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# Plasticity of brain in normal adult some earth-dwelling Anamia and terrestrial Amniota: further review on the trend of seasonal environmental influence on encephalic proliferation, as revealed by immunohistochemistry

Vito Margotta[1], Claudio Chimenti[2,*]

Departments of [1]Animal and Human Biology, and [2]Biology and Biotechnology "Charles Darwin", University "La Sapienza", Roma, Italy

## Abstract

Further immunohistochemical evidence have been provided by the present authors about the persistence of latent natural proliferative potentially in adult brain of some low vertebrates and its response to cyclic seasonal environmental fluctuations (temperature, photoperiod) has been reviewed. These stimuli elicit an otherwise hidden mitotic activity thanks to stem cells still present especially in less high vertebrates like *Triturus carnifex*, *Rana bergeri*, *Podarcis sicula*. The evidence gathered from specimens caught in the wild in spring, summer and autumn has been compared with previous evidence on specimens of newts, frogs and lizards. Signs of proliferation were mainly observed in the typical sites (olfactory bulbs/peduncles, telencephalic hemispheres) occupied by cells in mitotic stand-by. The findings have shown increasing labelling from spring to summer to autumn with minor differences among species, and have confirmed that in adulthood the proliferative therefore the reparative and even the regenerative power of brain cells is highest in urodela (the vertebrates best equipped with quiescent cells), intermediate in anura and lowest in lacertilian Reptiles.

## Keywords

Neural stem cells, matrix cells, matrixareas, Amphibians, lacertilian Reptiles.

## Review

Since about a half century detailed awareness has been acquired on the plasticity of the brain in some adult heterothermic vertebrates: fresh water, earth-dwelling Anamnia and terrestrial Amniota (Margotta and Morelli, 1996).

Among these investigations a thread of research has been devoted to study if in adults the impact of cyclic seasonal environmental fluctuations (consisting in temperature and photoperiod variations) could exert any influence on brain in terms of physiological cell proliferative answer, or unmask a latent spontaneous proliferative power thus making apparent reparative and even regenerative potentialities due to an otherwise hidden mitotic activity of stem cells still present in the adult brain of some vertebrate species, mainly in lower ones.

* Corresponding author. E-mail: claudio.chimenti@uniroma1.it


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Such events have been correlated with the persistence in the adult of a stock of brain stem cells which have some own characteristics: morphological (being small and basophilic), physiological (able to proliferate), behavioural (persistence of the tendency to proliferate).

Usually these undifferentiated cells appear as scattered ("matrix areas"), as clusters of grouped cells, sometimes layered, in circumscribed areas ("matrix areas"), once nicknamed *Matrixzonen* (according to Kirsche, 1967) typically located among the ependymal cells lining each encephalic cavity and in the sub-ependymal layer, or elsewhere in cerebral tissues.

The number and size of such stem cells can vary among the different vertebrate groups and species; generally speaking, they appear much more numerous and large in lower than in higher species. In a gradual scale of value regarding the adult fresh water, earth-dwelling Anamnia and heterothermic terrestrial Amniota, such cells in mitotic stand-by appear to be relatively abundant in the urodelan Amphibians (the best provided with these cells among vertebrates), intermediate in the Teleosts, and relatively scarce in the anuran Amphibians and above all in the lacertilian Reptiles.

The number of such sleeping cells can vary according to different encephalic districts and is persistently high in the forebrain (olfactory bulbs/peduncles , telencephalic hemispheres), where they are mainly distributed in characteristic, mirror-like sites: the matrix cells in proximity of the olfactory cavities, the matrix areas at the edge, latero-dorsally and ventrally at the bottom of the sickle-shape, hallow ventricular surface of each telencephalic hemisphere: *zonae germinativae dorsales* and *ventrales*, respectively. In particular, only in lacertilian Reptiles each *zona germinativa dorsalis* appear sub-divisible in two portions, *lateralis* and *medialis*, like in *Lacerta viridis* (Minelli and Del Grande, 1980).

