Determination of the embryonic origin of the mesencephalic nucleus of the trigeminal nerve in birds

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SUMMARY

The precise site of origin and the cells of origin of the mesencephalic nucleus of the trigeminal nerve in birds have been studied by the method of growing embryonic fore-, mid- and hind brain grafts inside a millipore filter chamber filled with amniotic fluid, upon a host chorioallantoic membrane, and by the method of transplantation of cranial neural crest of the mesencephalon between quail embryo-donors and duck embryo-hosts.

The growth and differentiation of grafts grown in the chambers resemble very closely those of normal embryos at corresponding stages of development. Data obtained from a total of 42 grafts representative of the three species of birds used in this study, show that the cells of the mesencephalic nucleus are present only in grafts of embryonic midbrain and totally absent in forebrain and hindbrain grafts. The presence of cells of the mesencephalic nucleus in midbrain grafts suggests that these neurons arise chiefly from the developing mesencephalon. Histological examination of such grafts has shown that these neurons are observed in the leptomeningeal tissue overlying the midbrain region and extend into the various laminae of the optic tectum. A progressive increase in the size of the cells from an immature state confined to the mesodermal tissue to fully mature neurons in the stratum griseum periventriculare of the optic tectum is observed. This is also indicative of a downward migration of these cells.

In interspecific transplantation experiments of cranial neural crest of the mesencephalon, the quail cells occur in the form of clusters and appear to migrate towards the ventricular surface independent of the migration of the cells of the tectum which takes place simultaneously. Three stages in the development of the grafted neural crest material of the quail are observed based on the perikaryal diameter of the cells, and the accumulation of Nissl material in the cytoplasm. The smallest cells are located more superficially near the pial surface, and the larger mature neurons are observed in the stratum griseum periventriculare of the optic tectum. All the layers of the optic tectum including the neuroepithelium on the experimental side are comprised entirely of duck cells. The data presented in this study provide direct evidence that the precursor neurons of the mesencephalic nucleus of the

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trigeminal nerve are derivatives of the cranial neural crest of the mesencephalon, and that the layers of the optic tectum are formed through the proliferative activity in the neuroepithelium.

INTRODUCTION

The mesencephalic nucleus of the trigeminal nerve has long been a convenient group of neurons for purely anatomical, histological and physiological investigations from the time of Meynert (1872), since they have been recognized as the largest sensory neurons in the central nervous system. This is particularly reflected by the vast data that have been accumulated since then on all the above aspects concerning the nucleus (Johnston, 1909; May & Horsley, 1910; Willens, 1911; Valkenberg, 1911; Allen, 1919; Weinberg, 1928). However, the precise site of origin of the cells of the mesencephalic nucleus has not been clearly defined or established for any of the vertebrate species. Incidental references have been made to these cells being derived from either a separate germinal population found in the alar plate of the midbrain and pons (Alley, 1973, 1974), or, in contrast to other nuclei of the brain stem, from the neural crest of the related neural folds (Piatt, 1945; Rogers & Cowan, 1973).

Although several attempts have been made in the past (Weston, 1962, 1963; Johnston, 1966) to trace the migration and differentiation of neural crest cells using autoradiographic procedures, the latter method has since proved impracticable for several reasons. The labeling becomes diluted over a number of cell divisions and cannot be strictly localized making both quantitative and qualitative estimates very difficult. In addition, there is always the question whether all of the cells showing the label are indeed neural crest derivatives. Of particular interest in this context are the interspecific transplantation experiments between chick and quail embryos by Le Douarin (1969, 1973), who has used this technique effectively and extensively in the analysis of the developmental fate and migration pattern of grafted neural crest cells derived from the quail. As pointed out by Le Douarin (1973), the natural quail nuclear marker labeling technique is not only stable, but also stands out in sharp contrast from those of the host cells in unparalleled cleanliness and constancy. In spite of the paucity of information on the precise origin of these cells, there is general agreement, based on careful morphological studies, concerning the close similarity in appearance between the cells of the mesencephalic nucleus of the trigeminal nerve and those of the dorsal root ganglia.

