

SOME OBSERVATIONS ON THE EARLY DEVELOPMENT OF THE
VESTIBULAR NUCLEI IN THE WHITE RAT.

BY

GEORGE A. WALKER.

Med.
B.S., University of Kansas, 1930.

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Approved by

Henry C. Gray

Department of Anatomy.

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I. Introduction.

Although the literature dealing with the vestibular mechanism is quite voluminous, there are relatively few papers to be found dealing with the morphological aspects of the nuclei of termination for the vestibular part of the eighth cranial nerve, and there are no references in the literature to the embryological history of these nuclei. Their gross anatomy, relations and connections were worked out in detail in man and some other mammals as early as 1865. An excellent summary of the literature up to 1909 may be found in Barker's "Nervous System". The connections of the various nuclei were determined from Golgi, Pal Weigert, Nissl and other preparations by the earlier authors, and a very welcome confirmation and extension of the previously held views came as a result of the application of the Marchi technique following extirpation procedures, as applied by Thomas. More recently Muskies and Gray using essentially the same technique, have studied the connections in the cat with somewhat divergent results. Fuse has added to the work of the earlier authors, distinguishing seven parts in the lateral vestibular nucleus and two or three cell groups in the superior nucleus.

For suggesting the problem which leads to the observations here presented the writer is indebted to Dr. Henry C. Tracy. The author wishes to express his appreciation of the kind and helpful suggestions continually given by Dr. Tracy and Dr. Homer B. Latimer, and of the technical assistance rendered by Miss Eleanor Henderson.

The present paper aims to supply a brief account of the developmental history of the vestibular nuclei, and to establish the sequence and the time at which the more significant histological and morphological

changes make their appearance. It is intended, at a later date, to correlate with these observations the changes in behavioral capability with particular reference to the vestibular mechanism, with which these changes are associated.

II. Material and Methods.

The material consists of a graded series of rat embryos of from fourteen to thirty-six days of age. The age of the members of the series is reckoned from the copulation time to the time at which the embryos were removed from the uterus and fixed. Since the time elapsing between copulation and actual fertilization is not accurately known, and since this time is in all probability variable, there is a certain error in the above reckoning but it is considered to be less than would obtain if the age of the embryos under observation were considered to be proportional to the longitudinal dimension of the embryo, since this dimension cannot be accurately determined owing to variation in body curvature, biological variations from individual to individual and the difficulty of making accurate measurements. Our error is at most only a matter of a few hours, depending upon the variation in time required for actual fertilization to occur.

The embryos were fixed at once after removal from the uterus. Fixatives used were Bouin's, Mueller's, 10 % formol, 96 % alcohol with 2 % acetic acid, and pyridine. The embryos after fixation were sectioned in various planes, the most useful being those in the transverse and longitudinal axes, though series in odd planes are of value in some cases. The staining techniques used were hemotoxylin and eosin, Ranson Pyridine silver method, Cajal's reduced silver nitrate

method designated by him as number 5 (a), a modified reduced silver method to be described below, and a modified Nissl method, also given below. Thirty-seven series were studied in all.

In attempting to secure well impregnated sections by the Cajal method mentioned above, which is recommended for rat embryos by various authors, the writer found that uniform results could not be secured. It occurred to the author that fixation in neutral formalin, followed by immersion in dilute silver nitrate should cause a slow reduction of the silver salt in the tissues as the solution diffused into it; this reduction should then be susceptible of intensification by subsequent treatment with any of the photographic developers. Accordingly embryos were fixed in 10 % formol for 9 days, rinsed in water and then immersed in $1\frac{1}{2}$ % silver nitrate until the embryos turned tobacco brown in color; this usually required about 2 days at 37°C. in the dark. The embryos were then rinsed in water and placed in the following reducing agent:

Hydroquinone	2 grams
Water	100 cc.
Formol (40%)	8 cc.

The material was left in this reagent for about 24 hours, in the dark at room temperature, until the entire embryo had turned a metallic black in color. Dehydration in graded alcohols was then followed by imbedding in paraffin and sections were made as thin as desired (10 microns in most cases), mounted and then the paraffin was removed with xylol, balsam applied and cover glasses placed. This technique was found to be quite uniformly successful, giving material in which cell

processes may be traced for some distance, and in which the general cell morphology is about as clear as by the Golgi method.