The *zonae ventrales* are the best provided with undifferentiated cells; intra-specific difference can be found among the brain of the earth-dwelling Anamnia and poikilothermal Amniota studied. Matrix cells can be observed here and there in the diencephalon. Such cells appear absent from the midbrain, in the *truncus cerebri*, and the *cerebellum* (in both sites, with the only exception of Teleosts in which it is possible to recognize symmetrical, additional areas provided with proliferative potential: the *zonae germinativae caudales* in the midbrain and scattered cells in the cerebellar deep tissue. Sometimes mitotically dormant cells can be found in the *medulla oblongata*.

Both telencephalic *zonae germinativae* are extended antero-posteriourly and active with different time courses: the *zonae dorsales* appear to exhaust their self-maintaining potential earlier than the *zonae ventrales*, which are generally wider and richer in cells (Kirsche, 1967).

All these information has been acquired and expanded through observations on untreated animals and upon experimental intervention, more frequently represented by brain surgery, ablations of encephalic plugs or wider portions sometimes with subsequent hetero- and rarely homo-transplatation (even of the whole brain), and *in vitro* culture of cerebral tissues. Analytical techniques were at first traditional histology, then autoradiography and immunohistochemistry, seldom electron microscopy.

These quiescent cells are remnants of the neural layer which forms in the early embryo and responsibles for the morphogenesis of the central nervous system (Kahle, 1951; Fujita, 1963; Kirsche, 1967), which may explain why the number of such cells decreases during the life of the organism: going from earlier to more advanced embryonic stages, then through the subsequent larval ones - if present – and eventually into adult life.

The greater part of the information on this subject is the consequence of the exhaustive studies of Kirsche (1967, 1983), who investigated adult non-mammalian vertebrates by traditional histological methods. The studied species ranged from Teleosts to Birds, passing through urodelan and anuran Amphibia and lacertilian Reptiles. This author must be credited for the generation of most data on the features and localization of these putative precursor or stem cells in the adult brain and the recognition that they can be normally silent but are cable of self-reproduction and can start cycling again giving rise to descendants which undergo differentiation into neuronal or glial cells (Kirsche, 1967, 1983).

The persistence also in adult life of such cells in mitotic stand-by, thanks to their proliferative power, sustains both the physiological and experimental proliferative events and explains the reparative and even regenerative potentialities still present in the adult brain of many vertebrate species, especially in the less high ones.

In the last years investigations have been carried on adults of some earth-dwelling Anamnia (frogs and newts) and terrestrial Amniota (lizards) to investigate the spontaneous brain proliferative answer to cyclic seasonal environmental changes (temperature and photoperiod variations). These investigations were performed mainly by us with the help of immunohistochemistry for Proliferating Cell Nuclear Antigen (PCNA), expressed by proliferating cells (Miyachi et al., 1978; for further details on this method see Margotta and Chimenti, 2016).

By other authors in the past and by ourselves (Margotta, 2012, 2014a; Margotta and Chimenti, 2016, 2017, 2018, 2019a, 2019b) attention was paid to spring, summer and autumnal seasonal environment, while no attention was devoted to winter season conditions. In the last conditions autoradiographic studies were done by Minelli et al. (1982) on injured and uninjured brain of adult *Rana esculenta*, observing a post-autumnal arrest of encephalic proliferation that reached a nadir in full winter.

Immunohistochemical studies were done by Ramirez et al. (1997) on adult brain-damaged *Podarcis hispanica*, who referred that ”…cold (winter) temperature prevented migration of the newly generated immature neurons”.

An explanation on the “winter” outcomes both of Minelli et al. (1982) and Ramirez et al. (1997) could be found in a report by Margotta and Morelli (1997) which on the basis of the literature sources, furnished the matter of a critical review of the past acquired data on the analyzed relationships between contribution and persistence of radial glial cells in physiological neurogenesis and in post-traumatic conditions, advancing a hypothesis on the role of the glial cells in the plasticity of the central nervous system in the adults of several vertebrate species. Peraps, this information could to clarify and to justified the previous “winter” findings of Minelli et al. (1982) and Ramirez et al. (1997).

