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Zebrin II Is Expressed in Sagittal Stripes in the Cerebellum of Dragon Lizards (*Ctenophorus* sp.)

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Cerebellum · Zebrin · Aldolase C · Purkinje cells · Lizards · Central netted dragon

Abstract
Aldolase C, also known as zebrin II (ZII), is a glycolytic enzyme that is expressed in cerebellar Purkinje cells of the vertebrate cerebellum. In both mammals and birds, ZII is expressed heterogeneously, such that there are sagittal stripes of Purkinje cells with high ZII expression (ZII+) alternating with stripes of Purkinje cells with little or no expression (ZII–). In contrast, in snakes and turtles, ZII is not expressed heterogeneously; rather all Purkinje cells are ZII+. Here, we examined the expression of ZII in the cerebellum of lizards to elucidate the evolutionary origins of ZII stripes in Sauropsida. We focused on the central netted dragon *(Ctenophorus nuchalis)* but also examined cerebellar ZII expression in 5 other dragon species (*Ctenophorus* spp.). In contrast to what has been observed in snakes and turtles, we found that in these lizards, ZII is heterogeneously expressed. In the posterior part of the cerebellum, on each side of the midline, there were 3 sagittal stripes consisting of Purkinje cells with high ZII expression (ZII+) alternating with 2 sagittal stripes with weaker ZII expression (ZIIw). More anteriorly, most of the Purkinje cells were ZII+, except laterally, where the Purkinje cells did not express ZII (ZII–). Finally, all Purkinje cells in the auricle (flocculus) were ZII–. Overall, the parasagittal heterogeneous expression of ZII in the cerebellum of lizards is similar to that in mammals and birds, and contrasts with the homogenous ZII+ expression seen in snakes and turtles. We suggest that a sagittal heterogeneous expression of ZII represents the ancestral condition in stem reptiles which was lost in snakes and turtles.

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Introduction

Amongst tetrapods, there is considerable variation in the gross anatomy of the cerebellum. In amphibians, snakes, and turtles, the cerebellum is a thin dome or sheet of cells, whereas in mammals and birds, the cerebellum is highly developed and divided into several transverse lobules or folia [Larsell, 1967; Voogd and Glickstein, 1998]. In mammals and birds, it has been shown that the cerebellum is organized into "zones" that lie in the sagittal plane [Voogd and Bigare, 1980; Arends and Zeigler, 1991]. These sagittal zones are apparent with respect to several aspects of cerebellar anatomy and physiology: (i) climbing fibres from particular subnuclei in the inferior

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olive project to sagittal zones in the cerebellar cortex, (ii) mossy fibres from particular nuclei project to sagittal zones, (iii) Purkinje cells within sagittal zones have common projections to the cerebellar and vestibular nuclei, and (iv) Purkinje cells within sagittal zones have similar response properties and tend to fire synchronously [Voogd, 1967; Ekerot and Larson, 1973; Andersson and Oscarsson, 1978; Llinas and Sasaki, 1989; De Zeeuw et al., 1994; Voogd and Glickstein, 1998; Wu et al., 1999; Ruigrok, 2003]. The cerebellum in reptiles is also organized into sagittal zones, although there are only a few studies in this regard [Bangma et al. 1983; Bangma and Ten Donkelaar, 1984].

Numerous molecular markers also show a sagittal expression pattern [for reviews, see Herrup and Kuemerle, 1997; Apps and Garwicz, 2005; Apps and Hawkes, 2009; Cerminara et al., 2015]. The most extensively studied of these markers is zebrin II (ZII), which is expressed in Purkinje cells. The expression of ZII across the cerebellum is heterogeneous such that there are sagittally oriented stripes of Purkinje cells that strongly express ZII alternating with sagittal stripes of Purkinje cells that show little or no ZII expression. Those Purkinje cells that show strong ZII expression are generally referred to as ZII+, whereas those that show little or no ZII expression are referred to as ZII– [Brochu et al., 1990; Hawkes, 1992; Hawkes and Herrup, 1995]. The expression of ZII has been studied in several mammalian species and revealed a consistent pattern of ZII+ and ZII– stripes [Leclerc et al., 1992; Ozol et al., 1999; Armstrong and Hawkes, 2000; Sanchez et al., 2002; Larouche et al., 2003; Marzban et al., 2003; Sillitoe et al., 2003a, b; Marzban and Hawkes, 2011; Marzban et al., 2011; Marzban et al., 2015]. Alternating sagittal ZII+ and ZII– stripes are found in lobules I–V, and lobules VIII and dorsal IX. These are referred to as the anterior region (AR) and the posterior region (PR), respectively. Stripes are not present in lobules VI–VII or ventral IX and X; instead most Purkinje cells are ZII+. These are known as the central region (CR) and the nodular region (NR), respectively.

ZII has also been studied in several species of birds [Pakan et al., 2007; Iwaniuk et al., 2009; Marzban et al., 2010; Corfield et al., 2015, 2016]. As in mammals, ZII is expressed heterogeneously such that there are ZII+ and ZII– sagittal stripes, and the pattern of expression is remarkably similar to that observed in mammals: alternating ZII+ and ZII– stripes are apparent in lobules II–V (AR) and VIII and IX (PR), whereas most Purkinje cells in lobules VI, VII (CR), and X (NR) are ZII+. The only obvious difference between mammals and birds with respect to the pattern of ZII expression is in lobule I, which has stripes in mammals (except for bats [Kim et al., 2009]), but is uniformly ZII+ in birds (lingular region). The remarkable degree of similarity between avian and mammalian cerebella with respect to the alternation in ZII+ and ZII– regions suggests it is a homologous character that was present in stem reptiles.