The experiments reported here are concerned with two interrelated problems: the precise site of origin of the cells of the mesencephalic nucleus of the trigeminal nerve; and the origin of the precursor neurons of the mesencephalic nucleus. The modified method of chorioallantoic membrane grafting described by Kato (1970) suggested itself as a more feasible approach to study the localization of the cells in terms of the exact embryonic level from which the cells originate, and also to follow their migration into the stratum griseum
Origin of the mesencephalic nucleus

periventriculare of the optic tectum and among the fibers of the posterior com-
misure. The second problem was approached with the knowledge gained from
the first series of experiments in order to determine the cells of origin of the
mesencephalic nucleus. Le Douarin's work prompted us to try to apply the same
techniques to follow through the cranial neural crest cells of the mesencephalon,
if indeed they represent the precursor neurons of the mesencephalic nucleus.
The basic approach has been to transplant a fragment consisting almost entirely
of neural crest of the mesencephalon from donor quail embryos to host duck
embryos, and to look for the distribution of the donor quail cells in a closely
timed series of developmental stages in the hope of identifying precisely the cells
of origin of the mesencephalic nucleus.

MATERIALS AND METHODS

Experiment I: Chorioallantoic membrane grafts (CAM) of embryonic brain
regions

Three species of birds were chosen for this study, namely, the Japanese quail,
Coturnix coturnix japonica; the Babcock strain of fowls and the Peking duck,
Anas platyrhynchos. Fertile eggs from quails and fowls were obtained from birds
maintained in the animal care facility of this Medical Center, while eggs of the
Peking duck were obtained from C. & R. Duck Farm, Inc., New York. The
eggs were incubated in forced draft incubators maintained at 37.5 °C and
relative humidity at 65–70 %. In order to ensure that the embryos of the three
species used in this study would be of the same stage at the time of operation
corresponding approximately to chick embryo stage 10, somites 10, 33–38 h of
incubation (Hamburger & Hamilton, 1951), the eggs were set for incubation
times as follows: 33–38 h for the chick; 27–30 h, stage 7 for the quail; 45–50 h,
stage 8, for the duck. Corresponding stages for the quail were determined
according to the stage series of Zacchei (1961), and for the duck according to
Koecke (1958). The method of opening the eggs and preparing the embryos for
microsurgery have been described fully by Narayanan (1970).

GRAFTING PROCEDURE

The modified method of growing a graft inside a Millipore filter chamber
placed upon a host chorioallantoic membrane (CAM), and filled with amniotic
fluid as described by Kato (1970), was followed. The chamber consisted of a ring
cut out from a small size Beem capsule with an inner diameter of approximately
6 mm, and 5 mm high, to the bottom of which a Millipore filter disc (H-A type)
was glued with Millipore cement (Fig. 1). A hole was then made in the center
of the disc with a hot needle. The chamber was sterilized with ultraviolet light
irradiation before use. Chick embryos of approximately 9–11 days of incubation
age were used as hosts for grafting. After incubating for the desired period, eggs
Fig. 1. Schematic drawing of the chorioallantoic membrane grafting procedure for growing grafts of embryonic brain regions from a stage 9-10 chick embryo inside a Millipore filter chamber. FB, forebrain; MB, midbrain; HB, hindbrain; MFV, Millipore Filter Chamber; CAM, chorioallantoic membrane.

of each donor species were provided with a 'window' (Narayanan, 1970). The shell membrane in each case was picked away with watchmaker's forceps, and the outside of the shell was sterilized with a cotton swab soaked in absolute alcohol. Each egg prepared in this manner was then transferred to an eggholder (Wenger, 1951). For the quail egg, however, a special platform made of plexiglas with a hole cut in the center resembling the general elliptical shape of the egg was placed between the clamps of the egg holder (Narayanan, 1970). The surgical procedure was carried out in a two-egg operating carousel (Narayanan, 1970) in order to expedite the rapid transfer of organ primordia from donor to host, and also, to keep both donor and host embryos under optimal temperature and humidity conditions during the operation.