Therefore, was supposed that the different seasonal inputs on the proliferative rhytms could elucidate the controversial results reached by some authors in previous researches on the regenerative capacities of the central nervous system in the adult anurans.

Therefore, adult normal brain of *R. bergeri*, *Triturus carnifex*, *Podarcis sicula* – once, respectively, *R. esculenta* (Capula, 2000 a) *T. cristatus carnifex* (Bonifazi, 2000) and *L. viridis* (Capula, 2000 b) – were again investigated by Margotta (2012, 2014a), Chimenti and Margotta (2015), Margotta and Chimenti (2016. 2017, 2018, 2019a). Stain-

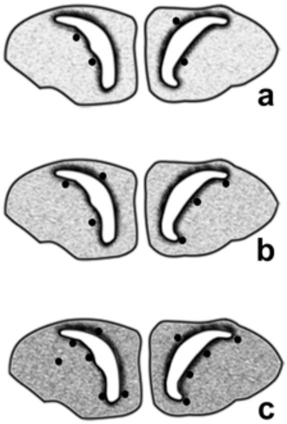


**Fig. 1.** Drawings (not to scale) of a transverse view of adult normal brain of *Triturus carnifex*. Olfactory bulbs in specimens caught in the wild in spring (a), summer (b), autumn (c). The dots represent PCNA immunolabelled matrix cells, isolated in the ependyma and periependymal grey matter.

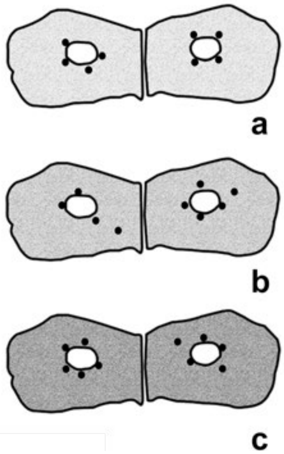


**Fig. 2.** Drawings (not to scale) of a transverse view of adult normal brain of *Rana bergeri*. Olfactory bulbs in specimens caught in the wild in spring (a), summer (b), autumn (c). The dots represent PCNA immunolabelled matrix cells, isolated in the ependyma and periependymal grey matter.

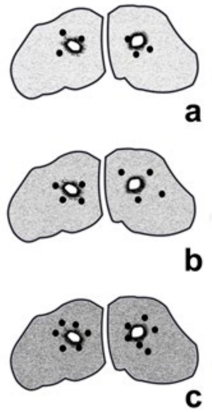


**Fig. 3.** Drawings (not to scale) of a transverse view of adult normal brain of *Podarcis sicula*. Olfactory bulbs in specimens caught in the wild in spring (a), summer (b), autumn (c). The dots represent PCNA immunolabelled matrix cells, isolated in the ependyma and periependymal grey matter.

ing was observed in the typical localization of putative stem cells, as "matrix cells" in the olfactory (Figs. 1; 2; 3) and diencephalic districts or as "matrix areas" in the telencephalic hemispheres (Figs. 4; 5; 6) where labelling was in correspondence of the ventricular edges of *zonae germinativae dorsales* (Figs. 4; 5), which in lizards are subdivided, as already said, each in *lateralis* and *medialis* (Fig. 6), and also in relationship with the bottom of the ventricular cavities in *zonae germinativae ventrales* (Figs. 4; 5; 6). Labelled cells were found among the ependymal cells and in the sub-epedymal layer. This pattern emerged also from further immunohistochemical studies on the same earth-dwelling Anamnia (Margotta and Chimenti, 2016, 2017, 2018, 2019a) and terrestrial Amniota (Margotta and Chimenti, 2019b). The findings were more evident in *T. carnifex* (Figs. 1, 4), less so in *R. bergeri* (Figs. 2, 5) and least in *P. sicula* (Figs. 3, 6).

In the investigated species the immunohistochemical signs of proliferation show an ascending trend from spring (Figs. 1a, 2a, 3a, 4a, 5a, 6a), through summer (Figs. 1b, 2b, 3b, 4b, 5b, 6b), to autumn (Figs. 1c, 2c, 3c, 4c, 5c, 6c), with only minor differences linked to the position of each species in the zoological/evolutionary scale.

