

Gerhard Roth^a
Kiisa C. Nishikawa^b
David B. Wake^c

^a Brain Research Institute,
University of Bremen, Bremen, Germany
^b Physiology and Functional Morphology
Group, Department of Biological Sciences,
Northern Arizona University,
Flagstaff, Ariz.,
^c Department of Integrative Biology and
Museum of Vertebrate Zoology,
University of California,
Berkeley, Calif., USA

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Genome Size, Secondary Simplification, and the Evolution of the Brain in Salamanders

Abstract

Compared to other vertebrates, even including lampreys and hagfishes in some respects, salamanders exhibit a relatively simple organization of brain and sense organs which is illustrated here using the visual system as an example. The greatest simplicity is found in the bolitoglossine salamanders, yet all bolitoglossines possess highly projectile tongues and rely on vision for survival; furthermore, some species are agile and acrobatic. The unusual features of the visual system of salamanders include small numbers of large neurons, a low degree of morphological differentiation among neurons, a small proportion of myelinated axons in the optic nerve, and an optic tectum consisting essentially of a periventricular cellular layer and a superficial fiber layer. Similar features are found throughout the central nervous system of salamanders and in the lateral line, auditory and olfactory systems as well. Phylogenetic analysis shows that the most parsimonious interpretation of these data is that the simple organization of the brain and sense organs of salamanders was derived secondarily from a more complex ancestral state. We hypothesize that increased genome size has led to simplification of the nervous system in salamanders. Increased genome size appears to have had profound effects on neural development in salamanders, leading to paedomorphosis, the retention of juvenile or even embryonic characteristics into adulthood. In particular, large genome size is associated with large cell size and reduced rates of cell proliferation, migration and differentiation. Secondary simplification has constrained the function of the salamanders' visual system, primarily by increasing cell size and decreasing cell numbers. However, it also has provided an opportunity for the evolution of compensating mechanisms, which have helped to restore or even enhance visual function. Most apparent among the compensatory mechanisms of bolitoglossine salamanders is the presence of well developed ipsilateral retinotectal projections, which apparently enhance depth perception. It is difficult to explain the unusual history of the nervous system in salamanders solely in terms of natural selection and adaptation. Increasing genome size through selfish replication appears to have played a major role in the evolution of salamander brains by imposing functional constraints as well as creating opportunities for overcoming them.

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Introduction

For several decades, comparative neuroanatomists have recognized that the brains and sense organs of salamanders are simpler in their morphology and organization than those of sharks and bony fishes [Clairambault and Mansour-Robaey, 1992], even simpler than the nervous systems of hagfishes and lampreys in some respects. For example, Herrick [1948] and Leghissa [1962] believed that the brain of salamanders resembled the bauplan of the vertebrate brain more closely than the brain of any other living species of vertebrate. However, Herrick [1948] and others realized that the evolutionary position of salamanders, as tetrapods, conflicted with the hypothesized ancestral morphology of salamander brains. Especially with respect to the auditory system, Herrick [1948] had begun to suspect that the brains and sense organs of salamanders, simple as they might be, had been derived from a more complex ancestral state. The problem that we address in this essay concerns this paradox, that the brains and sense organs of salamanders are more simple in their morphology than would be expected on the basis of their phylogenetic relationships within the vertebrate lineage.

The evolution of the vertebrate nervous system is traditionally portrayed as a story of progress, beginning with the innovative but lowly jawless fishes and improving gradually over time. However, recent work has demonstrated that adaptive radiation of brain and sense organs has occurred within each of the vertebrate classes independently [Northcutt, 1984]. Salamanders are unusual because they appear to represent an exception to the rule that brain and sense organs have increased in complexity during their evolution. Even within the amphibian order Caudata, salamanders show a trend in which greater simplicity of neural structures is found in more derived taxa. For example, the salamander family Plethodontidae is the most speciose family of salamanders and possesses many uniquely derived characteristics. Yet, its members exhibit a greater simplicity of neural structures than is found generally among salamanders. The tribe Bolitoglossini of the family Plethodontidae has radiated extensively within the Neotropics [Wake and Lynch, 1976; Wake, 1987]. Many bolitoglossine salamanders are acrobats that occupy diverse, three dimensional habitats including bromeliads and the walls of caves. They exhibit many derived features, including direct development and the presence of a highly projectile tongue which can be aimed with great accuracy at fast-moving prey [Wake, 1966, 1982]. Yet, in many respects, the bolitoglossine salamanders exhibit the most simple nervous systems found among vertebrates [Roth et al., 1993].