ZII expression has been studied previously in 2 non-avian reptiles: the western diamondback rattlesnake (*Crotaus atrox*) [Aspden et al., 2015] and the red-eared terrapin (*Trachemys scripta elegans*) [Sillitoe et al., 2005]. In both species, ZII stripes are not present, and all Purkinje cells are uniformly ZII+. This suggests that ZII stripes have been lost in both turtles and snakes, or that they evolved convergently in birds and mammals. The cerebellum of both turtles and snakes is relatively small and simple compared with lizards and crocodilians, and consists of a single unfolded dome [Larsell, 1967]. Turtles and snakes are also both highly derived, and the uniform ZII+ expression in the cerebellum may not be representative of the expression pattern in extinct stem reptiles or other extant reptiles. To aid in resolving the question of the evolutionary history of ZII stripes in reptiles, we examined ZII expression in the cerebellum of dragons (*Ctenophorus* spp.), a genus of agamid lizards (Agamidae) endemic to Australia. We concentrated primarily on the central netted dragon (*Ctenophorus nuchalis*), but also examined brains from 5 other *Ctenophorus* species.

Materials and Methods

Ten adult male lizards were field collected in Australia and transported to the Australian National University (ANU) in Canberra, ACT, Australia. The animals were then perfused and their brains stored in 4% paraformaldehyde [Hoops, 2015]. Five of the brains were from central netted dragons (*C. nuchalis*) in addition to 1 specimen each of 5 other species from the same genus: painted dragon (*Ctenophorus pictus*), peninsula dragon (*Ctenophorus fionni*), crested bicycle dragon (*Ctenophorus cristatus*), red-barred dragon (*Ctenophorus vadnappa*), and ochre dragon (*Ctenophorus tjanjalka*). All animals were collected under permit from the Government of South Australia (No. U25961-1) and processed with the approval of the ANU Animal Ethics Executive Committee (No. A2011/49).

The brains were equilibrated in a 30% sucrose solution (0.1 M PB), embedded in gelatin, and serially sectioned at a thickness of 40 μm. All but 1 of the brains were sectioned in the coronal plane: 1 central netted dragon brain was cut in the sagittal plane. All sections were collected in 0.1 M PBS and divided into 4 alternate series. Sections from 1 series were Nissl-stained (cresyl violet), the 2nd and 4th series were processed for ZII expression, and the 3rd series was processed for both ZII and calbindin expression. Calbindin is expressed in all Purkinje cells [Bastianelli, 2003] and commonly

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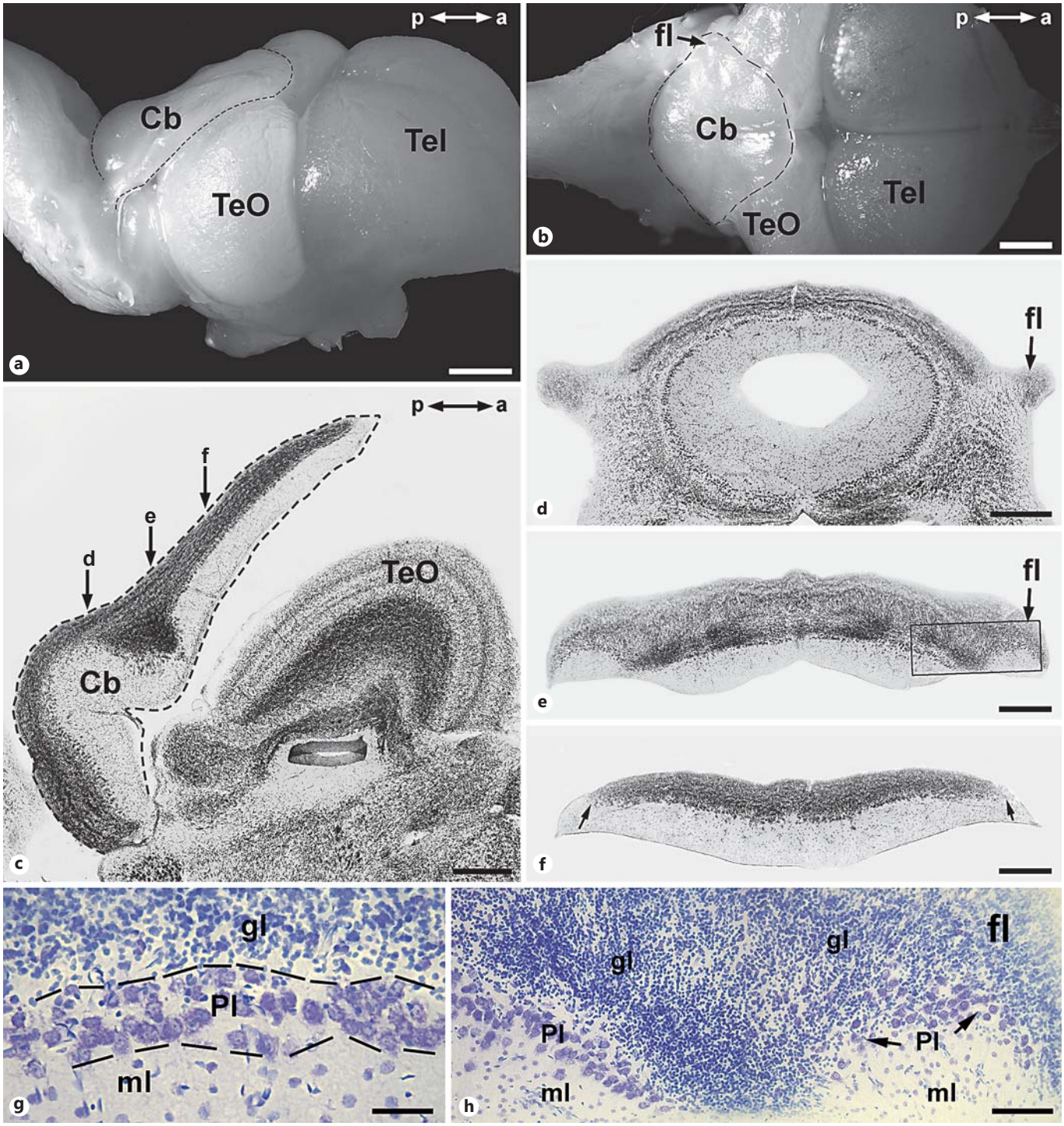

