The Development of the Diencephalon in *Trichosurus vulpecula*

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*Introduction*

Kuhlenbeck (1931) described the development of the diencephalic nuclei in the Lacertilia, Chelonia, and Crocodilia. He also included some notes on the plan of development of the diencephalic nuclei in the ophidian brain. Rendahl (1924) and Kuhlenbeck (1936) described the development of the diencephalon of the bird. The latter author (Kuhlenbeck, 1937) dealt with the development of the avian diencephalic nuclei in considerable detail. Gronberg (1901) briefly described the development of the diencephalon in the European hedgehog (*Erinaceus europeus*), which is one of the most primitive of the insectivorous mammals. Tilney (1932) investigated the development of the forebrain, in the albino rat, including some observations on the development of the diencephalic nuclei.

By Miura (1933) the development of the diencephalon in the rabbit was described in great detail. Tilney and Kubie (1931), in their description of the development of the telencephalon in the cat, included some notes on the development of the diencephalon. In the past there have been few if any systematic studies of the development of the diencephalon of the Australian marsupials. It was therefore deemed advisable to make a systematic study of the development of the diencephalon in an Australian marsupial, *Trichosurus vulpecula*.

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Materials and Methods

For this work two series of slides of *Perameles nasuta* and twenty-five series of slides of *Trichosurus vulpecula* were available for study. These marsupial embryos were embedded in paraffin, sectioned in the transverse plane, and stained with hematoxylin and eosin. Most of the series of embryos were of *Trichosurus vulpecula*. However, as certain crucial embryonic stages of this species were not available for study, the 16 mm and the 23 mm stages of a distantly related marsupial, *Perameles nasuta*, were used to illustrate these stages. Inasmuch as no sagittal series of slides of either *Trichosurus vulpecula* or *Perameles nasuta* were available for study, a sagittal series of another Australian marsupial, *Phascolarctos phascolomys*, was used to illustrate certain points in the early development of the diencephalon. However, this study deals mainly with the development of the diencephalon of *Trichosurus vulpecula*.

Description

*Trichosurus vulpecula* (8.5 mm stage)

The diencephalon in an 8.5 mm *Trichosurus vulpecula* embryo shows the diencephalic wall to be divided into the epithalamic, dorsal thalamic, ventral thalamic and the hypothalamic zones. The diencephalic vesicle at this stage shows quite clearly a well defined sulcus dorsalis (fig. 1, S.d.). This sulcus demarcates the epithalamic from the dorsal thalamic zones. Ventral to this sulcus, a sulcus medius is seen which demarcates the dorsal thalamic zone from the ventral thalamic zone (fig. 1, S.m.). More ventral to this sulcus a sulcus ventralis is present, demarcating the ventral thalamic from the hypothalamic zone (fig. 1, S.v.). KUHLENBECK (1931) has similarly noted these corresponding sulci in the diencephalon of *Lacerta agilis* embryos. Within the dorsal part of the diencephalic ventricle, the synencephalic recess can be seen, and in the ventral part of the diencephalon the parencephalic recess may be noted. At the middorsal extremity of the diencephalon, the anlage of the epiphysis is shown (fig. 1, ep.). The diencephalic wall in all its zones, namely the epithalamic, dorsal thalamic, ventral thalamic and the hypothalamic zones, consists of an inner layer or the matrix zone and an outer layer, the primitive mantle zone. This stage of diencephalic development, in *Trichosurus vulpecula*, would correspond closely to that of the 97 hour chick embryo as described by KUHLENBECK (1937).
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**Trichosurus vulpecula** (14.5 mm stage)

This stage of development of the diencephalon in *Trichosurus vulpecula* still shows the diencephalic wall to be clearly subdivided into the epithalamic zone, the dorsal thalamic zone, the ventral thalamic zone and the hypothalamic zones. The sulcus dorsalis can be readily seen, it demarcates the epithalamic from the dorsal thalamic zone. At this level the sulcus synencephalicus is fused with the sulcus dorsalis (fig. 2, S.d.+Sy.). The sulcus medius and the sulcus ventralis are clearly seen also at this stage, demarcating the dorsal thalamic, ventral thalamic and the hypothalamic zones (fig. 2, S.m. and S.v.). In the ventral part of the hypothalamus the sulcus lateralis infundibuli is observed (fig. 2, Sn.). At the middorsal extremity of the diencephalon, the anlage of the epiphysis can be seen (fig. 2, ep.). The diencephalic wall at this stage shows a slightly more advanced stage of development as compared with that of the previously described stage. Thus, in the dorsal thalamus and ventral thalamus, the primordial matrix cell layer shows evidence of a differentiation of a dense periventricular inner layer which is the area cellularis interna (fig. 2, i.c.d. and i.v.c.).

External to this layer, a more loosely arranged cell layer is noted, which forms the area cellularis externa of the dorsal and ventral thalamus. This cell layer at this stage forms a thin cell mass (fig. 2, e.c.d. and e.v.c.). In the hypothalamus likewise a similar dense inner periventricular cell layer of the area cellularis interna can be seen. An area of more loosely arranged cells which lies peripheral to the inner cell layer forms a fairly well defined area cellularis externa (fig. 2, i.h.c. and e.h.c.). In the epithalamus an area cellularis externa cannot yet be definitely demarcated. This stage of *Trichosurus vulpecula* shows a stage of development only slightly more advanced than that of the 120 hour chick embryo as described by Kuhlenbeck (1936).

