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Development of the dorsal thalamus in a reptile: identification of subdivisions and their associated nuclei

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ABSTRACT How the dorsal thalamus of amniotes (reptiles, birds, and mammals) is organized remains an important but incompletely answered question. Identification of meaningful subdivisions would greatly aid in its understanding. Because the dorsal thalamus is more simply organized during development, studies have examined this structure during embryogenesis. Most reports using this approach have examined the developing dorsal thalamus in mammals and birds. Only rarely has the development of the dorsal thalamus been investigated in reptiles. Regardless, any approach to identify subdivisions, the presumed building blocks of the dorsal thalamus, should include representatives of all three classes of vertebrates. To fill this gap in knowledge, the development of the dorsal thalamus was investigated in *Alligator mississippiensis*, a member of the reptilian group most closely related to birds. As the first detailed study of its kind, cytoarchitecture and calretinin expression were used to examine dorsal thalamus development. Three subdivisions, termed tiers, and the individual nuclei originating from each tier, were identified. These three tiers were similar to the subdivisions found in birds and, to a limited extent, in mammals. Taken together, these early subdivisions may represent the common building blocks of the dorsal thalamus and provide clues to understand how evolution has sculpted this structure in amniotes.

KEYWORDS: calretinin, immunohistochemistry, cytoarchitecture, evolution, tiers

Introduction

The dorsal thalamus is present in all amniotes (reptiles, birds, and mammals) (Nieuwenhuys *et al*., 1998; Butler and Hodos, 2005). As defined in the present analysis, the dorsal thalamus is characterized by two features. One is its origin from the alar portion of prosomere 2, the posterior parencephalon (Puelles, 2001a; Redies *et al*., 2000; Puelles and Martinez, 2013; Amat *et al*., 2022). The other is its connection with the telencephalon (Jones, 2007). Furthermore, not only does the dorsal thalamus interconnect the brainstem with the telencephalon but it also processes information independently (Sherman, 2007; Rikhe *et al*., 2018). These features make the dorsal thalamus an essential brain structure.

Despite its importance, how the dorsal thalamus is organized remains incompletely understood. Without this knowledge, comparisons of the dorsal thalamus and its respective nuclei among amniotes are problematic. In order to understand dorsal thalamus organization, identification of meaningful subdivisions should prove useful. These divisions could provide a common reference point, a so-called 'bauplan', which could then serve as the building blocks of the dorsal thalamus in any amniote (Bayer and Altman, 1995a; Amat *et al*., 2022). Viewed from this perspective, these core components and their derivatives could be compared among various species to determine what has changed, what has been lost, and what is new and unique.

A variety of subdivisions of the dorsal thalamus has been proposed in adult animals. In mammals, where the vast majority of studies have been performed, categories have been devised based on features such as thalamo-telencephalic connections (Macchi, 1983), immunohistochemical characters (Jones, 1998; 2007) and certain anatomical and circuitry properties (Sherman and Guillery, 2006; 2013). However, none of these schemes have accounted for all known nuclei across mammals. To date, the best categorization of dorsal thalamic subdivisions in mammals remains the classical description based on type of information transmitted and location (Jones, 2007).

Since subdivisions in adult amniotes have yet to uncover a universal plan, other approaches to understand dorsal thalamic organization have been explored. One avenue of investigation has been examination of its development. This choice is based on the notion that the dorsal thalamus is more simply organized during embryogenesis when compared with adult forms and therefore more easily understood. Accordingly, multiple reports have described the development of the dorsal thalamus based on cytoarchitecture in a variety of amniotes. These studies have determined when individual dorsal thalamic nuclei can be identi-

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fied and what morphological changes occur as development proceeds (Wyeth, 1924, *[Sphenodon punctatus]*); Rendahl, 1924, [*Gallus*]); Kuhlenbeck, 1937, *[Gallus]*); Rose, 1942 [Lagomorpha]); Cooper, 1950, *[Homo sapiens]);* Bergquist, 1953, [*Lepidochelys olivacea*; *Chrysemys marginata*]); Ströer,1956, *[Rattus]*); Niimi *et al.*, 1962, *[Mus]);* Coggeshall, 1964, *[Rattus]*); Keyser, 1972, [*Cricetulus griseus*]); Senn, 1979, [*Lacerta sicula; Natrix natrix; Chelydra serpintina; Alligator mississippiensis*]); Hergueta *et al*., 1993, *[Emys orbicularis]*). However, reports examining the developing amniote dorsal thalamus that specifically document subdivisions and their derived nuclei are few. These categories have been described in mammals and birds where they have been termed pronuclei (Rose,1942), tiers (Redies *et al*., 2000; Puelles *et al*., 2019; Puelles and Martinez, 2013), and cytogenetic subdivisions (lobes, lobules, sublobules) (Altman and Bayer, 1988).

Besides providing the building blocks for understanding dorsal thalamus organization, other potential benefits of recognizing developmental subdivisions have been proposed. These include the possibility that these subdivisions form the basis for distinguishing different types of sensory systems. This, in turn, might influence the evolution of these circuits (Butler, 1994; 2022).

Subdivisions of the developing dorsal thalamus have been investigated in mammals (Rose, 1942; Altman and Bayer, 1988; Gezelius and López-Bendito, 2017; González *et al*., 2002); and birds (Redies *et al*., 2000; Martinez-de-la-Torre *et al*., 2002). Nevertheless, any attempt to provide a fundamental bauplan of dorsal thalamic subdivisions during development in amniotes should include reptiles. Divisions of dorsal thalamic nuclei in adult lizards have been suggested to reflect an embryonic pattern (Dávila *et al*., 2000). However, to my knowledge, no developmental study has specifically investigated this problem in detail in any reptile. To fill this gap in knowledge, the present analysis was undertaken in *Alligator mississippiensis*, a member of the group of reptiles most closely related to birds (Whetstone and Martin, 1979; Hedges, 1994). I sought to determine if subdivisions were indeed present in the *Alligator* during dorsal thalamus development, and, if so, which nuclei originated from which compartments. This analysis begins when the dorsal thalamus is a single unit and ends with hatching.

Results

Dorsal thalamic nuclei in juvenile/adult *Alligator*

To provide a background for the present analysis, a short summary of morphologic features of individual nuclei in juvenile *Alligator* is presented. A more detailed description of nuclei in the alar thalamus can be found elsewhere (Huber and Crosby, 1926; Pritz, 2024). However, these two analyses did not distinguish tiers as subdivisions of the dorsal thalamus nor did they group these nuclei according to tiers. These prior studies (Huber and Crosby, 1926; Pritz, 2024) described a variety of morphologic characters of thalamic nuclei. The present account focuses on the dorsal thalamus and groups the 13 nuclei that project to the telencephalon in crocodiles (Pritz, 2024) according to their respective subdivisions, termed tiers, which are discussed subsequently. These tiers are ventral, intermediate, and dorsal.

Before describing the tiers in the dorsal thalamus, two further comments clarify the terminology used in the present study. One is that the dorsal thalamus as defined in the introduction refers to that part of the alar thalamus in prosomere 2 termed dorsal thalamus. This area is separate from the epithalamus which is the other component of the alar thalamus. The other is the alar ventral thalamus which is the same as the alar prethalamus. These latter two terms, alar ventral thalamus and alar prethalamus, are interchangeable and are referenced together in the text and figure

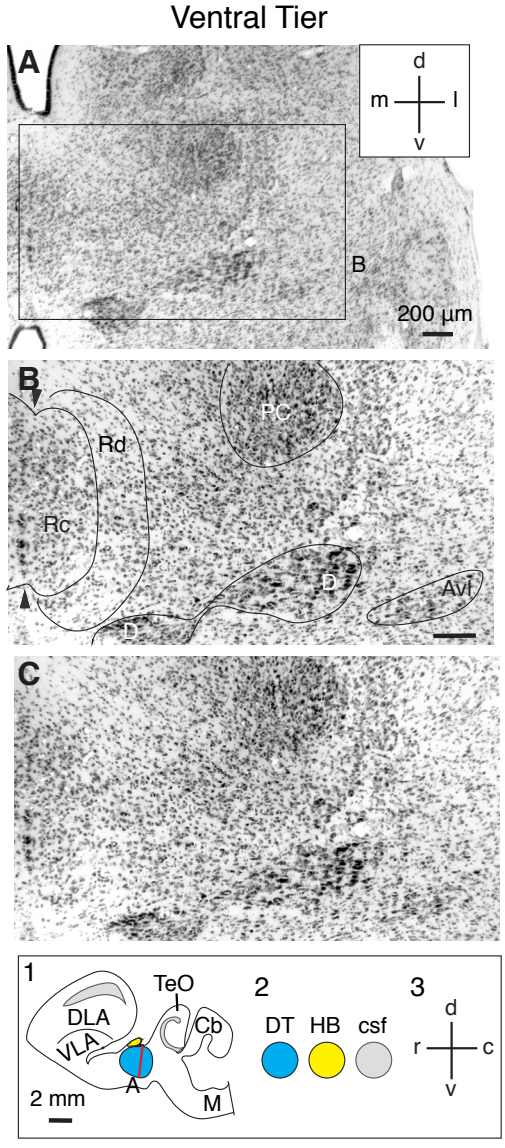


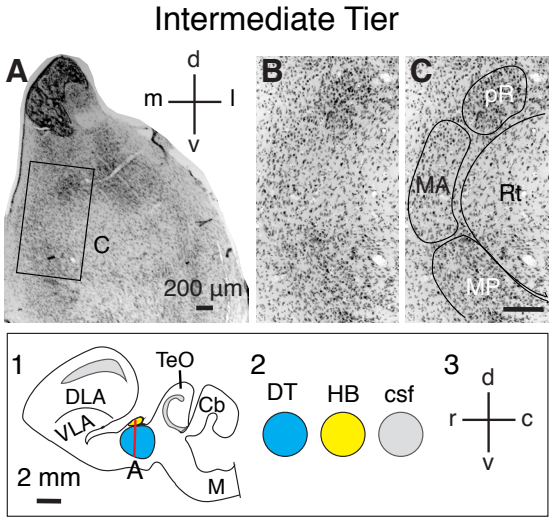
Fig. 1. Ventral tier nuclei in a juvenile *Alligator*. Cresyl violet transverse sections, 25 µm thick, illustrate the nuclei in the ventral tier (A-C). Two identical photos show marked (B) and unmarked (C) nuclei. Orientation of transverse sections (A-C) is indicated (A). Value of scale bar for all photos is the same (A). Inset 1 shows a schematic parasagittal outline of a juvenile brain that indicates the approximate location and orientation of the transverse section (A). Inset 2 indicates the color-coding scheme, while inset 3 shows the orientation of the schematic line drawing. Arrowheads (B) mark the midline. Abbreviations: Avl, area ventrolateralis; c, caudal; Cb, cerebellum; csf, cerebrospinal fluid; d, dorsal; D, nucleus diagonalis; DLA, dorsolateral area; DT, dorsal thalamus; HB, habenula; l, lateral; m, medial; M, medulla; PC, nucleus posterocentralis; r, rostral; Rc, nucleus reuniens pars centralis; Rd, nucleus reuniens pars diffusa; TeO; optic tectum; v, ventral; VLA, ventrolateral area.

legends. These definitions provide explanations for understanding these terms as used in the present analysis.