Fig. 1. The cerebellum of the central netted dragon (*C. nuchalis*). Lateral (**a**) and dorsal views (**b**) of the brain highlighting the cerebellum (Cb) enclosed by the dashed lines. Midsagittal section (**c**) through the cerebellum stained for Nissl. **d–h** Nissl-stained coronal sections through the cerebellum. **d–f** Lower magnification sections are from posterior to anterior, at levels indicated by the arrows in **c**. **g** The borders of the Purkinje layer (Pl) are indicated by the broken lines. **h** A higher magnification of the area indicated by the black rectangle in **e** including the lateral flocculus (fl). **f** The arrows indicate the extent of the Purkinje cell layer. a, anterior; gl, granular layer; ml, molecular layer; p, posterior; Tel, telencephalon; TeO, optic tectum. Scale bars, 1 mm (**a**, **b**), 400 μm (**c–f**), 50 μm (**g**), and 100 μm (**h**).

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used in conjunction with ZII to determine whether ZII– stripes are devoid of Purkinje cells or simply lack expression of ZII [Pakan et al., 2007]. Those sections processed for ZII were first rinsed in wells containing 0.1 M PBS and then incubated in blocking serum (10% normal donkey serum; Jackson ImmunoResearch Laboratories) for 1 h at room temperature. The sections were then incubated at 4°C for 5 days in 0.9% NaCl in 0.1 M PBS (pH 7.4) containing 0.1% Triton X-100 and an antibody to aldolase C (1:1,000; goat polyclonal antibody; sc-12065, Santa Cruz Biotechnologies, Santa Cruz, CA, USA). Ahn et al. [1994] concluded that the protein carrying the ZII epitope is the metabolic enzyme aldolase C. After 5 rinses in 0.1 M PBS, the sections were incubated for 4 h at room temperature in Alexa Fluor 594 conjugated donkey anti-goat antibody (Jackson ImmunoResearch Laboratories; diluted 1:100 in PBS, 2.5% normal donkey serum, and 0.4% Triton X-100). After 4 h, sections were rinsed 5 times in 0.1 M PBS, mounted on gelatinized slides, and briefly left to dry in the open air. For the series processed for both ZII and calbindin, the above procedure was followed except, after the blocking step, the tissue was incubated in a solution containing both anti-aldolase C and anti-calbindin (1:2,000; rabbit polyclonal; CB38, Swant) antibodies. The secondary antibody to anti-calbindin was an Alexa Fluor 488 conjugated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories; diluted 1:200 in PBS, 2.5% normal donkey serum, and 0.4% Triton X-100). For negative controls, a section from each series was processed without the primary antibody. In addition, we processed a section from a pigeon (*Columba livia*) cerebellum with each series as a positive control.

Microscopy and Image Analysis

Sections were viewed with a compound light microscope (Leica DMRE) equipped with the appropriate fluorescence filters for visualization. Images were acquired using a Retiga EXi FAST Cooled Mono 12-bit camera (QImaging) and analyzed with OpenLab imaging software (Improvision). Photos were then stitched together in PTGui (New House Internet Services BV) for visualization of the entire sections. Adobe Photoshop (San Jose, CA, USA) was used to adjust for brightness and contrast.

Nomenclature

Although we use an antibody to aldolase C, and not the ZII antibody originally used by Hawkes and Herrup [1995], we refer to the resultant labelling as ZII expression. Ahn et al. [1994] clearly showed that the protein carrying the ZII epitope is the metabolic enzyme aldolase C, and the patterns of expression by Purkinje cells to both the ZII antibody and an antibody to aldolase C were identical. Following Voogd and Bigare [1980], we refer to the sagittal organization of the cerebellum observed with respect to anatomical connections and Purkinje cell response properties as "zones." Although ZII shows a sagittal expression pattern as well, we refer to this pattern of ZII expression as "stripes" after Hawkes and Herrup [1995]. They referred to transverse areas of the cerebellum showing similar patterns of ZII expression as "transverse zones." To avoid confusion with sagittal zones, we refer to the former as "transverse regions." The lateral protrusion of the cerebellum, which is present in most vertebrates, is referred to as both the auricle and the flocculus [Larsell, 1967].