**Trichosurus vulpecula** (13.5 mm stage)

The diencephalon of the 13.5 mm *Trichosurus vulpecula* shows a stage of diencephalic development which is more advanced than that noted in the 14.5 mm stage. Thus, the primordium of the epiphysis may be noted at the dorsomedial extremity of the diencephalic wall (fig. 3, ep.). The anlage of the epiphysis is more evaginated, and shows a slightly more advanced degree of development than at the
previously described stage (cf. fig. 1). Laterally the wall of the diencephalon is divided into the epithalamic, dorsal thalamic, ventral thalamic and the hypothalamic zones by the sulcus dorsalis, sulcus medius and the sulcus ventralis, respectively (fig. 3, et., t.d., t.v. and hy.).

In the epithalamus a quite thick inner densely packed periventricular cell layer, the area cellularis interna, can be noted. However, in contrast to that observed in the previously described stage, a thin layer of rather loosely arranged cells can be seen, lying just peripheral to the inner cell layer of the epithalamus. This cell layer forms the beginning of the area cellularis externa of the epithalamic zone (fig. 3, Ep. m. and Ep. l.). In the dorsal thalamic, ventral thalamic and the hypothalamic zones of the diencephalic wall, a fairly thick area cellularis interna layer, and a quite well defined, outer loosely arranged area cellularis externa layer can be demonstrated (fig. 3, i.c.d., i.v.c., i.h.c., and e.c.d., e.v.c., e.h.c.). The area cellularis externa is thickest in the ventral thalamic and hypothalamic diencephalic zones. In point of the level of development of the diencephalon, the 13.5 mm stage would correspond in a general way to that of the chick embryo of 5 days and 16 hours incubation as described by KUHLENBECK (1937).

*Phascolarctos phascolomys* (13.5 mm stage)

Inasmuch as no sagittal series of slides of either *Trichosurus vulpecula* or of *Perameles nasuta* were available for study, a sagittal series of a 13.5 mm *Phascolarctos phascolomys* embryo was used in this study to illustrate certain salient points. *Phascolarctos phascolomys* is Australian diprotodont marsupial belonging to the family of the Phascolomidae. The 13.5 mm *Phascolarctos phascolomys* embryo is comparable in point of degree of diencephalic development to that of the previously described 13.5 mm stage of *Trichosurus vulpecula*. The 13.5 mm *Phascolarctos* sagittal section used to illustrate this stage is close to the midplane, yet rather remarkably in this section no epiphyseal anlage is observed. The two subdivisions of the diencephalon, namely the parencephalon, and just caudal to it, the synencephalon, which constitute the transitory neuromeres of the diencephalon, can still be noted (fig. 4, Par. and Syn.). According to KUHLENBECK (1937), in later stages only the rostral half of the synencephalon merges with the parencephalon to form the definitive diencephalon. He also indicated, that the caudal half of the synen-
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The diencephalon merges with the mesencephalon. The rostral end of the basal plate or tegmentum is also seen in this section.

The hypothalamus may also be observed in this figure. The optic fibers have developed at this stage, and the optic chiasma is shown at the ventral extremity of the anterior part of the hypothalamus (fig. 4, Opt. ch.). The velum transversum, which is a groove lying dorsally in the midline, can likewise be observed. Its tip forms the anterior boundary of the diencephalon (fig. 4, v.t.). Lying ventrally at the same level, in the midplane a thickening of the hemisphere well represents the torus transversus, described by KUHLENBECK (1936) in the bird (fig. 4, t.t.). According to KUHLENBECK the torus transversus later forms the bed of the anterior commissure.

*Perameles nasuta* (16 mm stage)

In the 16 mm *Perameles nasuta* the diencephalic wall still shows clearly the sulcus dorsalis, sulcus medius and the sulcus ventralis (fig. 5, S.d., S.m. and S.v.). These sulci clearly demarcate the epithalamic, dorsal thalamic, ventral thalamic and the hypothalamic zones of the wall of the diencephalon (fig. 5, et., t.d., t.v. and hy.). In the wall of the diencephalon the differentiation of the area cellularis interna and the area cellularis externa layers can be clearly observed. In the epithalamus a well defined area cellularis externa is present which however, is not quite as thick as that of the dorsal thalamus (fig. 5, Ep. l.). The area cellularis interna forms a fairly thick cell layer in the epithalamus (fig. 5, Ep. m.).

In the dorsal thalamus and the ventral thalamus the area cellularis externa is quite thick, and consists of an area of rather loosely arranged cells (fig. 5, e.c.d. and e.v.c.). The area cellularis externa is well formed and can be readily noted as a well defined layer of loosely arranged cells in the hypothalamus (fig. 5, e.h.c.). The sulcus lateralis infundibuli can be seen lying at the medial border of the lower part of the hypothalamus (fig. 5, Sn.). The sulcus lateralis infundibuli subdivides the pars posterior of the hypothalamus into a hypothalamus inferior and superior. In the dorsal thalamus and the ventral thalamus a fairly thick area cellularis interna is well defined (fig. 5, i.c.d. and i.v.c.). In the hypothalamus the presence of an area cellularis interna is likewise obvious. It forms a quite thick layer of densely arranged cells. Just lateral to this level, a thin membranous structure is seen: the lamina affixa (fig. 5, l.
The lamina affixa is a part of the ependymal lining of the lateral ventricle which is superimposed upon the lateral part of the thalamus.

Trichosurus vulpecula (19 mm stage)

The 19 mm Trichosurus vulpecula embryo shows a stage of development of the diencephalon which is comparable to that of the 15 mm rabbit embryo, as noted by MIURA (1933). Thus, the epithalamus can be clearly demarcated from the dorsal thalamus by a well defined sulcus dorsalis. However the sulcus dorsalis is fused with and coincides at this level with the bottom of the synencephalic recess (fig. 6, S.d.+Syn. Rec.). Thus, the bottom of the synencephalic recess at this point may also be termed sulcus synencephalicus. The cell groups of the epithalamus are now differentiated into a fairly thick outer area cellularis externa which is more loosely arranged than the inner cell layer of the epithalamus (fig. 6, Ep. 1.). The inner cell layer or area cellularis interna of the epithalamus forms a quite thick, densely packed cell layer (fig. 6, Ep. m.).