The present authors' findings in different species and conditions have expanded those of Minelli et al. (1982) and Ramirez et al. (1997) and strongly support the hypothesis proposed by present authors, that the entity of the spontaneous proliferative processes along seasons accounts for the reparative or even regenerative phenomena obtained by previous authors, the extent of which could depend on syner-

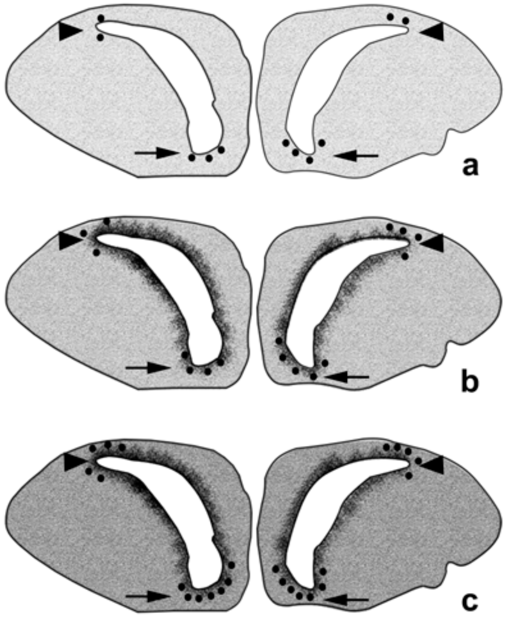


**Fig. 4.** Drawings (not to scale) of a transverse view of adult normal brain of *Triturus carnifex*. Telencephalic hemispheres in specimens caught in the wild in spring (a), summer (b), autumn (c). The dots represent PCNA immunolabelled matrix cells, clustered in the walls of telencephalic symmetrical ventricles to form the *zonae germinativae dorsales* (arrowheads) and *ventrales* (arrows).

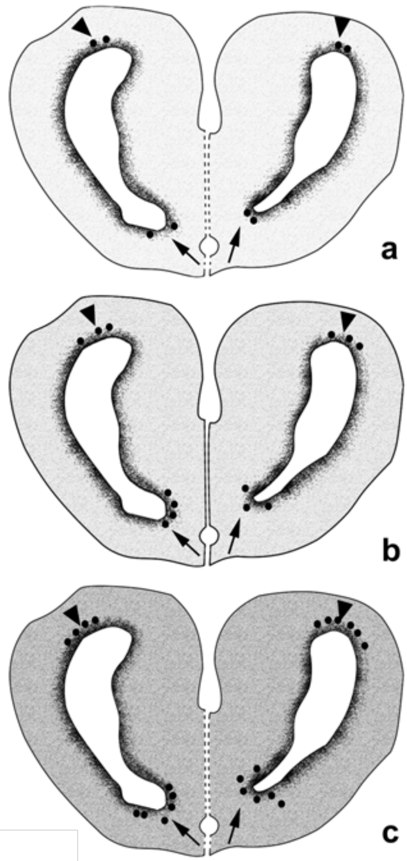


**Fig. 5.** Drawings (not to scale) of a transverse view of adult normal brain of *Rana bergeri*. Telencephalic hemispheres in specimens caught in the wild in spring (a), summer (b), autumn (c). The dots represent PCNA immunolabelled matrix cells, clustered in the walls of telencephalic symmetrical ventricles to form the *zonae germinativae dorsales* (arrowheads) and *ventrales* (arrows).

gic stimulation by various stressful stimuli: extreme temperature, surgery, other types of trauma. This was also supported by a comparison between the findings of previous authors on adult *R. esculenta* in autumn (Minelli et al. 1982) and on adult *P. hispanica* in summer (Ramirez et al. 1997) and those of present authors on uninjured adult *R. bergeri* (Margotta, 2012; Margotta and Chimenti, 2017, 2018), *P. sicula* (Margotta, 2014a; Margotta and Chimenti, 2019b), *T. carnifex* (Margotta and Chimenti, 2019a) and on adult poikilothermal earth-dwelling and terrestrial vertebrates subjected to cold shock (Chimenti and Margotta, 2013, 2015; Margotta, 2014b, 2015).