Results

Figure 1 shows the basic anatomy of the cerebellum of *C. nuchalis*. The cerebellum is not large or complex, but consists of a single sheet (Fig. 1a, b). Unlike other reptiles, or amphibians, the cerebellum in lizards is inverted, such that it is effectively folded backwards and lies atop the optic tectum, leaving the granule cell layer exposed dorsally [Larsell, 1967] (Fig. 1a–c). As in birds and mammals, the flocculus (a.k.a. auricle) protrudes laterally (Fig. 1b, d). Throughout most of the cerebellum, the Purkinje cell layer is 1–3 cells in depth (Fig. 1g). Midway through the cerebellum, there is a distinct swelling that stains very dark for Nissl (Fig. 1c). This swelling consists of clusters of Purkinje cells apparently enmeshed with granule cells (Fig. 1e). Purkinje cells are clearly present in the flocculus (Fig. 1h).

ZII was expressed heterogeneously in Purkinje cells of *C. nuchalis,* such that some had strong ZII expression (ZII+), some had no ZII expression (ZII–), and others had a weak amount of ZII expression (ZIIw). Figure 2 shows 3 coronal sections through the cerebellum of *C. nuchalis*. The sections are processed for both ZII (left, red) and calbindin (green, middle), and the overlays are also shown (right). Figure 2a–c shows a section from the posterior part of the cerebellum where clear sagittally oriented ZII stripes were readily apparent. On either side of the midline, there were 3 ZII+ stripes, alternating with

Fig. 2. Zebrin II (ZII) expression in the Purkinje cells of the cerebellum in lizards. Photomicrographs of coronal sections are shown as triptychs, with ZII immunoreactivity shown in red (**a**, **d**, **g**, **j**, **m**, **p**), calbindin (Calb) in green (**b**, **e**, **h**, **k**, **n**, **q**), and the overlays (**c**, **f**, **i**, **l**, **o**, **r**). **a–l** The 4 sections shown are from *C. nuchalis*. **a–c** A lower magnification photomicrograph section from the posterior cerebellum. Part of this section (indicated by the white rectangle in **b**) is shown at higher magnification (**d–f**). In this section, there are clear sagittal stripes of high ZII (ZII+) expression alternating with regions of weaker ZII expression (ZIIw). **g–i** A coronal section through the flocculus (fl). Note that medially the Purkinje cells are ZII+, but those in the flocculus do not express ZII (ZII–). **j–l** A more anterior section where the medial Purkinje cells are also ZII+ and the lateral Purkinje cells are ZII–. **m–o** Posterior cerebellum in *C. fionni* at a level similar to the sections shown in **a–c**. **p–r** Cerebellum of *C. cristatus* at a level similar to the sections shown in **g–i**. Note again that the Purkinje cells in the flocculus are ZII–. **q** Dotted line indicates the midline. l, lateral; m, medial. Scale bars, 250 μm (**c**; applies for **a–c**) and 100 μm (**f**, **i**, **l**, **o**, **r**; applies for **d–r**).