First, the host embryo was prepared for grafting following the procedure described by Hamburger (1960). A sterilized Millipore filter chamber (MFC) was carefully positioned on the CAM in such a way that the hole in the disc was directly on the ‘Y’ fork of a bifurcating membrane blood vessel to promote vascularization of the graft. The chamber was filled with chick Ringer's solution (New, 1966). Next, the donor embryo was moved in place under the microscope for extirpation of embryonic brain regions. The donor embryo was stained with neutral red impregnated in agar. The vitelline membrane over the head region was carefully incised with a glass needle. The desired embryonic brain region corresponding to each of the primary brain vesicles including non-neural tissues of that level was isolated in ovo by clean transverse incisions as shown in Fig. 1, using a vibrating needle. The excised brain region was loosened and allowed to
float with the addition of Ringer’s solution and was transferred to the chamber on the CAM of the host embryo with a Spemann micropipette. The Ringer’s solution in the chamber was gradually withdrawn by suction, and the graft was affixed to the desired site. The window on the host egg was then sealed with a round cover glass and hot paraffin. Between 12 and 18 h following grafting, the seal was removed and fresh chick amniotic fluid was added to the chamber. The egg was resealed and returned to the incubator. The grafts were allowed to grow in the chamber undisturbed and recovered 8, 10 and 12 days after grafting. They were then fixed in Bouin’s fluid overnight, dehydrated in ethanol, processed for paraffin embedding, serially sectioned at 12 μm, and stained with Haematoxylin-eosin-orange-G (Humason, 1962).

Experiment II: Interspecific transplantation of cranial neural crest from the level of the mesencephalon between quail embryo-donors and duck embryo-hosts

This series of experiments were performed on duck embryos as hosts and quail embryos as donors. We chose the duck embryo to serve as the host for various reasons. First, the posterior commissure in the duck appears as a conspicuous bundle of fibers between the optic tecta which are farther apart than in other species of birds. Therefore contributions from both donor and host embryos in the development of the optic tectum and also the mesencephalic nucleus may be clearly identified in a histologic section. Second, the cells of the mesencephalic nucleus appear to be clearly delimited into medial and lateral cell clusters among the fibers of the posterior commissure and within the stratum griseum periventriculare of the optic tectum respectively, which may be important in identifying cells derived from the donor quail graft.

Fertile duck eggs for this experiment were purchased from C. & R. Duck Farm, Inc., New York, and the quail eggs were collected from birds maintained in the animal care facility at this medical center. Both duck and quail eggs were incubated in the laboratory in large forced draft incubators at 37.5 °C and at a relative humidity of 65-70 %. To ensure that the embryos of the two species would be of the same stage of development at the time of operations, the duck eggs were incubated for 40–45 h, and the quail eggs, 27–30 h prior to the operation. Incubation times and stages for the operation were determined according to the normal stage series by Zacchei (1961) for the quail, and by Koecke (1958), for the duck (Fig. 2).

The method of opening the egg and preparing the embryo for microsurgery using a two egg operating carousel was similar to that previously described (Narayanan, 1970). The operations were carried out aseptically. Briefly, the vitelline membrane and the fragment of neural crest at the level of the mesencephalon to be excised from the donor quail embryo were lightly stained with Nile blue sulfate impregnated in agar, while the corresponding level of the mesencephalon of the duck embryo host was stained with neutral red
Fig. 2. Scheme showing the excision of a fragment of neural crest from the midbrain region of a quail embryo to replace a similar fragment previously removed from a duck embryo.

impregnated in agar. This differential staining of graft tissue as pointed out in a previous study (Narayanan & Hamburger, 1971) facilitated the exact positioning and orientation of the graft while being maneuvered into place in the host embryo.