The dorsal thalamus lies ventral to the sulcus dorsalis (fig. 6, t.d.). The diencephalic wall of the dorsal thalamus has become thicker than that noted at the previously described stage. The area cellularis interna of the dorsal thalamus has become considerably thicker than at the 16 mm stage (fig. 6, i.c.d.). The outer cell mass or the area cellularis externa of the dorsal thalamus has also become thickened. It now forms a somewhat thicker cell layer than the inner cell layer (fig. 6, e.c.d.). In the section used to illustrate the diencephalon of this stage a sulcus medius is not seen. This is possibly due to the obliqueness of this section. In the ventral thalamus, also, the area cellularis interna is considerably thickened (fig. 6, i.v.c.), as is the outer cell mass or area cellularis externa of the ventral thalamus (fig. 6, e.v.c.). The sulcus ventralis can be noted demarcating the ventral thalamic from the hypothalamic region of the diencephalic wall (fig. 6, S.v.). In the hypothalamic zone the inner cell mass is greatly thickened and consists of a layer of densely arranged cells (fig. 6, i.h.c.). The external layer, or the area cellularis externa of the hypothalamic region of the diencephalic wall, forms a thick cell layer of rather loosely arranged cells (fig. 6, e.h.c.).
**Trichosurus vulpecula (24 mm stage)**

The diencephalic wall at this stage shows a considerable advance in the degree of development as compared with that of the stage previously described. Thus, the diencephalic wall is considerably thicker, particularly in its dorsal and ventral thalamic zones, as compared with that of the 19 mm stage. However, the sulcus dorsalis, sulcus medius and the sulcus ventralis can still be readily noted dividing the diencephalic wall into the epithalamic, dorsal thalamic, ventral thalamic, and the hypothalamic zones.

In the epithalamus a quite thick outer cell layer or area cellularis externa, and a relatively thinner internal cell layer, or area cellularis interna are observed (fig. 7, Ep. l. and Ep. m.). These cell zones vaguely foreshadow the future medial and lateral habenular nuclei. In the dorsal thalamus an area of cell condensation of its inner cell mass is seen which bulges somewhat into the third ventricle. The outer cell zone of the dorsal thalamus can be readily delimited. It is slightly thicker than the inner cell mass (fig. 7, i.c.d. and e.c.d.). At this stage for the first time the lamina medullaris externa can be observed, which forms a cell free area lying between the medial parts of the dorsal and ventral thalamus (fig. 7, l. med. ext.).

At this level of the diencephalon the sulcus dorsalis joins the synencephalic recess (fig. 7, S.d.+Syn. Rec.). The parencephalic recess may also be seen, lying ventral to the sulcus medius (fig. 7, Par. Rec.). In the ventral thalamus the inner cell mass has become considerably thickened as has the outer cell zone of the ventral thalamus (fig. 7, i.v.c. and e.v.c.). However, the cells of the area cellularis externa of the ventral thalamus are more loosely arranged than those of the inner cell mass of this zone. In the hypothalamic region both the inner cell mass and the external cell zone, are quite thick. These cell areas form well defined cell masses in the ventral part of the diencephalic (fig. 7, i.h.c. and e.h.c.). At the medial border of the middle part of the hypothalamic zone a distinct sulcus, the sulcus lateralis infundibuli is seen which divides the hypothalamus into a dorsal and ventral subdivision (fig. 7, Sn.). KUHLENBECK (1931) has described a similar sulcus lateralis infundibuli in *Tropidonotus, Lacerta* and *Chrysemys*. The 24 mm stage of *Trichosurus vulpecula* would correspond, in a general way, in point of diencephalic development to that of the chick embryo of 6 days, as described by KUHLENBECK (1936).
Perameles nasuta (23 mm stage)

In point of degree of diencephalic development, the 23 mm Perameles nasuta represents a stage of diencephalic development more advanced than that of the 24 mm Trichosurus vulpecula. Thus, in the epithalamus a quite thick outer layer or area cellularis externa is observed, and a somewhat thinner inner cell layer or area cellularis interna (figs. 8 and 9, Ep. m. and Ep. i.). These cell masses vaguely foreshadow the future medial and lateral adult habenular nuclei. The diencephalic wall in the dorsal and ventral thalamus has become somewhat thicker than in the previously described stage. The sulcus dorsalis can still be noted, and demarcates the epithalamus from the dorsal thalamus (figs. 8 and 9, S.d.). In the dorsal thalamus the area cellularis interna forms a quite thick layer of densely packed cells (figs. 8 and 9, i.c.d.). The outer cell mass or the area cellularis externa of the dorsal thalamus is now a layer of rather loosely arranged cells which is slightly thicker than the area cellular interna (figs. 8 and 9, e.c.d.). The sulcus medius can be observed, and the sulcus ventralis may still be seen lying ventral to it (figs. 8 and 9, S.m. and S.v.).

The lamina medullaris externa is larger and better defined than at the previously described stage (figs. 8 and 9, l. med. ext.). It forms a well defined cell free zone lying between the inner and outer cell groups of the dorsal and the ventral thalamus. In the ventral thalamus the area cellularis interna has become fairly thick. It is separated from the outer cell zone of the ventral thalamus by an area in which the cells are rather sparsely arranged (figs. 8 and 9, i.v.c.). The outer cell zone or the area cellularis externa of the ventral thalamus has become thicker than at the previously described stage (figs. 8 and 9, e.v.c.). In the hypothalamic region the area cellularis interna has also become thickened, and forms a layer of rather densely packed cells. The outer layer or area cellularis externa of the hypothalamic zone now also forms a thick layer which, however, consists of loosely arranged cells (figs. 8 and 9, i.h.c. and e.h.c.).