The usual Nissl methods being somewhat difficult to apply to serial sections the modification suggested by Ley was made use of. This consists in alcohol fixation (96 % with 2 % acetic acid), paraffin imbedding, sectioning and mounting by the albumen water method. The slides with sections attached were then placed in xylol, graded alcohols down to water and then left in a 1 % solution of Toluidin blue for six hours, differentiated in absolute alcohol after dehydration and in some cases were counter stained with erythrosin or eosin, which also improved the differentiation of Nissl bodies. Embryos of all ages were subjected to exactly the same technique, particularly with regard to the length of time during which they were allowed to remain in the fixative, since it was planned to make certain comparisons as to cell size etc., in material obtained by this technique.

III. Observations and discussion.

The histological changes in developing neuroblasts and the sequence in which they occur are essentially the same for all nerve cells. The differences between adult nerve cells involve, therefore, only differences in degree of development, ultimate size, number and complexity of processes, abundance and arrangement of Nissl substance and neurofibrillae. It is not surprising, therefore, that in the early stages all cells in the neural tube look much alike, and it is only when some group of cells begins to forge ahead of its neighbors that one can be certain that a nucleus is being born..

The earliest indications of nuclear masses are to be seen in the neural tube of embryos of 15 days development. Previous to this time the neural tube in the region of the pontine flexure exhibits an extreme degree of flattening out. The roof plate is thin, membranous and non-nervous in structure, being only one cell layer in thickness. The basal and alar plates are greatly thickened; the basal plate is the thicker as a result of the more rapid rate of proliferation of the cells in the nuclear layer of the median ventral half of the neural tube as a whole. The basal and alar plates are joined at the shallow but well defined sulcus limitans. The marginal zone of both plates has already been extensively invaded by developing neuroblasts, so that there is only a very narrow band of the original marginal zone which remains free of cells. In the more ventral part of the tube, medial to the sulcus limitans, an advanced degree of development is apparent. Here the mantle layer is as thick as the nuclear layer and differentiation of neuroblasts is proceeding rapidly; most of the neuroblasts are in the monopolar stage, decussating processes are fairly numerous at the midline and there is some indication of nuclear masses, as indicated by the presence of larger cells occurring in groups at the level of entrance of the 5th, 7th and 12th nerves, in the mid ventral region of the neural tube, but there is no indication of nuclear masses lateral to the sulcus limitans in embryos of less than 15 days development.

It is expedient to consider the advance in development from two viewpoints. One may follow the differentiation of individual neuroblasts until the adult condition is reached and one may then consider

the development of the vestibular nuclei as a whole in relation to their changing topographical relations and general history.

1. Differentiation of individual neuroblasts.

The earlier phases of differentiation through which the neuroblasts pass while they are as yet in the nuclear and mantle zones have long been known. The work of His, Cajal, and more recently that of Hardesty, Harrison, Speidel and others has served to make clear most of the details involved in the process and but little can be added to this work. The present observations have as their only purpose that of determining the relative time at which some of these better known processes make their appearance.

In embryos of 15 days development, in which it is for the first time possible to be certain that one is observing neuroblasts in the position of the future vestibular nuclei, the cerebellar plates, which have developed in the alar plate of the rhombencephalon, arch over the fourth ventricle and approach each other. Cranially, they are fused together in the midline, and to the rostral margin of this fused portion is attached the anterior medullary velum, while to the caudal margin is attached the posterior medullary velum. Proliferation of the ependymal cells in the posterior medullary velum has caused that structure to buckle into the cavity of the fourth ventricle from both the lateral attachments of the velum and the posterior portion, thus producing the first indications of the choroid plexus of the fourth ventricle, (Fig.3). The basal plate presents features indicating an advanced degree of development as compared with the alar plate in