The excision of the neural crest fragment from the donor quail embryo was carried out with the vibrating needle (Wenger, 1968). First, a mid-dorsal incision was made along the length of the mesencephalon, and the right half of the neural tube of this region was separated. Next, the neural crest material of the right half was then carefully separated from the underlying neuroepithelium by running the very fine point of the vibrating needle between it and the neural crest, while being viewed under a Reichert binocular stereomicroscope at a magnification of ×40. Every effort was made to dissect out the neural crest fragment cleanly with as little of extraneous material as possible. Similarly, utmost care was taken to avoid injury to the neuroepithelium of the level of the mesencephalon of the host embryo while preparing the gap to receive the graft tissue. The excised fragment of neural crest was transferred with a Spemann micropipette from donor quail to the duck embryo host (Fig. 2). A procedure which we consider critical in in ovo operations at very early stages is the addition of a mixture of Ringer's solution and albumen to the host after surgery. The albumen in this case was drawn from another incubated duck egg. As pointed out by Silver (1960), the removal of the vitelline membrane in the area of surgery
leads to the loss of a thin film of albumen over the surface of the embryo which is normally responsible for keeping the vitelline membrane moist. Unless this is replenished by the addition of the albumen mixture, it invariably results in the formation of adhesions between the embryo and the vitelline membrane due to desiccation of the membrane, and as a consequence to the death of the embryo (New, 1966). The unoperated side of the host embryo was used as controls for the experiment. All operated embryos were raised for periods ranging from 9 to 15 days of incubation age. They were fixed in Zenker’s fluid by immersion or by transcardiac perfusion. The brains were processed for paraffin embedding, serially sectioned at 12 μm in a transverse plane, and stained according to Feulgen and Rossenbeck’s procedure.

**OBSERVATIONS**

The following observations are based on a total of 42 embryonic brain grafts representative of three avian species used in this investigation. In general, grafts of embryonic brain regions grew well within the chamber and differentiated normally. Figure 3 shows a typical case of a midbrain graft excised from a quail embryo of approximately 27–30 h of incubation age which has grown to the extent of almost completely filling the chamber, and showing excellent differentiation with feather follicles period. One feature common to the majority of grafts was the degree of vascularization indicative of good growth of the graft within the chamber (Fig. 3). The grafts were allowed in each case to grow in the chamber for approximately 9–12 days. CAM grafts of the embryonic forebrain showed good differentiation of structures typical of the forebrain region. The extent of differentiation of the graft which was studied microscopically showed features resembling grossly that of a normal embryo of that species at corresponding stages of development. The telencephalic hemispheres showed expansion of their lateral walls representing the corpus striatum, prominent lateral ventricles and the choroid plexus. The optic primordium was normal in appearance and location. A ring of scleral cartilage, complete and normal in thickness was present. Short thick extrinsic eye muscles, lens and a much folded membrane of pigment cells were identifiable. Cells of the mesencephalic nucleus of the trigeminal nerve were not observed in any of the grafts of this series.

The development and differentiation of the structures in CAM grafts of the embryonic hindbrain were typical of the rhombencephalic region of normal embryos at corresponding stages of development. An enlarged fourth ventricle and structures characteristic of the inner ear were observed. Cells of the mesencephalic nucleus of the trigeminal nerve were not observed in any of the embryonic hindbrain grafts.
Fig. 3. Graft of the midbrain of quail showing rich vascularization of graft. Developmental age – 7 days.

Fig. 4. Section of graft embryonic midbrain of a quail embryo grown in the chamber for 11 days, showing symmetrical development of the optic tecta and the various layers of the tectum.

**CAM grafts of the midbrain**

In grafts of this series, the tectum was well developed and appeared to be organized into clearly identifiable strata as in normal embryos at corresponding stages of development (Fig. 4). Figure 4 shows section of a graft of the embryonic midbrain of a quail embryo grown in the chamber for 11 days. The

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**Figures 5-8**

Fig. 5. Section through midbrain graft of a quail embryo showing immature cells of the mesencephalic nucleus of V (arrow) migrating through the leptomeningeal tissue. Developmental age – 11 days.

Fig. 6. Section through midbrain graft of a quail embryo showing medium sized migrating cells of the mesencephalic nucleus of V (arrows) in the stratum album centrale of the optic tectum. Developmental age – 11 days.

Fig. 7. Section through midbrain graft of a quail embryo showing a large mature cell of the mesencephalic nucleus of V (arrow) in the leptomeninges which had failed to migrate.