In the medial wall of the hypothalamic zone the sulcus lateralis infundibuli is still observed (figs. 8 and 9, Sn.). In the peripheral part of the outer cell zone of the dorsolateral part of the hypothalamic region a small rather oval shaped cell mass can be seen. This forms the primordium of the future subthalamic nucleus (figs. 8 and 9, Cst. Pr.). Thus, the subthalamic nucleus is derived from the dorsolateral part of the area cellularis externa of the hypothalamic zone.
At the level of the caudal extremity of the hypothalamus the primordium of the mamillary body may be noted at the ventromedial part of the caudal extremity of the diencephalon (fig. 9, mam. Pr. R.). The primordium of the mamillary body forms a group of densely packed cells lying just ventromedial to the caudal extremity of the hypothalamus. The primordium of the mamillary nuclei is derived from the caudal extremity of the area cellularis interna of the hypothalamic zone. The 23 mm Perameles nasuta embryo would correspond, in point of diencephalic development, to that of the 7 day old chick as described by KUHLENBECK (1937).

**Trichosurus vulpecula** (30 mm stage)

In the diencephalon of the 30 mm Trichosurus vulpecula embryo the sulcus dorsalis is still distinct. In the wall of the epithalamus a fairly distinct medial habenular cell mass can be delimited in the medial cell mass of this zone. The lateral habenular nucleus can also be distinguished, forming a fairly thick band-like cell mass lying in the outer cell mass of the epithalamus (fig. 10, hm. and hl.). In the dorsomedial part of the inner cell mass of the dorsal thalamus a rather large area of cell condensation can be noted which forms the primordium of the future nucleus medialis thalami (fig. 10, Pr. m.). The sulcus medius is still seen. The sulcus ventralis is noted lying ventral to the sulcus medius. The lamina medullaris externa is readily observed as a well defined cell free zone lying between the inner and outer cell zones of the dorsal and ventral thalamus (fig. 10, l. med. ext.).

In the outer cell zone or area cellularis externa of the dorsal thalamus, in its peripheral part, a rather thin band-like group of cells is present which forms the pars dorsalis of the lateral geniculate nucleus (fig. 10, c.g.l.d.). The pars dorsalis of the lateral geniculate nucleus takes origin from the lateral part of the area cellularis externa of the dorsal thalamus. In the lateral part of the outer cell zone of the ventral thalamus, a small cell group rather densely arranged is seen which forms the primordium of the nucleus reticularis thalami (fig. 10, Pr. n. ret.). Thus, the nucleus reticularis thalami is derived from the ventrolateral part of the area cellularis externa of the ventral thalamus.

At the level of the caudal extremity of the diencephalon the caudal extremity of the dorsal thalamus may be noted. In the dorsal part of the caudal extremity of the dorsal thalamus an area of cell
condensation is observed, which forms the primordium of the future pretectal nuclei. At the caudal level of the diencephalon a small, rather loosely arranged cell mass, in the medial part of the ventral thalamus, forms the primordium of the future zone incerta nucleus (fig. 11, Pr. Zi.). This nucleus takes origin from the medial and ventral part of the area cellularis externa of the ventral thalamus. In the lateral part of the caudal extremity of the ventral thalamus, the caudal part of the primordium of the nucleus reticularis thalami is observed (fig. 11, Pr. n. ret.). In the periphery of the area cellularis externa of the ventral thalamus a small band-like group of cells forms the ventral division of the lateral geniculate nucleus. The pars ventralis of the lateral geniculate nucleus is derived from the peripheral part of the area cellularis externa of the ventral thalamus (fig. 10, c.g.l.v.). In the ventromedial part of the hypothalamic zone a fairly well defined oval shaped ventromedial nucleus of the hypothalamus can be delimited (fig. 10, Nu. hvm.). At the caudal level of the diencephalon the caudal extremity of the sulcus medius is still noted as is also the sulcus ventralis which is fused with the sulcus parencephalicus at this level (fig. 11, S.m. and S.v. + S. Par.).

Ventral to the sulcus ventralis the tegmental cell cord forming a thin band-like group of cells, is lying just ventrolateral to the third ventricle (fig. 11, t.g.c.). In the peripheral part of the dorsal area of the caudal extremity of the hypothalamus a fairly compact, elliptical shaped small group of cells can be delimited which forms the subthalamic nucleus of LUYs (fig. 11, Cst.). In the ventromedial part of the diencephalon the nucleus of the mamillary body is seen (fig. 11, Nu. mam.). This nucleus is considerably more differentiated than at the previously described stage and forms a densely packed compact cell mass. The tuberculum posticum, which marks the caudal end of the diencephalon, can be observed at this level of the diencephalon (fig. 11, tb. p.).

*Trichosurus vulpecula* (40 mm stage)

At the 40 mm stage the sulcus dorsalis still demarcates the epithalamus from the dorsal thalamus. In the epithalamus a definite medial habenular cell mass can be delimited in the medial cell group of this area. The medial habenular nucleus forms a fairly thick band-like, densely packed cell mass (fig. 12, hm.). The lateral habenular nucleus can likewise be readily demarcated. It forms a fairly large loosely arranged cell mass, lying lateral to the medial habenular
nucleus (fig. 12, hl.). In the medial part of the dorsal thalamus a large oval-shaped mass of cells, quite densely arranged, has differentiated from the dorsal part of the inner cell mass of the dorsal thalamus. It forms a well-demarcated nucleus medialis thalami (figs. 12, 13 and 14, m.). The massa intermedia is a poorly defined area consisting of loosely arranged cells lying on each side of the mid-plane, just medial and ventral to the nucleus medialis thalami. The lamina medullaris externa is still well defined (figs. 12, 13 and 14, l. med. ext.). It forms a cell free zone, lying between the nuclear derivatives of the dorsal and ventral thalamus. In the lateral part of the dorsal thalamus the pars dorsalis of the lateral geniculate nucleus is observed. This nucleus forms a thin band-like cell group (figs. 13 and 14, c.g.l.d.).

