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NEUROGENESIS, CELL DEATH AND REGENERATION IN THE ADULT GYMNOTIFORM BRAIN

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Summary

Gymnotiform fish, like all teleosts examined thus far, are distinguished by their enormous potential for the production of new neurons in the adult brain. In *Apteronotus leptorhynchus*, on average 10^5 cells, corresponding to approximately 0.2 % of the total population of cells in the adult brain, are in S-phase within any period of 2 h. At least a portion of these newly generated cells survive for the rest of the fish's life. This long-term survival, together with the persistent generation of new cells, leads to a continuous growth of the brain during adulthood.

Zones of high proliferative activity are typically located at or near the surface of the ventricular, paraventricular and cisternal systems. In the central posterior/prepacemaker nucleus, for example, new cells are generated, at very high rates, in areas near the wall of the third ventricle. At least some of these cells differentiate into neurons, express immunoreactivity against the neuropeptide somatostatin and migrate into more lateral areas of this complex.

Approximately 75 % of all new brain cells are generated in the cerebellum. In the corpus cerebelli and the valvula cerebelli, they are produced in the molecular layers, whereas in the eminentia granularis the newborn cells stem from proliferation zones in the pars medialis. Within the first few days of their life, these cells migrate towards specific target areas, namely the associated granule cell layers. At least some of them develop into granule neurons.

The high proliferative activity is counterbalanced by apoptosis, a mechanism that resembles the processes known from embryonic development of the vertebrate brain. Apoptosis also appears to be used as an efficient mechanism for the removal of cells damaged through injury in the brain of adult *Apteronotus leptorhynchus*. Since apoptosis is not accompanied by the side effects known from necrosis, this 'clean' type of cell death may, together with the enormous proliferative activity in the brain, explain, at least partially, the tremendous capability of teleost fish to replace damaged neurons with newly generated ones.

One factor that appears to play a major role in the generation of new cells and in their further development is the neuropeptide somatostatin. In the caudal cerebellum of the gymnotiform brain, somatostatin-binding sites are expressed, at extremely high densities, at sites corresponding to the areas of origin, migration and differentiation of the newborn cells. This pattern of expression resembles the expression pattern in the rat cerebellum, where somatostatin immunoreactivity and somatostatin-binding sites are transiently expressed at the time when the granule cells of the cerebellum are generated. Moreover, after mechanical lesions of the corpus cerebelli, the expression of somatostatin-like immunoreactivity is tremendously increased in several cell types (presumably astrocytes, microglia and granule cell neurons) near the path of the lesion; the time course of this expression coincides with the temporal pattern underlying the recruitment of new cells incorporated at the site of the lesion.

Key words: salvage pathway, pyrimidine synthesis, cell proliferation, postembryonic neurogenesis, apoptosis, neuronal regeneration, somatostatin, cerebellum, central posterior/prepacemaker nucleus, Gymnotiformes, *Apteronotus albifrons*, *Apteronotus leptorhynchus*, *Eigenmannia* sp.

Introduction

In mammals, several types of tissue of peripheral organs retain their ability to generate new cells from precursors during adult life, whereas the brain is severely limited in this respect. In all mammalian species examined thus far, postnatal neurogenesis is either completely absent (Rakic, 1985) or this phenomenon has been observed in only a very few brain regions, with the number of newborn cells being extremely low (Altman, 1962, 1963, 1969a,b; Altman and Das, 1965; Mareš and Lodin, 1974; Kaplan and Hinds, 1977; Kaplan, 1981; Bayer et al., 1982; Kaplan and Bell, 1983; Corotto et al., 1993;

Lois and Alvarez-Buylla, 1993, 1994; Gould et al., 1998). As a consequence, replacement of neurons lost as a result of neurodegenerative diseases or injuries is usually impossible in the adult mammalian brain.

These limitations contrast with the enormous potential of teleost fish for neurogenesis and gliogenesis during adulthood. Earlier studies in these vertebrates had indicated that the capability to produce new neurons and glial cells, as well as the ability to regenerate neural tissue after injuries, is very pronounced and, in at least a few brain regions, persists over long periods of life (Rahmann, 1968; Richter and Kranz, 1970a,b; Kranz and Richter, 1970a,b; Johns, 1977; Meyer, 1978). This proliferative capacity parallels the enormous ability of fish to regenerate axons and even whole neurons in the central nervous system after injuries or experimentally induced lesions (Kirsche, 1950; Botsch, 1960; Kirsche and Kirsche, 1961; Pflugfelder, 1965; Bernstein, 1968; Meyer et al., 1985; Waxman and Anderson, 1986; Stuermer et al., 1992).

While the earlier investigations had succeeded in establishing evidence for the continuous production of brain cells beyond the embryonic stages of development, the elucidation of the details of this phenomenon, including the further fate of these cells and the mechanisms underlying this phenomenon, had to await the advent of a suitable model system and of appropriate techniques. At the beginning of the 1990s, such a model system became available when Maler et al. (1991) published their stereotactic atlas of the gymnotiform brain. This atlas, still unique in terms of both its accuracy and nuclear resolution, enabled researchers, for the first time, to localize exactly and to map zones of proliferative activity in the adult teleost brain. Moreover, the topographical data provided by the brain atlas were greatly supplemented by the wealth of information accumulated over the past 30 years on the neuroanatomy and neurophysiology of many of the brain regions involved in the production and sensory processing of electric signals in gymnotiform fish (see Bullock and Heiligenberg, 1986). My group, therefore, started, in collaboration with several other laboratories, to examine postembryonic neurogenesis in the brain of the two gymnotiform fish *Eigenmannia* sp. and *Apteronotus leptorhynchus*. The major results of these findings will be summarized and discussed in this review.