this region. The nuclear, mantle and marginal zones are still present but in modified form; great numbers of longitudinally directed fibers in the mantle zone have converted that part of the neural tube into what may now be recognized as the lateral reticular formation. These longitudinal fibers are diffusely distributed throughout the extent of the mantle layer, permeating through the inter-cellular spaces, but in some parts they form rather distinctly circumscribed bundles; thus the fasciculus solitarius, the restiform body and the spinal tract of the trigeminal nerve are well defined and stand out prominently in sections through this region (Fig 1). These bundles are placed in essentially their adult relation and serve as valuable landmarks. Lateral to the fibers of the restiform body there is a mass of cells into which centrally directed fibers from the spiral ganglion may be traced; these cells are the neuroblasts which are destined to become the dorsal and ventral cochlear nuclei. At this stage the cells in the region of the future vestibular nuclei can be differentiated from those of the surrounding territory chiefly by their larger size. These larger cells form a rather well defined group which, when it is first identifiable, lies at the level of entrance of the eighth cranial nerve, medial and dorsal to the dorsal most border of the spinal tract and nucleus of the trigeminal nerve. The group is situated about midway between the lateral border of the neural tube and the sulcus limitans in embryos of 15 days; in those of 16 days of age the group has increased its transverse dimension so that it extends medially beyond the sulcus limitans. The group of neuroblasts lies rather deeply in the mantle layer, and is

separated from the floor of the fourth ventricle by a number of layers of less differentiated cells, which, together with the vestibular anlage, produce a considerable bulge in the floor of the medulla at this point, (Fig.1). The deeply placed situation of this group of neuroblasts when first defined suggests the conclusion that the vestibular nuclei develop as modifications of the intersegmental neurone system of the cephalic region of the medulla, rather than as specializations of the special somatic sensory column of cells, which lies more laterally and dorsally than these nuclei, comprising only the cochlear nuclei at this level. This observation is of interest in view of the intimate relation of the vestibular nuclei to the medial longitudinal fasciculus. There is no indication that these nuclei develop from the extreme dorsolateral part of the basal plate as has been suggested by Strong in Bailey's Textbook of Embryology, in an effort to explain the presence of a motor type of cell in two of these nuclei.

The difference in size referred to above is not actually very great, being only about three microns in both the long and short axes of the nuclei of these cells. This fact is what makes the group stand out from the surrounding regions, since the larger, lighter stained nuclei constituting the group are easily discernable under low magnification. In addition to their greater size, however, the cells of this group are ahead of those nearby in other respects; the least differentiated of them show elongated or fusiform nuclei surrounded by a thin film of cytoplasm which is finely granular, basophilic, and extends out into a single wide blunt process with a concave terminal border (Fig 4.), which from later observations may be identified as the

axone. The available material is not sufficiently finely graded to make it possible to state whether or not this axone is discernible before or after ingrowing processes from the bipolar cells of the vestibular ganglion may be seen making their way into the region of the nucleus of termination for the eighth nerve. Embryos of fifteen days development exhibit many monopolar neuroblasts of large size and fusiform shape lying among the central processes of cells whose bodies lie in the vestibular ganglion, from which these processes may be traced into the dorso-lateral region of the anterior part of the medulla, (Fig.1). In embryos of fourteen days development no such relation can be made out.

The axone of the cells of the vestibular nuclei are directed variously. In some cases it is plain that the axone points in a direction at right angles to the incoming vestibular root fibers, pointing either directly dorsalward or ventralward, but in most cases it is equally plain that the axones of these neuroblasts are not oriented in any particular manner with respect to that fascicle, for they may be found lying in every possible plane.

In embryos of fifteen days development the nuclei of most of the cells in the vestibular anlage are fusiform, rather than round as are the undifferentiated cells, the chromatin material is scattered, basophilic and there are no nucleoli. Further changes involving the nucleus are a gradual change in shape from fusiform to oval, then to the very nearly exactly circular shape of the adult. Embryos of fifteen days show oval and fusiform nuclei in the vestibular anlage in about equal numbers, those of twenty days development have only round

nuclei and those of 36 days have round or only slightly oval nuclei. Since the primitive nucleus is round, the series of changes in shape from round to fusiform, to oval to round again are, therefore, an expression of the advancing development of this part of the cell. The chromatin material is at first scattered (14-15 days), then becomes concentrated into delicate threads on which nodal points can be seen and nucleoli appear (16 days). Throughout the course of development all nuclei of the cells of the vestibular anlage are eccentric in position; this is true even in the adult, with few exceptions. There is a progressive increase in the size of the nuclei from the 14th day to the 36th when the adult size is reached. Accompanying this increase in the size of the nucleus there is a progressive increase in the amount of cytoplasm which the cells possess.