The simplicity of salamander nervous systems raises several questions that have important implications for understanding the evolution of nervous systems in general: (1) Were salamanders left behind by natural selection, retaining primitively simple brains while other vertebrate classes evolved more complex brains independently, or is the simple brain of salamanders derived from a more complex ancestral state? (2) If salamander brains are secondarily simplified, what developmental mechanisms are responsible for decreasing their complexity? (3) What are the functional consequences of decreased complexity? and (4) Is there any evidence that salamanders in general, and bolitoglossines in particular, have compensated for any negative functional consequences associated with a decrease in the complexity of their nervous systems?

The purpose of this essay is to provide evidence for the unusual simplicity of salamander nervous systems, using the visual system as one example of a trend that is evident throughout the brain and in the lateral line, auditory and olfactory systems as well [Roth et al., 1992, 1993; Roth and Schmidt, 1993]. We then explore the evolutionary history, developmental mechanisms and functional consequences of simplification of the visual system in salamanders. Lastly, we explore possible mechanisms that may compensate for the negative consequences of secondary simplification.

The bolitoglossine salamanders exhibit the most reduced visual system found among vertebrates that depend upon vision for survival [Roth, 1987]. Phylogenetic analysis demonstrates that the simplified brain and sense organs of salamanders were derived from a more complex ancestral state. We hypothesize that increased genome size has led to secondary simplification of the brain and sense organs of salamanders. This hypothesis is supported by phylogenetically independent trends in other amphibians and lungfishes. Lastly, we suggest that secondary simplification itself has provided a means by which bolitoglossine salamanders have been able to restore and apparently even improve the function of their visual system, despite the profound functional constraints that have been imposed by secondary simplification.

The Salamander Visual System: Evidence for Simple Morphology

In this section, we compare the anatomy of the visual system of salamanders with that of other vertebrates, concentrating on the retina and the optic tectum, respectively.

The Retina

Because visual acuity depends critically upon the size and number of photoreceptors, perhaps the most surprising feature of the retina in salamanders is the small number and relatively huge size of retinal neurons, including photoreceptors, interneurons and retinal ganglion cells, compared to those in other vertebrates that depend on vision for survival. As estimated from counts of optic nerve fibers, the number of retinal ganglion cells (RGCs) varies by approximately three orders of magnitude among vertebrates, from fewer than two thousand in hagfishes [H. Wicht, pers. commun.] to more than one million in birds [Binggeli and Paule, 1969; O'Flaherty, 1971] and mammals [Vaney and Hughes, 1976; Hughes, 1977]. Among salamanders, the number ranges from 25,000 in the bolitoglossine genus *Batrachoseps* [Linke and Roth, 1990] to 75,000 in the salamandrid *Notophthalmus viridescens* [Ball and Dickson, 1983]. Only vertebrate species with reduced eyes and poor visual acuity have fewer optic nerve fibers than bolitoglossine salamanders. In addition to hagfishes (see above), other species with low numbers of optic nerve fibers include lampreys with 35,000 [Öhman, 1977], lepidosirenid lungfishes with approximately 1,500 [Northcutt, 1977], and caecilians with about 4,000 optic nerve fibers [Fritzsch et al., 1985]. Salamanders also possess fewer morphologically distinct types of retinal ganglion cells than are found in teleost fishes and amniotes [Linke and Roth, 1989].

Another unusual feature of salamander visual systems is the small proportion of myelinated axons in the optic nerve. In teleosts [Tapp, 1973, 1974], birds [Binggeli and Paule, 1969] and mammals [Ogden and Miller, 1966; Forrester and Peters, 1967], virtually all optic nerve fibers possess a myelin sheath, whereas lampreys [Öhman, 1977] and hagfishes [Wicht and Northcutt, 1990] have only unmyelinated optic nerve fibers. The proportion of myelinated optic nerve fibers varies from 0–7% among salamanders, with the bolitoglossine genera possessing the smallest proportions of myelinated optic nerve fibers [Linke and Roth, 1989, 1990]. Among vertebrates, only agnathans possess a smaller proportion of myelinated optic nerve fibers than salamanders [Roth et al., 1993]. Furthermore, the glial cells in the optic nerve of *Batrachoseps* are relatively undifferentiated, showing characteristics of both astrocytes and oligodendrocytes [Linke and Roth, 1989].

The Optic Tectum

The optic tectum is the major visuomotor integration center of salamanders [Roth, 1987]. The optic tectum of salamanders differs from that of other vertebrates in several important respects. Like the retina, the optic tectum

of salamanders contains fewer neurons and fewer morphologically distinct types of neurons than are found in vertebrates with good vision [Roth et al., 1990a]. For example, salamandrid salamanders possess approximately 86,000–92,000 tectal neurons, and bolitoglossine salamanders possess 37,000–85,000, whereas the frog *Rana esculenta* possesses approximately 800,000 tectal neurons [Roth et al., 1990a].