Divergence of substrate specificity in the salvage pathway of pyrimidine synthesis

Studies on cell proliferation in the brain of adult gymnotiform fish have, in particular, been advanced by the exploitation of the salvage pathway of pyrimidine synthesis. In this biochemical pathway, thymidine is, in the presence of ATP and a divalent cation, phosphorylated by thymidine kinase to thymidine monophosphate. In a subsequent reaction catalyzed by the enzyme thymidilate kinase, thymidine monophosphate is phosphorylated to its triphosphate analogue, thymidine 5′-triphosphate, which is available for synthesis of DNA. In our studies on gymnotiform fish, we have administered tritiated thymidine, or its analogue 5-bromo-2′-deoxyuridine (BrdU), intraperitoneally. Mitotically active cells in S-phase have been detected by [^{3}H]thymidine autoradiography, biochemical assays or immunohistochemical techniques using antibodies directed against BrdU (for methodological details, see Zupanc and Zupanc, 1992; Zupanc and Horschke, 1995, 1996).

Since all vertebrates tested thus far are able to utilize [^{3}H]thymidine for biosynthesis of nucleotides *via* the salvage pathway in the brain, it was especially interesting to find a divergence in substrate specificity in *Apteronotus leptorhynchus* and *Eigenmannia* sp. While brain cells of both species are able to use BrdU for pyrimidine synthesis, only *Eigenmannia* sp. can incorporate [^{3}H]thymidine into DNA during the S-phase of the cell cycle (Zupanc and Horschke, 1996). This inability of *Apteronotus leptorhynchus* to utilize thymidine for nucleotide synthesis is likely to be caused either by a defect in the transport system mediating the uptake of thymidine or by a deficiency in the thymidine kinase. Similar deficiencies have been reported for several mutant cell lines and strains of bacteria (Adair et al., 1980; Nairn et al., 1982; Hanson and Ullman, 1989).

Cell proliferation in the adult brain

BrdU, which can be utilized for incorporation into replicating DNA by both *Eigenmannia* sp. and *Apteronotus leptorhynchus*, is metabolically available to label mitotically active brain cells for approximately 4 h after a single intraperitoneal injection (Zupanc and Horschke, 1995). This determination of the clearance time of BrdU was an essential prerequisite to enable us to analyze cell proliferation quantitatively in the brain of gymnotiform fish.

On the basis of this information, survival times of 2 h were chosen for mapping of the proliferation zones. Consequently, BrdU was metabolically available for incorporation into newly synthesized DNA during the entire survival period. Quantitative analysis employing 2 h of post-injection survival showed that, on average, 10^5 cells are generated in the brain of adult individuals of *Apteronotus leptorhynchus* within this period (Zupanc and Horschke, 1995). Since using cell suspensions, the total number of cells in the brain has been estimated to be of the order of 5×10^7, and the number of cells produced within any 2 h corresponds to approximately 0.2 % of the total population of cells in the brain of adult *Apteronotus leptorhynchus*. Such a proliferative activity points to a tremendous degree of structural change taking place in the brain of this fish.

In the telencephalon, diencephalon, mesencephalon and rhombencephalon, the total number of these cells is low, making up approximately 25 % of all mitotically active cells in the brain. Many of these cells are scattered over wide areas. Otherwise, zones of high proliferative activity are typically located at or near the surface of the ventricular, paraventricular and cisternal systems (Zupanc and Horschke, 1995).

The central posterior/prepacemaker nucleus is of special interest to the neuroethologist because part of it acts as an

important motor centre controlling modulations of the electric organ discharges in gymnotiform fish (for a review, see Zupanc and Maler, 1997). The vast majority of cell divisions take place in a narrow zone immediately adjacent to the wall of the third ventricle (Zupanc and Zupanc, 1992; Zupanc and Horschke, 1995; Stroh and Zupanc, 1996). On average, approximately 100 cells undergo mitosis in this cell group within any period of 2 h. As the number of cells comprising this nucleus has been estimated to total approximately 10^4 cells (Stroh and Zupanc, 1996), approximately 1 % of the cell population in the central posterior/prepacemaker nucleus enters the S-phase of mitosis within any 2 h period. Thus, this percentage is significantly higher than the average percentage (0.2 %) of mitotic cells in the brain. It has been hypothesized that this enormous mitotic activity forms the structural basis for a possible 'refreshment' of information stored in the underlying neural network (Zupanc and Maler, 1997).

Approximately 75 % of all cells in the gymnotiform brain labelled by BrdU after a post-injection survival time of 2 h are situated in the three subdivisions of the cerebellum, namely the corpus cerebelli, the valvula cerebelli (with its medial and lateral parts) and the eminentia granularis (with its medial and posterior parts) (Zupanc and Horschke, 1995). Zones displaying proliferative activity are restricted to small areas, such as narrow strips around the midline in the molecular layer of the corpus cerebelli and valvula cerebelli, around the boundary between the corpus and valvula, and in a large portion of the area covered by the eminentia granularis pars medialis.

