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[Short Communication]

Immunohistochemical Mapping of Serotonin-containing Neurons in the Brain of the Senegal Bichir, *Polypterus senegalus* (Brachiopterygii, Osteichthyes)

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ABSTRACT—The distribution of serotonin-immunoreactive (5HT-IR) neurons was studied in the brain of a primitive bony fish, *Polypterus senegalus*. The diencephalon contained a prominent 5HT-IR cell group (G2) consisting mainly of liquor-contacting neurons in the hypothalamic periventricular wall including the paraventricular organ. Their ependymofugal processes arborized profusely and gave rise to dense networks of varicose fibers in the hypothalamus. Other groups of 5HT-IR cells were situated in the preoptic area including the periventricular preoptic nucleus (G1) and in the dorsal thalamus (G3). In the brain stem, 5HT-IR cells were found in the medial part, mainly in the superior raphe nucleus (G4). No 5HT-IR cells were demonstrated in other regions of the brain. The cell groups referred to as G1 and G4 in the present study seem to be new components comprising the serotonergic systems in the *Polypterus* brain.

INTRODUCTION

The distribution of serotonin (5HT)-containing cells and fibers in the central nervous system (CNS) has been studied in a variety of vertebrates by means of fluorescence histochemical and immunohistochemical techniques (Baumgarten, 1972; Parent, 1981). In fishes, anatomical data on the serotonergic neuron system have been accumulated for some representative groups, e.g., cyclostomes (Baumgarten, 1972; Steinbush and Niewenhuys, 1979; Harris-Warrick *et al*., 1985; Brodin *et al*., 1988; Kadota, 1991), cartilaginous fishes (Richie *et al*., 1983, 1984; Stuesse and Cruce, 1991; Yamanaka *et al*., 1990), and teleosts (Kah and Chambolle, 1983; Ekström and van Veen, 1984; Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Nagatsu *et al*., 1984; Margolis-Kazan *et al*., 1985; Meek and Joosten, 1989). However, information is still limited or absent with respect to nonteleostean bony fishes, e.g., Holostei (Parent and Northcutt, 1982; Chiba and Oka, 1999), Brachiopterygii (Reiner and Northcutt, 1992; Chiba, 1997), and Dipnoi (Reiner and Northcutt, 1987). Parent (1981) pointed out that the central 5HT neuronal systems of vertebrates are well developed across the phylogenetic scale and display a strikingly constant feature in the terminal arborization of 5HT systems from one class to another, suggesting that the central serotonergic systems are phylogenetically ancient and subserve similar fundamental brain function in all vertebrates. Nevertheless, detailed mapping data on the central serotonergic systems are still scanty for primitive bony fishes of great phylogenetic significance. This is true in the Brachiopterygii, i.e., the bichir and the reedfish.

The present study was conducted to provide overall mapping data of serotonergic neurons in the brain of the Senegal bichir, *Polypterus senegalus*, by mean of light microscopic immunohistochemistry, thus contributing to the comparative neuroanatomy of the vertebrate CNS.

MATERIALS AND METHODS

Six individuals of either sex of *P. senegalus*, 25–38 cm in total length and purchased from a local dealer, were used in the present study. The fish were anesthetized with 0.2% *m*-aminobenzoate-methanesulfonate and perfused through the heart initially with teleost saline and subsequently with acid-free Bouin's fluid. Paraffin sections were cut serially 8 or 15 μm thick in transverse and sagittal planes, and stained immunohistochemically by use of a commercial kit (Nichirei, Tokyo, Japan) employing the streptavidin-biotin method. A polyclonal antibody (anti-5HT antibody, No. 1234, donated by Dr. Yui, Zenyaku Kohgyo Co. Ltd., Tokyo, Japan; Yui *et al*., 1990) was used as the primary antibody, at a dilution of 1:8000. The peroxidase complex was visualized by incubation with diaminobenzidine and mounted in Canada balsam. Some sections were counterstained with Mayer's hematoxylin. Controls were prepared by replacement of the primary antiserum with (1) normal rabbit serum and (2) antiserum preabsorbed for 48 hr at 4°C with 5HT hydrochloride (Research Biochemicals Inc., Natick, USA) conjugated with bovine serum albumin at 1 and 10 μM concentrations. Immunostaining was negative in all sections treated by either of the control procedures. The nomenclature used for desig-

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nating cell masses or other structures in the present study was adopted from Northcutt (1981) and Nieuwenhuys (1983).