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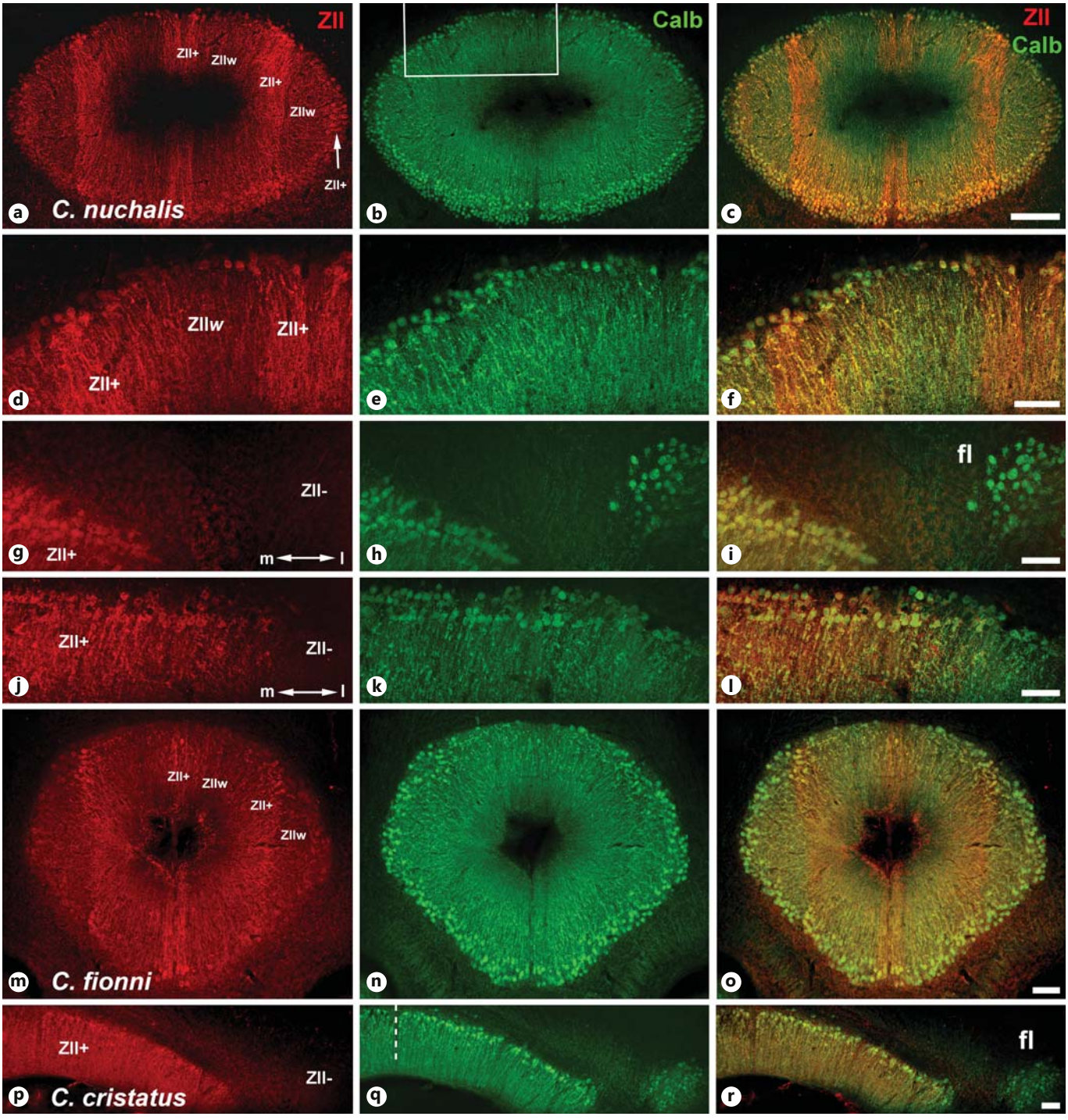
ZIIw stripes. In more anterior sections, ZII– Purkinje cells were clearly seen laterally (Fig. 2j–l), including in the flocculus (Fig. 2g, h).

This same pattern of ZII expression was observed in all the cerebella of all 5 dragon species. A section from *C. fionni* is shown to indicate the ZII+ and ZIIw stripes in the posterior cerebellum (Fig. 2m–o). A section from *C. cristatus* at a more anterior level is shown, emphasizing that Purkinje cells in the flocculus were ZII– (Fig. 2p–r).

The distribution of ZII+, ZIIw, and ZII– Purkinje cells across the entire cerebellum of *C. nuchalis* is shown in Figure 3. This is shown with a series of coronal sections



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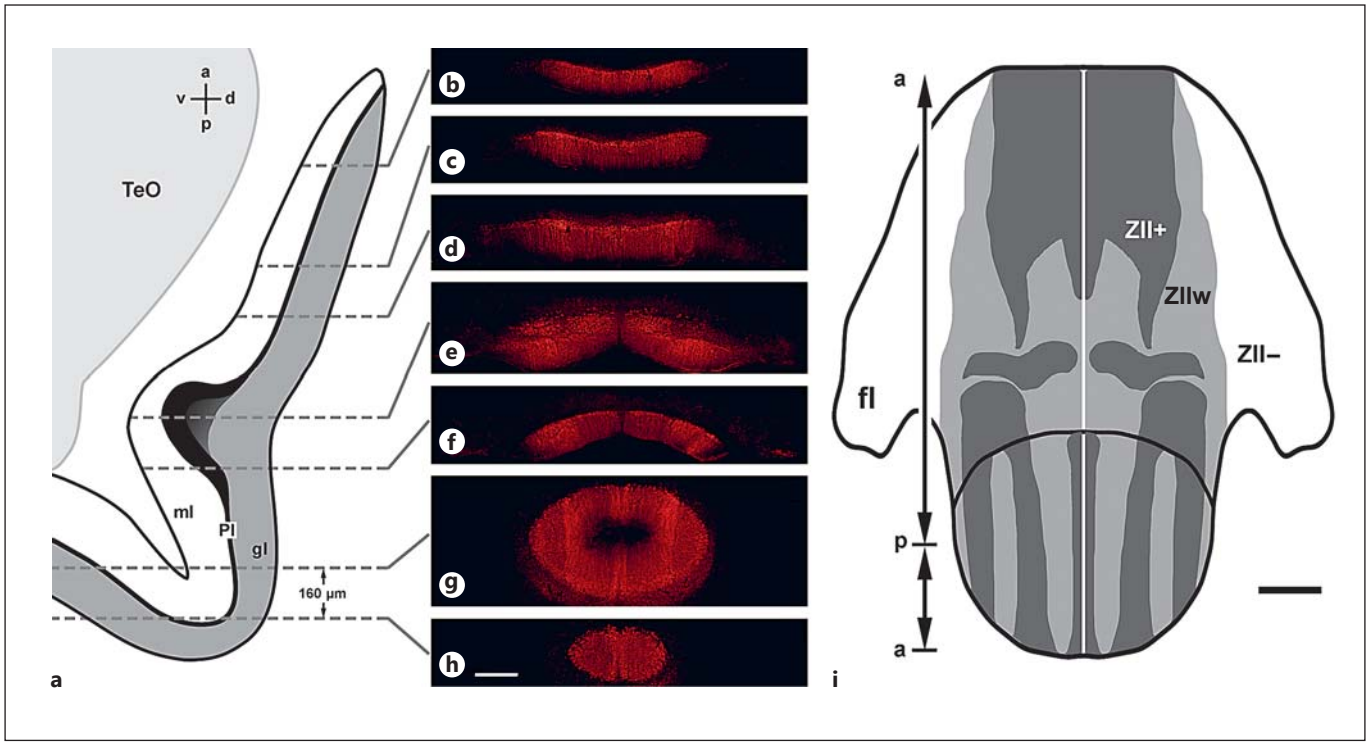