In embryos of 16 days, in which the first blood vessels are seen invading the neural tube from practically every side, and in which these blood vessels are particularly numerous in the region just lateral to the vestibular anlage, dendrites are also present. The first process to be put forth by the cells of this group is, as has been mentioned above, the axone; twenty-four hours after the axone begins to push out into the interlacing network of processes from other cells it is possible to make out other shorter, narrower, and more delicate processes. The first of these usually projects from the side of the cell opposite to that on which the axone takes origin. At first only one of these latter processes may be seen, and like the axones, these processes cannot be said to have any particular orient-

ation; they project from different cells in every possible direction, no matter what the plane of section may be. The dendrites do not appear until immediately after the neural tube is well supplied with blood vessels and it is clear that only those cells in regions which are well supplied with blood vessels are pushing out dendrites. The appearance of dendrites, therefore, appears to be conditioned by the establishment of a blood supply. This suggests that the change in environment which results from the invasion of the neural tube by blood vessels has some significant relation to the first appearance of dendrites, which, of course, depends upon further growth of the neurocytoplasm. This observation and suggestion was made long ago by His and is confirmed in the present material.

The further history of the cells of the vestibular anlage with respect to the dendrites is comparatively simple. They undergo a gradual increase in number and length with increasing age of the embryos under observation; the adult morphology is attained in every respect except size by the twenty-seventh day. The increase in the size of the cells in the vestibular group is shown in tabular form below:

Age in days	Shape of the nucleus.	Long diameter in microns	Short diameter in microns
14	oval	5.50	4.85
15	oval	10.60	7.85
16	oval	10.60	9.0
17	oval	11.20	5.9
18	oval	13.24	7.95
20	round	13.24	—
22	oval	15.89	13.24
23	oval	15.90	13.24
27	oval	15.89	14.00
30	oval	18.49	14.00
32	oval	18.60	14.1
36	oval	20.00	15.80

The dimensions given are the average of measurements made upon ten cells in embryos of each age. It is to be noted that the rate of growth through the period from the 14th to the 36th day is not uniform. Comparison of the dimensions for succeeding days shows that the rate of growth from the 14th day to the 18th is much greater than that for later periods, and it is during this earlier period that the histological differentiation of the cells is proceeding most rapidly also.

The observation that the neuroblasts of the more lateral parts of the vestibular anlage are from the first of much greater size than those of any other parts, is of interest in connection with the well known fact that the nucleus of Deiter, which is characterized in the adult by the large size of its cells, lies in this territory. It would

seem that this group of neuroblasts is early subjected to some influence which causes this greater relative size to be attained. The only observable factor which might play this part is the presence of more and larger blood vessels in this lateral region, though the possibility that the individual cells concerned may have from the first greater potentialities as to differentiation must be borne in mind.

Nissl substance makes its appearance simultaneously with that of the dendrites, both of which appear somewhat later than the axones. In the monopolar stage, to which all of the neuroblasts have attained before or just after they have reached the mantle layer, the cytoplasm takes a diffuse basophilic stain, with either hemotoxylin and eosin or the Nissl method described previously. The cytoplasm in the earlier stages has the appearance of ground glass of light bluish tint in which no discrete granules can be made out, with the highest magnifications available, (about 1500 x). The general somatic cells of the embryo also have this appearance, hence it is not characteristic of either spongioblasts or neuroblasts. This appearance prevails until the 15th day of intra uterine life at which time it is possible in favorable sections to see fine deep blue stained granules in the cytoplasm of the neuroblasts in the vestibular anlage, but not in the spongioblasts, in fact this difference in the cytoplasm is one of the chief means by which it is possible to differentiate these two types of cells although there are other differences, as shape, size, amount of cytoplasm, depth of staining with reduced silver technique, etc.