RESULTS AND DISCUSSION

The distribution of 5HT-IR cells and fibers in the brain of the Senegal bichir is illustrated in Figs. 1–3. As shown in the diagram (Fig. 1), 5HT-IR cell groups tentatively referred to as G1–G4 were found in the telencephalon (G1), in the diencephalon (G2 and G3), and in the upper brain stem (G4). No 5HT-IR cells could be seen in other brain regions. The most prominent cell group, G2, occupied a relatively large area of the hypothalamic periventricular wall including the paraventricular organ, extending caudally from the dorsal hypothalamus cranially to the infundibular recess (Fig. 1B–G), and was composed mainly of liquor-contacting neurons (Fig. 2B, C). In the preoptic area, a small 5HT-IR cell group, G1, was present (Figs. 1A, 2A). This cell group contained small bipolar or multipolar neurons scattered in the area including the periventricular preoptic nucleus. None of the cells projected their dendritic processes into the ventricular cavity. The G3 group appeared as a small cell mass in the dorsal thalamic nucleus, being a little apart from the ependymal layer and lying ventrolaterally to the fasciculus retroflexus (Figs. 1D, 2D). The cells of this group displayed a relatively weak immunoreaction for 5HT and their processes were indistinct. The last group, G4, was situated in the medial region of the upper brain stem, mainly in the superior raphe nucleus. It extended laterally at the level of the interpeduncular nucleus (Figs. 1H–I, 2E). The cells of this group were less intensely immunostained, and their processes were difficult to trace. The distribution of the immunoreactive fibers in selected areas of the brain is also outlined in Fig. 1. As shown there, 5HT-IR fibers were considerably dense in the hypothalamus (Fig. 1B–G), where ependymofugal processes of the liquor-contacting neurons were oriented laterally and ventrolaterally (Fig. 1B), arborized profusely, and gave rise to dense networks in the hypothalamus. Here, thick bundles of fine 5HT-IR fibers were demonstrated, although their destination was undetermined (Fig. 1B, C). In the hypothalamo-hypophysial complex, immunostained fibers were sporadically seen apposed to the capillary plexus of the median eminence (Fig. 3), but no 5HT-IR structures were demonstrated in the pars distalis or pars nervosa.

The present immunohistochemical results on the bichir brain confirmed the previous findings on the same species (Reiner and Northcutt, 1992) and expanded our knowledge about the central serotonergic neuronal systems. The newly recognized loci for the 5HT-IR cells in the bichir brain are the preoptic area (G1) and the upper brain stem, i.e., the superior raphe nucleus (G4). The previous study on the bichir telencephalon (Reiner and Northcutt, 1992) did not show the cells or cell cluster corresponding to the G1 in the present study. This discrepancy may be ascribed to differences in the sensitivity of the antibodies used or in the quantity of the tissue antigen probably due to the different physiological conditions of the fish, although the exact causal factor(s) were not determined in the present study. Such an example of discrepancy has been noted in the literatures (Grant *et al*., 1989; Meek and Joosten, 1989) with respect to the 5HT-IR neurons in the preoptic area of the mormyrid brain. On the other hand, the newly found G4 has not been missed in the previous study (Reiner and Northcutt, 1992), for it is just that Reiner and Northcutt (1992) did not study the extratelencephalic areas. The occurrence of 5HT-IR cells in the preoptic area is not surprising; a corresponding cell mass was reported in the stingray (Richie *et al*., 1983), although it is apparently absent in the dogfishes (Yamanaka *et al*., 1990; Stuesse *et al*., 1991) and the ratfish (Stuesse and Cruce, 1991). With respect to the actinopterygians, no such cell mass was observed in teleosts (Kah and Chambolle, 1983; Ekström and van Veen, 1984; Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Nagatsu *et al*., 1984; Margolis-Kazan *et al*., 1985) or lepisosteids (Parent and Northcutt, 1982; Chiba and Oka, 1999). However, there is a discrepancy about this subject in the mormyrid fish, *Gnathonemus petersii*, the weak-electric teleost (Grant *et al*., 1989; Meek and Joosten, 1989). The occurrence of the cell masses corresponding to the G2 and G3 in the bichir brain is consistent among different actinopterygian groups, i.e., the teleosts (Kah and Chambolle, 1983; Ekström and van Veen, 1984; Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Nagatsu *et al*., 1984; Margolis-Kazan *et al*., 1985; Meek and Joosten, 1989), the lepisosteids (Chiba and Oka, 1999), and the brachiopterygians (the present study), although there are minor differences or discrepancies among the species concerning the terminology and the anatomical locations of these cell masses. In addition, the prominent cell group corresponding to the G2, i.e., 5HT-IR liquor-contacting neurons in the hypothalamic periventricular wall, occurs constantly in the brains of cyclostomes (Baumgarten, 1972; Steinbush and Niewenhuys, 1979; Kadota, 1991) and cartilaginous fishes (Richie *et al*., 1983; Yamanaka *et al*., 1990; Stuesse and Cruce, 1991; Stuesse *et al*., 1991). It is possible to point out that fish brains contain well-developed 5HT systems in the forebrain, mainly in the thalamo-hypothalamic area, in addition to conservative raphe 5HT system (Parent, 1981).