Fig. 3. Zebrin II (ZII) expression throughout the cerebellum of the central netted dragon *(C. nuchalis)*. **a** Drawing of a midsagittal section of the cerebellum. **b–h** Photomicrographs of ZII expression in coronal sections from rostral (**b**) to caudal (**h**). The sections are either 160 or 320 µm apart, and the rostrocaudal locations are indicated by the dashed lines. **i** Reconstruction of the ZII expression across the entire cerebellum. ZII+, ZIIw, and ZII– regions are shown. a, anterior; d, dorsal; p, posterior; v, ventral. For other abbreviations, see caption to Figure 1. Scale bars, 400 µm (**h**; applies to **b–h**) and 500 µm (**i**).

from the same individual (Fig. 3a–h). In addition, the patterns of ZII expression across the entire "unfolded" cerebellum are shown in Figure 3i based on a reconstruction using all sections in the three series that were processed for ZII (Fig. 3i). The reconstruction followed the same procedure used by Vibulyaseck et al. [2015]. Two brains were reconstructed in this manner, and the same pattern of ZII expression was observed. Posteriorly, 3 pairs of alternating ZII+ and ZIIw stripes were observed (Fig. 3g, h). Moving anteriorly, the medial ZII+ stripe was replaced with ZIIw Purkinje cells, and the 2 lateral ZII+ stripes merge into a single wider ZII+ stripe (Fig. 3f). At this rostro-caudal level, the Purkinje cells in the flocculus are clearly ZII– (Fig. 3f; see also Fig. 2g–i). At the level of the Purkinje cell clusters, the medial Purkinje cells are ZII+, whereas laterally they are ZII– (Fig. 3e). More anteriorly, more Purkinje cells are ZIIw, there appear to be two ZII+ stripes on either side of the midline (Fig. 3d), and ZII– Purkinje cells are found laterally. Finally, in the most anterior 25% (approximately) of the cerebellum, most Purkinje cells are ZII+, although the ZII– Purkinje cells persist laterally (Fig. 3b, c). Between the medial ZII+ Purkinje cells and the lateral ZII– stripes, there appear to be a few ZIIw Purkinje cells.

Discussion

In birds and mammals, the cerebellum is relatively large and highly fissured, consisting of several lobules or folia. In non-avian reptiles, the cerebellum is relatively smaller, and much simpler, consisting of just a "sheet" or "dome" above the 4th ventricle. The largest and most complex cerebellum among the non-avian reptiles is found in the crocodilians, where there are two shallow transverse fissures that divide the cerebellum into 3 lobes [Larsell, 1967]. Lizards might have the most unusual cerebellum insofar as it is inverted (Fig. 1) [Larsell, 1967]. Despite these differences in gross morphology, the basic circuitry of the cerebellum is highly similar. It consists of