McCallum and Scott found that in very early stages the ventral

horn cells in embryo pigs consist almost entirely of a nucleus rich in chromatin, while the protoplasm of the cells is poorly developed. At a later period the cell elongates, the nucleus becomes less rich in chromatin and close to the nucleus a "cap" of peculiar nature, stainable with toluidin blue, makes its appearance. Still later in development this substance seems to be uniformly distributed throughout the cytoplasm, and finally the aggregations of this substance in the form of spindles met with in the adult are encountered. McCallum and Scott are therefore, of the opinion that the Nissl substance is derived from the nucleus of the nerve cell. It is not intended to enter into the controversy as to the nature of the Nissl substance in this paper, but it is clear that the environment in the immediate vicinity of the nucleus is most favorable for the formation of the stainable substance of Nissl, whatever may be its actual source.

The above description quoted from McCallum and Scott may be taken over bodily, as it is a quite accurate account of the conditions as seen in rat embryos; the only facts which can be added are those relating to the time at which the conditions described above obtain. In fifteen day embryos the paranuclear "caps" may first be made out; these persist to the end of the seventeenth day, at which time the entire cytoplasm of the cells becomes uniformly filled with fine granules of bluish staining material by enlargement of the "caps". Aggregation of this material leading to the formation of discrete clumps begins on the eighteenth day and the rearrangement is complete on the twenty-seventh day, (about six days after birth).

An additional observation is the fact that there is a notable tendency to the formation of chromophilic cells, that is, cells in which the entire cytoplasm stains a deep blue with toluidin blue or any of the anytin dyes, particularly noticeable in embryos of more advanced ages.

In examining the series of slides from which the observations here recorded were made, it was noticed that in the younger embryos the Nissl technique used stains quite well both neuroblasts and their processes, making it possible to follow cell processes for some distance, but in the older specimens, as differentiation advances, the stain is confined more and more to the cell bodies, until in specimens stained by this technique, of more than twenty-five days of age, only cell bodies are stained and processes can no longer be followed. Just the opposite situation may be observed with regard to the reaction to the reduced silver technique, the younger embryos show cell bodies well outlined by the method while processes are poorly stained and cannot be followed far, while in older specimens the cell bodies react less and less well to the method and their processes tend to stand out more clearly. The ages at which these changes occur correspond for the two techniques, Since care was exercised to apply each technique in exactly the same way to the different embryos, this observation indicates that accompanying the histological differentiation of the neuroblasts there is an intracellular change in the chemical constitution of the protoplasm. The nature of this change is, of course, as yet only a matter for speculation.

III. Development of the vestibular anlage as a whole.

As has been stated above, at the stage in development when it is for the first time possible to be certain that one is observing neuro-

blasts in the position of the future vestibular nuclei, it may be seen that the cells of this region can be distinguished from those of the surrounding territory by their larger size, though this is not the only way in which they differ, as has been discussed. These larger cells form a rather well defined group which, when it is first to be seen (in embryos of fifteen days of age) lies at the level of entrance of the vestibular part of the eighth nerve medial and dorsal to the dorsal most border of the spinal tract of the fifth nerve. The group is situated about midway between the lateral border of the neural tube and the sulcus limitans and is quite deeply placed, being separated from the ependymal layer of the floor of the fourth ventricle by about six layers of undifferentiated cells, which together with the vestibular anlage produce a considerable bulge in the floor of the ventricle at this point, (Fig.1). Proof that this group of cells represents the earliest indication of the nuclei of termination for the eighth nerve is found in the fact that it is at this age possible to trace fibers from the proximal part of the acustico-facial ganglion complex into the dorso lateral aspect of the medulla and into the territory indicated. This group of cells lies just posterior to the pontine flexure, (Fig 3), and immediately lateral to the shallow sulcus limitans extending laterally to a point about midway between the sulcus limitans and the lateral border of the neural tube. The mass is elongated and oval in shape; it is flattened dorso ventrally, and is about eighty-five microns in depth, four hundred and sixty microns long and one hundred and eighty-five microns wide. There are no subdivisions apparent, though the cells in the lateral part of the group

are distinctly larger than those in other parts, representing the first step in the differentiation of Deiter's nucleus. At this stage the pontine flexure is a sharp bend in the long axis of the tube, (Fig. 3), and the restiform body consists of a well defined fascicle which can be traced around the pontine flexure into the thickened roof plate which represents the cerebellum. From the superior lateral part of the vestibular anlage, a few cells form a chain extending upward toward the cerebellum along the medial side of the restiform body; these cells are undifferentiated as yet but they are destined to become the superior vestibular nucleus.

