was determined according to: i) the visual examination of the gonads following a VI stage scale, and ii) the gonadosomatic index (Morales-Nin and Moranta, 1997). Two fish developmental groups were identified: immature (virgin to pubescent) corresponding to fishes with a body weight range of 180-360 g and mature (2329-5550 g), were all fishes have undergone at least one spawnning (Morales-Nin and Moranta, 1997).

Immunoblots were performed as described previously (Beitner-Johnson *et al*. 1993; Busquets *et al*. 1996). Briefly, 200 mg of whole brain were homogenised (1:20 w/v) in 40 mM Tris buffer, pH 6.7, containing 1% Triton X-100, 1 mM EDTA, 1 mM phenylmethylsulfonylfluoride (PMSF) and leupeptin (40 mg/ml). The samples were centrifuged at 40000 g for 45 min at 4°C and the supernatant was subjected to 10% SDS-polyacrylamide gel electrophoresis. Proteins were determined by the method of Bradford (Bradford, 1976).

Separated proteins were transferred to nitrocellulose membranes blocked with phosphate-buffered saline containing 5% non-fat dry milk and 0.1% tween-20 (blocking solution) and incubated at room temperature for 2h in blocking solution containing the primary antibody (anti-GFAP monoclonal antibody, Sigma; clone GA5) at a 1:5000 dilution. The secondary antibody, (horseradish peroxidase-linked sheep anti-mouse IgG; Amersham International; Buckinghamshire, U.K.) was used at 1:5000 dilution. Immunoreactivity was detected with an enhanced chemiluminiscence (ECL) western blot detection system (Amersham International) followed by exposure to Amersham Hyperfilm ECL. Films were scanned in the image analyser Bio Image (Millipore, Ann Arbor, MI). The integrated optical density (IOD) corrected by the protein content (in the range of linearity) is termed IOD units.

Statistics: Pearson's correlation coefficients were calculated to test for possible association among variables. The level of significance was chosen as $p<0.05$.

RESULTS AND DISCUSSION

Brain tissue of *Dentex dentex* L. express a main GFAP immunoreactive band with a relative molecular weight of 52-53 kDa (Fig. 1), which is inside of the range previously described in other marine fish (*Sparus aurata* L.) and mammal neural tissues (Eng, 1985; Beitner-Johnson *et al*., 1993; Busquets *et al*.,1996). This result indicates the specificity of the GFAP immunoreactivity signal in brain tissue of Common Dentex. Common Dentex GFAP immunoreactive band showed a relative molecular weight slightly heavier compared with *Sparus aurata* L (50-51 kDa) (Fig. 1). This heavier molecular weight could represent a species specific glycosilation pattern of the protein.

The levels of GFAP appear to be modulated during the Common Dentex life cycle: the density of brain GFAP immunoreactivity in juvenile animals (body weight range: 180-360 g) is relatively low compared with developing-mature specimens (body weight range: 2329-3260 g) but similar to heavier specimens (body weight range: 4170-5550 g) (Table 1). The possible relationship between maturation stage (body weight) and GFAP levels in brain tissue was assessed through correlation analysis. There was a significant negative correlation between the expression of GFAP and the body weight of mature specimens ($r=-0.785$; $p<0.05$; $n=8$)(Fig. 2). This result seems to indicate a loss of GFAP levels in

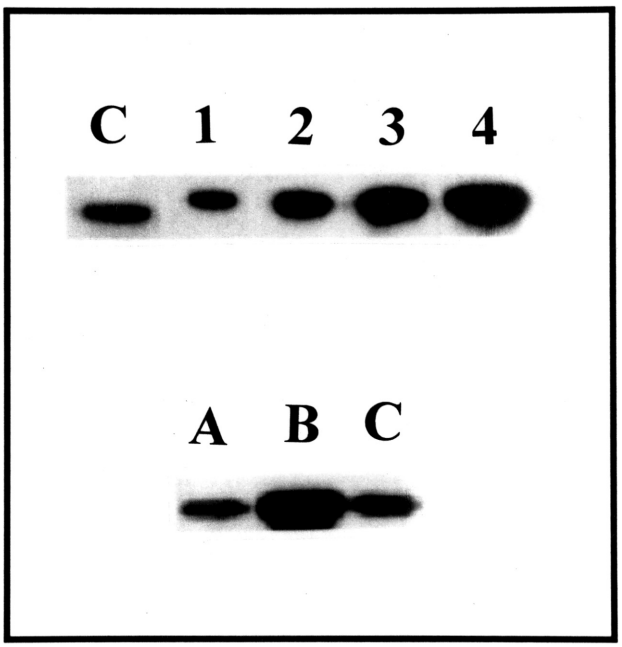

FIG. 1. – Top: *Dentex dentex* brain tissue GFAP immunoreactivity signal intensity versus protein content. The amount of total protein loaded per gel was 7 μg (lane 1), 15 μg (lane 2), 30 μg (lane 3) and 60 μg (lane 4). Lane C: Control of GFAP immunoreactivity from brain tissue of *Sparus aurata* L. Bottom: A representative immunoblot showing the common dentex brain GFAP as a function of body weight. A: Juvenile (body weight: 280 g; GFAP: 1.51 IOD units). B: Development-mature (body weight: 2329 g; GFAP: 52.43 IOD units). C: Development-mature (body weight: 5550 g; GFAP: 3.05 IOD units).