Fig. 8. Section through midbrain graft of a quail embryo showing two large, mature cells of the mesencephalic nucleus of V. One is located in the leptomeninges (A); the other is located in stratum griseum preventriculare (B).
Origin of the mesencephalic nucleus
optic tecta appear symmetrical and the various layers are clearly seen. Prominent in the tegmental region, the trochlear and oculomotor nuclei were seen in some of the grafts. Neurons of the mesencephalic nucleus were observed in all of the midbrain grafts, and this was seen consistently in grafts derived from chick, duck and quail embryos. Based on area measurements made of cells of the mesencephalic nucleus in the grafts, there appeared to be a developmental trend with respect to relative size, and maturity of the cells. This trend is clearly brought out in Figs 5–8. For instance, very immature cells are observed migrating through the leptomeninges into the optic tectum (Fig. 5). Larger migrating cells with large pale nuclei and cytoplasm with practically no Nissl appear scattered throughout the various layers of the optic tectum. In Figure 6, clusters of large migrating cells are observed in the stratum album centrale, and one conspicuously large mature mesencephalic cell with abundant Nissl in its cytoplasm is seen located in the stratum griseum periventriculare. Perhaps of even greater interest is the finding that in some instances conspicuously large mature mesencephalic cells with abundant Nissl are seen in the leptomeningeal tissue. Figure 7 shows one such cell in the leptomeninges which has apparently failed to migrate, while Fig. 8 shows fully developed mesencephalic cells, one of which is located in the leptomeninges while the other has reached its normal destination in the stratum griseum periventriculare. A gradation in the size of the cells was readily apparent proceeding from the pial surface inwards and could be classified into: (a) an early neuroblast stage which comprised small cells with perikaryal diameter between 5 and 7 µm mostly observed in the leptomeningeal tissue; (b) migratory stage of cells 8–10 µm in the layers of the optic tectum chiefly in the stratum album centrale and (c) mature cells 12–16 µm in the stratum griseum periventriculare. This distribution of the cells was also highly suggestive of a downward migration of the cells of the mesencephalic nucleus during development. The cells of the mesencephalic nucleus are distinguishable from the rest of the cells in the different laminae of the optic tectum in that they appear mostly in clusters, particularly in later stages of development, as well defined medial and lateral groups of cells. The medial group of cells are found chiefly among the fibers presumably of the tectal commissure in the grafts (Fig. 12) while the

**Figures 9–12**

Fig. 9. Section through the optic tectum of a 10-day normal quail embryo showing the lateral group of cells of the mesencephalic nucleus of V (arrows).

Fig. 10. Section through graft of midbrain of a quail embryo showing the lateral group of cells of the mesencephalic nucleus of V (arrows). Developmental age – 11 days.

Fig. 11. Section through the tectal commissure of a 10-day normal quail embryo showing the medial group of cells of the mesencephalic nucleus of V (arrows).

Fig. 12. Section through graft of midbrain of a quail embryo showing the medial group of cells of the mesencephalic nucleus of V (arrow) in the tectal commissure. Developmental age – 11 days.
Origin of the mesencephalic nucleus
lateral group of cells are arranged as a single row confined chiefly to the stratum griseum periventriculare of the optic tectum (Fig. 10). The distribution of mature cells follows the same pattern into medial and lateral groups as is commonly observed in sections through the midbrain of control quail embryos (Figs. 9, 11).

Cell differentiation and organization in control versus experimental optic tecta with transplanted cranial neural crest from quail embryo-donors

The following observations are based on 14 experimental cases which showed good fusion of the grafted neural crest fragment from quail embryo-donors, with the neuroepithelium at the level of the mesencephalon of duck embryo-hosts. The optic lobes of both the unoperated control side and operated (right) side, developed in normal fashion. The tecta were approximately of the same size and shape based on external appearance as criterion. The operated region showed good healing and was covered with skin.

Histological examination of the experimental material confirmed that the grafting procedure was successful in the cases reported in this study. There were no developmental aberrations. The external limiting membrane was intact, and there was no evidence of any cellular extensions or overgrowth due to the experimental intervention. To study the effect of transplantation of neural crest material on the organization of the cells in the various strata of the optic tectum, we have followed the description and terminology of LaVail & Cowan (1971a) on the normal cytoarchitectonic structure and cellular morphology for the mature chick optic tectum. The pattern of distribution of cells in the optic tectum is shown in Figs. 13 and 15 of the normal quail embryo of approximately 12 days incubation age, and in Figs. 14 and 16 of a normal duck embryo of 14 days incubation age. From this it can be seen that the stratification is identical in both species. A comparison of sections with those illustrated by

**Figures 13-16**

Fig. 13. Section through the optic tectum of a normal quail embryo of 12 days incubation age to show the typical laminated arrangement of tectal cells. The neural epithelium (ne) is to the left bottom corner of the picture. Note the distinct nucleoli of the quail cells. Arrows indicate the cells of the mesencephalic nucleus of V.