It can be said, therefore, that in adults the proliferative and therefore the reparative and even the regenerative power of the brain increases progressively from the lacertilian Reptiles to anuran Amphibians, Teleosts and eventually urodelan Amphibians, due to the respective, available stock of cells in mitotic stand-by of which the brain is espe-

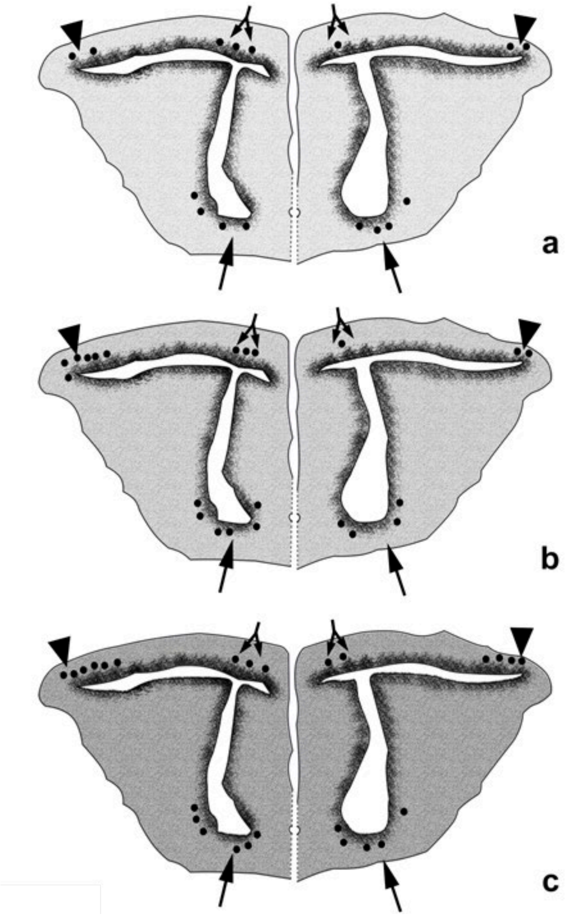


**Fig. 6.** Drawings (not to scale) of a transverse view of adult normal brain of *Podarcis sicula*. Telencephalic hemispheres in specimens caught in the wild in spring (a), summer (b), autumn (c). The dots represent PCNA immunolabelled matrix cells, clustered in the walls of telencephalic symmetrical ventricles to form the *zonae germinativae latero-dorsales* (arrowheads), *medio-dorsales* (arrowforkeds) and *ventrales* (arrows).

cially equipped in urodelans. The latter group of Amphibians is, among vertebrates, that which occupies the most privileged position, since these animals are the richest in undifferentiated cells, therefore those most endowed with proliferative potentiality and hence the best equipped for reparative or even regenerative processes.

Recently, in an immunohistochemistry report we exposed the influence of seasonal variations alone or coupled with an induced cold shock on spontaneous brain proliferation in adult poikilothermal earth-dwelling Anamnia and terrestrial Amniota; similar results were found in frogs, newts and lizards (Margotta and Chimenti, 2016).

Also now appear reasonable, as formely according to an our past opinion: is unthinkable that the spontaneous glimpsed proliferative phenomena owing to a seasonal input, referred in the actual report, could be able by oneself to justify the entity of the reparative or even the regenerative processes referred as obtained by previous authors. the degree of which could be explained attaching a stimulating value to further experimental various (thermal, surgical, traumatic) stressful stimulus, subjected by the employed samples.

The content of the actual exposition forms with that of the previous reports an *unicum* with which we have attempted to furnish a whole seasonal panorama of the natural cell proliferative trend as consequence of the impact of the cyclic seasonal environmental fluctuations (made of temperature and photoperiodic variations), revealed by the immunoreactive patterns, in the adult brain of heterothermic some vertebrates.

So we have satisfied an our recent wish: take cognizance of the overall annual view of the interrelations among such behavioural stimulus on adult encephalic cell proliferations in some earth-dwelling Anamnia and poikilothermal Amniota.

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