It has been hypothesized that the proliferation zones in the molecular layer at the midline of the corpus cerebelli and valvula cerebelli pars medialis, where most of the cells of these two cerebellar subdivisions are born during adulthood, represent remnants of embryonic proliferation zones (Zupanc et al., 1996). This is in agreement with studies on the embryonic development of the cerebellum in the trout (Pouwels, 1978a). Stem cells (termed secondary matrix cells) at embryonic proliferation zones are situated at ventricular surfaces. Later, when the two halves of the cerebellum fuse in the median plane by a medially directed outgrowth, the ventricular cavity in this region becomes obliterated. The persistence of mitotic activity of secondary matrix cells during adult life has been suggested by indirect evidence obtained in the trout (Pouwels, 1978a,b). In contrast, the corresponding germinal matrix in mammals, the so-called external granule cell layer, is expressed only during embryogenesis and a short period of postnatal development (Fujita et al., 1966; Fujita, 1967; Altman, 1972).

Development of the cells born postembryonically

The employment of various survival times after the administration of [^{3}H]thymidine or BrdU revealed the further development of cells born postembryonically in the central posterior/prepacemaker nucleus (Zupanc and Zupanc, 1992; Stroh and Zupanc, 1996). In this complex, the new cells migrate laterally within a few days after their generation, where at least some of them adopt morphological characteristics typical of neurons (Zupanc and Zupanc, 1992). A minor fraction (approximately 5 %) of the BrdU-labelled cells start in the period between 2 days and 3.5 days after birth to adopt immunoreactivity against the neuropeptide somatostatin (Stroh and Zupanc, 1996). This percentage matches well the fraction of somatostatin-positive cells in the entire complex (Sas and Maler, 1991; Zupanc et al., 1991a,b; Stroh and Zupanc, 1995, 1996; Zupanc et al., 1997).

Techniques similar to those used in the central posterior/prepacemaker nucleus revealed details of the postembryonic development in the cerebellum (Zupanc et al., 1996; Ott et al., 1997). In the corpus cerebelli and the valvula cerebelli, the cells generated in the molecular layers migrate within the first few days of their life towards specific target areas, namely, the respective granule cell layers (Fig. 1). In the caudal part of the cerebellum, the cells migrate through the adjacent molecular layer to the granule cell layer of the eminentia granularis pars posterior (Fig. 2). In the latter granule cell layer of *Apteronotus leptorhynchus*, N-type Ca^{2+} channels (as detected through binding of ω-conotoxin GVIA) are expressed particularly on the plasmalemmal surface of the somata of granule cells (Tharani et al., 1996). This expression pattern resembles that observed in the developing mouse cerebellum (Komuro and Rakic, 1992). In the mouse cerebellum, selective blockade of these Ca^{2+} channels by addition of ω-conotoxin curtails cell movement, suggesting that these channels play a role in the directed migration of newborn neurons. A similar function may be exerted in the adult cerebellum of gymnotiform fish during postembryonic brain development. Interestingly, the distribution of nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase in Purkinje and granule cells of the eminentia granularis pars posterior is also similar to that in the developing, but not the adult, mammalian cerebellum (Turner and Moroz, 1995). Taken together, these result suggest that the gymnotiform cerebellum during adulthood most closely resembles the mammalian cerebellum during its development shortly before and after birth.

A combination of retrograde *in vitro* tracing and immunohistochemistry for BrdU techniques (for methodological details, see Zupanc, 1998) was used to establish the identity of the cells born in the cerebellum of *Apteronotus leptorhynchus*. These experiments demonstrated that at least a portion of the newly generated cells develop into granule neurons (Zupanc et al., 1996). This is further supported by cell culture studies showing that these cells have morphological and immunohistochemical characteristics that are consistent with those of granule cells (Kotecha et al., 1997).

Survival times of up to 440 days demonstrated that, after a pronounced reduction of their number within the first few weeks following arrival at their target areas, many of the newborn cells remain alive for extremely long periods and, most likely, for the rest of the fish's life (Zupanc et al., 1996; Ott et al., 1997) (Fig. 3). This long-term survival, together with the continuous production of new cells, leads to a permanent