From a morphological perspective, perhaps the most striking feature of the optic tectum of salamanders is the weak development or even absence of multiple lamination [Roth et al., 1990a; Schmidt and Roth, 1993]. In salamanders, nearly all of the tectal neurons are periventricular, and these periventricular neurons are surrounded by a superficial fiber layer (fig. 1a, b) [Roth et al., 1990a]. In all other vertebrates except lepidosirenid lungfishes and a few derived species of frogs and caecilians, the optic tectum is multilaminated in its morphology. In most frogs, for example, there are four cellular layers above the ependymal layer, and these layers are separated from each other by intervening fiber layers (fig. 1c) [Potter, 1969]. The absence of lamination in salamander tecta is due to the fact that few neurons migrate into the superficial fiber layer [Schmidt and Roth, 1993]. In salamandrid salamanders, 3–5% of tectal neurons have migrated into the superficial layer, whereas only 1–2% are migrated in bolitoglossine salamanders. In the frog *Rana esculenta*, approximately 30% of tectal neurons have migrated into superficial fiber layers [Roth et al., 1990a].

A final major difference between the optic tecta of salamanders and other vertebrates is that there is no incompatibility between ipsilateral and contralateral retinotectal fibers in salamanders, whereas this incompatibility is well developed in other vertebrates that have been investigated. In bony fishes [S. Collin, pers. commun.], frogs [Constantine-Patton, 1981], and birds [O'Leary et al., 1983], the axons of retinal ganglion cells grow from the retina out toward the tectum during development. Early in development, retinofugal projections from both eyes grow into each tectal hemisphere, but the fibers from the ipsilateral eye are eliminated progressively through competition with contralateral fibers. In mammals [Huerta and Harting, 1984] and lungfishes [Northcutt, 1980], the ipsilateral fibers are retained throughout life, but the ipsi- and contralateral fibers segregate into columns and puffs, respectively. In salamanders, not only are ipsilateral and contralateral fibers retained throughout life, but they never segregate from each other [Rettig and Roth, 1986].

Despite these differences, there are several important similarities between the optic tecta of salamanders and