The bichir and reedfish with some unique and primitive chraracters are grouped in the Brachiopterygii, being ranked as a sister group of the Actinopterygii. With respect to their central nervous system, some interesting features were reported, e.g., the telencephalon shows a marked differentiation with strong eversion (Nieuwenhuys, 1982); the extraordinary distribution of urotensin I, a caudal neurosecretory peptide, in the brain stem and the spinal cord (Oka *et al*., 1995). Nevertheless, the present study has shown that the 5HT neuron systems in the bichir brain represent a general or basic pattern common to those of other actinopterygian groups. This fact may support the previous view (Parent, 1981) that the central 5HT systems are phylogenetically ancient. However, it is obvious that further study is necessary to elucidate the evolutionary aspect and functional significance of the central 5HT systems in the brain of vertebrates.

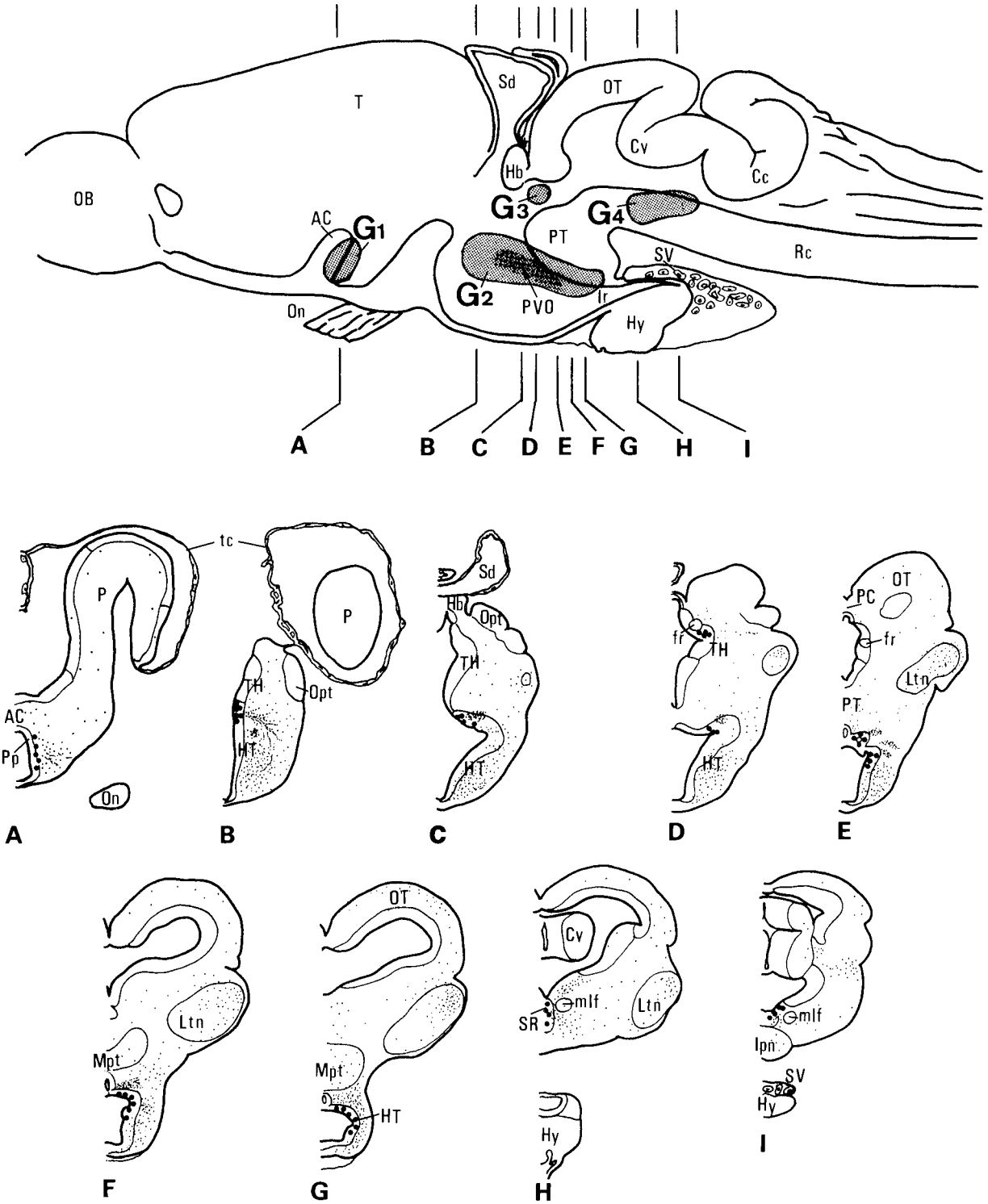

Fig. 1. Diagrammatic illustration of 5HT-IR structures in the brain of the Senegal bichir, *Polypterus senegalus*. Top figure shows the location of 5HT-IR cell groups, G1–G4, in the median section and the levels of transverse sections indicated by the lettered vertical lines. (A)–(I) show 5HT-IR cells (dots) and fibers (stippling) in different levels of a right-half brain. AC = anterior commissure; Cc = cerebellar corpus; Cv = cerebellar valve; fr = fasciculus retroflexus; Hb = habenula; HT = hypothalamus; Hy = hypophysis; Ipn = interpeduncular nucleus; Ir = infundibular recess; Ltn = lateral toral nucleus; mlf = medial longitudinal fasciculus; Mpt = median nucleus of the posterior tubercle; OB = olfactory bulb; On = optic nerve; Opt = optic tract; OT = optic tectum; P = pallium; PC = posterior commissure; Pp =periventricular preoptic nucleus; PT = posterior tubercle; PVO = paraventricular organ; Rc = rhombencephalon; Sd = saccus dorsalis; SR = superior raphe nucleus; SV = saccus vasculosus; T = telencephalon; tc = tela choroidea; TH = thalamus.