The central nucleus in the ventral tier is nucleus reuniens (Fig. 1). Two separate nuclei have been distinguished: a pars centralis and a pars diffusa. The pars centralis contains medium size neurons that are more closely packed when compared with those in the pars diffusa. The latter surrounds the pars centralis. During development when these two separate nuclei cannot be differentiated, they are referred to as the nucleus reuniens complex. Nucleus diagonalis is located ventral to the reuniens nuclei in transverse and sagittal planes. It contains small to medium size, darkly stained neurons. Area ventrolateralis and nucleus posterocentralis are located lateral and caudal to the reuniens nuclei and nucleus diagonalis. Area ventrolateralis is a thin nucleus whose main orientation in the transverse plane is from medial to lateral. It contains relatively small neurons that are darkly stained and loosely packed. Nucleus posterocentralis is a round nucleus with mostly medium size neurons that are relatively loosely packed. Its border is indistinct.

Nuclei located in the intermediate tier surround the core nucleus, nucleus rotundus (Fig. 2). These include; nucleus medialis anterior; nucleus medialis posterior, and the perirotundal nucleus. While several parts of nucleus rotundus have been identified in a related crocodilian, *Caiman crocodilus* (Pritz, 1997), no distinction was made in the embryonic material. Nucleus rotundus is a distinct, large, oval-shaped nucleus with small to medium neurons that are loosely arranged. Its border is distinct. The perirotundal nucleus is a small, round nucleus located at the dorsomedial border of nucleus rotundus. Its neurons are more closely packed and smaller than those in the nucleus rotundus. Nucleus medialis anterior and nucleus medialis posterior surround nucleus rotundus on its dorsal, medial, and ventral sides. Nucleus medialis anterior is a small, round to oval-shaped nucleus that is located immediately lateral to the caudal pole of nucleus dorsomedialis anterior. Nucleus medialis posterior is a moderate size nucleus that is larger than nucleus medialis anterior. It is located medial and ventral to nucleus rotundus and contains small, closely packed neurons. The border between nuclei medialis anterior and posterior is indistinct. In the developmental material that follows, this pair of nuclei is referred to as the medialis complex when they cannot be differentiated.

Nuclei that comprise the dorsal tier include: nucleus dorsomedialis anterior, area ventralis anterior, nucleus dorsolateralis anterior, and the dorsal geniculate nucleus (Fig. 3). Nucleus



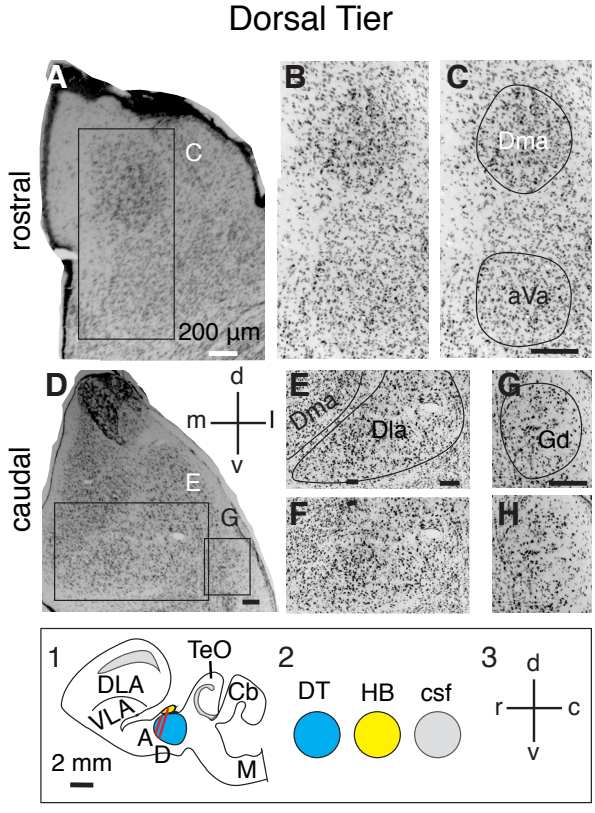


Fig. 2 (Left). Intermediate tier nuclei in a juvenile *Alligator*. Cresyl violet transverse sections, 25 µm thick, illustrate the nuclei in the intermediate tier (A-C). Two identical photos show marked (C) and unmarked (B) nuclei. Orientation of transverse sections (A-C) is indicated (A). Value of scale bar for all photos is the same (A). Inset 1 shows a schematic parasagittal outline of a juvenile brain that indicates the approximate location and orientation of the transverse section (A). Inset 2 indicates the color-coding scheme while inset 3 shows the orientation of the schematic line drawing. Abbreviations: c, caudal; Cb, cerebellum; csf, cerebrospinal fluid; d, dorsal; DLA, dorsolateral area; DT, dorsal thalamus; HB, habenula; l, lateral; m, medial; M, medulla; MA, nucleus medialis anterior; MP, nucleus medialis posterior; pR, perirotundal nucleus; r, rostral; Rt, nucleus rotundus; TeO; optic tectum; v, ventral; VLA, ventrolateral area.

Fig. 3 (Right). Dorsal tier nuclei in a juvenile *Alligator*. Cresyl violet transverse sections, 25 µm thick, illustrate the nuclei in the dorsal tier at rostral (A-C) and caudal (D-H) levels. In the rostral part, two identical photos show marked (C) and unmarked (B) nuclei. In the caudal portion, two identical photos indicate marked (E, G) and unmarked (F, H) nuclei. Orientation of transverse sections (A-H) is indicated (D). Value of scale bar for all photos is the same (A). Inset 1 shows a schematic parasagittal outline of a juvenile brain that shows the approximate location and orientation of the transverse sections (A,D). Inset 2 indicates the color-coding scheme while inset 3 shows the orientation of the schematic line drawing. Abbreviations: aVa, area ventralis anterior; c, caudal; Cb, cerebellum; csf, cerebrospinal fluid; d, dorsal; Dla, nucleus dorsolateralis anterior; DLA, dorsolateral area; Dma, nucleus dorsomedialis anterior; DT, dorsal thalamus; Gd, dorsal geniculate nucleus; HB, habenula; l, lateral; m, medial; M, medulla; r, rostral; TeO; optic tectum; v, ventral; VLA, ventrolateral area.

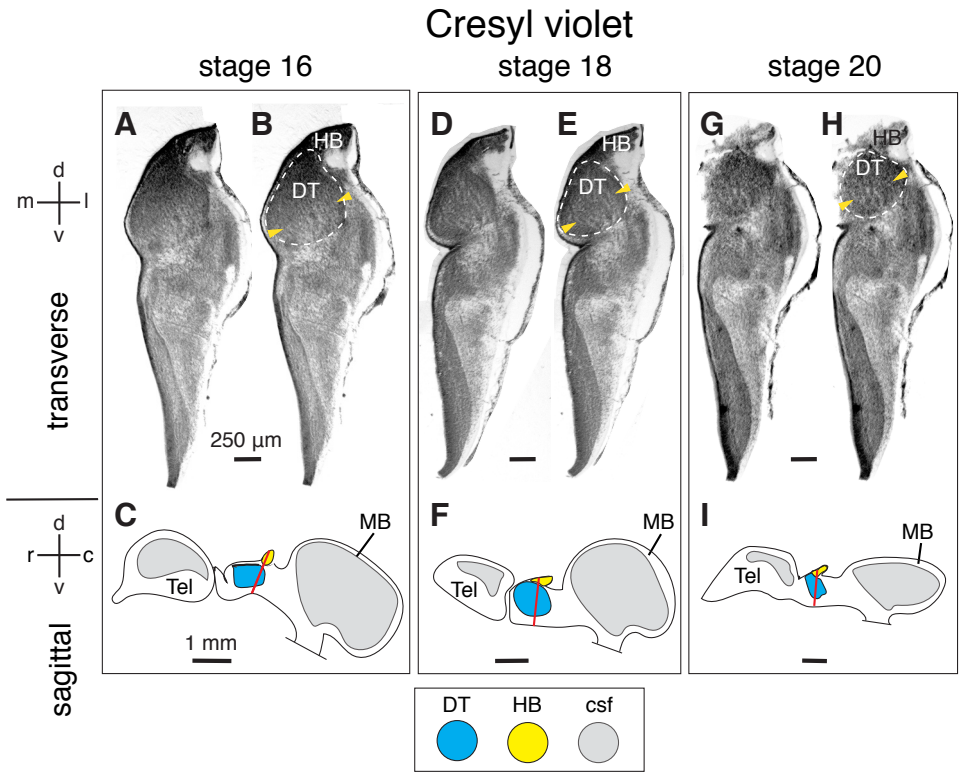


Fig. 4. Unitary dorsal thalamus cytoarchitecture. Cresyl violet stained transverse sections at stages 16 (A-C), 18 (d-f), and 20 (G-I) show the morphology of the dorsal thalamus when it is a single structure. For each pair of identical photos, one is unlabeled (A,D,G) while the other image is marked (B,E,H). Parasagittal schematic line drawings (C,F,I) indicate the approximate transverse plane of section (red line). Value of scale bars for all photos is the same and is marked (A,B). Value of scale bar for all the sagittal schematics is the same and is marked (C). Broken lines (B,E,H) outline the dorsal thalamus. Arrowheads (B,E,H) mark presumed fibers associated with the dorsal thalamus. Inset shows the color-coding scheme. Abbreviations: c, caudal; csf, cerebrospinal fluid; d, dorsal; DT, dorsal thalamus; HB, habenula; l, lateral; m, medial; MB, midbrain; r, rostral; Tel, telencephalon; v, ventral.