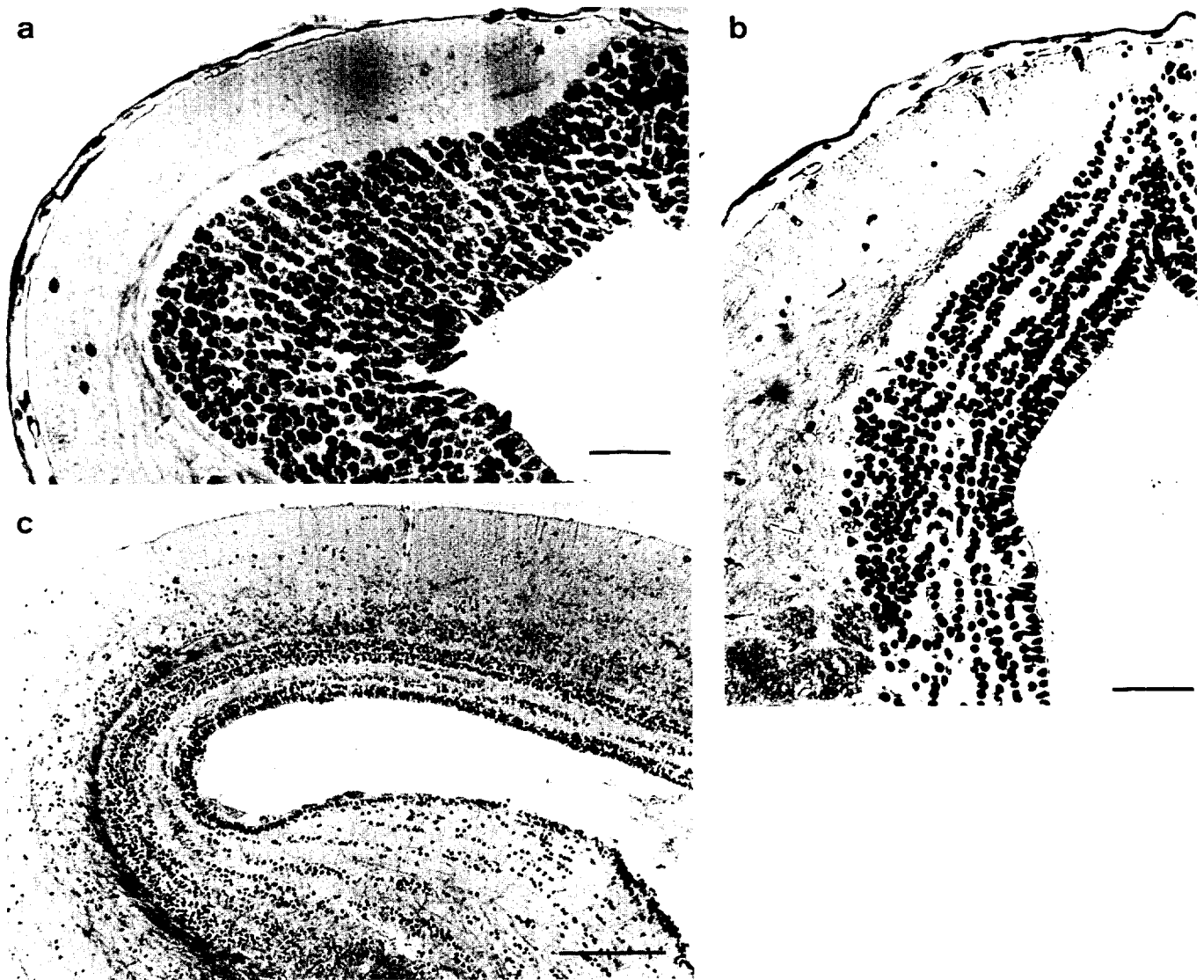


Fig. 1. Cross sections through the brain at the level of the optic tectum, the dorsal tegmentum and the torus semicircularis in three amphibians. **a** The bolitoglossine salamander *Hydromantes italicus*. **b** The ambystomatid salamander *Ambystoma mexicanum*. **c** The frog *Rana temporaria*. Scale bar = 50 μ m. The brains of *Hydromantes* and *Ambystoma* represent the simplest and most complex brain morphologies found among salamanders, respectively. The complexity of brain morphology found in *Rana temporaria* is typical for most anurans. Note the differences in cell size, which are positively correlated with genome size and negatively correlated with morphological differentiation of the brain.

other vertebrates. In general, the expression of cell-surface molecules, including NCAM and polysialic acid, during development is similar [Becker et al., 1993], as is the distribution of several neurotransmitters in the optic tectum, including bombesin, leucine-enkephalin, substance P and acetylcholine [Schmidt et al., 1989]. The distribution of neurotransmitters is laminated in salamanders [Schmidt et al., 1989], and the pattern of lamination is very similar to that of frogs [Kuljis and Karten, 1982]. The fact that the

neurotransmitters present in the nerve terminals of tectal neurons exhibit a laminated distribution, whereas the cell bodies themselves do not, suggests that the lack of lamination in the cell bodies of the tectum in salamanders is secondary.

Other similarities between salamanders and frogs include the electrophysiological response properties and the neuroanatomical connections of neurons in the optic tectum. Although the neurons of the optic tectum are less dis-

tinct morphologically in salamanders than in frogs, there is a general correspondence between the location of dendritic arbors and axonal projections of particular cell types [Roth et al., 1990a]. In addition, the visual response properties of tectal neurons are similar in frogs and salamanders [Ewert, 1984; Roth, 1986]. There are more similarities in tectal response types between frogs and salamanders that have similar prey preferences than between members of either group that have different prey preferences [Roth 1986, 1987].

The major anatomical differences between the optic tecta of salamanders and frogs involve reduced numbers of tectal neurons, a reduced degree of morphological differentiation of neurons, and reduced numbers of neurons that have migrated into superficial fiber layers in salamanders compared to frogs and other vertebrates. In contrast, the neurotransmitters, the electrophysiological response properties, and the axonal projections of tectal neurons in salamanders are similar to those of other vertebrates. A recent study [Schmidt and Roth, 1993] suggests that, while the pattern of tectal development is generally similar in frogs and salamanders, rates of cell proliferation and migration in the tectum during development are very low in salamanders compared to frogs, which accounts for the major morphological differences. The results of these recent studies suggest that similarities between the brains of salamanders and the embryonic brains of other vertebrates represent convergence rather than homology.

Is the Simple Morphology of the Visual System Primitive or Derived in Salamanders? Evidence from Phylogenetic Analysis

The previous section showed that many characteristics of the visual system are simpler in salamanders than they are in other vertebrate groups, particularly those groups that depend upon vision for survival. The morphology of the visual system of salamanders is simpler in general than that of cartilaginous and bony fishes, and even simpler than that of agnathans in some respects. Why is the visual system of salamanders so simple? There are two alternative hypotheses: (1) salamanders have retained a primitively simple structure of the visual system, while other vertebrate lineages have evolved increasingly complex visual systems independently; or (2) the simple organization of the visual system in salamanders has evolved from a more complex ancestral state.