In the peripheral part of the outer cell zone of the ventral thalamus a band-like group of cells can be noted, which forms the ventral part of the lateral geniculate nucleus (fig. 13 and 14, c.g.l.v.). This nucleus is demarcated, at its dorsal extremity, from the dorsal part of the lateral geniculate nucleus. In the lateral part of the ventral thalamus, a loosely arranged cell mass is seen forming the nucleus reticularis thalami (figs. 12, 13 and 14, n. ret.). The nucleus zonae incertae can also be readily delimited. This nucleus forms a small oval-shaped cell group which lies in the medial part of the ventral thalamus (figs. 13 and 14, Z.i.). The sulcus ventralis may still be observed, demarcating the ventral thalamus from the hypothalamic zone of the diencephalon.

The subthalamic nucleus of LUYs is represented by an elliptical group of cells lying at the dorsolateral extremity of the caudal part of the hypothalamic zone (fig. 14, Cst.). It forms a well defined nucleus at this stage. In the hypothalamic zone an area of cell condensation may be noted in the locus of the future dorsomedial nucleus of the hypothalamus. The ventromedial nucleus of the hypothalamus forms a large oval-shaped cell mass in the ventromedial part of the hypothalamus (fig. 13, Nu. hvm.). At the 40 mm stage of Trichosurus vulpecula the mamillary nucleus can be demarcated as a definitive nucleus which is better defined than at the previously described stage (fig. 14, Nu. mam.). However, the mamillary nucleus is not as yet differentiated into definitive medial and lateral mamillary nuclei. No other hypothalamic nuclei can as yet be delimited. In the ventral part of the inner wall of the middle of the hypothalamic zone the sulcus lateralis infundibuli is still observed (figs. 13 and 14, Sn.). The sulcus lateralis infundibuli at
the more caudal level of the hypothalamus divides into two separate component sulci. The 40 mm *Trichosurus vulpecula* would correspond, in point of diencephalic development, to that of the 30 mm rabbit embryo as described by MIURA (1933).

*Trichosurus vulpecula* (44 mm stage)

In the diencephalon of the 44 mm *Trichosurus vulpecula* embryo most of the diencephalic nuclei of the adult state are present. In epithalamus a well defined medial and a lateral habenular nucleus can be readily demarcated, resembling those of the adult form (figs. 16, 17 and 18, hl. and hm.). The medial habenular nucleus forms a fairly large, compactly arranged cell mass, while the lateral habenular nucleus consists of a quite large, rather loosely arranged cell group lying just lateral to the medial habenular nucleus. At the anterior extremity of the dorsal thalamus can be demarcated a small oval-shaped cell mass of rather loosely arranged cells which forms the nucleus anterodorsalis thalami (fig. 15, N. ad.). Lying just ventral to the anterodorsal thalamic nucleus a small cell mass is seen, consisting of small and medium sized cells. This cell mass forms the anteroventral nucleus of the dorsal thalamus (fig. 15, N. av.). Both of these nuclei belong to the anterior group of the dorsal thalamic nuclei which are quite well developed in the thalamus of *Trichosurus vulpecula*. The rostral extremity of the nucleus reticularis thalami can also be noted at this level, lying just ventral and slightly lateral to the anteroventral nucleus of the thalamus (fig. 15, n. Ret. R.).

In the medial and dorsal part of the dorsal thalamus a well defined nucleus medialis thalami is observed. This cell mass forms a large oval-shaped group of densely packed cells which resembles the nucleus medialis thalami of the adult state (figs. 16, 17 and 18, m.). Just ventral to the medial part of the nucleus medialis thalami, a small cell mass consisting of compactly arranged cells can be noted. This cell group forms the nucleus parafascicularis thalami (figs. 17 and 18, Pf.). The nucleus parafascicularis is derived from the medial part of the area cellularis interna of the dorsal part of the dorsal thalamus. Just dorsal and slightly lateral to the nucleus medialis thalami, a fairly large oval-shaped cell mass can be demarcated forming the mediolateral nucleus thalami (figs. 16, 17 and 18, nml.). The nucleus mediolateralis thalami is derived from the area cellularis interna of the dorsal part of the dorsal thalamus. The nucleus
medialis thalami, the nucleus mediolateralis thalami and the nucleus parafascicularis thalami form the medial group of nuclei of the dorsal stage of the dorsal thalamus.

In the lateral part of the dorsal thalamus a large oval-shaped group of medium sized densely packed cells can be distinguished. This lies just lateral to the nucleus mediolateralis thalami and forms the nucleus lateralis dorsalis thalami (figs. 16, 17 and 18, ld.). It takes origin from the area cellularis externa of the dorsal thalamus. Just dorsal and slightly medial to the dorsolateral nucleus of the thalamus, a fairly large cell mass can be delimited consisting of large and medium sized cells rather densely arranged. This forms the nucleus posterior thalami. The nucleus posterior thalami also takes origin from the area cellularis externa of the dorsal thalamus (figs. 17 and 18, npo.). Lying lateral and ventral to the dorsolateral nucleus of the thalamus, at the peripheral part of the dorsal thalamus, a band-like group of cells can be seen which constitute the pars dorsalis of the lateral geniculate nucleus. This cell mass begins to resemble that of the adult state (figs. 16, 17 and 18, c.g.l.d.). The lamina medullaris externa can still be noted at this stage (fig. 16, l. med. ext.). At the caudal level of the diencephalon the rostral extremity of the pars dorsalis of the medial geniculate nucleus is noted. It forms a small cell group, lying at the periphery of the ventral stage of the dorsal thalamus (fig. 18, Cgm. d.r.).