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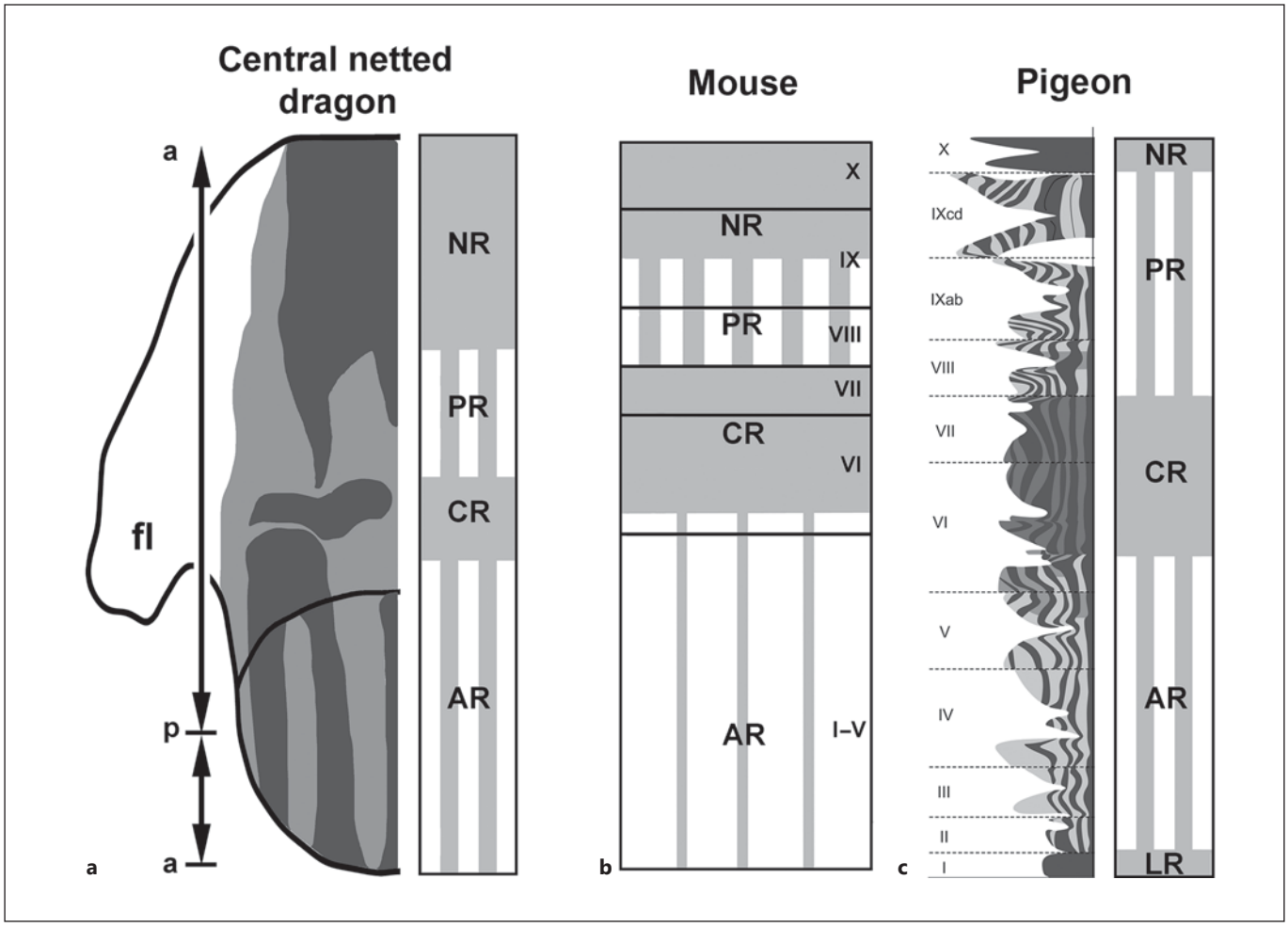

Fig. 4. A comparison of ZII expression in the central netted dragon with that in birds and mammals. A reconstruction of the ZII expression is shown for the central netted dragon *(C. nuchalis)* (**a**) and pigeon (*C. livia*) (**c**; adapted from Corfield et al. [2015]). Schematics of the basic transverse zones of ZII expression are also shown for the lizard (right side of **a**), pigeon (right side of **c**), and mouse (**b**; adapted from Marzban et al. [2011]). In these basic schematics, the stripes in the anterior (AR) and the posterior region (PR) for the lizard and pigeon are not meant to represent the relative size or number of the ZII stripes, rather just that the regions contain stripes. a, anterior; CR, central region; NR, nodular region; p, posterior. See text for details.

2 major afferent systems (mossy and climbing fibres), a single output (Purkinje cells), and a few interneurons, and the connectivity is much the same in birds, mammals, and reptiles [Llinas and Hillman, 1969; ten Donkelaar, 1998; Butler and Hodos, 2005; Glickstein et al., 2009].

In mammals and birds, numerous studies have shown that the basic unit of cerebellar organization is the sagittal zone [Voogd and Bigare, 1980]. This sagittal organization was reflected by the expression of molecular markers such as ZII [Hawkes, 1992, Herrup and Kuemerle, 1997; Apps and Hawkes, 2009] and other aspects of cerebellar anatomy and physiology: climbing and mossy fibre afferentation, Purkinje cell projections, and Purkinje cell response properties [Voogd, 1967, Ekerot and Larson, 1973: Andersson and Oscarsson, 1978; Llinas and Sasaki, 1989; De Zeeuw et al., 1994; Voogd and Glickstein, 1998; Wu et al., 1999; Ruigrok, 2003, Winship and Wylie, 2003; Wylie et al., 2003; Apps and Garwicz, 2005; Horn et al., 2010; Pakan et al., 2011]. Although there are only a few studies, the cerebella in lizards, turtles, and snakes are also organized in sagittal zones [Bangma et al., 1983; Bangma and ten Donkelaar, 1982, 1984].

In the present study, we found that ZII is expressed heterogeneously in the cerebellum of lizards in the genus

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Ctenophorus, such that there are sagittal stripes that vary in the intensity of the degree of ZII expression. We identified three degrees of expression: Purkinje cells that showed high, weak, and no ZII expression, which we have designated as ZII+, ZIIw, and ZII–, respectively. Traditionally, in studies of ZII heterogeneity, Purkinje cells with weak or no ZII expression are designated as ZII– [Hawkes, 1992; Pakan et al., 2007; Marzban et al., 2010]. Thus, traditionally, the ZIIw identified in the present study would be identified as ZII–. However, because there was such a clear distinction, in all individuals, between the Purkinje cells that expressed little ZII versus those that expressed no ZII (e.g., Fig. 2), we opted to differentiate ZIIw from ZII– cells.