These hypotheses concern evolutionary history and therefore can only be addressed using the methods of phylogenetic analysis, or cladistics, in which outgroup analysis

is used to determine whether the characteristics of a group are ancestral or derived [Hennig, 1966]. Roth et al. [1993] presented a phylogenetic analysis of 23 characteristics of the brain and sense organs of vertebrates, including many of the characteristics of the visual system listed above. Of the 23 characters that were analyzed, only one (the presence of migrated neurons in the medial pallium) was most parsimoniously interpreted as primitive in salamanders. Only one character (the presence of well-developed ipsilateral retinotectal projections) was more elaborate in salamanders than in vertebrates generally (see below for a more detailed discussion of this character). Two characters (the small number of types of retinal ganglion cells and the small proportion of myelinated optic nerve fibers) were equally parsimonious under the two alternative hypotheses (primitive simplicity and secondary simplification). The remaining 19 characters, including the small number of retinal ganglion cells and the unlayered morphology of the optic tectum, were most parsimoniously interpreted as secondarily simplified. The primitive simplicity hypothesis required 128 evolutionary steps to explain the distribution of characters among taxa, whereas the secondary simplification hypothesis required only 56 events. The analysis overwhelmingly supported the hypothesis that the brain and sense organs of salamanders, including the visual system, have been simplified secondarily from a more complex ancestral state [Roth et al., 1993].

This analysis also showed that the visual system of salamanders is just one example of a syndrome that has affected most parts of the brain and many other sensory systems. In addition to the optic tectum, numerous other areas of the central nervous system of salamanders exhibit low cell numbers and an absence of lamination, including the superior olive, torus semicircularis, diencephalic and synencephalic nuclei, nucleus ruber and spinal cord [Roth et al., 1993]. The lateral line, olfactory and auditory systems also exhibit reduction in salamanders, with the extreme of reduction occurring in the bolitoglossines [Roth et al., 1993].

In addition to salamanders, lepidosirenid lungfishes, some caecilians and frogs exhibit a parallel reduction of complexity in brain and sense organs [Roth et al., 1993]. Lepidosirenid lungfishes show independent reduction in 11 of 23 characters, including absence of cell migration in the optic tectum, torus semicircularis, diencephalic and synencephalic nuclei, and spinal cord. In contrast, the Australian lungfish *Neoceratodus* shows reduction in only 3 of 23 characteristics [Roth et al., 1993].

Independent reduction of brain and sense organs also appears to have occurred within the amphibian orders Gym-

nophiona [Roth et al., 1993] and Anura [G. Roth, J. Blanke and D. Wake, unpubl. observ.]. As a group, caecilians show reduction in 15 of the 23 neural characters [Roth et al., 1993]. Within caecilians, some species exhibit a greater simplification of neural structures than others. For example, the basal caecilian *Epicrionops* possesses a multilaminated optic tectum, whereas lamination is reduced in *Typhlonectes* [Himstedt and Manteuffel, 1985; A. Schmidt and M. Wake, pers. commun.]. A similar range of variation is found among frogs, in which most species possess a multilaminated optic tectum [Potter, 1969], but the tectum is nearly unlaminate in *Bombina* [W. Walkowiak and G. Roth, unpubl. observ.] and *Arenophryne* [G. Roth, J. Blanke and D. Wake, unpubl. observ.].

Within salamanders, the degree of morphological complexity of the brain and sense organs is inversely correlated with genome size [Roth et al., 1993]. Desmognathine salamanders with relatively small genomes exhibit greater morphological complexity than bolitoglossine salamanders with large genomes [Roth et al., 1990b]. The other taxa that exhibit an independent reduction of brain and sense organs also appear to possess large genomes. Salamanders and lungfishes exhibit the largest genomes found among vertebrates, but within lungfishes *Neoceratodus* has a smaller genome size than the lepidosirenids [Olmo, 1983]. Within frogs and caecilians, the taxa with the simplest brains also have the largest genome sizes. For example, *Arenophryne rotunda* has the simplest brain and sense organs [G. Roth, J. Blanke and D. Wake, unpubl. observ.] and the largest genome size found among frogs [Mahony, 1986]. Based on these observations, we hypothesize that increased genome size may be involved in secondary simplification.

Developmental Mechanisms for Secondary Simplification

The available data strongly suggest that the central nervous system and many sensory systems, including the visual system, have been simplified secondarily from a more complex ancestral state in salamanders [Roth et al., 1993]. We now seek to explain the developmental basis for secondary simplification, which may help us to understand why the nervous systems of salamanders, as well as those of lungfishes, caecilians, and some frogs have become more simplified during their evolution, whereas increasing complexity appears to have evolved in many other vertebrate taxa.

We hypothesize that secondary simplification of the nervous system in salamanders is the result of an increase in

genome size, which has occurred in parallel among lungfishes, caecilians and frogs. This hypothesis is based on the fact that these taxa possess genome sizes that are 1–2 orders of magnitude larger than those found in most vertebrates [Olmo, 1983] and on the observation that large genome size is correlated with large cell size [Roth et al., 1990b] and reduced rates of cell proliferation and differentiation [Sessions and Larson, 1987].