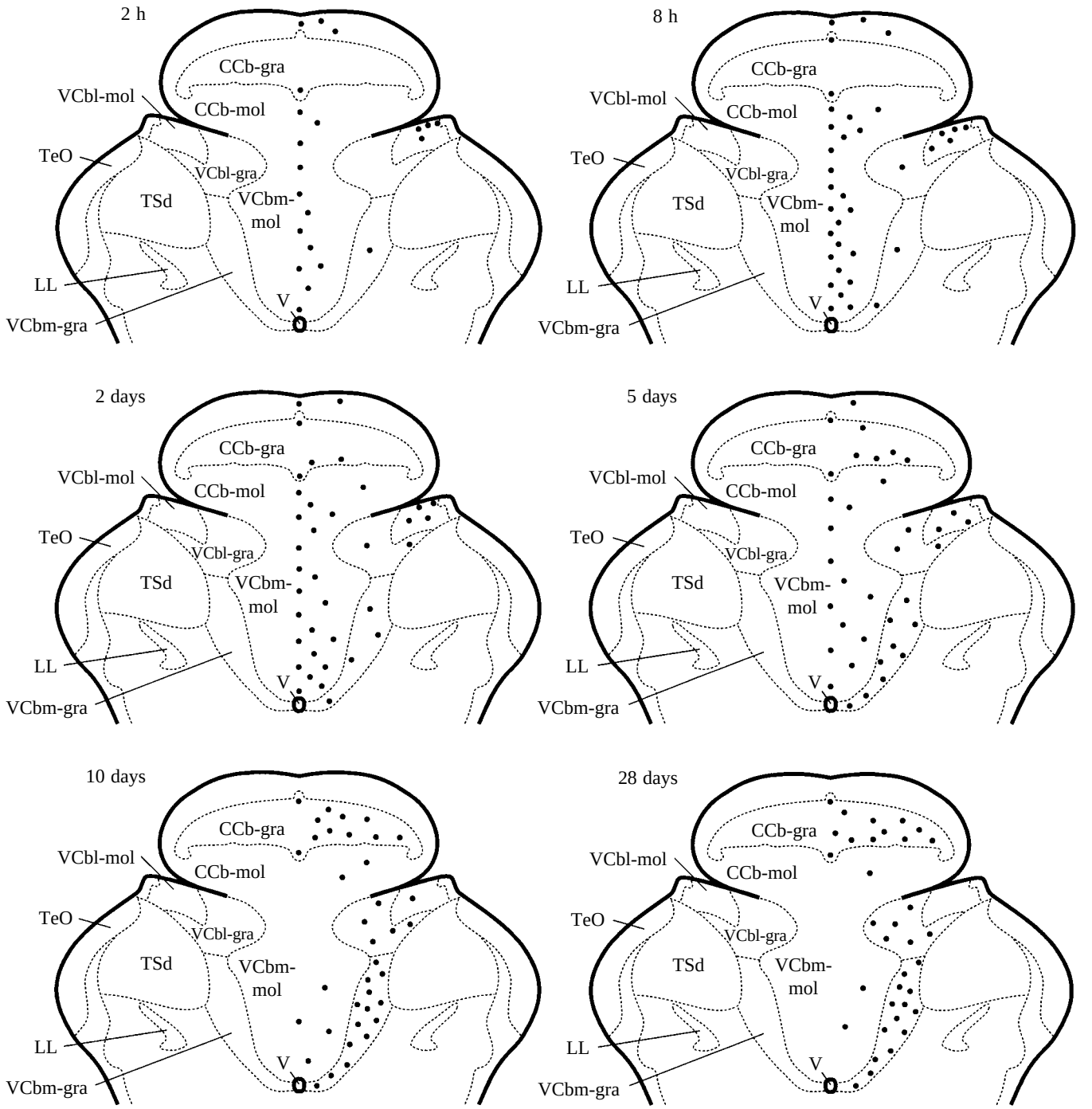

Fig. 1. Schematic representation of the distribution of 5-bromo-2′-deoxyuridine (BrdU)-labelled cells in the corpus cerebelli and valvula cerebelli of *Apteronotus leptorhynchus* after various post-injection survival times. The location of labelled cells is indicated by black dots. The number of dots represents approximately the density of labelled cells. CCb-gra, granule cell layer of corpus cerebelli; CCb-mol, molecular layer of corpus cerebelli; LL, lateral lemniscus; TeO, optic tectum; TSd, dorsal subdivision of torus semicircularis; V, ventricle; VCbl-gra, granule cell layer of valvula cerebelli pars lateralis; VCbl-mol, molecular layer of valvula cerebelli pars lateralis; VCbm-gra, granule cell layer of valvula cerebelli pars medialis; VCbm-mol, molecular layer of valvula cerebelli pars medialis.

growth of the entire brain. While the body mass of the fish increases from 1 g to 16 g, the total number of brain cells approximately doubles from 5×10^7 to 10^8 (Zupanc and Horschke, 1995).

As expected, concomitant with the overall growth, an increase in the volumes of the target areas within the cerebellum is observed. Somewhat unexpected, however, was the finding that the cerebellar molecular layers not only