Fig. 14. Section through the optic tectum of a normal duck embryo of 14 days incubation age. The neural epithelium (ne) is to the left bottom corner of the picture. Note the less well defined nucleoli of the duck cells compared to those of the quail in Fig. 2. Arrows show the cells of the mesencephalic nucleus of V.

Fig. 15. Higher power view of a portion of the optic tectum of a 12-day normal quail embryo to show the large nucleolus characteristic of quail cells. Arrows indicate the cells of the mesencephalic nucleus of V. Feulgen–Rossenbeck’s staining.

Fig. 16. Higher power view of a portion of the optic tectum of a 14-day normal duck embryo. The cells are appreciably pale staining, and the mass of chromatin appears dispersed. Arrows show cells of the mesencephalic nucleus of V. Feulgen–Rossenbeck’s staining.
Origin of the mesencephalic nucleus

[Images of tissue sections with black arrows pointing to cells]
LaVail & Cowan (1971a) for the chick suggest that the general pattern is very similar to that of the chick, and for this reason no attempt will be made to describe the various strata of either the duck or quail embryo in any detail.

In the experimental cases, locating the boundaries of the graft by the presence of quail cells posed no serious problem, since in the quail nucleus the chromatin appears as a large central mass that is strongly Feulgen positive when stained by the Feulgen–Rossenbeck’s technique (Le Douarin, 1973). This unique property of the quail cells as pointed out by Le Douarin (1973) enables one to follow the quail cells wherever they migrate and in whatever part or structure they eventually become located. The graft was usually found midway in the roof of the cerebral aqueduct extending laterally into the optic tectum of the right side. A low-power photomicrograph of a section through the optic tectum in one of our experimental cases (QDNC 18; 16-day embryo) is shown in Fig. 17. From this it can be seen that the major strata as described by LaVail & Cowan (1971b) are recognizable and comprised chiefly duck cells. Along the ventricular surface of the tectum, the neuroepithelium is rather thin and is composed of densely packed cells to form a pseudo-stratified epithelium at this stage. Proceeding towards the pial surface, the fairly wide cellular zone immediately adjoining the neuroepithelium representing the stratum griseum periventriculare, the fiber zone representing the stratum album centrale, and the cellular zone – stratum griseum centrale are recognizable in the section.

In contrast to the disposition of duck cells in the optic tectum on the grafted side into clearly distinguishable strata, the quail cells of the graft did not appear to conform to the laminated arrangement. Instead, the quail cells were seen in closely packed clusters near the pial surface (Figs. 20, 21) becoming scattered in the deeper layers of the optic tectum and finally are seen distributed more or less evenly as a row of cells within the stratum griseum periventriculare. The same three stages of development of these cells as described in the preceding pages under Experiment I, are readily recognizable on the basis of form, size and the amount of Nissl material accumulated in the cell. Quail cells closer to the pia are small with perikaryal diameter of approximately 5–7 \( \mu m \); medium-sized cells with diameter between 8 and 11 \( \mu m \) are present in the deeper layers of the optic tectum and mainly in the stratum album centrale, and large cells with perikaryal diameters of 12–18 \( \mu m \) occur mostly in the stratum griseum periventriculare.

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Fig. 17. A low-power photomicrograph of a transverse section to show the general appearance of the optic tectum in a 16-day experimental duck embryo (QDNC-18) in which quail neural crest was grafted. The arrows indicate the course of migration of the quail neural crest cells towards the stratum griseum periventriculare and among the fibers of the tectal commissure.
Mesencephalic nucleus of the trigeminal nerve

Two significant features emerge from the foregoing analysis of our material with respect to the composition and distribution of cells in the optic tectum of the experimental side, in addition to the various strata being comprised exclusively of duck cells. First, is the presence of distinctive quail cells in the stratum griseum periventriculare, which on the basis of their size and location represent the lateral group of cells of the mesencephalic nucleus (Fig. 18). Secondly, is the presence of quail cells in compact masses closer to the midline and among the fibers of the tectal commissure (Fig. 19) representing the medial group of cells of the mesencephalic nucleus in the grafted side. Qualitatively, the quail cells of both medial and lateral groups are large, with considerable amounts of Nissl material scattered throughout the cytoplasm of the cell and large centrally placed vesicular nuclei, resembling very closely cells of the mesencephalic nucleus of normal quail embryos at corresponding stages of development.