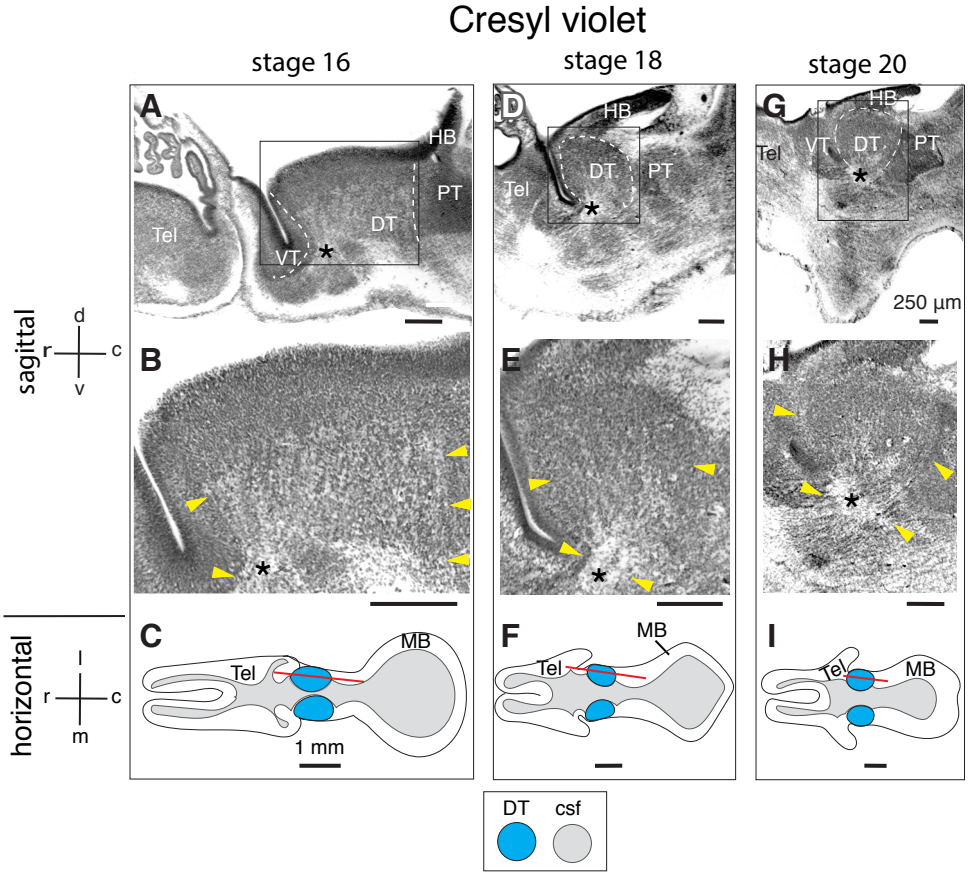


Fig. 5. Unitary dorsal thalamus cytoarchitecture. Cresyl violet stained sagittal sections at stages 16 (A,B), 18 (D,E), and 20 (G,H) show the morphology of the dorsal thalamus when it is a single structure. The enclosed box in the low power views (A,D,G) is shown at higher magnification (B,E,H). Schematic horizontal line drawings (C,F,I) indicate the approximate sagittal plane of section (red line). Value of scale bar for all photos is the same and is marked (G). Value of scale bar for all the horizontal schematics is the same and is marked (C). Broken lines (A,D,G) outline the dorsal thalamus. Inset shows the color-coding scheme. Arrowheads (B,E,H) point to borders of presumed fibers whereas asterisks (A,B,D,E,G,H) mark a compact bundle of presumed fibers. Abbreviations: c, caudal; csf, cerebrospinal fluid; d, dorsal; DT, dorsal thalamus; HB, habenula; l, lateral; m, medial; MB, midbrain; PT, pretectum; r, rostral; Tel, telencephalon; v, ventral; VT, ventral thalamus (prethalamus).

dorsomedialis anterior and area ventralis anterior are found at the rostral pole of the diencephalon (Fig. 3 A-C). While a difference between the rostral and more caudal portion of nucleus dorsomedialis anterior is suggested in juvenile crocodiles (Pritz, 2024), no distinction is made in the embryonic *Alligator* material. Nucleus dorsomedialis anterior contains small to medium, darkly stained neurons that are closely packed. Area ventralis anterior is a poorly defined nucleus located ventral to nucleus dorsomedialis anterior in the transverse plane. Its small neurons are more loosely arranged and not as darkly stained as those in nucleus dorsomedialis anterior (Fig. 3 A-C). Nucleus dorsolateralis anterior, the largest nucleus in the dorsal thalamus, is located lateral to nucleus dorsomedialis anterior and contains larger and more loosely organized neurons than is present in nucleus dorsomedialis anterior (Fig. 3 D-F). The dorsal geniculate nucleus is a round nucleus located internal to the optic tract with loosely packed neurons (Fig. 3 D,G,H).

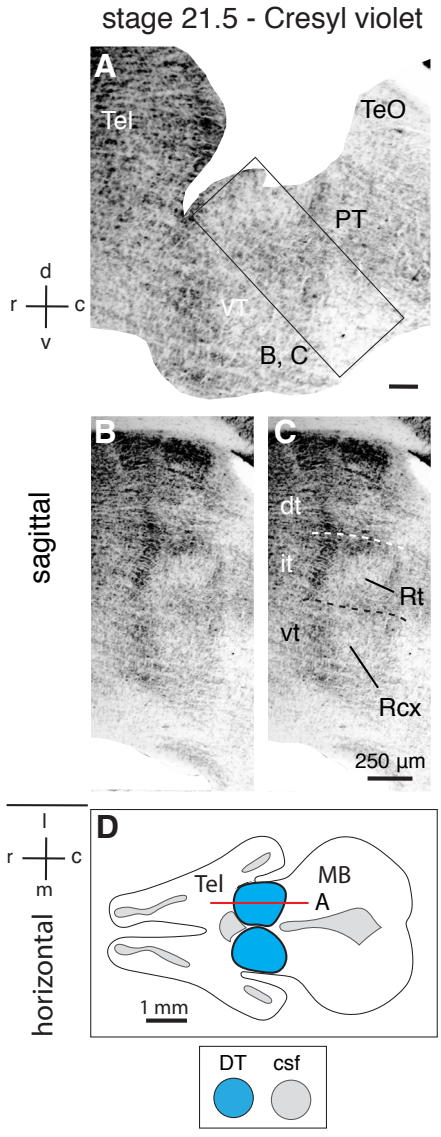


Fig. 6. Dorsal thalamus differentiation. Cresyl violet stained sagittal sections at stage 21.5 identify subdivisions in the dorsal thalamus in three tiers: dorsal, intermediate and ventral. A low power image (A) shows the location of higher magnification views (B,C) from the enclosed box (A). The enlarged views (B,C) are identical. One is labeled (C) while the other is not (B). A schematic horizontal drawing (D) indicates the approximate location and plane of section (red line) for the photos (A-C). Broken lines indicate approximate borders between subdivisions. Value of scale bar for all photos is the same and is indicated (C). Inset shows the color-coding scheme. Abbreviations: c, caudal; csf, cerebrospinal fluid; d, dorsal; dt, dorsal tier; DT, dorsal thalamus; it, intermediate tier; l, lateral; m, medial; MB, midbrain; PT, pretectum; r, rostral; Rt, nucleus rotundus anlage; Rcx, nucleus reuniens complex anlage; Tel, telencephalon; TeO, optic tectum; v, ventral; vt, ventral tier; VT, ventral thalamus (prethalamus).

Stages 16, 18, 20- cresyl violet

The appearance of the dorsal thalamus at stages 16, 18, and 20 are shown in tissue sectioned transversely (Fig. 4) and sagittally (Fig. 5). At this developmental time, the dorsal thalamus remains a unitary structure without internal differentiation. In both transverse and sagittal sectioned material at these stages in development, gaps in cell staining are considered to represent fibers. These fibers are associated with the dorsal thalamus but not with the alar ventral thalamus (alar prethalamus). These presumed fibers can best seen along the ventral border of the dorsal thalamus. This is better visualized in sagittal sectioned material (arrowheads and asterisks, Fig. 5 B,E,H) but is also present in transverse sections (arrowheads Fig. 4 B,E,H). These presumed fibers were not present along the ventral border of the alar ventral thalamus (alar prethalamus). This feature serves as a marker to distinguish the dorsal thalamus from the alar ventral thalamus (prethalamus).

Stage 21.5- cresyl violet

The first indication of subdivisions is seen at stage 21.5 (Fig. 6). Three compartments, termed tiers, were identified. The intermediate tier is marked by the presence of the anlage of nucleus rotundus. The ventral tier landmark is the anlage termed the nucleus reuniens complex. This terminology is used because no distinction between nucleus reuniens pars centralis and pars diffusa could be made at this time. In the sagittal plane, the dorsal tier is located above the intermediate tier and the ventral tier is located below this subdivision (Fig. 6 B,C). The anlage of nuclei rotundus and the reuniens complex were marked by their comparative lack of cell staining when compared with the surrounding areas. A similar, central area lacked cell staining in the dorsal tier (Fig. 6 B,C). However, it was not clear what developmental field this represented. The respective borders of nuclei rotundus and the reuniens complex were indistinct, as were the boundaries between tiers, which is why they were marked by broken lines (Fig. 6C). While the terminology used for tiers identifies them in transverse and sagittally sectioned material, this nomenclature does not entirely reflect that the dorsal tier in *Alligator* is located not only dorsal to the intermediate tier but rostral as well. Similarly, the ventral tier not only is situated ventral to the intermediate tier but extends caudally as well. These features are best seen in tissue sectioned horizontally (see Fig. 7 D,F).

Stage 22- calretinin

The immunoreactivity to the calretinin antibody better defined the tiers of the dorsal thalamus than did the cresyl violet stained material (Fig. 7). This was not simply because the observations

on calretinin stained sections were made at a slightly later developmental stage than the cresyl violet material. Cresyl violet stained sections at stage 22 demonstrated these three tiers but not nearly as well the calretinin stained material (data not shown). In calretinin stained material, the anlage for nucleus rotundus and for the reuniens complex were each outlined by the relative paucity of calretinin immunopositive cells in these respective nuclei when compared with the surrounding dorsal thalamus. This was observed in all tissue planes (Fig. 7 B,D,F). In addition, horizontal sections stained for calretinin demonstrated that the dorsal tier occupied a rostral part of the dorsal thalamus and that the inferior tier was located caudally (Fig. 7F).

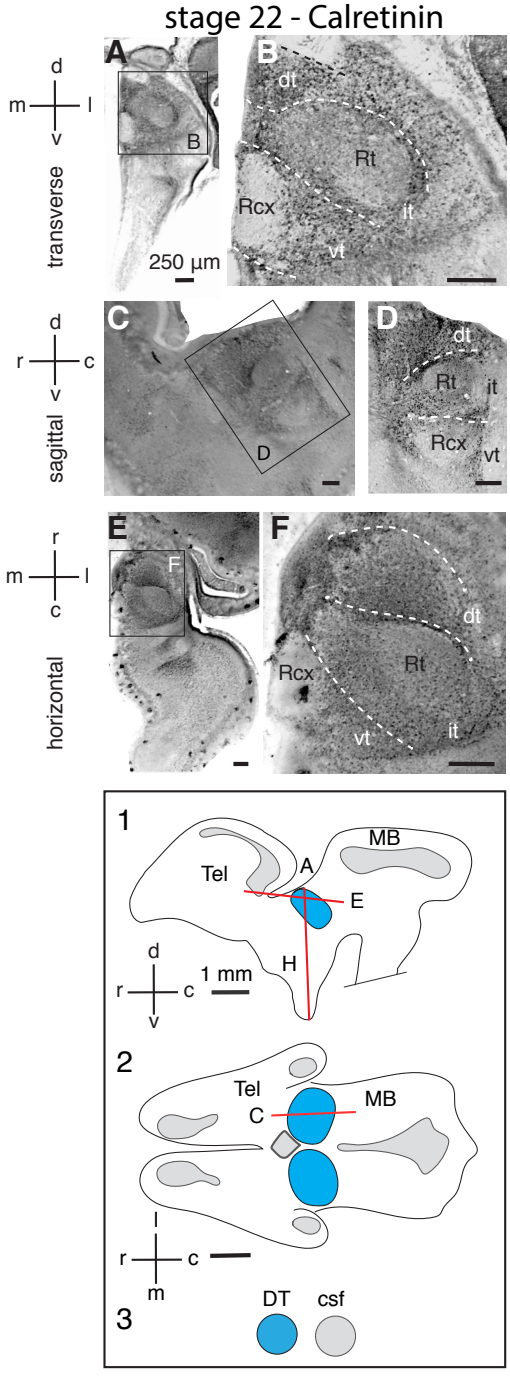


Fig. 7. Calretinin immunoreactivity at stage 22. The expression of calretinin at stage 22 shows the divisions of the dorsal thalamus. The anlage of nucleus rotundus and the reuniens complex are marked by their relative paucity of calretinin (+) cells. These observations are presented in transverse (A,B), sagittal (C,D) and horizontal (E,F) sections. The approximate boundaries of the three tiers are indicated by broken lines (B,D,F). Enlarged views from enclosed boxes (A,C,E) illustrate these tiers and their respective components (B,D,F). Value of scale bar for each photo is the same and is shown (A). The approximate plane of section and location (red lines) of the transverse and horizontal images (inset 1, A,E) and the sagittal images (inset 2, C) are indicated on a schematic outline of embryo brains at similar stages. Value of scale bar of insets 1 and 2 is the same and is shown (inset 1). The color-coding scheme is indicated (inset 3). Abbreviations: c, caudal; csf, cerebrospinal fluid; d, dorsal; dt, dorsal tier; DT, dorsal thalamus; it, intermediate tier; l, lateral; m, medial; MB, midbrain; r, rostral; Rcx, nucleus reuniens complex anlage; Rt, nucleus rotundus anlage; Tel, telencephalon; v, ventral; vt, ventral tier.