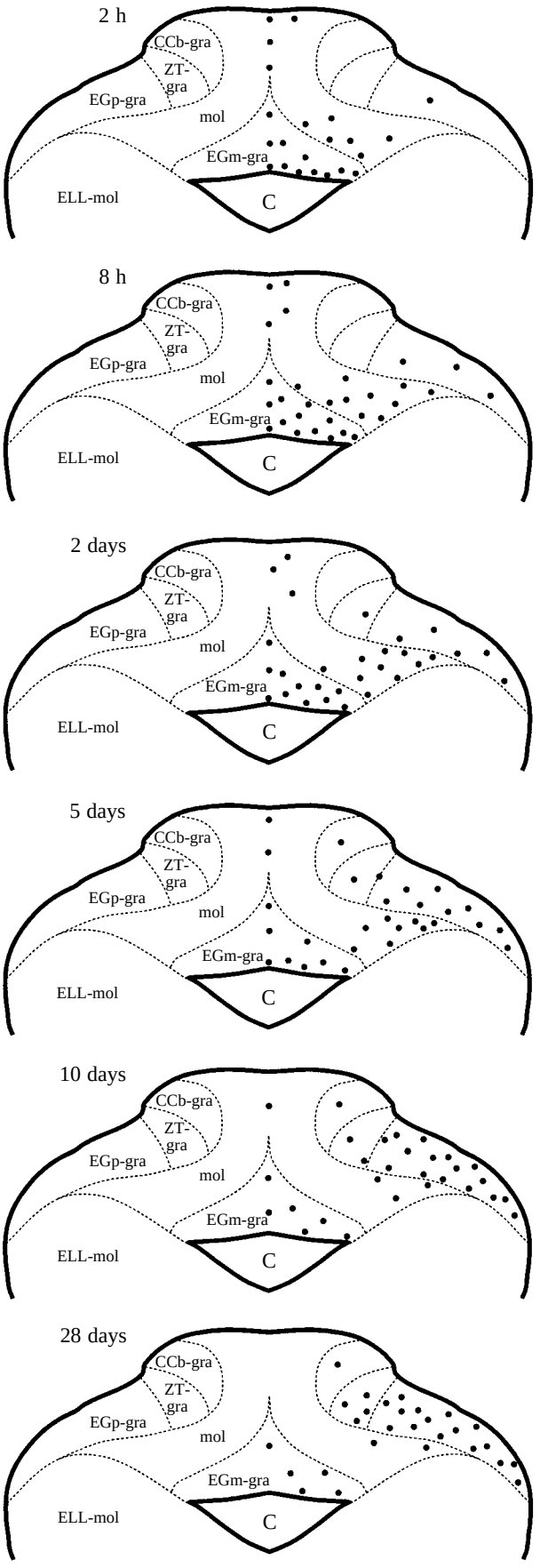

Fig. 2. Schematic representation of the distribution of 5-bromo-2′-deoxyuridine (BrdU)-labelled cells in the eminentia granularis after various post-injection survival times. The location of labelled cells is indicated by black dots. C, cerebello-medullary cistern; CCb-gra, granule cell layer of corpus cerebelli; EGm-gra, granule cell layer of eminentia granularis pars medialis; EGp-gra, granule cell layer of eminentia granularis pars posterior; ELL-mol, molecular layer of electrosensory lateral line lobe; mol, molecular layer of corpus cerebelli, eminentia granularis pars anterior, eminentia granularis pars medialis and eminentia granularis pars posterior; ZT-gra, granule cell layer of the transitional zone.

increase in size, but also show a positive allometric growth relative to the granule cell layers (Zupanc et al., 1996), even though the target areas to which the newborn cells migrate are the granule cell layers and not the molecular layers. The molecular layers consist predominantly of parallel fibres formed by the axons of the granule cells, the dendrites of the Purkinje cells and of the eurodendroid neurons, and the cell bodies and dendrites of stellate cells (for a review, see Finger, 1983). Since only a negligible number of newborn cells appear to stay in the molecular layer, these results imply drastic changes occurring in the structure of the elements of the molecular layers as well.

The role of cell death in postembryonic development

Until recently, cell death had been thought to be absent from the brain of fish in stages beyond embryogenesis (Fox and Richardson, 1982; Crapon de Caprona and Fritzsch, 1983; Galeo et al., 1987; Fine, 1989; Waxman and Anderson, 1985). This assumption was based on the failure of these studies to detect pyknotic nuclei or on the absence of a period of declining cell number in neural systems of various species of fish examined. However, since new cells are born continuously, a continuous reduction of a specific fraction of these cells would not be apparent by performing cell counts over time in their target areas. Moreover, such an interpretation is in conflict with the apparent fate of the newborn cells after they have reached their target areas within the cerebellum of *Apteronotus leptorhynchus* (Fig. 3). The reduction in their areal density in the period 4–7 weeks after their generation clearly suggests that they undergo cell death (Zupanc et al., 1996) since, at these stages of their development, there is no evidence that these cells migrate out of the cerebellum.

This hypothesis could, indeed, be confirmed using more direct approaches (Soutschek and Zupanc, 1995, 1996). Such methods included the identification of DNA fragmentation (a feature characteristic of programmed cell death, also called apoptosis) through terminal deoxynucleotidyl-transferase-mediated dUTP-biotin nick end-labelling (TUNEL) of 3′-OH ends of DNA (Modak and Bollum, 1972; Gavrieli et al., 1992; Surh and Sprent, 1994) and morphological characterization of the TUNEL-positive cells by fluorescence scanning confocal microscopy.

Studies using these approaches have shown that in many brain areas, including the prepacemaker nucleus (Soutschek