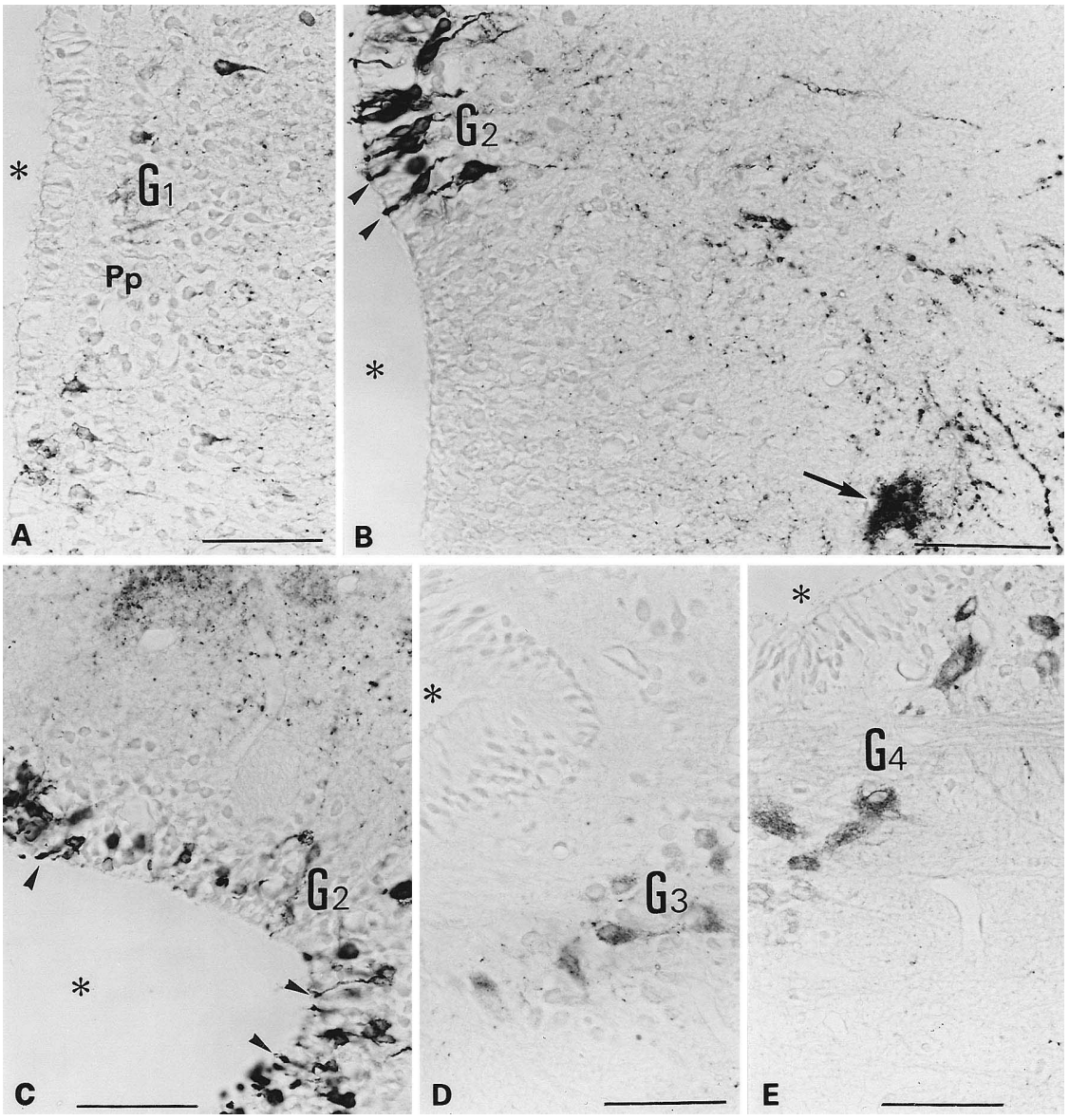

Fig. 2. 5HT-IR structures in various parts of the bichir brain: (A), 5HT-IR cell mass (G1) in the preoptic area; (B) and (C), 5HT-IR cell mass (G2) and varicose fibers in the hypothalamic periventricular wall; (D), 5HT-IR cell mass (G3) in the dorsal thalamus; (E), 5HT-IR cell mass (G4) in the raphe region. Arrow, a thick bundle of 5HT-IR fibers; arrowheads, intraventricular processes of the 5HT-IR liquor-contacting neurons; asterisks, the brain ventricle; Pp, the periventricular preoptic nucleus. Scale bar = 50 μm.

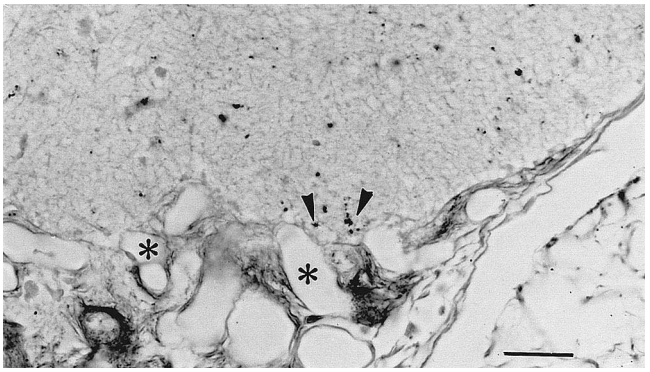

Fig. 3. 5HT-IR varicose fibers (arrowheads) closely apposed to the capillary plexus of the portal system (asterisks) in the median eminence. Scale bar = 10 μm.

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