Ventral Tier

The development of nuclei that comprise the ventral tier is shown in cresyl violet stained sections beginning at stage 23 and continuing through stage 26-28 (Fig. 8). Nucleus diagonalis and area ventrolateralis were identified at stage 23 (Fig. 8 B,C). However, the division of the nucleus reuniens complex anlage into its two individual nuclei, the pars centralis and the pars diffusa, was not clear until stage 24. At this stage, nucleus reuniens pars centralis is fused at the midline (Fig. 8 F,G). At this same stage, a faint outline of nucleus posterocentralis is suggested; however, its borders remained indistinct (broken line, Fig. 8F). Still later, at stage 26-28 (Fig. 8 J,K), individual nuclei have increased in size and nucleus posterocentralis was identified.

Intermediate Tier

Differentiation of nuclei in the intermediate tier was first observed at stage 23 where the anlage of the medialis complex was seen as separate from nucleus rotundus in cresyl violet stained sections (Fig. 9 B,C). Not until stage 26-28 could a distinction between nucleus medialis anterior and nucleus medialis posterior be identified in cresyl violet stained sections (Fig. 9 J,K). The other nucleus in the intermediate tier, the perirotundal nucleus, was first seen at stage 24 in both calretinin (Fig. 10 B,C) and cresyl violet (Fig. 10 F,G) stained sections. Both nucleus rotundus and the perirotundal nucleus were marked by their relative paucity of calretinin (+) cells when compared with the dense calretinin (+) cells surrounding these two nuclei (Fig.10 B,C). What these more densely staining calretinin (+) cells represent is unknown. In cresyl violet stained sections at stage 26-28, the perirotundal nucleus sits as a cap on top of the dorsomedial border of nucleus rotundus in a similar position to the calretinin stained sections observed earlier at stage 24 (Fig. 10 F,G).

Dorsal Tier

Nuclei that comprise the dorsal tier were first seen at stage 23 (Figs. 11, 12) in tissue stained with cresyl violet. At this time, the rostral pole of nucleus dorsomedialis anterior is identified by its closely packed, darkly stained cells. (Fig. 11 B,C). Area ventralis anterior could not be reliably outlined at stage 23 (Fig. 11 B,C). Even at stage 24, only a hint of its borders was suggested (Fig. 11 E,F). At the pre-hatchling stage, stage 26-28, area ventralis anterior is identified. However, even at this stage, its borders were not sharply marked in cresyl violet stained material (Fig. 11 H,I). Nev-

ertheless, this feature is similar to its appearance in juvenile/adult crocodiles (Pritz, 2024). The dorsal geniculate nucleus, nucleus dorsolateralis anterior, and nucleus dorsomedialis anterior were identified at stage 23 (Fig. 12). Their appearance remained unchanged over time except for a qualitative increase in size (Fig 12 B,C; E,F; H,I). The border between nucleus dorsomedialis anterior and the medial part of nucleus dorsolateralis anterior is marked by a relatively cell free zone at stage 23 (Fig. 12 B,C) which persists through the remainder of development (Fig. 12 E,F; H,I).

Discussion

This study has documented two significant features of the developing dorsal thalamus in *Alligator*. One is how and when the dorsal thalamus is transformed from a unitary structure into subdivisions, termed tiers. The other is the identification of individual nuclei that belong to each tier and when each of these nuclei assume their adult morphology (see Fig. 13).

Before discussing these results, several notes of caution need to be addressed. The present analysis has relied predominantly on cytoarchitecture to analyze dorsal thalamus development. Other methods (see below) to identify subdivisions might reveal a different parcellation and/or a different assignment of nuclei to individual tiers. The stages examined covered most of the time periods of dorsal thalamus development. Accordingly, it is unlikely that changes were overlooked at 'in-between' time periods. However, as noted in the summary figure (Fig.13), the time course from initial identification of tiers until hatching could span up to a month. Lastly, the present analysis does take account for the possibility that neuron migration (Golden and Cepko, 1996; Golden *et al*., 1997; Wong *et al.,* 2018) might influence these observations. For example, neurons originating from two or more separate neuro-epithelial primordia might contribute to the same nucleus.

A number of studies using a variety of methods and approaches have examined the development of the dorsal thalamus and have determined subdivisions. Examples of these are the following: cytoarchitecture (Rose, 1942; Niimi *et al*., 1962); migration area determination (Bergquist, 1953; Bergquist and Källén, 1954); 'birth dating' using both short (Bayer and Altman, 1995b) and long survival times (Angevine, 1970; Wong *et al*., 2018; McAllister and Das, 1977; Bayer and Altman, 1995b; Lynn *et al*., 2015; Xi *et*

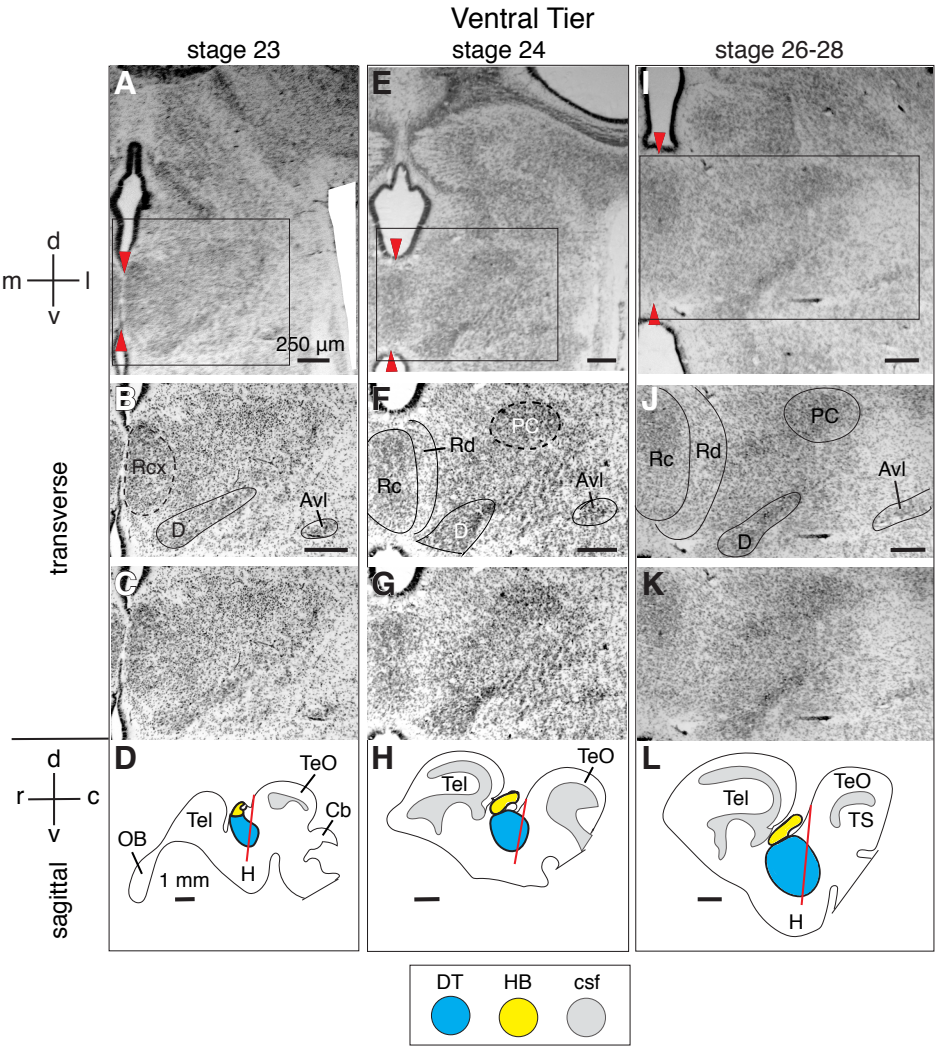


Fig. 8. Ventral tier development. The development of nuclei that comprise the ventral tier are shown in vertical panels at stages 23 (A-C), 24 (E-G), and 26-28 (I-K) in transverse cresyl violet stained sections. The enclosed boxes (A,E,I) are shown at higher magnification in two pairs of identical photos. One is labeled (B,F,J) while the other is unmarked (C,G,K). Solid lines outline nuclei borders whereas broken lines indicate the uncertain border of the nucleus reuniens complex (B). Value of scale bar is the same for all photos and is indicated (A). Schematic line drawings of parasagittal sections at each respective stage (D,H,L) show the approximate location and plane of section (red lines) for the photos. Value of scale bar for each line drawing is the same and is indicated (D). The color-coding scheme is indicated (inset). Arrowheads (A,E,I) mark the midline. Abbreviations: Avl, area ventrolateralis; c, caudal; csf, cerebrospinal fluid; Cb, cerebellum; d, dorsal; D, nucleus diagonalis; DT, dorsal thalamus; H, hypothalamus; HB, habenula; l, lateral; m, medial; OB, olfactory bulb; PC, nucleus posterocentralis; r, rostral; Rc, nucleus reuniens pars centralis; Rd, nucleus reuniens pars diffusa; Rcx, nucleus reuniens complex anlage; Tel, telencephalon; TeO, optic tectum; TS, torus semicircularis; v, ventral.