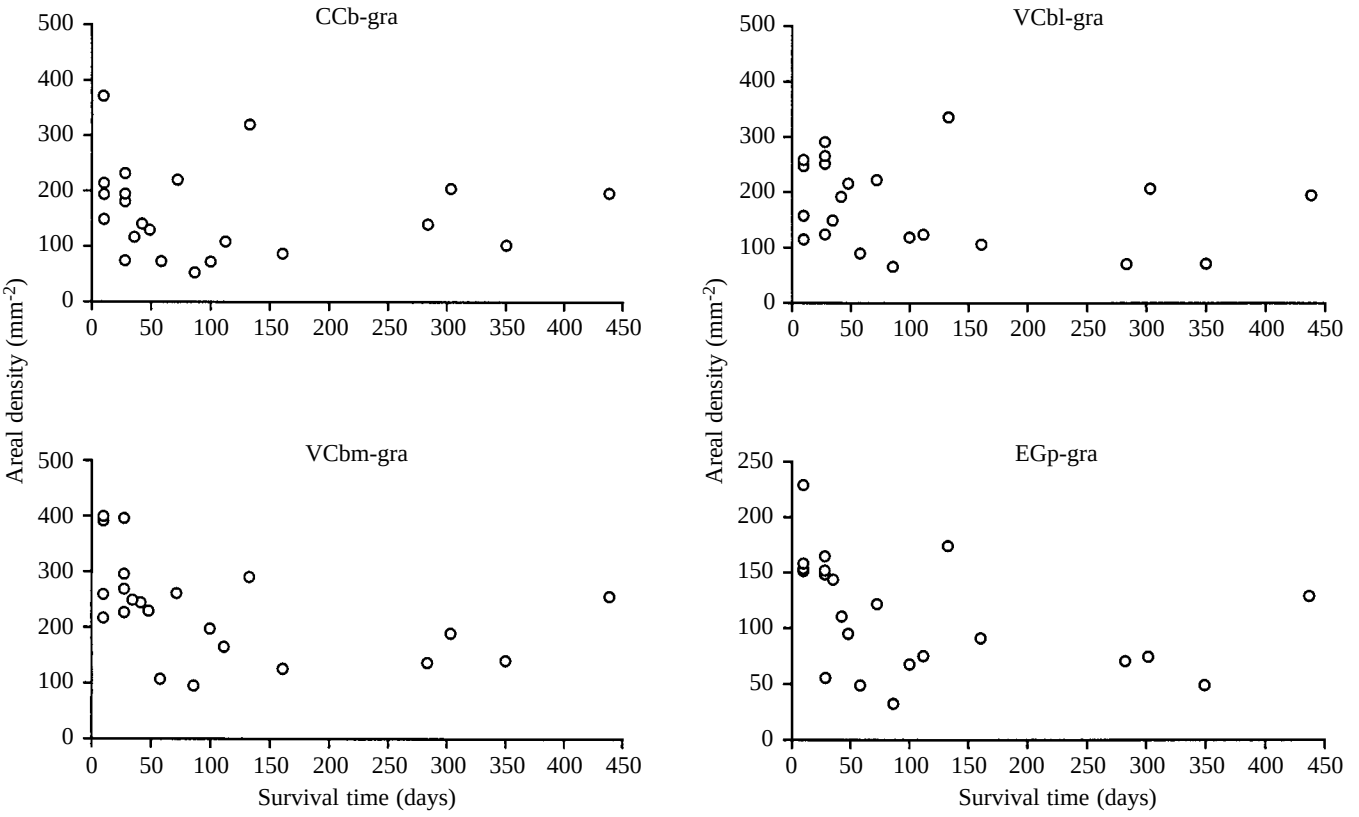

Fig. 3. Areal density of 5-bromo-2′-deoxyuridine (BrdU)-labelled cells as a function of post-injection survival time in the granule cell layers of four subdivisions of the cerebellum of *Apteronotus leptorhynchus*. CCb-gra, granule cell layer of corpus cerebelli; VCbl-gra, granule cell layer of valvula cerebelli pars lateralis; VCbm-gra, granule cell layer of valvula cerebelli pars medialis; EGp-gra, granule cell layer of eminentia granularis pars posterior. These four granule layers are the main target regions into which cerebellar cells generated postembryonically migrate. Note the pronounced decrease in areal density of the newborn cells approximately 4–7 weeks after their birth. This decrease indicates the occurrence of massive cell death at this stage of development. After this decrease, the normalized cell number stays rather constant over the period of the study.

and Zupanc, 1995) and the cerebellum (Soutschek and Zupanc, 1996), a large number of cells undergo apoptosis. The density of apoptotic cells is significantly higher in the granule cell layers of the various cerebellar subdivisions than in the corresponding molecular layers, implying that cell death may mainly be involved in the regulation of the cell number of the newborn cells after they have reached their target areas.

Interestingly, in the granule cell layers of two cerebellar subdivisions, the corpus cerebelli and the valvula cerebelli pars medialis, the areal density of apoptotic cells displays a significant negative correlation with body mass (Soutschek and Zupanc, 1996). Since body mass and age are highly positively correlated in *Apteronotus leptorhynchus*, this result demonstrates an age-dependent reduction in apoptotic events. This appears to be in sharp contrast to the situation in mammals. An age-related reduction in cell number has been found in various neural systems of rats and primates (Terry et al., 1987; Fischer et al., 1989; Han et al., 1989; Stroessner-Johnson et al., 1992; Swaab et al., 1993; Smith and Booze, 1995; Zhang et al., 1995b), which suggests an increase in the number of cell death events occurring. Furthermore, the relative number of striatal cells undergoing apoptosis is greater in older rats than in younger individuals, as shown by the TUNEL method (Zhang et al., 1995a), possibly reflecting an increase in the degree of ‘senility’.

Apoptosis in the brain of *Apteronotus leptorhynchus*, in contrast, appears to represent a rather different mechanism. One of its functions may be to eliminate new neurons that, after arrival at the target site, have failed to make proper connections with other neurons and to receive adequate amounts of specific survival factors produced by the cells in this target area. A similar function has been suggested for apoptosis during embryonic development (Raff, 1992; Raff et al., 1993).

Neuronal regeneration

The ability of gymnotiforms, and probably all fishes, to generate new cells in the brain during adulthood is paralleled by their enormous potential to regenerate axonal processes (axonal regeneration) and even to replace whole neurons (neuronal regeneration) after injuries. Stab-wound lesions applied to the corpus cerebelli heal very fast and, presumably, also completely. This is indicated by histological observations showing that the path caused by the stab wound becomes gradually reduced over

the period following the lesion, until, several weeks after the injury, it finally disappears. Signs of inflammation, cavitation and scarring do not occur (G. K. H. Zupanc, S. O'Kane and M. W. J. Ferguson, unpublished observations).