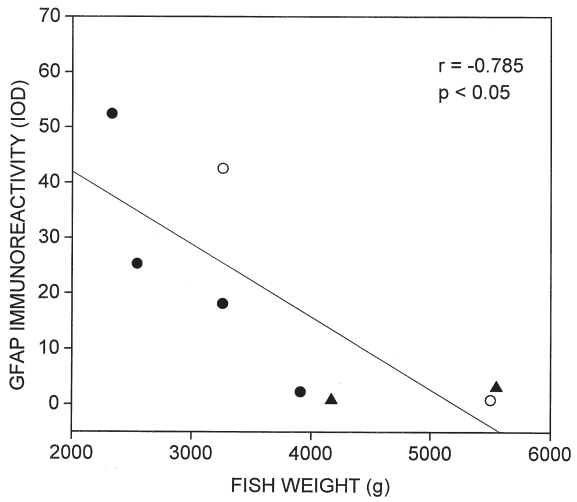

FIG. 2. – Scatterlog and linear regression line showing the relationship between the expression of GFAP in brain tissue of *Dentex dentex* and its body weight. Open circles: males. Closed circles: females. Triangles: not determined.

brain tissue of Common Dentex as a result of maturation-ageing physiological processes.

Glia cells play an important role in the physiology of the developing and adult brain: preserve the ionic composition of the extracellular space, maintain the blood-brain barrier, and in the developing brain, neurons migrate to their specific targets using radial glia projections (Arenander and De Vellis, 1989; Kimelberg and Noremberg, 1989; Raine, 1989). In addition, astrocytes can release various neuron growth factors (Englele and Bohn, 1991) and also provide an aid in synaptic transmission (Martin, 1992).

TABLE 1. – Relationship between animal weight and brain GFAP immunoreactivity during *Dentex dentex* development.

Developmental group	GFAP Immunoreactivity (IOD units)	Weight (g)
Juvenile immature	2.27	180
	6.05	187
	1.514	280
	0.97	360
Developing-mature	52.43	2329
	25.32	2542
	18.14	3260
	42.56	3260
	2.28	3910
	0.77	4170
	0.84	5500
	3.05	5550

In fish CNS (and other phylogenetically lower vertebrates), new neurons are continuously being added throughout life, nevertheless the rate of neurogenesis seems to decline as the fish ages (Easter *et al.*,1981). In this context astrocytes play an important role in the control of neuronal proliferation in brain cell cultures of goldfish (*Carassius auratus*) by (i) direct astrocyte-neurone contact and (ii) by secreting soluble factor(s) (Sivron *et al.* 1993)

Our results suggest that Common Dentex at an early stage of maturity (body weight: 2329-3260 g, > 6 years old) present a relatively abundant mass of astrocytes (high values of GFAP immunoreactivity) to sustain a high rate of neurogenesis compared with juvenile sexually immature or fully developed older specimens (Table 1). Older specimens may present relatively lower rates of neuronal proliferation and therefore low mass of astrocytes or astrocytic projections to sustain it.

On the other hand, there is a relationship between the increase of glia cell activity and sexual maturity, and that might be related to the differentiation and expression of the areas related to sexual hormone production. In this context, modulation of GFAP immunoreactivity has been detected in brain tissue during the oestrus cycle in female rats (García-Segura *et al.,* 1994). The regulation and onset of puberty

in fishes is related to the synthesis and accumulation of GTH (gonadotropin) in the pituitary (Billard, 1978).

In this regard, there is evidence that at least in mammals gonadotropin releasing hormone immunoreactive axons are associated with glial (GFAP immunoreactive) processes (Silverman *et al*., 1991). A possible relationship between sexual maturation and GFAP modulation in the brain of Common Dentex may exist. However, there is limited information available and a large number of questions remain regarding the role of glia and its association with the neurohormonal system in fishes. Our study shows a down-regulation of brain GFAP immunoreactivity in fishes during a maturation-ageing physiological process. We previously demonstrated similar results in a marine fish (*Sparus aurata* L.) reared in an aquaculture facility. Thus, the present work shows that the down-regulation of brain GFAP occurs in fishes in a natural environment which rules out developmental artifacts or genetic factors caused by rearing fishes in captivity. However the effect of aeging, with lower growth and metabolic rates, and of sexual development and maturity upon the fish brain and glia fish cells is poorly known. Thus, further studies considering both factors should be carried out in the future.

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