Genome size varies by more than two orders of magnitude among vertebrates, from less than 1 to 142 pg of DNA per haploid nucleus [Olmo, 1983]. Average genome sizes for reptiles, birds and mammals are 2.5, 1.5 and 3.3 pg, respectively [Olmo, 1983]. Genome sizes in salamanders range from 13.4–83 pg [Sessions and Larson, 1987]; frogs range from 0.75–19 pg, with an average of 3.3 [Olmo, 1983; Mahony, 1986]; and caecilians may have genome sizes as large as 13.2 pg [M. Wake, pers. commun.]. Lungfishes have the largest genome sizes found among vertebrates, with 80 pg in the Australian lungfish *Neoceratodus*, 111 pg in the South American lungfish *Lepidosiren*, and 142 pg in the African lungfish *Protopterus* [Olmo, 1983].

Why do salamanders and lungfishes have so much DNA? Differences among species in the amount of DNA can arise from polyploidy, in which all of the chromosomes are represented by multiple copies. However, in salamanders the increase is due to the amount of non-transcribed, middle to highly repetitive sequences of DNA within each chromosome [Horner and MacGregor, 1983]. The selfish DNA hypothesis holds that genome size will tend to increase until the tendency is halted by countervailing selection [Orgel and Crick, 1980]. One explanation for the large genomes of amphibians and lungfishes is that they have been less resistant physiologically to increasing genome size than other vertebrates.

There are a number of important functional and morphological correlates of genome size, including cell size, rate of cell division, rate of differentiation and cellular metabolic rate [Sessions and Larson, 1987]. Roth et al. [1988] found high positive correlations between genome size and neuron size among salamanders. Salamanders with genome sizes of 77 pg had neurons that were six times as large as species with genome sizes of 13.4 pg [Roth et al., 1988]. Sessions and Larson [1987] studied limb regeneration in salamanders with varying genome sizes. They found high negative correlations between genome size and the rates of growth and differentiation of the regenerating limb bud. Their results suggest that there is a negative exponential relationship between genome size on the one hand and cell proliferation and differentiation rates on the other.

The morphological variables that are correlated with genome size are the same variables in the nervous systems of salamanders, some caecilians and frogs, and lepidosirenid lungfishes that have been secondarily simplified during their evolution. Characteristics of the visual system in salamanders include small numbers of large neurons in the retina and optic tectum, which appear to result from reduced rates of cell proliferation [Schmidt and Roth, 1993]. Reduced morphological differentiation of neurons and glia, and reduced rates of cell migration (which give the central nervous system an unlayered appearance) are also characteristic of the visual system in salamanders. We hypothesize that all of these features are the result of increased genome size and that the independent simplification of these characteristics in other amphibians and lepidosirenid lungfishes is due to increased genome size within these groups as well.

Increased genome size has profoundly affected developmental processes in salamanders, not only in the visual system but in the brain and other sensory systems as well. By slowing down the rates of cell proliferation and differentiation, increased genome size results in a truncated pattern of development in which the terminal stages of differentiation fail to occur. This, in turn, leads to paedomorphosis, in which sexually mature adults resemble juveniles or even embryos of ancestral taxa in many features. Many of the unusual features of the visual system in salamanders can be explained by paedomorphosis. For example, the small number of myelinated axons in the optic nerves of salamanders apparently is due to failure of glial precursors to differentiate into mature astrocytes and oligodendrocytes which form myelin sheaths [Linke and Roth, 1989]. In bolitoglossine salamanders, glial precursors in the optic nerve remain in an undifferentiated state throughout adult life [Linke and Roth, 1989].

Paedomorphosis is found in numerous non-neural characteristics of salamanders as well [Wake, 1966]. Perhaps the best known form of paedomorphosis is perennibranchiation, in which sexual maturity is attained in individuals that retain a larval morphology throughout their lives because metamorphosis fails to occur [Gould, 1977]. Perennibranchiation itself does not have a detectable influence on brain morphology. The axolotl (*Ambystoma mexicanum*), a neotenic salamander, exhibits one of the most complex brain morphologies found among salamanders. Remarkably, the brain of the axolotl is even more complex than that of the metamorphosing *Ambystoma macrodactylum* [G. Roth, J. Blanke and D. Wake, unpubl. observ.]. These observations suggest that perennibranchiation is fundamentally different from paedomorphosis in its effects on morphology.

In contrast to perennibranchiation, an even more extreme type of paedomorphosis is observed in bolitoglossine salamanders, in which many characteristics never develop beyond early embryonic stages [Wake and Roth, 1989]. Thus, it appears that the presence of large amounts of DNA in the genome may result in an organism-wide syndrome of paedomorphosis by retarding, disturbing or inhibiting genetic expression during development. However, the cellular mechanisms through which large genome size affects gene expression remain to be elucidated.