In embryos of sixteen days of age a subdivision of the original group into a lateral large celled group and a medial small celled group is apparent. The two groups have increased their combined transverse dimensions so that at this stage the cell mass extends from the medial border of the restiform body to a point well medialward of the sulcus limitans. Beginning at the seventeenth day the restiform body enlarges by accretion of fibers on its dorsal aspect; these fibers do not follow the pontine flexure but take a short cut, so to speak, passing from the floor of the fourth ventricle to the overhanging cerebellum by way of a bridge of tissue formed by fusion of the midregions of the lateral ridges defined by the sulcus limitans medially and the lateral border of the neural tube laterally. This fusion and subsequent enlargement of the restiform body cut off a lateral part of the cavity of the fourth ventricle from the main cavity. This is the lateral recess (Fig 5), which retains, however, a connection with the main

cavity of the fourth ventricle behind the restiform body. The pontine flexure is obliterated by progressive fusion of the longitudinal ridges in the dorsal angle of the flexure and a general thickening of the medullary floor both caudal and cephalad to the apex of the bend. It is the fusion of the lateral pair of the four longitudinal ridges which is the site of the restiform body, which at first has only a very narrow connection with the cerebellum and then becomes progressively wider until in embryos of 20 days the restiform body and the bridge of cells medial to it (superior nucleus) are about one third as wide as the entire neural tube at this stage. This widening of the connection between the medulla and the overhanging cerebellar plates involves not only an increase in the absolute size of the restiform body but also is due in great part to the great increase in the number of cells which lie just medial to the restiform body, which cells are continuous with the superior and lateral part of Deiter's nucleus; these cells constitute the superior vestibular nucleus, which, therefore, develops as an extension superiorly and cephalically of the cephalic extremity of Deiter's nucleus, and may, therefore, be regarded as an appendage to that nucleus; in fact, it is difficult to tell just where Deiter's nucleus ends and the superior nucleus begins. Since there is not any very great difference in the type of cells in the two, both consisting of large multipolar cells. The superior vestibular nucleus in the rat does not consist of medium sized multipolar cells, as has been claimed for it by various investigators in other mammals; in fact some of the constituent cells are even larger than those of Deiter's nucleus, and their tinctorial re-

actions are identical, particularly the reaction to Nissl technique. This group of cells, which is differentiated by its position only, forms from the first a bridge of cells extending from the original vestibular anlage upward and forward into continuity with the more deeply situated cells of the cerebellum, those which are destined to become the dentate, emboliform, globose and fastigial nuclei of the adult cerebellum. This continuity is apparent in embryos of fifteen days.

It is clear, therefore, that the lateral part of the original vestibular anlage undergoes differentiation, producing the large celled group in its lateral part; this differentiation then involves the chain of cells which from the first connect the cerebellar nuclei with the vestibular anlage, leading to the development of the superior vestibular nucleus by the differentiation of these cells. The differentiation of Deiter's nucleus begins on the fifteenth day and that of the superior vestibular nucleus begins on the seventeenth day. Both of these nuclei undergo both absolute and relative increase in size so that in the adult about half of the lateral extent of the so called restiform body consists of the superior vestibular nucleus, the cells of which lie in the network of heavy fibers which enter the restiform body from various sources.

The medial and descending vestibular nuclei show no obvious difference in their rates of differentiation; both may be said to exist from the fifteenth day. Their differentiation involves an increase in the size of the two groups of cells as a whole; this increase in size is relative and absolute for both nuclei until the

twentieth day, when the adult topography is reached by both nuclei; after this period there is increase in absolute size only, while the relations and limits of both groups do not change in any essential features.