In the ventral etage of the medial group of the dorsal thalamus a fairly large cell mass consisting mainly of medium sized cells, rather closely arranged, can be demarcated in its medial part. This forms the nucleus ventralis medialis thalami (figs. 16, 17 and 18, vm.), which takes origin from the ventral part of the area cellularis interna of the dorsal thalamus. Lying just lateral to this nucleus, in the ventral etage of the dorsal thalamus, a large oval-shaped cell mass is observed consisting of closely packed medium sized cells. This cell group forms the nucleus ventralis lateralis thalami which derives from the ventral part of the area cellularis externa of the dorsal thalamus (figs. 16, 17 and 18, lv.). Just ventral to the nucleus ventralis lateralis a rather spherical group of cells can be observed, which forms the nucleus reticularis thalami (figs. 16, 17 and 18, n. ret.). It belongs to the ventral thalamus, and forms a well defined nucleus at this stage.

At the lateral extremity of the ventral thalamus a band-like cell group is noted. This constitutes the pars ventralis of the lateral geniculate nucleus (figs. 16, 17 and 18, c.g.l.v.). In the medial part
of the ventral thalamus a small oval-shaped cell mass is seen lying medial to the nucleus reticularis thalami. This cell mass forms the nucleus zonae incertae which begins to resemble that of the adult state (figs. 16, 17 and 18, Zi.). Just ventral and slightly lateral to the nucleus reticularis thalami, in the subthalamus, an elliptical shaped cell group is observed consisting of large and some medium sized cells. This cell mass is the subthalamic nucleus of Luys (figs. 16, 17 and 18, Cst.). It forms a compact and quite well defined nucleus which begins to resemble that of the adult state. At the level of the caudal extremity of the diencephalon the tegmental cell cord can be noted. This is situated just ventral and lateral to the ventral extremity of the third ventricle and forms a thin band-like group of compactly arranged cells (figs. 17 and 18, t.g.c.). The sulcus dorsalis and the sulcus ventralis are still observed at this stage.

A fairly well defined nucleus paraventricularis hypothalami can be demarcated in the medial part of the anterior level of the hypothalamus (fig. 16, N. par. Hy.). The same is true for a well defined nucleus ventromedialis hypothalami and a dorsomedial nucleus, in the medial part of the hypothalamus (fig. 16, N. hdm. and Nu. hvm.). A nucleus lateralis hypothalami can also be noted in the lateral part of the hypothalamus, consisting of a large group of loosely arranged cells (fig. 16, la. hl.). At the caudal extremity of the hypothalamus the mamillary body is seen forming a well defined cell mass. Thus a quite definitive medial mamillary nucleus and a well defined lateral mamillary nucleus can be delimited in the mamillary body (fig. 16, Nu. mam. L. and Nu. Med. mam.). The 44 mm Trichosurus vulpecula embryo would correspond in point of degree of diencephalic development to a stage slightly more advanced than that of the 45 mm rabbit embryo, as described by Miura (1933).

**Discussion**

In *Trichosurus vulpecula, as in reptiles and birds (Kuhlenbeck, 1931, 1937), the various diencephalic nuclei take origin from the inner and outer cell zones of the four longitudinal zones of the diencephalon which are demarcated by sulci. All the respective epithalamic, thalamic and hypothalamic nuclei differentiate from the area cellularis interna and externa of these longitudinal zones. Thus, the medial and the lateral habenular nuclei differentiate from the inner and outer cell masses of the epithalamus. At the anterior part of the dorsal thalamus, the anterior extremity of the area cellularis...**
interna and externa of the dorsal thalamus fuse to form a single homogeneous cell layer.

From this fused homogeneous cell layer the definitive areas of cell condensation arise which constitute the nucleus anterodorsalis thalami and the nucleus anteroventralis thalami. These nuclei, which form the nuclear cell masses of the anterior part of the dorsal thalamus, appear in a definitive form in the 44 mm stage of *Trichosurus vulpecula*. They cannot be delimited with certainty at earlier stages. MIURA (1933) similarly found in the rabbit a nucleus anterodorsalis thalami and a nucleus anteroventralis thalami, which originated from the fused anterior extremity of the area cellularis interna and externa of the dorsal thalamus. However, a nucleus medialis anterior thalami, a nucleus parataenialis and a nucleus paramedianus thalami of the rostral group of the dorsal thalamic nuclei, such as MIURA found in the new-born rabbit, could not be demarcated in the 44 mm *Trichosurus vulpecula* embryo, which was the latest stage studied.

Further caudally the nucleus parafascicularis thalami and the nucleus mediolateralis thalami differentiate from the inner cell mass of the medial group of the dorsal stage of the dorsal thalamus. The nucleus medialis thalami likewise takes origin from the area cellularis interna of the medial part of the dorsal stage of the dorsal thalamus. It should be kept in mind however, that, although the differences between the area cellularis interna and externa are very pronounced at certain early transitory stages, these two cell areas have a tendency to merge with further differentiation. Thus, certain difficulties arise in definitely assigning the origin of some diencephalic nuclei exclusively to one or the other of these cell areas. MIURA (1933) similarly found in the rabbit that the medial group of the dorsal stage of the dorsal thalamic nuclei, namely the nucleus medialis thalami, the nucleus mediolateralis thalami, as well as the nucleus parafascicularis, took origin from the inner cell mass of the dorsal thalamus. However, in the 44 mm *Trichosurus vulpecula*, a nucleus reunions 1 and 2, in the dorsal stage of the medial group of the dorsal thalamic nuclei, such as MIURA found in the new-born rabbit, could not be demarcated.