It is somewhat surprising that we observed sagittal stripes of alternating ZII immunoreactivity in the lizards given that the only previous studies on non-avian reptiles, a snake [Aspden et al., 2015] and a turtle [Sillitoe et al., 2005], found that all Purkinje cells were ZII+. Although it is possible that the presence of ZII stripes in *Ctenophorus* is unique among lizards or even among non-avian reptiles, we suggest that ZII stripes were lost in snakes and turtles. Previously, it has been argued that the pattern of ZII stripes across the cerebellum is highly similar in birds and mammals (Fig. 4). By examining ZII expression in the cerebella of several mammals [Leclerc et al., 1992; Ozol et al., 1999; Armstrong and Hawkes, 2000; Sanchez et al., 2002; Larouche et al., 2003; Marzban et al., 2003; Sillitoe et al., 2003a, b; Marzban and Hawkes, 2011; Marzban et al., 2011; Marzban et al., 2015], it was argued that the vermis consists of 4 transverse regions (Fig. 4b): an AR covering lobules I–V and consisting of alternating ZII+ and ZII– stripes; a CR covering lobules VI and VII where all Purkinje cells are ZII+; a PR that includes lobule VIII and part of IX and consisting of ZII stripes; and an NR covering lobule X and the posterior part of lobule IX where all Purkinje cells are ZII+. A strikingly similar pattern occurs in birds [Pakan et al., 2007; Iwaniuk et al., 2009, Marzban et al., 2010; Vibulyaseck et al., 2015; Corfield et al., 2015, 2016]. The pattern of ZII expression in the pigeon is shown on an unfolded cerebellum [based on Corfield et al., 2015] and as a schematic (Fig. 4c). As in mammals, there is an NR consisting of folium X where all Purkinje cells are ZII+, a PR covering folia VIII and IXcd consisting of sagittal ZII stripes, a CR where all Purkinje cells are ZII+ in folia VI and VII, and an AR of ZII stripes covering folia II–V. In birds, folium I, the lingula, is uniformly ZII+. A ZII+ lingular region is not found in mammals, with the exception of microchiropteran bats [Kim et al., 2009]. In Figure 4a, we show the pattern of ZII expression in the lizard with a schematic of the medial regions, to illustrate its similarity to that of birds and mammals. Note that as the cerebellum in lizards is inverted, the anterior portion that is folded back over the optic tectum actually corresponds to the posterior cerebellum in birds and mammals. Considering the medial part of the lizard cerebellum, there appear to be the same 4 regions observed in mammals. In the anterior part of the lizard cerebellum, there are ZII stripes, as in mammals. At the level of the Purkinje cell clusters, all cells tend to be ZII+, and as such this resembles the mammalian CR. This is followed by a ZII striped region resembling the PR, and finally a region where most Purkinje cells are ZII+, resembling the NR.

Given that ZII is heterogeneously expressed in the cerebellum of *Ctenophorus,* such that there are sagittally oriented ZII+/– stripes, and that the pattern of the ZII stripes is similar to that of mammals and birds, we surmise that ZII stripes were present in the cerebella of stem reptiles. In turtles and snakes, ZII stripes seem to have been lost, as all Purkinje cells are ZII+. Given that recent studies have placed the turtles as a sister group to the archosaurs [Crawford et al., 2012; Fong et al., 2012; Lu et al., 2013], the trait appears to have been lost twice. In a previous report [Aspden et al., 2015], we speculated that loss of ZII stripes in snakes and turtles could result from some simple changes. As both the NR and CR in birds and mammals consist of uniformly ZII+ Purkinje cells, the cerebellum of snakes and turtles may have been reduced to an NR, or a CR. A reduction to NR is more plausible because the vestibulocerebellum (IXcd and X) is considered to be the most highly conserved [Larsell, 1967; Voogd and Glickstein, 1998], and there are cerebellar projections to the vestibular nuclei in turtles [Bangma et al., 1983]. Functionally, these changes in cerebellar organization likely reflect locomotion and general body morphology in both snakes and turtles. Snakes lack limbs and turtles have little to no axial mobility due to the structure of the carapace. These changes in locomotor structures could have resulted in a decrease in cerebellar processing requirements for coordinated movement, leading to the observed changes in cerebellar anatomy. But what are the consequences of having a cerebellum consisting of only ZII+ cells? The functional significance of the heterogeneous expression of ZII is still a matter of debate [Cerminara, et al., 2015]. Recent research suggests that ZII+ and ZII– Purkinje cells differ with respect to the underlying mechanism of synaptic plasticity during motor learning; ZII+ cells rely more on long-term potentiation, whereas ZII– cells rely more on long-term depression

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[Wadiche and Jahr, 2005; Zhou et al., 2014]. Thus, the requirement for cerebellar zones relying on long-term depression more so than long-term potentiation may have been lost in turtles and snakes, which have only ZII+ Purkinje cells.

Conclusions

We showed that there is a heterogeneous expression of ZII in Purkinje cells in the cerebellum in lizards of the genus *Ctenophorus,* such that there are sagittal stripes of Purkinje cells with high ZII expression (ZII+) alternating with stripes with little or no ZII expression (ZIIw, ZII–). This contrasts with previous studies in snakes [Aspden et al., 2015] and turtles [Sillitoe et al., 2005] in which all Purkinje cells are ZII+. We suggest that the pattern of ZII expression across the cerebellum is similar to what is observed in birds and mammals, and that ZII stripes represent the ancestral condition that was subsequently lost in turtles and snakes.

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Disclosure Statement

None of the authors has any conflict of interest.

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