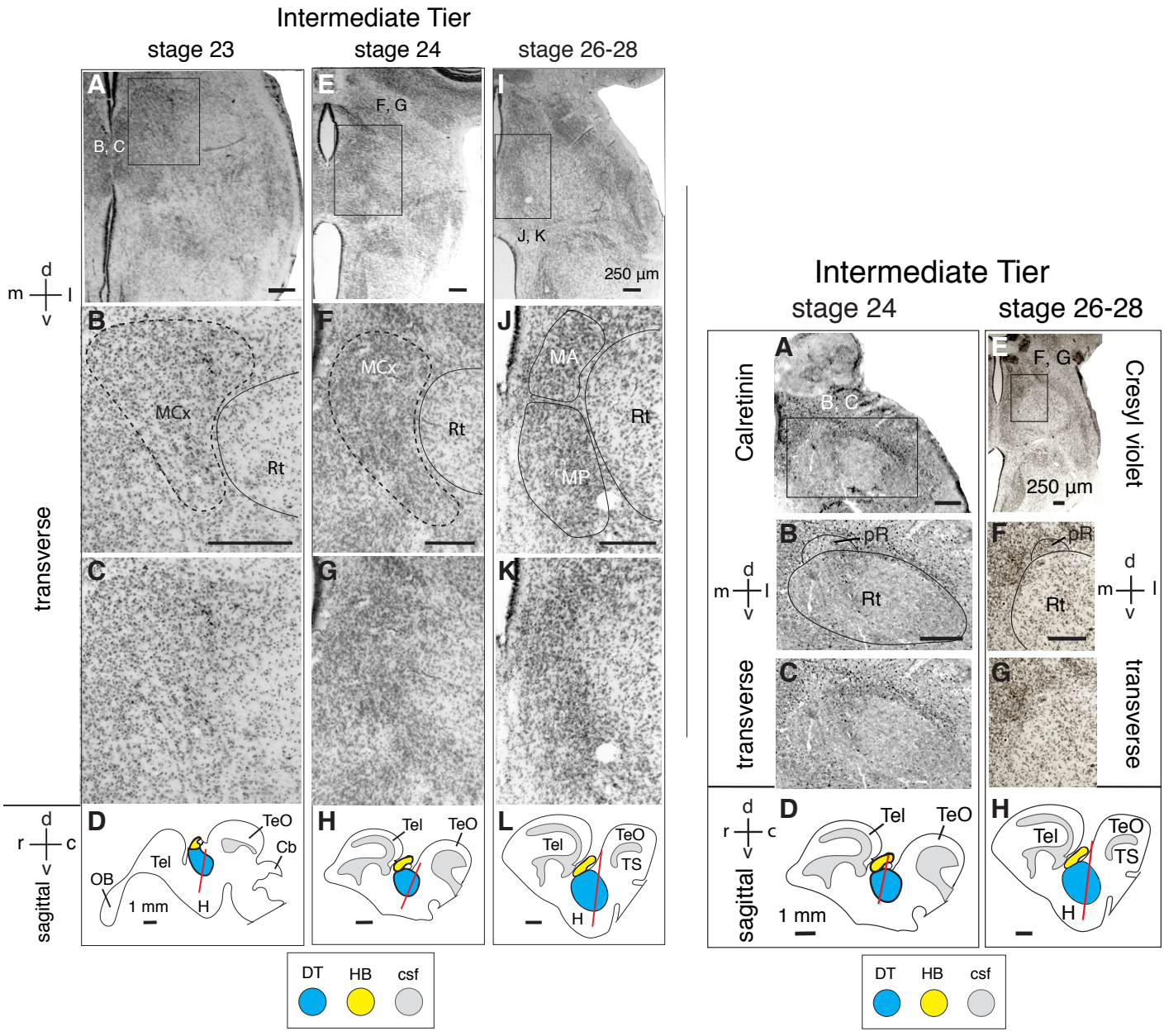


Fig. 9 (Left). Intermediate tier development. The development of some of the nuclei that comprise the intermediate tier are shown in vertical panels at stages 23 (A-C), 24 (E-G), and 26-28 (I-K) in transverse cresyl violet stained sections. The enclosed boxes (A,E,I) are photographed at higher magnification in two pairs of identical photos. One is labeled (B,F,J) while the other is unmarked (C,G,K). Solid lines outline nuclei whereas broken lines mark uncertain borders (B,F). Value of scale bar is the same for all photos and is indicated (I). Schematic line drawings of parasagittal sections at each respective stage (D,H,L) show the approximate location and plane of section (red lines) for the photos. Value of scale bar for each line drawing is the same and is indicated (D). The color-coding scheme is indicated (inset). Abbreviations: c, caudal; csf, cerebrospinal fluid; Cb, cerebellum; d, dorsal; DT, dorsal thalamus; H, hypothalamus; HB, habenula; l, lateral; m, medial; MCx, medialis complex anlage; MA, nucleus medialis anterior; MP, nucleus medialis posterior; OB, olfactory bulb; r, rostral; Rt, nucleus rotundus; Tel, telencephalon; TeO, optic tectum; TS, torus semicircularis; v, ventral.

Fig. 10 (Right). Intermediate tier development. The development of the perirotundal nucleus is illustrated in vertical panels at stages 24 (A-C) and 26-28 (E-G). Transverse sectioned tissue was processed for calretinin immunohistochemistry (A-C) and for cresyl violet (E-G). The enclosed boxes (A,E) are photographed at higher magnification in two pairs of identical photos. One is labeled (B,F) while the other is unmarked (C,G). Solid lines outline nuclei. Value of scale bar is the same for all photos and is indicated (E). Schematic line drawings of parasagittal sections at each respective stage (D,H) show the approximate location and plane of section (red lines) for the photos. Value of scale bar for each line drawing is the same and is indicated (D). The color-coding scheme is indicated (inset). Abbreviations: c, caudal; csf, cerebrospinal fluid; d, dorsal; DT, dorsal thalamus; H, hypothalamus; HB, habenula; l, lateral; m, medial; pR, perirotundal nucleus; r, rostral; Rt, nucleus rotundus; Tel, telencephalon; TeO, optic tectum; TS, torus semicircularis; v, ventral.

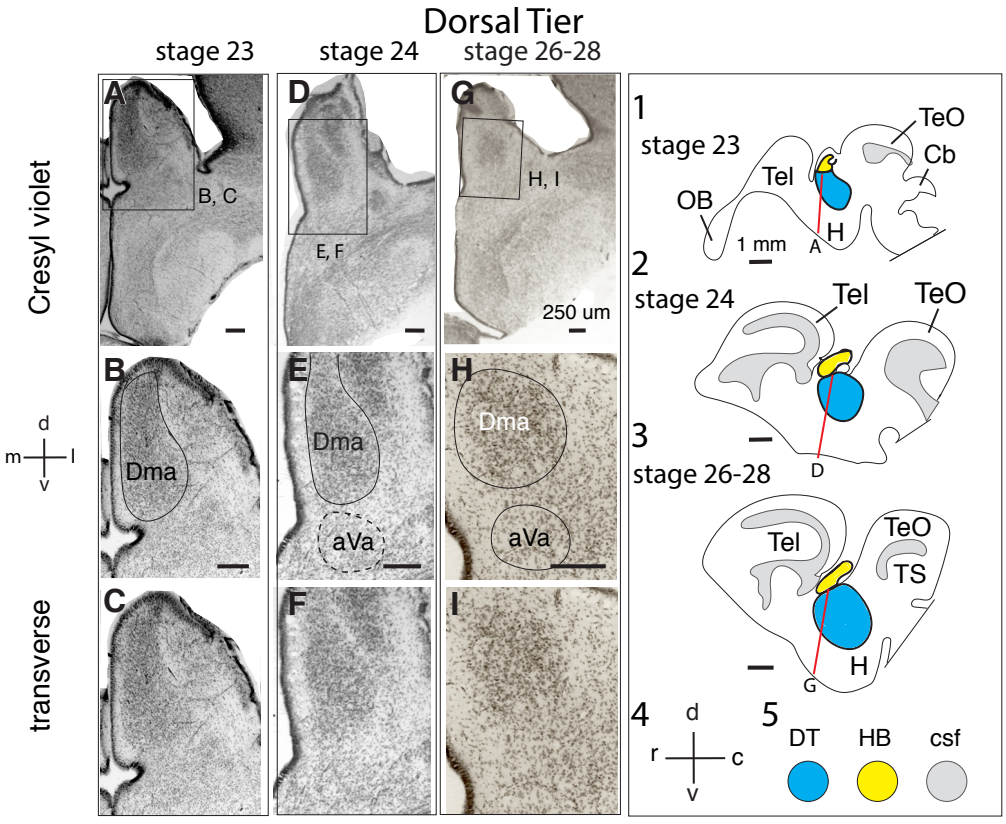


Fig. 11. Dorsal tier development. The development of the anterior part of nucleus dorsomedialis anterior and the area ventralis anterior are shown in vertical panels at stages 23 (A-C), 24 (D-F), and 26-28 (G-I). The enclosed boxes (A,D,G) are photographed at higher magnification in two pairs of identical photos. One is labeled (B,E,H) while the other is unmarked (C,F,I). Solid lines (B,E,H) outline nuclei whereas broken lines (E) mark the indistinct borders of area ventralis anterior. Value of scale bar is the same for all photos and is indicated (G). Insets show: schematic line drawings of parasagittal sections at stages 23 (inset 1); 24 (inset 2); and 26-28 (inset 3); orientation of the parasagittal line drawings (inset 4); and the color-coding scheme (inset 5). The approximate location and plane of section (red lines) for the photos is indicated on the sagittal outlines (insets 1-3). Value of scale bar for each line drawing is the same and is indicated (inset 1). Abbreviations: aVa, area ventralis anterior; c, caudal; Cb, cerebellum; csf, cerebrospinal fluid; d, dorsal; Dma, nucleus dorsomedialis anterior; DT, dorsal thalamus; H, hypothalamus; HB, habenula; l, lateral; m, medial; OB, olfactory bulb; r, rostral; Tel, telencephalon; TeO, optic tectum; TS, torus semicircularis; v, ventral.

al., 2008); immunohistochemistry (Redies *et al*., 2000; González *et al*., 2002); and gene and transcription factor expression (Lim and Golden, 2002; González *et al*., 2002; Martinez-de-la-Torre *et al*., 2002; Hashimoto-Torii *et al*., 2003; Gezelius and López-Bendito, 2017; Nagalski *et al*., 2016; Shi *et al*., 2017; Govek *et al*., 2022; Lo Giudace *et al*., 2024). Despite determining subdivisions of the dorsal thalamus, none of these studies traced individual nuclei from their initial identification to their adult form as was done in the present report.

This study benefitted from the observations of others who described subdivisions of the adult dorsal thalamus in lizards (Díaz *et al*., 1994; Dávila *et al*., 2000) and chicks (Puelles *et al*., 2019) and in the developing dorsal thalamus in chicks (Redies *et al*., 2000; Puelles, 2001b; Puelles and Martinez, 2013). In adult chicks, the ventral tier is highlighted by the presence of nucleus ovoidalis whereas nucleus rotundus is the defining nucleus in the intermediate tier (Puelles *et al*., 2019). Nucleus ovoidalis and nucleus rotundus in birds are homologous to nucleus reuniens pars centralis and nucleus rotundus respectively in crocodilians (Bruce, 2007; Butler, 1994, 2022; Puelles, 2001b).

These three tiers in *Alligator* were ventral, intermediate and dorsal. Each tier followed a similar pattern: a core nucleus which was surrounded by several nuclei. For the ventral tier, the core nucleus was nucleus reuniens pars centralis. This central nucleus was surrounded by nucleus reuniens pars diffusa, nucleus diagonalis, nucleus posterocentralis, and area ventrolateralis. The intermediate tier was anchored by nucleus rotundus which was enveloped by the perirotundal nucleus and nuclei medialis anterior and posterior. The dorsal tier central nucleus was nucleus dorsolateralis anterior. This nucleus was surrounded by nucleus dorsomedialis anterior, area ventralis anterior, and the dorsal geniculate nucleus. However, one difference between the present results and those in chick was that an anteroventral tier noted in chick (Redies *et al*., 2000; Puelles *et al*., 2019) was not identified in *Alligator* because the nuclei in *Alligator* that corresponded to these nuclei described in chick could not be determined.

The results of the present report provide a framework that can be used for further analysis of dorsal thalamus development in *Alligator*. Most likely, the coarse outline presented here will be modified by future studies using other techniques such as genotyping and transcriptomics. These latter methodologies will even better define the dorsal thalamic nuclei and, perhaps, uncover ones that have yet to be identified.

Furthermore, the present development scheme that divides the dorsal thalamus into tiers can serve as a basis to investigate the interconnections between the dorsal thalamus and the telencephalon. This approach has been explored by others (see Fig. 5 in Puelles, 2001b). However, comparable dorsal thalamic developmental data are needed not only in other sauropsids (reptiles and birds) but also in mammals. While several studies have investigated forebrain interconnections during development in sauropsids (Cordery and Molnar, 1999; Wu *et al*., 2000; Bielle *et al*., 2011; Tosa *et al*., 2015; Reyes-Pinto *et al*., 2024), such analyses would be aided by a similar developmental approach to the telencephalon. Just such an investigation is planned for the future. Taken together, a combination of these two studies would provide a solid foundation to examine the formation of thalamotelencephalic connections in *Alligator*.