The nuclei of the medial group of the ventral stage of the dorsal thalamus, which could be first noted in the 44 mm *Trichosurus vulpecula* embryo are the nucleus ventralis medialis thalami and the nucleus ventralis lateralis thalami which are in quite definitive form. The nucleus ventralis medialis thalami is derived from the area cellularis interna of the ventral part of the dorsal thalamus, while
the nucleus ventralis lateralis thalami originates from the area cellularis externa of the ventral part of the dorsal thalamus. In the new-born rabbit, MIURA (1933) similarly noted a nucleus ventralis medialis thalami and a nucleus ventralis lateralis thalami in the medial group of nuclei of the ventral etage of the dorsal thalamus. In the 44 mm *Trichosurus vulpecula* embryo a nucleus ventralis anterior, a nucleus reuniens 3 and 4, or a nucleus laminaris, such as described by MIURA in the ventral etage of the dorsal thalamus of the new-born rabbit could not be delimited.

In *Trichosurus vulpecula* the lateral group of dorsal thalamic nuclei originates from the area cellularis externa of the dorsal thalamus. This group consists of the nucleus lateralis dorsalis thalami, the nucleus posterior thalami, and the pars dorsalis of the lateral geniculate nucleus. MIURA (1933) also described in the lateral group of dorsal thalamic nuclei of the rabbit a nucleus lateralis dorsalis thalami, a nucleus posterior thalami and a pars dorsalis of the lateral geniculate nucleus. This group, according to MIURA, is derived from the area cellularis externa of the dorsal thalamus. However, in the 44 mm *Trichosurus vulpecula* a nucleus magnocellularis thalami, such as MIURA found in the lateral group of the dorsal thalamic nuclei of the new-born rabbit could not be distinguished. Thus, the origin and development of the various dorsal thalamic nuclei in *Trichosurus vulpecula* is quite similar to that of the rabbit as observed by MIURA (1933).

According to KUHLENBECK (1954) the zona incerta in mammals originates from the ventral thalamic zone. In *Trichosurus vulpecula* this zone is derived from the medioventral part of the cellularis externa of the ventral thalamus with perhaps an addition from the adjacent area cellularis interna of the ventral thalamus. The pars ventralis of the lateral geniculate nucleus differentiates from the peripheral part of the area cellularis externa of the ventral thalamus. In addition, the nucleus reticularis thalami differentiates from the ventrolateral part of the area cellularis externa of the ventral thalamus. MIURA (1933) similarly described in the ventral thalamus of the rabbit a nucleus reticularis thalami and a pars ventralis of the lateral geniculate nucleus, both originating from the area cellularis externa of the ventral thalamus. However, it was not possible to demarcate in the ventral thalamus of the 44 mm *Trichosurus vulpecula* a nucleus taeniae, or a nucleus reuniens 5, such as MIURA described in the ventral thalamus of the new-born rabbit. The new-born rabbit represents in point of degree of diencephalic development a
more advanced stage than the 44 mm *Trichosurus vulpecula* embryo.

Only the rostral extremity of the pars dorsalis of the medial geniculate nucleus was noted in the 44 mm stage of *Trichosurus vulpecula*. It is a small cell mass, situated in the periphery of the ventral stage of the dorsal thalamus. According to Legros Clark (1932, 1933) and Kuhlenbeck (1954), the pars dorsalis of the medial geniculate nucleus is differentiated from the posterior ventral nucleus of the thalamus. Inasmuch as a complete description of the development of the medial geniculate nucleus in *Trichosurus vulpecula* is planned in a future paper, no further mention will be made of this nucleus in this paper.

In the rostral part of the hypothalamus the lateral preoptic nucleus differentiates from the area cellularis externa. In the more caudal part of the hypothalamus the outer cell mass gives origin to the lateral hypothalamic nucleus. The area cellularis interna of the hypothalamic region differentiates into the medial preoptic nucleus, the dorsomedial hypothalamic nucleus, the ventromedial hypothalamic nucleus, the nucleus paraventricularis hypothalami, as well as into the nucleus posterior hypothalami. The medial and lateral mamillary nuclei differentiate from the caudal part of the area cellularis interna of the hypothalamic zone. Miura's (1933) interpretation of the origin and development of the various nuclei of the hypothalamus of the rabbit holds also for *Trichosurus vulpecula*.

The subthalamic nucleus of Luys is derived, in *Trichosurus vulpecula*, from the dorsolateral part of the area cellularis externa of the hypothalamic zone. This nucleus can be regarded as homologous to the nucleus entopeduncularis posterior of Sauropsida. The entopeduncular nuclei (anterior and posterior) represent the interstitial nuclei of the peduncular radiation of fiber masses. At the 44 mm stage, it begins to resemble the subthalamic nucleus of the adult state. The origin of the subthalamic nucleus of Luys as described by Miura (1933) in the rabbit is quite similar to that noted in *Trichosurus vulpecula*. The plan of diencephalic development in the marsupials as exemplified by *Trichosurus vulpecula* resembles in a general way Kuhlenbeck's observations on the development of the diencephalon in reptiles (1931) and in birds (1936, 1937). This plan and the manner of differentiation of the various thalamic and hypothalamic nuclei is also similar to that of the rabbit as described by Miura (1933).
Summary

1. The diencephalon of Trichosurus vulpecula shows at its early stages of development, the well known four longitudinal zones, i.e., the epithalamic, dorsal thalamic, ventral thalamic, and the hypothalamic zone. These zones are demarcated from each other by a sulcus dorsalis, a sulcus medius, and a sulcus ventralis, respectively.