DISCUSSION

It is apparent from the chorioallantoic membrane transplantations of embryonic brain regions of the three avian species detailed above that the cells of the mesencephalic nucleus of the trigeminal nerve are derived mainly from the mesencephalon. It has enabled us to extend and clarify the results of previous investigators (Pearson, 1949; Rogers & Cowan, 1973). As yet, there have been comparatively few studies devoted to the origin of the cells of the mesencephalic nucleus. The experiments of Piatt (1945) on Amblystoma favor a neural crest origin of these neurons, although the possible origin of these neurons from other sources such as the neuroblasts in the neural folds, which later differentiate in the outer margin of the central gray of the alar plates of the mesencephalon has been proposed by Pearson (1945). Alley (1974) in his studies on the morphogenesis of the trigeminal mesencephalic nucleus in the hamster produced indirect

Figures 18–21

Fig. 18. Higher power view of area A in Fig. 17 to show the cells of the mesencephalic nucleus of V (marked by arrows) derived from the grafted quail neural crest. The stratum griseum periventriculare (sgp), and the neural epithelium (ne) comprised duck cells.

Fig. 19. Higher power view of area B in Fig. 17 to show the cells of the mesencephalic nucleus of V (marked by arrows) derived from the grafted quail neural crest among the fibers of the tectal commissure.

Fig. 20. A photomicrograph of a portion of the optic tectum close to the pial surface in a 16-day experimental duck embryo (QDNC-17), to show migrating quail cells (marked by arrows) derived from the quail neural crest graft.

Fig. 21. A photomicrograph of a portion of the optic tectum close to the ventricular surface in a 17-day experimental duck embryo (QDNC-13), to show migrating quail cells (marked by arrows) in the stratum griseum periventriculare (Sgp).
evidence suggesting that these cells arise from a germinal population other than the ventricular zone of the alar plate. Rogers & Cowan (1973) in a study on the origin of the mesencephalic nucleus of V in the chick based on autoradiographic procedures, have suggested a neural crest or neural fold origin from the midbrain. Hamburger & Narayanan (1969) observed a severe depletion in the number of cells in the mesencephalic nucleus of V following radical extirpation of the neural crest of embryonic midbrain of rostral hindbrain, also suggesting a neural crest origin for these cells from midbrain or rostral hindbrain levels. Most of these studies have provided only indirect evidence in support of their views on the probable site of origin of these neurons.

The resemblance of cells in the leptomeninges to those of the deeper layers of the optic tecta are in general agreement with Rogers & Cowan (1973) and is borne out by our data. A detailed discussion of this work is beyond the scope of the present study, but a few points deserve special comments. As pointed out by Rogers & Cowan (1973) if the neurons of the mesencephalic nucleus are derived from the neural folds one might expect to find all of these neurons in all stages of development to remain within the external limiting membrane. On the other hand, if they are derived from neural crest some of these neurons might be observed in the loose leptomeningeal tissues. In our CAM transplants immature neurons have been observed to migrate through the leptomeningeal tissues suggestive of a neural crest origin for these cells. One other feature which provides additional evidence in support of their neural crest origin is the presence of clearly identifiable mature cells of the mesencephalic nucleus in the leptomeningeal tissue, and hence, outside of the external limiting membrane. Its presence in the mesodermal tissue can only be explained as due to failure on the part of these cells to migrate which is not an unusual occurrence in the migratory behavior of neural crest cells.