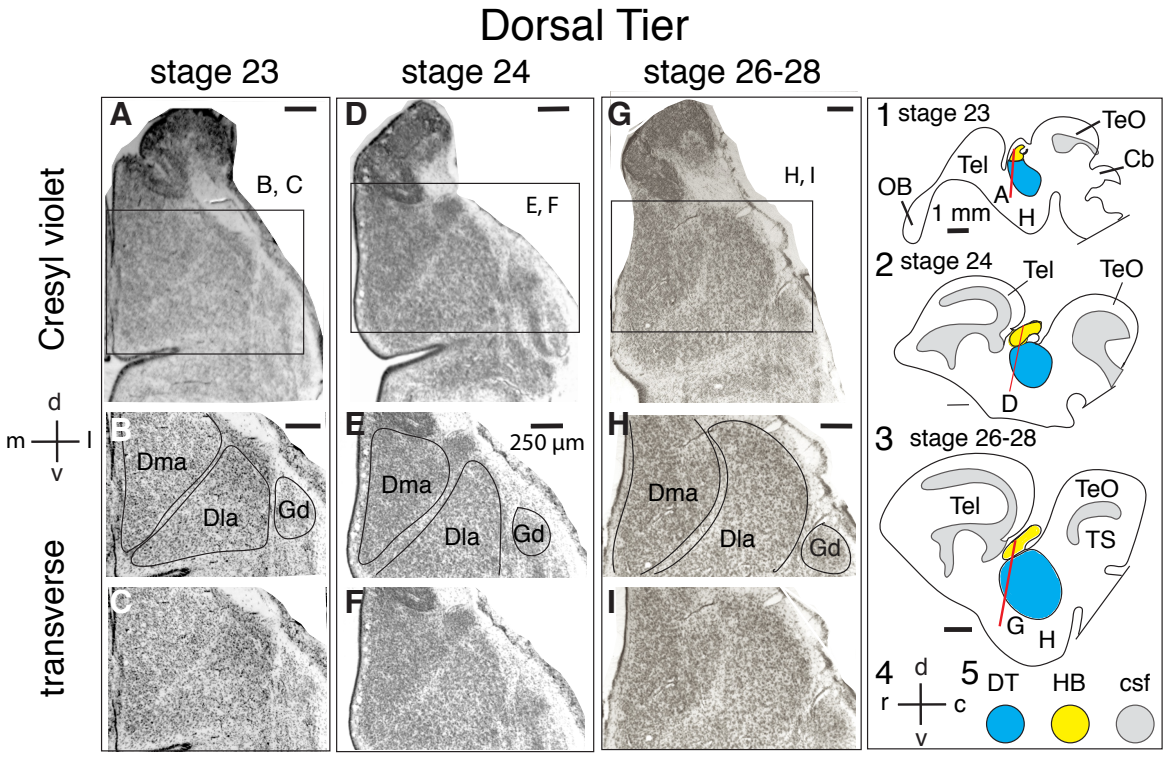


Fig. 12. Dorsal tier development. The development of nucleus dorsomedialis anterior, nucleus dorsolateralis anterior, and the dorsal geniculate nucleus are shown in vertical panels at stages 23 (A-C), 24 (D-F), and 26-28 (G-I). The enclosed boxes (A,D,G) are photographed at higher magnification in two pairs of identical photos. One is labeled (B,E,H) while the other is unmarked (C,F,I). Solid lines outline nuclei. Value of scale bar for all photos is the same and is indicated (E). Insets show: schematic line drawings of parasagittal sections at stages 23 (inset 1); 24 (inset 2); and 26-28 (inset 3); orientation of the sagittal line drawings (inset 4); and the color-coding scheme (inset 5). The approximate location and plane of section (red lines) for the photos is indicated on the sagittal outlines (insets 1-3). Value of scale bar for each line drawing is the same and is indicated (inset 1). Abbreviations: c, caudal; Cb, cerebellum; csf, cerebrospinal fluid; d, dorsal; Dla, nucleus dorsolateralis anterior; Dma, nucleus dorsomedialis anterior; DT, dorsal thalamus; Gd, dorsal geniculate nucleus; H, hypothalamus; HB, habenula; l, lateral; m, medial; OB, olfactory bulb; r, rostral; Tel, telencephalon; TeO, optic tectum; TS, torus semicircularis; v, ventral.

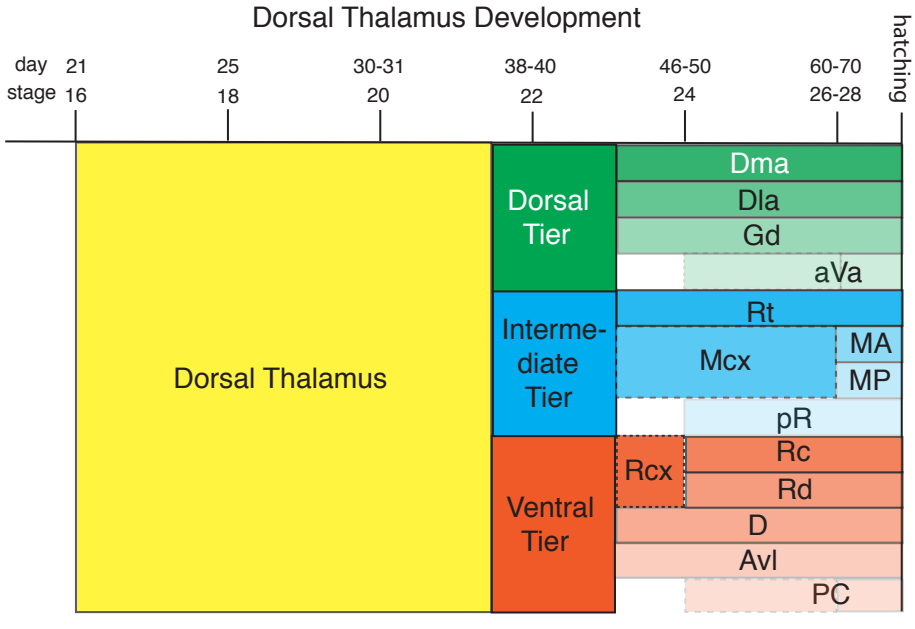


Fig. 13. Summary of dorsal thalamus development. Time course for the formation of tiers and their associated nuclei is illustrated schematically. Subdivision sizes and nuclei are not drawn to scale. Broken lines indicate possible nucleus formation when borders are indistinct. Abbreviations: aVa, area ventralis anterior; Avl, area ventralis lateralis; Dla, nucleus dorsolateralis anterior; D, nucleus diagonalis; Dma, nucleus dorsomedialis anterior; Gd, dorsal geniculate nucleus; MA, nucleus medialis anterior; MCx, medialis complex; MP, nucleus medialis posterior; PC, nucleus posterocentralis; pR, perirotundal nucleus; Rc, nucleus reuniens pars centralis; Rcx, nucleus reuniens complex; Rd, nucleus reuniens pars diffusa; Rt, nucleus rotundus.

Concluding remarks

This study describes the development of the dorsal thalamus in *Alligator*. A brief report with limited figures and text described and compared the development of dorsal thalamic nuclei between turtle and chick (Bösel, 1971/1972). Nevertheless, the present analysis represents the first detailed study of the development of subdivisions and their respectively derived nuclei in the dorsal thalamus of any reptile. This analysis begins when the dorsal thalamus is unitary and continues through hatching. Subdivision into three tiers was initially noted at stage 21.5 based on cytoarchitecture. This was supported by observations on the differential expression of a calretinin antibody at a slightly later time, stage 22. Nuclei associated with each tier were subsequently identified. Furthermore, when borders could not separate individual nuclei, these indistinct boundaries were considered to represent the anlage of the derived nuclei. These results are summarized schematically (Fig. 13).

Several advantages accrue from using *Alligator* in this study of dorsal thalamus development. Among reptiles, *Alligator* eggs are available yearly and the developmental stages based on external body features are well described and reproducible (Ferguson, 1985). Because gestation in *Alligator* is much longer than in the case of rodents or birds, subtle changes, such as identification of the anlagen of the reuniens complex and the medialis complex were identified. These changes might have been overlooked in species where gestation was shorter or if sampling of stages was limited and incomplete. Lastly, because crocodiles are the reptilian group most closely related to birds (Whetstone and Martin, 1979; Hedges, 1994), comparisons with chick will reveal evolutionary relationships. The parcellation scheme used in the present analysis is one example and points to yet another feature shared by *Alligator* and chick.

Materials and Methods

General

This report used the same cytoarchitectural data set that was employed in a previous study that examined epithalamus development (Pritz, 2025). The materials and methods used in that analysis are summarized below. Additional details can be found in that report.

Animals

Alligator mississippiensis eggs were provided by the Rockefeller Wildlife Refuge located in Grand Chenier, Louisiana. Viable embryos were placed in an incubator (Model 1535, VWR Scientific, Philadelphia, PA) at 30^{0}C. Only females will be produced at this temperature (Ferguson and Joanen, 1982). However, the sex of the embryo was not determined by dissection.

Overall details for tissue processing of the cytoarchitecture used for the juvenile *Alligator* that was the basis for photographs (Figs. 1-3) have been described previously (Pritz, 2023). Nevertheless, the specifics for this case are presented for completeness. The animal weighed 98 grams and measured 16.2 cm in snout-vent length. Euthanasia was performed by an overdose of sodium pentobarbital. Sodium heparin, 0.5cc, was then given through intraperitoneal and intracardiac routes. Transcardiac perfusion used the following solutions sequentially: 0.1 M phosphate buffer at pH 7.2 (PBS), 10% formalin in PBS, and 30% sucrose/10% formalin in PBS. The calvarium over the dorsal brain was removed and the brain was placed in 30% sucrose/10%formalin/PBS. The brain was then blocked in a standard transverse plane (Pritz, 2023); embedded in albumin-gelatin; and sectioned at 25 µm on a freezing, sliding microtome (AO Optical, Buffalo, NY). Sections were collected in 2% formalin, mounted on chrome alum coated slides, and stained with 0.1% cresyl violet. Air dried slides were then dehydrated through a series of graded alcohols, cleared in xylene, and cover slipped with Permount (Fisher Chemical, Waltham, MA).

Embryo harvesting

Prior to sacrifice, embryo position was identified. Embryos were then dissected free using standard microsurgical technique under a dissecting microscope (Zeiss, Germany) and staged (Ferguson, 1985). Embryos were sacrificed by transection at the cervico-medullary junction, divided at the isthmus, and placed in a variety of fixatives. When neurologic function was observed, embryos were cooled until neurologic function ceased.

Cytoarchitecture library

Embryos between stages 16 and 26-28 (Ferguson, 1985) were stained with cresyl violet to examine the cytoarchitecture of the dorsal thalamus. The earliest stages, stages 16, 18, and 20, were chosen for examination because this time in development was well before presumed dorsal thalamic differentiation occurred. Subsequently, even numbered stages were examined. Depending on these results, stages in between these even numbered stages were investigated.

Because the morphology of embryonic brains between stages 26 and 28 was so similar, brains at these stages were considered as a single stage. In instances where the external morphology displayed features of two stages, e.g. stages 22 and 23, embryos were labeled as stage 22.5.