2. In each of these four longitudinal diencephalic zones an inner area cellularis interna and an outer area cellularis externa develop.

3. The differentiation of the various cell masses of the diencephalon can be traced from the inner and outer cell groups of these zones.

4. The plan of development of the diencephalic nuclei in Trichosurus vulpecula resembles in a general way that in reptiles and birds (Kuhlenbeck, 1931, 1936, 1937) and more closely that in the rabbit (Miura, 1933).

Bibliography

———, The ontogenic development of the diencephalic centers in a bird's brain (chick) and comparison with the reptilian and mammalian diencephalon. J. Comp. Neur. 68: 23-63 (1937).
Key to Abbreviations

ad. anlage of adenohypophysis
am. pr. primordium of nucleus amygdala
Bs. vlp. periamygdalar cortex
Cer. Pl. cerebellar plate
c. g. l. d. pars dorsalis of lateral geniculate nucleus
c. g. l. v. pars ventralis of lateral geniculate nucleus
Cgm. d. r. rostral extremity of the pars dorsalis of medial geniculate nucleus
Ch. Pl. IV. chorioid plexus anlage of fourth ventricle
Cl. claustrum
Cst. subthalamic nucleus of Luys
Cst. Pr. primordium of subthalamic nucleus of Luys
D(2+1)c pyriform cortex
e. c. d. area cellularis externa thalami dorsalis
e. h. c. area cellularis externa hypothalami
e. v. c. area cellularis externa thalami ventralis
fde. dentate fascia
hl. lateral habenular nucleus
hm. medial habenular nucleus
hy. hypothalamus
i. c. d. area cellularis interna thalami dorsalis
i. h. c. area cellularis interna hypothalami
inf. st. infundibular stalk
i. v. c. area cellularis interna thalami ventralis
l. af. lamina affixa
la. hl. lateral hypothalamic nucleus
ld. nucleus lateralis dorsalis thalami
l. med. ext. lamina medullaris externa
lv. nucleus ventralis lateralis thalami
m. nucleus medialis thalami
mam. Pr. R. rostral part of primordium of mamillary body
mes. mesencephalon
mi. massa intermedia of thalamus
m. ad. nucleus anterodorsalis thalami
N. av. nucleus anteroventralis thalami
N. am. nucleus amygdala
N. hdm. dorsomedial nucleus of hypothalamus
nml. nucleus mediolateralis thalami
npo. nucleus posterioris thalami
n. ret. nucleus reticularis thalami
n. Ret. R. rostral extremity of nucleus reticularis thalami
N. tb. Hy. nucleus tuberis of hypothalamus
Nu. hvm. ventromedial nucleus of hypothalamus
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<tr>
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<tr>
<td>Nu. mam.</td>
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Plate

I—V
Explanation of Figures

Fig. 1. Transverse section of diencephalon of an 8.5 mm *Trichosurus vulpecula* embryo, 12 μ. This and all other sections illustrated have been stained with hematoxylin and eosin.

Fig. 2. Transverse section of diencephalon of a 14.5 mm *Trichosurus vulpecula* embryo, 12 μ.

Fig. 3. Transverse section of diencephalon of a 13.5 mm *Trichosurus vulpecula* embryo, 12 μ.

Fig. 4. Sagittal section of the diencephalon of a 13.5 mm *Phascolarctos phascolomys* embryo, close to the midplane, 12 μ.
Explaination of Figures

Fig. 5. Transverse section of diencephalon of a 15 mm *Perameles nasuta* embryo, 12 μ.

Fig. 6. Transverse section of diencephalon of a 10 mm *Trichosurus vulpecula* embryo, 12 μ.

Fig. 7. Transverse section of diencephalon of a 24 mm *Trichosurus vulpecula* embryo, 12 μ.

Fig. 8. Transverse section of diencephalon of a 23 mm *Perameles nasuta* embryo, 12 μ.
Explanation of Figures

Fig. 9. Transverse section of diencephalon of a 23 mm *Perameles nasuta* embryo, at a level slightly caudal to that of fig. 8, 12 μ.

Fig. 10. Transverse section of diencephalon of a 30 mm *Trichosurus vulpecula* embryo, 12 μ.

Fig. 11. Transverse section of the caudal part of the diencephalon of a 30 mm *Trichosurus vulpecula* embryo, 12 μ.

Fig. 12. Transverse section of the rostral level of the diencephalon of a 40 mm *Trichosurus vulpecula* embryo, 12 μ.
Explanation of Figures

Fig. 13. Transverse section of diencephalon of a 40 mm *Trichosurus vulpecula* embryo, at the level of the middle part of the diencephalon, 12 μ.

Fig. 14. Transverse section of diencephalon of a 40 mm *Trichosurus vulpecula* embryo, at a level slightly caudal to that of fig. 13, 12 μ.

Fig. 15. Transverse section of the rostral extremity of the diencephalon of a 44 mm *Trichosurus vulpecula* embryo, 12 μ.

Fig. 16. Transverse section of the middle level of the diencephalon of a 44 mm *Trichosurus vulpecula* embryo, 12 μ.


**Explanation of Figures**

Fig. 17. Transverse section of the diencephalon of a 44 mm *Trichosurus vulpecula* embryo, at a level caudal to that of fig. 16, 12 μ.

Fig. 18. Transverse section of the diencephalon of a 44 mm *Trichosurus vulpecula* embryo, at a level slightly caudal to that of fig. 17, 12 μ.