The method of interspecific transplantation of neural crest material between quail and duck embryos has enabled us to clarify earlier observations on the cells of origin, migration and final localization of the neurons of the mesencephalic nucleus of V. The presence of cells of the mesencephalic nucleus derived from the grafted neural crest fragment of the quail is perhaps the most striking observations of this study. The migration of the presumptive neurons of the mesencephalic nucleus is of considerable interest in view of the migration of the tectal cells themselves which occurs simultaneously. Especially notable, in this context, are the studies of La Vail & Cowan (1971a, b) on the normal cyto-architectonic structure of the chick optic tectum, using autoradiographic procedures. Since most of this work has been described in detail by these authors, attention will be limited in this discussion to the pattern of migration of the tectal cells during development in contrast to the presumptive neurons of the mesencephalic nucleus of V. Three fairly distinct phases have been defined by La Vail & Cowan (1971b). A first phase extending from day 3 to 6 of incubation, characterized by a rapid proliferation of cells in the neuroepithelium.
Origin of the mesencephalic nucleus

A second phase, lasting till day 12 of incubation marked by the outward migration of cells from the neuroepithelium, and a final phase after day 12, characterized by growth of cells in each layer and the realization of the cytoarchitectonic structure of the mature optic tectum. According to them, the cellular layers of the chick optic tectum are formed as a result of three successive migrations of differentiated neurons and glial cells; the more superficial layers are formed at a relatively late stage in development and migrate in successive waves through the deeper layers of the optic tectum.

Although the present study is primarily concerned with the origin of the mesencephalic nucleus of V, the fact that in our experimental material the tectal cells in all of the layers of the tectum on the grafted side comprised duck cells would support the observations of LaVail & Cowan (1971b) on the origin of these cells through proliferative activity in the neuroepithelium. In our experimental material the neuroepithelium unquestionably is of the duck-host, and the cells of the mesencephalic nucleus of V, on the other hand, are quail cells. There is evidence of an inward migration from the surface of precursor neurons of the mesencephalic nucleus of V derived from the quail neural crest graft, which seemingly takes place independent of the outward migration of the tectal cells. Based on this evidence alone the possibility of the origin of the cells of the mesencephalic nucleus of V from the same population of cells as the rest of the tectal cells, the neuroepithelium, is no longer tenable.

The timing and length of the proliferative period from the neuroepithelium and the migratory pattern of the tectal cells have far reaching implications when considered in the light of what has been described for the mesencephalic nucleus of V by Rogers & Cowan (1973). In a study on the development of the mesencephalic nucleus of the chick using autoradiographic methods, they have shown that virtually the entire population of neurons in the mesencephalic nucleus of V is formed by the fourth day of incubation, and can readily be identified in the region of the tectal commissure and in the future stratum griseum periventriculare immediately superficial to the neuroepithelium in contrast to the late appearing tectal cells. It has been fairly well documented that alar plate derivatives are generated later than those of the basal plate (Hamburger, 1948; Cowan & Wenger, 1968). Thus, the disparity in the time of development of the tectal cells which are alar derivatives and the cells of the mesencephalic nucleus of V finds a ready explanation if it is assumed that they are derived from entirely two different populations of cells. As stated by Rogers & Cowan (1973), the surprisingly early formation of the cells of the mesencephalic nucleus provides indirect evidence for their origin from the neural crest or neural folds rather than from the alar plate neuroepithelium.

Our discussion to this point has focused on the migratory pattern of the tectal cells versus the cells of the mesencephalic nucleus of V, and the time of origin of the latter based on autoradiographic studies of Rogers & Cowan (1973). However, our main objective has been to determine by interspecific
transplantation of neural crest material between quail and duck embryos whether the cells of the mesencephalic nucleus of V are, in fact, derived from cranial neural crest. In the present study, chorioallantoic membrane grafting of embryonic brain levels has shown: (1) that the precursor neurons of the mesencephalic nucleus are found in the leptomeningeal tissue of the level of the mesencephalon and are not confined within the external limiting membrane; (2) that they do pass through the leptomeninges in clusters and (3) that the cells on the leptomeningeal tissue are in fact the precursor neurons as shown by the presence of mature cells in the leptomeninges which had, apparently, failed to migrate, and resembling in all respects the fully developed neurons of the mesencephalic nucleus of V in the stratum griseum periventriculare. Perhaps the most significant aspect of this experiment of transplanting neural crest lies in the demonstration that the cells of the mesencephalic nucleus of V are derived from donor quail cells providing confirmatory evidence of a cranial neural crest origin from the mesencephalon. All other cells forming the various layers of the optic tectum are derived through proliferative activity of the alar plate neuroepithelium.

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Origin of the mesencephalic nucleus


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