Two types of experiments provided brains for this analysis. In the majority of cases, cresyl violet stained material came from sections that were adjacent to experiments that investigated the expression of a variety of antibodies. Most of this tissue was processed in a delayed fashion and the embryos were initially frozen in a -80^{0}C refrigerator. For these cases, in order to preserve tissue morphology, storage required embryo placement in either 100% methanol or Dent's solution (80% methanol, 20% dimethyl sulfoxide).

For brains that were processed solely for cytoarchitecture, tissue was stored in 4% paraformaldehyde and, in rare cases, in Bouin's solution. When sections were subsequently examined, 4% paraformaldehyde gave superior results and became the preferred fixative.

Further processing of frozen brains required thawing. Tissue was gradually warmed to 2-4^{0}C, placed in decreasing concentrations of methanol beginning at 100% methanol, and finally put into PBS before embedding in gelatin (22 ml distilled water; 6.25 g sucrose; 3.13 g gelatin). The resulting gelatin blocks were hardened in 30%sucrose/2%formalin/PBS or 30%sucrose/4% paraformaldehyde/PBS and stored at 2-4^{0}C until sectioning. Brains were sectioned on a freezing, sliding microtome at 25, 40, or 60 µm in one of three planes (transverse, sagittal, horizontal) and collected in PBS. Sections were mounted on chrome alum coated slides, stained with 0.1% cresyl violet, air-dried, dehydrated

through a series of graded alcohols, cleared in xylene, and cover slipped with Permount.

Brains of embryos that were examined solely for cytoarchitecture were stored at 2-4^{0}C until further processing. Brains were embedded in gelatin. The resulting blocks were placed in 30% sucrose/4% paraformaldehyde/PBS and stored at 2-4^{0}C until further processing. Gelatin blocks were sectioned on a freezing, sliding microtome at 25 µm in one of three planes and the resulting sections were stored in 2% formalin. The remainder of tissue processing was identical to the above description.

Calretinin immunohistochemistry

Embryos were processed for calretinin immunohistochemistry because calretinin expression identified certain dorsal thalamic nuclei in a related juvenile crocodilian, *Caiman crocodilus (*Pritz, 2024). Embryos between stages 16 and 26-28 were examined. Embryos were sectioned in one of three planes at either 40 or 60 µm. The anti-calretinin antibody was a rabbit polyclonal antibody (Swant, Bellinzona, Switzerland; AB_2619710) that was used at concentrations 1:500 to 1:4000. In one instance, the concentration of antibody was 1:500 and in three instances, the concentration was 1:1000. Of the remaining cases, a concentration of 1:2000 was used in 67% while a concentration of 1:4000 was employed in 33%. Generally, higher concentrations (1:2000) were used for thicker sections (60 µm) whereas lower concentrations (1:4000) were employed for thinner sections (40 µm).

Free-floating sections were first washed thoroughly in PBS before placement into the following solutions sequentially: (1) 3% or 30% hydrogen peroxide in PBS for 10 or 30 minutes; (2) 5% normal goat serum (Chemicon, Temecula, CA) in PBS for 1 hour; (2) primary antibody solution diluted in PBS containing 5% normal goat serum and 0.5% Triton-X-100 (TX) at 2-4^{0}C overnight; (3) goat anti-rabbit IgG (Chemicon) for 2 hours; (4) avidin-biotin complex (Vector labs, Newark, CA) at a concentration of 1:100 for 2.5 hours. When normal goat serum did not precede placement in the primary antibody solution, 2% normal goat serum and 0.25% TX were added to the primary antibody solution. Between all steps and after the last one, sections were washed thoroughly in PBS. Visualization of the reaction product used a diaminobenzidine substrate kit (Vector) and followed the listed instructions. Sections were transferred into PBS before mounting on chrome-alum coated slides. After satisfactory air-drying, slides were dehydrated through a series of graded alcohols, cleared in xylene, and coverslipped with either Permount or cytoseal 60 (Fisher Scientific, Pittsburgh, PA).

Controls included substitution of rabbit serum in place of the primary antibody at similar concentrations and omission of the secondary and tertiary (steps 3 and 4) reagents. None of these controls resulted in dorsal thalamus labeling. In addition, the immunohistochemical expression of the same calretinin antibody at the same concentrations in *Alligator* embryonic brains at these same time periods correlated with Western blots (Ruan *et al*., 2013). This documented the specificity of the antibody.

Morphological analysis

Processed sections were examined at a variety of magnifications under brightfield illumination using either an Olympus BH2 (Olympus Corporation, Lake Success, NY) or a Leitz Dialux 20 (Wetzlar, Germany) microscope. Photos of pertinent sections were obtained with a digital camera (Infinity 8-8C Color 8 MP microscope camera, Teledyne Lumenera, Evident Scientific, Ottawa, Ontario, Canada). All sagittal images were oriented with the forebrain facing left whereas all transverse and horizontal images were presented from a right sided perspective. Only brightness and contrast were adjusted. The resulting images were imported into Adobe Illustrator (v. 28.6, 2024) and arranged to make composite figures. Sections used for schematic brain outlines were drawn using a camera lucida attachment to the Leitz Dialux 20 microscope. The drawn images were then scanned, imported into Adobe Illustrator, and traced. The resulting line drawings were then added to photographs to make a composite figure.

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Conflict of interest

The author declares no conflicts of interest.

Ethical approval

The protocols and methods used in this study were in accordance with the guidelines of the National Institutes of Health. These were approved by the animal use committee at Indiana University School of Medicine.

Author contributions

The author performed the experiments, wrote the text, and compiled the figures.

Data Availability

Data sets are available upon request.

References

ALTMAN J., BAYER S. A. (1988). Development of the rat thalamus: I. Mosaic organization of the thalamic neuroepithelium. *Journal of Comparative Neurology* 275: 346-377. https://doi.org/10.1002/cne.902750304

AMAT J. A., MARTÍNEZ-DE-LA-TORRE M., TRUJILLO C. M., FERNÁNDEZ B., PUELLES L. (2022). Neurogenetic heterochrony in chick, lizard, and rat mapped with wholemount acetylcholinesterase and the prosomeric model. *Brain, Behavior and Evolution* 97: 48-82. https://doi.org/10.1159/000524216

ANGEVINE J. B. (1970). Time of neuron origin in the diencephalon of the mouse. An autoradiographic study. *Journal of Comparative Neurology* 139: 129-187. https://doi.org/10.1002/cne.901390202

BAYER S. A., ALTMAN J. (1995a). Principles of neurogenesis, neuronal migration, and neural circuit formation. In *The rat nervous system* (Ed. Paxinos G.). Academic Press/Elsevier, New York, pp. 1079-1098.

BAYER S. A., ALTMAN J. (1995b). Neurogenesis and neuronal migration. In *The rat nervous system* (Ed. Paxinos G.). Academic Press/Elsevier, New York, pp. 1041-1078.

BERGQUIST H. (1953). On the development of diencephalic and certain mesencephalic relations in Lepidochelys olivacea and other reptiles. *Acta Zoologica* 34: 155-190. https://doi.org/10.1111/j.1463-6395.1953.tb00370.x

BERGQUIST H. (1954). Ontogenesis of diencephalic nuclei in vertebrates. *Lund universitets arsskrift* 50: 1-34.

BERGQUIST H., KÄLLÉN B. (1954). Notes on the early histogenesis and morphogenesis of the central nervous system in vertebrates. *Journal of Comparative Neurology* 100: 627-659. https://doi.org/10.1002/cne.901000308

BIELLE F., MARCOS-MONDEJAR P., KEITA M., MAILHES C., VERNEY C., NGUYEN BA-CHARVET K., TESSIER-LAVIGNE M., LOPEZ-BENDITO G., GAREL S. (2011). Slit2 activity in the migration of guidepost neurons shapes thalamic projections during development and evolution. *Neuron* 69: 1085-1098. https://doi.org/10.1016/j.neuron.2011.02.026

BÖSEL R. (1971/1972). On the homologous development of the dorsal thalamic nuclear anlagen in turtle and fowl. *J Hirnforsch* 13: 173-179. https://doi.org/10.1515/9783112520000-002

BRUCE L. L. (2007). Evolution of the nervous system in reptiles. In *Evolution of nervous systems: A comprehensive reference* (Ed. Kaas J. H.). Academic Press, London, pp. 125-156. https://doi.org/10.1016/B0-12-370878-8/00130-0

BUTLER A. B. (1994). The evolution of the dorsal thalamus of jawed vertebrates, including mammals: Cladistic analysis and a new hypothesis. *Brain Research Reviews* 19: 29-65. https://doi.org/10.1016/0165-0173(94)90003-5

BUTLER A. B. (2022). Morphological, developmental, and functional evolution of the thalamus. In *The thalamus* (Ed. Halassa M. M.). Cambridge University Press, Great Britain, pp. 91-124. https://doi.org/10.1017/9781108674287.006

BUTLER A. B., HODOS W. (2005). Comparative vertebrate neuroanatomy: Evolution and adaptation. Second edition. John Wiley and Sons, Hoboken, NJ. https://doi.org/10.1002/0471733849

COGGESHALL R. E. (1964). A study of diencephalic development in the albino rat. *Journal of Comparative Neurology* 122: 241-269. https://doi.org/10.1002/cne.901220208

COOPER E.R.A. (1950). The development of the thalamus. *Acta Anatomica* 9: 201-226. https://doi.org/10.1159/000140439

CORDERY P., MOLNÁR Z. (1999). Embryonic development of connections in turtle pallium. *The Journal of Comparative Neurology* 413: 26-54. https://doi.org/10.1002/(SICI)1096-9861(19991011)413:1<26::AID-CNE2>3.0.CO;2-N

DÁVILA J. C., GUIRADO S., PUELLES L. (2000). Expression of calcium-binding proteins in the diencephalon of the lizard *Psammodromus algirus*. *The Journal of Comparative Neurology* 427: 67-92. https://doi.org/10.1002/1096-9861(20001106)427:1<67::AID-CNE5>3.0.CO;2-2

DÍAZ C., YANES C., TRUJILLO C. M., PUELLES L. (1994). The lacteridian reticular thalamic nucleus projects topographically upon the dorsal thalamus: Experimental study in *Gallotia galloti*. *Journal of Comparative Neurology* 343: 193-208. https://doi.org/10.1002/cne.903430202

FERGUSON M. W. J. (1985). Reproductive biology and embryology of the crocodilians. In *Biology of the Reptilia* (Ed. Gans C.). Vol. 14, Development A. John Wiley and sons, New York, pp. 329-491.