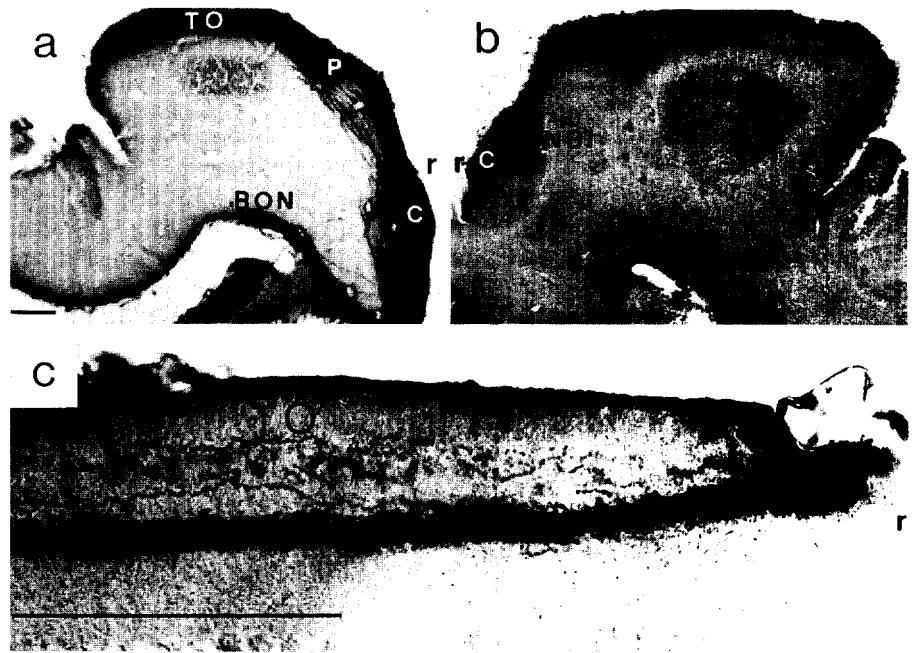
Functional Consequences of Secondary Simplification

The functional integrity of the visual system of salamanders has been affected profoundly by secondary simplification [Roth et al., 1988]. The most obvious detrimental feature is the large size and small number of neurons in the retina as well as in the optic tectum. The size of photoreceptors is directly related to the resolving power of the retina, so that larger photoreceptors will result in a decreased ability to resolve objects that are very close to each other. A smaller number of neurons may also impair visual acuity, may reduce the size of the visual field, and may impair the processing of visual signals in the retina and optic tectum to the extent that such processing depends upon a large population of neurons. In addition, the small proportion of myelinated axons in the optic nerve should reduce the rate of travel of action potentials from retina to optic tectum, which also may have negative consequences.

It is less clear whether absence of lamination in the optic tectum and other parts of the central nervous system has any functional consequences. Certainly, the nearly complete absence of lamination and cell migration has had a major influence on neuroanatomists, who have been misled into believing that many nuclei were absent in salamanders because their nuclei are periventricular and cytoarchitecturally indistinct. Many nuclei previously thought to be absent, such as the nucleus ruber, are present in salamanders but can be identified only by retrograde tracing [Naujoks-Manteuffel et al., 1988].

Bolitoglossine salamanders have the fewest neurons and the lowest degree of morphological differentiation found among salamanders. Yet, electrophysiological recordings from bolitoglossine salamanders show that the resolution power of their visual system is equivalent to that of frogs with 10–20 times more neurons [Roth, 1987; Wiggers and Roth, 1991]. This observation implies that the bolitogloss-

Fig. 2. Sagittal section through the brains of three species of plethodontid salamanders, showing the retinal afferents to the optic tectum stained with horseradish peroxidase. **a** Section through the contralateral thalamus and optic tectum of *Desmognathus ochrophaeus*. **b** Section through the ipsilateral diencephalon and optic tectum of *Eurycea bislineata*, showing ipsilateral retinotectal afferents extending in the layer of retinal afferents and essentially restricted to the rostral tectum. **c** Section through the ipsilateral tectum of *Batrachoseps attenuatus*, showing heavy retinotectal afferents extending throughout the tectum in the deep layer and, more indistinctly, in the intermediate layer of retinal afferents, as is typical of bolitoglossine salamanders. BON=Basal optic neuropil; C=neuropil of the corpus geniculatum thalamicum; NBI=neuropil Bellonci; P=praetectum; r=rostral; TO=tectum opticum. Scale bar=100 μ m. [After Rettig and Roth, 1986.]



sines have compensated somehow for the negative consequences of secondary simplification.

Compensation for Secondary Simplification

The visual system of salamanders exhibits many unique features which help to compensate for the negative effects of secondary simplification and may even enhance visual function. Several features compensate for reduced numbers of neurons in the retina, including an increase in eye size relative to head size [Roth et al., 1988], a shift in the proportion of rods and cones towards fewer rods and more of the much smaller cones [Roth et al., 1988], denser packing of photoreceptors [Roth et al., 1988], and an increase in the representation of the frontal visual field at the expense of the periphery [Roth, 1987]. In addition, there is an increase in the relative size of the optic tectum at the expense of the forebrain and tegmentum, as well as an increase in the density of tectal neurons [Roth et al., 1988]. Furthermore, there is a 1:1 ratio of photoreceptors to retinal ganglion cells throughout the retina, which makes the entire retina a functional fovea [Linke et al., 1986].