The path of the lesion is filled with newly generated cells, which are recruited from specific proliferation zones. The production of these cells is significantly increased in the area at and near the site of injury during the first few days following the lesion (G. K. H. Zupanc and R. Ott, unpublished observations). In addition, and this was a rather unexpected result, some cells that are recruited for this regenerative process are born prior to the application of the lesion. This finding is of particular interest, since it points to one possible function of the continuous generation of new cells in the adult brain: the possibility quickly to recruit cells from a pool of undifferentiated cells to replace cells damaged in case of an injury. At least some of the newly generated cells differentiate into granule cell neurons, as has been demonstrated by combining retrograde tracing techniques with labelling of mitotically active cells with BrdU (G. K. H. Zupanc and R. Ott, unpublished observations).

Other mechanisms essential for neuronal regeneration involve the elimination and removal of the damaged cells. In this process, apoptosis appears to play a major role (Zupanc et al., 1998). Following the application of a stab wound to the corpus cerebelli, the first TUNEL-positive cells at the site of the lesion appear as rapidly as 5 min after the insult. Thirty minutes after the lesion, the number of cells undergoing apoptosis reaches maximum levels. At 2 days of post-lesioning survival time, the number of TUNEL-positive cells starts to decline gradually, until this process reaches background levels 20 days after the lesion.

These findings in *Apteronotus leptorhynchus* are in contrast to the processes traditionally thought to take place in the mammalian brain. Cell death occurring during embryonic stages of development has been believed to be apoptotic in nature, whereas cell death caused by injuries has been categorized as necrotic. This concept has most clearly been put forward by Kerr and colleagues, who stated that 'necrosis ... is the inevitable outcome of irreversible disruption by injury of the processes that normally maintain the structural and functional integrity in the cell' (Kerr et al., 1987, p. 94). Although in the initial stages immediately following a lesion some cells may undergo necrosis, as suggested by ultrastructural studies (G. K. H. Zupanc and H. Schwarz, unpublished observations), at longer post-lesioning survival times starting at a few hours, apoptosis is the predominant, and presumably even the exclusive, type of cell death. Thus, gymnotiform fish appear to use a mechanism for the removal of damaged cells after injuries in the central nervous system that resembles the apoptotic cell death occurring in mammals during embryonic development.

In mammalian systems, necrosis is usually accompanied by an acute inflammatory response (for a review, see Kerr et al., 1995). The inflammation triggers further necrotic events, thus gradually transforming the site of injury into large cavities devoid of cells (Zhang et al., 1997). These cavities are typically bordered by scars which act as mechanical and biochemical barriers preventing the ingrowth of nerve fibres and the migration of cells into the site of the lesion. In contrast, these negative side effects are characteristically absent in apoptosis. The use of this 'clean' type of cell death by gymnotiform fish in the process of regeneration may, therefore, at least partially explain their enormous regenerative capability.

The role of somatostatin

One factor in the adult gymnotiform brain that appears to play a major role in the generation of new cells and in their further development is somatostatin. This term refers to a family of neuropeptides that are structurally related to the tetradecapeptide somatostatin-14 (for a review, see Patel, 1992). Somatostatin-like immunoreactivity, its mRNA and binding sites for the ligand are widely distributed in the adult gymnotiform brain (Sas and Maler, 1991; Zupanc et al., 1991b, 1994). Areas expressing somatostatin and somatostatin-binding sites include the central posterior/prepacemaker nucleus (Sas and Maler, 1991; Zupanc et al., 1991a,b, 1994, 1997; Stroh and Zupanc, 1996) and the cerebellum (Stroh and Zupanc, 1993; Zupanc et al., 1994). In the cerebellum, the somatostatin-binding sites are present at extremely high densities in the eminentia granularis pars medialis, the eminentia granularis pars posterioris and the molecular layer between these two granule cell layers (Zupanc et al., 1994). This pattern of distribution thus corresponds to the sites of origin, migration and differentiation of the cells born in the caudal cerebellum (Zupanc et al., 1996).

The discovery of somatostatin and somatostatin-binding sites in the cerebellum of adult gymnotiform fish was initially rather surprising, since in the cerebellum of adult mammalian species the number of somatostatin-positive structures is low. However, somatostatin-positive structures appear in the cerebellar primordium of foetal rats as early as gestational day 16 (Inagaki et al., 1982). From that time on, the somatostatin-positive structures increase in number and reach their maximum in the first week following birth; later, these structures decrease markedly in number (Inagaki et al., 1982; Villar et al., 1989).

This time course of the development of somatostatin-positive structures is paralleled by temporal events observed in the expression of somatostatin-binding sites. In the rat, somatostatin-binding sites have not been identified in the adult cerebellum (Reubi and Maurer, 1985; Uhl et al., 1985; Gonzalez et al., 1988; Martin et al., 1991). During ontogenesis, autoradiographic labelling is first seen at embryonic day 15. After birth (embryonic day 22), the density of somatostatin-binding sites increases drastically between postnatal days 4 and 13, while at postnatal day 23 the labelling disappears in most lobes of the cerebellum (Gonzalez et al., 1988, 1989). The binding sites are present in close association with the external

granule cell layer of the cerebellum, a transient germinal matrix located at the surface of the cerebellar cortex. The external granule cell layer contains essentially the stem cells of the granule cells. The decrease and disappearance of somatostatin-binding sites coincides with the involution of the external granule cell layer. In the course of the latter process, which starts at postembryonic day 10, the internal granule cell layer in the mature cerebellum is formed.