FERGUSON M. W. J., JOANEN T. (1982). Temperature of egg incubation determines sex in *Alligator mississippiensis*. *Nature* 296: 850-853. https://doi.org/10.1038/296850a0

GEZELIUS H., LÓPEZ-BENDITO G. (2017). Thalamic neuronal specification and early circuit formation. *Developmental Neurobiology* 77: 830-843. https://doi.org/10.1002/dneu.22460

GOLDEN J. A., CEPKO C. L. (1996). Clones in the chick diencephalon contain multiple cell types and siblings are widely dispersed. *Development* 122: 65-78. https://doi.org/10.1242/dev.122.1.65

GOLDEN J. A., ZITZ J. C., MCFADDEN K., CEPKO C. L. (1997). Cell migration in the developing chick diencephalon. *Development* 124: 3525-3533. https://doi.org/10.1242/dev.124.18.3525

GONZÁLEZ G., PUELLES L., MEDINA L. (2002). Organization of the mouse dorsal thalamus based on topology, calretinin immnunostaining, and gene expression. *Brain Research Bulletin* 57: 439-442. https://doi.org/10.1016/S0361-9230(01)00720-1

GOVEK K. W., CHEN S., SGOURDOU P., YAO Y., WOODHOUSE S., CHEN T., FUCCILLO M. V., EPSTEIN D. J., CAMARA P. G. (2022). Developmental trajectories of thalamic progenitors revealed by single-cell transcriptome profiling and Shh perturbation. *Cell Reports* 41: 111768. https://doi.org/10.1016/j.celrep.2022.111768

HASHIMOTO-TORII K., MOTOYAMA J., HUI C.C., KUROIWA A., NAKAFUKU M., SHIMAMURA K. (2003). Differential activities of Sonic hedgehog mediated by Gli transcription factors define distinct neuronal subtypes in the dorsal thalamus. *Mechanisms of Development* 120: 1097-1111. https://doi.org/10.1016/j.mod.2003.09.001

HEDGES S. B. (1994). Molecular evidence for the origin of birds.. *Proceedings of the National Academy of Sciences* 91: 2621-2624. https://doi.org/10.1073/pnas.91.7.2621

HERGUETA S., LEMIRE M., PIEAU C., WARD R., REPÉRANT J. (1993). The embryological development of primary visual centres in the turtle Emys orbicularis. *J Anat* 183: 404-367.

HUBER G. C., CROSBY E. C. (1926). On thalamic and tectal nuclei and fiber paths in the brain of the American alligator. *Journal of Comparative Neurology* 40: 97-227. https://doi.org/10.1002/cne.900400107

JONES E. G. (1998). Viewpoint: the core and matrix of thalamic organization. *Neuroscience* 85: 331-345. https://doi.org/10.1016/S0306-4522(97)00581-2

JONES E. G. (2007). The thalamus. Second edition. Cambridge University Press, Cambridge, United Kingdom.

KEYSER A. (1972). The development of the diencephalon of the Chinese hamster. An investigation of the validity of the criteria of subdivision of the brain. *Acta anatomica. Supplementum* 59: 1-178.

KUHLENBECK H. (1937). The ontogenetic development of the diencephalic centers in a bird's brain (chick) and comparison with the reptilian and mammalian diencephalon. *Journal of Comparative Neurology* 66: 23-75. https://doi.org/10.1002/cne.900660103

LO GIUDICE Q., WAGENER R. J., ABE P., FRANGEUL L., JABAUDON D. (2024). Developmental emergence of first- and higher-order thalamic neuron molecular identities. *Development* 151: dev202764. https://doi.org/10.1242/dev.202764

LIM Y., GOLDEN J. A. (2002). Expression pattern of cLhx2b, cZic1 and cZic3 in the developing chick diencephalon. *Mechanisms of Development* 115: 147-150. https://doi.org/10.1016/S0925-4773(02)00091-6

LYNN A. M., SCHNEIDER D. A., BRUCE L. L. (2015). Development of the avian dorsal thalamus: Patterns and gradients of neurogenesis. *Brain, Behavior and Evolution* 86: 94-109. https://doi.org/10.1159/000435803

MACCHI G. (1983). Old and new anatomo-functional criteria in the subdivision of the thalamic nuclei. In *Somatosensory integration in the thalamus* (Ed. Macchi G., Rustioni A., Spreafico R.). Elsevier, Amsterdam, pp. 3-15.

MARTÍNEZ-DE-LA-TORRE M., GARDA A. L., PUELLES E., PUELLES L. (2002). Gbx2 expression in the late embryonic chick dorsal thalamus. *Brain Research Bulletin* 57: 435-438. https://doi.org/10.1016/S0361-9230(01)00721-3

MCALLISTER J. P., DAS G. D. (1977). Neurogenesis in the epithalamus, dorsal thalamus and ventral thalamus of the rat: An autoradiographic and cytological study. *Journal of Comparative Neurology* 172: 647-686. https://doi.org/10.1002/cne.901720407

NAGALSKI A., PUELLES L., DABROWSKI M., WEGIERSKI T., KUZNICKI J., WISNIEWSKA M. B. (2016). Molecular anatomy of the thalamic complex and the underlying transcription factors. *Brain Structure and Function* 221: 2493-2510. https://doi.org/10.1007/s00429-015-1052-5

NIEUWENHUYS R., DONKELAAR H. J., NICHOLSON C. (1998). The central nervous system of vertebrates. Springer, New York. https://doi.org/10.1007/978-3-642-18262-4

NIIMI K., HARADA I., KUSAKA Y., KISHI S. (1962). The ontogenetic development of the diencephalon of the mouse. *The Tokushima Journal of Experimental Medicine* 8: 203-238.

PRITZ M. B. (1997). Some morphological features of a visual thalamic nucleus in a reptile: Observations on nucleus rotundus in *Caiman crocodilus*. *Brain, Behavior and Evolution* 49: 237-248. https://doi.org/10.1159/000112995

PRITZ M. B. (2023). Nuclei and tracts in the pretectum and associated tegmentum of crocodiles. *Journal of Comparative Neurology* 531: 415-450. https://doi.org/10.1002/cne.25433

PRITZ M. B. (2024). Nuclei and tracts in the thalamus of crocodiles. *Journal of Comparative Neurology* 532: e25595. https://doi.org/10.1002/cne.25595

PRITZ M. B. (2025). Development of the epithalamus in *Alligator mississippiensis*. *Brain Structure and Function* 230: 50. https://doi.org/10.1007/s00429-025-02913-9

PUELLES L. (2001a). Brain segmentation and forebrain development in amniotes. *Brain Research Bulletin* 55: 695-710. https://doi.org/10.1016/S0361-9230(01)00588-3

PUELLES L. (2001b). Thoughts on the development, structure and evolution of the mammalian and avian telencephalic pallium. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 356: 1583-1598. https://doi.org/10.1098/rstb.2001.0973

PUELLES L., MARTINEZ-DE-LA-TORRE M., MARTINEZ S., WATSON C., PAXINOS G. (2019). The chick brain in stereotaxic coordinates and alternate stains. Featuring neuromeric subdivisions and mammalian homologies. Second edition. Elsevier, San Diego, CA.

PUELLES L., MARTINEZ S. (2013). Patterning of the diencephalon. In *Patterning and cell type specification in the developing CNS and PNS: Comprehensive developmental neuroscience* (Ed. Rubenstein J., Rakic P.). Vol. 1. Academic Press/Elsevier Science and Technology, Cambridge, MA, pp. 151-172. https://doi.org/10.1016/B978-0-12-397265-1.00048-4

REDIES C., AST M., NAKAGAWA S., TAKEICHI M., MARTÍNEZ-DE-LA-TORRE M., PUELLES L. (2000). Morphologic fate of diencephalic prosomeres and their subdivisions revealed by mapping cadherin expression. *The Journal of Comparative Neurology* 421: 481-514. https://doi.org/10.1002/(SICI)1096-9861(20000612)421:4<481::AID-CNE3>3.0.CO;2-H

RENDAHL H. (1924). Embryologische und morphologische studien uber das zwischenhirn beim huhn. *Acta Zoologica* 5: 241-344. https://doi.org/10.1111/j.1463-6395.1924.tb00169.x

REYES-PINTO R., ROJAS M. J., LETELIER J. C., MARÍN G. J., MPODOZIS J. (2024). Early development of the thalamo-pallial stage of the tectofugal visual pathway in the chicken (*Gallus gallus*). *Journal of Comparative Neurology* 532: e25657. https://doi.org/10.1002/cne.25657

RIKHYE R. V., WIMMER R. D., HALASSA M. M. (2018). Toward an integrative theory of thalamic function. *Annual Review of Neuroscience* 41: 163-183. https://doi.org/10.1146/annurev-neuro-080317-062144

ROSE J. E. (1942). The ontogenetic development of the rabbit's diencephalon. *Journal of Comparative Neurology* 77: 61-129. https://doi.org/10.1002/cne.900770105

RUAN Y.W., SO K.F., PRITZ M.B. (2013). Calcium binding protein expression in the optic tectum of *Alligator* during development. *Developmental Neurobiology* 73: 899-910. https://doi.org/10.1002/dneu.22116

SENN D. G. (1979). Embryonic development of the central nervous system. In *Biology of the Reptilia* (Ed. Gans C., Northcutt R. G., Ulinski P.). *Vol.* 9. Neurology A. Academic Press, New York, pp. 173-243.

SHERMAN S. M. (2007). The thalamus is more than just a relay. *Current Opinion in Neurobiology* 17: 417-422. https://doi.org/10.1016/j.conb.2007.07.003

SHERMAN S. M., GUILLERY R. W. (2006). Exploring the thalamus and its role in cortical function. Second edition. MIT Press, Cambridge, Massachusetts. https://doi.org/10.7551/mitpress/2940.001.0001

SHERMAN S. M., GUILLERY R. W. (2013). Functional connections of cortical areas: A new view from the thalamus. MIT Press, Cambridge, Massachusetts. https://doi.org/10.7551/mitpress/9780262019309.001.0001

SHI W., XIANYU A., HAN Z., TANG X., LI Z., ZHONG H., MAO T., HUANG K., SHI S.H. (2017). Ontogenetic establishment of order-specific nuclear organization in the mammalian thalamus. *Nature Neuroscience* 20: 516-528. https://doi.org/10.1038/nn.4519

STRÖER W. F. H. (1956). Studies on the diencephalon. I. The embryology of the diencephalon of the rat. *Journal of Comparative Neurology* 105: 1-24. https://doi.org/10.1002/cne.901050102

TOSA Y., HIRAO A., MATSUBARA I., KAWAGUCHI M., FUKUI M., KURATANI S., MURAKAMI Y. (2015). Development of the thalamo-dorsal ventricular ridge tract in the Chinese soft-shelled turtle, *Pelodiscus sinensis*. *Development, Growth & Differentiation* 57: 40-57. https://doi.org/10.1111/dgd.12186

WHETSTONE K. N., MARTIN L. D. (1979). New look at the origin of birds and crocodiles. *Nature* 279: 234-236. https://doi.org/10.1038/279234a0

WONG S. Z. H., SCOTT E. P., MU W., GUO X., BORGENHEIMER E., FREEMAN M., MING G., WU Q. F., SONG H., NAKAGAWA Y. (2018). In vivo clonal analysis reveals spatiotemporal regulation of thalamic nucleogenesis. *PLOS Biology* 16: e2005211. https://doi.org/10.1371/journal.pbio.2005211

WU C. C., CHARLTON R. K., KARTEN H. J. (2000). The timecourse of neuronal connections of the rotundoectostriatal pathway in chicks (*Gallus gallus*) during embryogenesis: A retrograde transport study. *Visual Neuroscience* 17: 905-909. https://doi.org/10.1017/S0952523800176096

WYETH F. J. (1924). The development and neuromery of the forebrain in Sphenodon punctatus, with special reference to the presence and neuromeric significance of certain paired metameric diverticula of the central cavity of the forebrain. *Proceedings of the Zoological Society of London* 94: 923-959. https://doi.org/10.1111/j.1096-3642.1924.tb03323.x

XI C., ZENG S. J., ZHANG X. W., ZUO M. X. (2008). Neurogenic development of the visual areas in the Chinese softshell turtle (*Pelodiscus sinensis*) and evolutionary implications. *Journal of Anatomy* 212: 578-589. https://doi.org/10.1111/j.1469-7580.2008.00882.x