A unique feature of bolitoglossine salamanders is the presence of well developed ipsilateral retinotectal projections (fig. 2) [Rettig and Roth, 1986], which have evolved convergently within mammals in megachiropterans and primates [Pettigrew, 1986]. In bolitoglossine salamanders, the

ipsilateral retinotectal projections cover the whole tectum in a deep layer and the rostral tectum in a superficial layer [Rettig and Roth, 1986]. The function of the ipsilateral retinotectal fibers of bolitoglossine salamanders appears to be enhancement of stereopsis [Wiggers and Roth, 1991].

Most amphibians use retinal disparity to estimate the distance of objects from the retina [Roth, 1987]. In amphibian tecta, the disparity in the position of an object on the two retinas is measured between a direct image from the contralateral retina and an indirect image from the ipsilateral retina that is relayed to the optic tectum via the nucleus isthmi. The indirect ipsilateral image is approximately 30 ms older than the direct contralateral one, which leads to inaccuracy in depth perception for moving objects, such as prey [Wiggers and Roth, 1991]. Because bolitoglossine salamanders possess direct ipsilateral retinotectal fibers as well as contralateral ones, these salamanders can compare the direct contralateral image with a contemporaneous direct ipsilateral image in each tectal hemisphere. Bolitoglossines thereby may avoid the inaccuracy that is introduced by comparing the direct contralateral image with an older ipsilateral image from the nucleus isthmi [Wiggers and Roth, 1991].

The presence of ipsilateral retinotectal projections permits a substantial improvement of depth perception in bolitoglossine salamanders. Surprisingly, the development of this compensatory mechanism may itself be a product of secondary simplification. Most vertebrates possess ipsilat-

eral retinotectal projections transiently during development. However, the ipsilateral fibers are eliminated by activity-dependent competition with contralateral fibers at a relatively early developmental stage [Constantine-Paton, 1981]. Competition between ipsilateral and contralateral fibers is dependent upon N-methyl-d-aspartate (NMDA) glutamate receptors. In salamanders, competition between ipsilateral and contralateral fibers fails to occur, perhaps because the normal pattern of development of the glutamate receptors is truncated. Thus, while increased genome size and secondary simplification have constrained visual function significantly, they also have provided an opportunity for the evolution of mechanisms that have mitigated the effects of the constraints.

The development of ipsilateral retinotectal projections in salamanders is an example of ontogenetic repatterning, which involves non-terminal changes in the temporal sequence of ontogenetic processes [Roth and Wake, 1985; Wake and Roth, 1989]. Ontogenetic repatterning permits an organism to escape from developmental constraints associated with an ancestral ontogeny [Wake and Roth, 1989]. In salamanders, ipsilateral retinotectal projections develop much earlier during ontogeny than in outgroups. Furthermore, competition between ipsi- and contralateral fibers fails to occur, which results in an elaboration of the pathway in salamanders, compared to that in most other vertebrates. In the case of bolitoglossine salamanders, we believe that enhancement of stereopsis is the result of repatterning of the ancestral ontogeny of retinotectal fibers.

Conclusions

Whereas increasing brain complexity has evolved independently within most vertebrate classes, the nervous systems of salamanders, some other amphibians and lepidosirenid lungfishes exhibit secondary simplification. We

believe that an increase in genome size is primarily responsible for secondary simplification and that genome size itself has increased through a selfish replication mechanism. This history of the brain in salamanders, if correctly reconstructed, provides a challenge to the traditional view of brain evolution. Rather than a history of adaptation to the demands of a changing world through the process of natural selection, salamander brains tell a story in which internal constraints have arisen from non-adaptive processes, like the tendency for selfish DNA sequences to increase their representation in the genome. Natural selection may well have played an important role in limiting genome size, or in increasing the representation of individuals with compensatory mechanisms. However, it is difficult to invoke natural selection as the prime mover responsible for the evolutionary changes in salamander brains. Instead, it appears that natural selection has done its best to maintain visual function in spite of interference from non-adaptive processes.

It would have been convenient for developmental neurobiologists if salamanders had, in fact, been left behind by natural selection while the other vertebrates evolved increasing brain complexity independently. If salamander brains were primitively simple, then we would have a living bauplan available for study. However, because salamander brains are secondarily simplified, rather than primitively simple, and because several unique compensatory mechanisms have evolved among salamanders, it is dangerous to assume that any features of salamanders represent the primitive condition for vertebrates.

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