In the gymnotiform brain, the continuous expression of binding sites in the gymnotiform brain may be causally linked to the continuous production of new cells, including granule cells, during postembryonic stages of development. This may be mediated by nonsynaptic release and subsequent diffusion of the ligand over wide areas in which somatostatin receptors are expressed (for a review, see Zupanc, 1996), as suggested in the gymnotiform brain by results of studies in the central posterior/prepacemaker nucleus. In these investigations, peptide immunohistochemistry was combined with neuronal tract-tracing techniques. Using this method, no evidence for anterograde transport of the processed peptide could be found (Stroh and Zupanc, 1995; Zupanc et al., 1997).

Additional strong support for the hypothesis that somatostatin is causally involved in the generation and/or further development of cells generated in the adult gymnotiform brain comes from regeneration studies: subsequent to mechanical lesioning of the corpus cerebelli, the expression of somatostatin-like immunoreactivity is tremendously increased in cells near the path of the lesion (Zupanc, 1999). The number of cells expressing the neuropeptide peaks 1–5 days following the lesion; this corresponds to the time when the cells are born that later migrate to and become incorporated at the site of lesion (G. K. H. Zupanc and R. Ott, unpublished observations).

Future investigations aiming at further elucidating the role of somatostatin in the postembryonic development of the brain have recently received a major boost by the successful cloning, sequencing and pharmacological characterization of a fish-specific somatostatin receptor (Siehler et al., 1999; Zupanc et al., 1999). This receptor, cloned by reverse-transcription of mRNA obtained from brain tissue of *Apteronotus albifrons*, resembles in its sequence the mammalian somatostatin receptor 3. The wealth of molecular data obtained through these studies, together with the availability of fish-specific probes, now enables researchers to tackle questions that were, until very recently, inaccessible.

Functional considerations

As pointed out above, it is likely that the ability of fish to replace neurons damaged through injury or disease is causally linked to their capacity to generate new cells in the uninjured brain. Since a pool of undifferentiated cells is continuously filled through normal proliferative activity, recruitment of these cells enables the brain to replace damaged cells, in the event of an injury, much faster than would be the case by just inducing cell proliferation after injuries.

In addition to its possible causal link to the capability for neuronal regeneration, postembryonic neurogenesis in fish may be related to the fact that many fishes grow throughout adulthood. In mammals, muscle growth is based on an increase in size, but not in number, of muscle fibres (Rowe and Goldspink, 1969). In contrast, in fish the formation of new muscle fibres continues well into adult life (Koumans and Akster, 1995). Thus, it is possible that the increase in the number of peripheral motor elements prompts a concomitant increase in the number of central neuronal elements involved in the physiological control of the associated muscle activity.

Moreover, the number of sensory receptor cells, receptor organs or receptor units in the periphery has been shown to increase with age in several species of fish. Such a postembryonic production has been demonstrated, for example, for sensory hair cells in the inner ear of sharks (Corwin, 1981), for retinal cells in the eye of goldfish (Johns and Easter, 1977) and for electrosensory receptor organs in the gymnotiform fish *Sternopygus dariensis* (Zakon, 1984). In mammals, in contrast, the production of sensory cells appears to cease by the end of gestation (see, for example, Ruben, 1967). Thus, as described for motor systems, the continuous increase in the number of sensory structures in fish may necessitate the generation of additional central neurons to process the associated sensory information.

A further function of continuous neurogenesis in the fish brain may be to provide the neural substrate for accommodating long-term behavioral changes such as those observed in seasonally breeding animals (Zupanc, 1997). The addition of new neurons to the population of already existing ones may enable fish to accomplish a structural reorganization of the underlying neural network and thus alterations in the propensity to execute the associated behaviour. Similar structural changes, namely in dendritic morphology of neurons of the central posterior/prepacemaker nucleus, have been hypothesized to be responsible for seasonal changes in the execution of chirping behaviour in *Eigenmannia* sp. (Zupanc and Heiligenberg, 1989; Zupanc, 1991).

In conclusion, the findings summarized in this paper demonstrate that, in gymnotiform fish, neurogenesis continues beyond embryonic stages of development at a high rate and in many regions of the brain. The factors mediating promotion of this proliferative activity are unknown, as are the factors responsible for the suppression of mitotic activity in the mammalian brain. Elucidating these mechanisms is especially important, since stem cells exist in the mammalian brain that are normally quiescent, but which, under certain circumstances (e.g. by the addition of epidermal growth factor), can be activated (Reynolds and Weiss, 1992; Richards et al., 1992; Craig et al., 1996; for reviews, see Gage et al., 1995; Brüstle and McKay, 1996; Weiss et al., 1996). It is possible that factors that promote cell proliferation in the adult teleostean brain may also be able to stimulate cell division in the adult mammalian brain. Identification of these factors is, therefore, likely to open new horizons for the development of therapeutic agents to treat

neurodegenerative diseases and to restore neuronal function after injuries.

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