IV. Establishment of connections.

As has been mentioned above, the central processes from the cells in the vestibular ganglion reach the dorso-lateral aspect of the anterior part of the medulla on the fifteenth day, when they may be traced into the deeply situated group of cells which constitute the anlage of the vestibular nuclei, and since it is from this group of cells that all of the nuclei of termination for the vestibular nerves ultimately develop, it is evident that although functional synapses may not yet be present, the anatomical relation of vestibular ganglion and nuclei is established. As the four definitive nuclei develop out of the original mass, connections with the incoming root fibers is retained. The establishment of the secondary connections of these four nuclei occurs somewhat later. Thus on the seventeenth day bundles of fibers may be traced from Deiter's nucleus into the medial longitudinal fasciculus of the same and opposite sides, from Deiter's nucleus into the rectiform body and from this nucleus into the lateral reticular formation, though these last are scattered and cannot be followed very far. In addition a large bundle of fibers makes its appearance at this time (seventeenth day) extending from the superior vestibular nucleus superiorly and medially into the medial cerebellar nuclei. The connections of the medial and descending nuclei are more difficult to make out; the efferent connections are

by diffusely distributed fibers and cannot be followed very far, hence it is not until a much later period that one can be sure that such connections are established, and although these connections cannot certainly be made out until about the thirtieth day, presumably they are actually present much earlier, since there is no a priori reason for assuming that these latter two nuclei should be far behind the others in establishing their efferent connections.

Summary and conclusions

1. A modified reduced silver technique, suitable for application to rat embryos is described.
2. The sequence and the time at which certain histological characteristics of the neuroblasts constituting the vestibular nuclei are attained, is established. These characteristics and the time at which they appear are given in tabular form below:

- | | | |
|---|-------|---------------|
| (a) Increase in cell size (as compared to undifferentiated cells of the nuclear and mantle layers) | ----- | 15th day. |
| (b) Axones appear | ----- | 15th day. |
| (c) Dendrites appear | ----- | 16th day. |
| (d) Nissl substance appears | ----- | 16th day. |
| (e) Blood vessels are found (in the particular region involved) | ----- | 15th day. |
| (f) Primitive nuclei are round | | |
| change to fusiform | ----- | 15th day. |
| change to oval | ----- | 16th to 20th. |
| change to round | ----- | 20th day. |
| (g) There is a progressive increase in the amount of cytoplasm of individual cells from the 15th day to the 36th. | | |

(h) The observations of McCallum and Scott on the evolution of the Nissl substance, are confirmed in the present material.

(i) A change in the intracellular chemical constitution with advancing age is recorded.

3. The first indications of the vestibular nuclei are found in embryos of fifteen days development. From the mass of large neuroblasts which lie in the plane of entrance of the eighth nerve it is clear that all four of the nuclei of termination for this nerve in the adult, develop.

4. It is from the lateral region of this original group that Deiter's nucleus develops, being distinguishable by the sixteenth day.

5. From the superior lateral zone of this original group the superior vestibular nucleus develops by a process of extension along the medial side of the restiform body.

6. The medial and descending vestibular nuclei develops simultaneously from the medial and posterior regions of the original group of neuroblasts. These may be said to exist from the fifteenth day.

7. The original group of neuroblasts from which all of the vestibular nuclei develops is deeply situated, being separated from the floor of the fourth ventricle by six layers of undifferentiated cells, medial to the superior border of the spinal tract of the fifth nerve, along the dorsal border of the lateral reticular formation.

8. The central connections of the vestibular nerve are established by the fifteenth day of intra uterine life.

9. The secondary connections of the vestibular nuclei are established for the nucleus of Deiter and the superior nucleus by the seventeenth day. The secondary connections of the medial and descending nuclei are

probably also established by this time, though not definitely observed until the thirtieth day.

9. The adult topography and relations are attained by the twentieth day.

10. The pontine flexure is obliterated, in the white rat, by fusion of the longitudinal ridges in the dorsal angle of the flexure and a general thickening of the floor of the medulla, both caudal and cephalad to the apex of the bend.

Conclusions

1. The vestibular nuclei develop *in situ* by differentiation of neuroblasts derived from the nuclear layer in the immediate vicinity, all four of the adult nuclei being derivatives of a group of large cells which run well ahead of the cells in the surrounding territory in histological evolution.
2. The deep situation of the vestibular anlage when first identified indicates that the vestibular nuclei are a product of the specialization of the intersegmental neurone system of the cephalic part of the medulla.
3. The anatomical relations of the vestibular nuclei and the vestibular ganglion are established at least four days before vestibular function can be demonstrated, as determined by Lane (1916).

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DESCRIPTION OF FIGURES.

- Figure # 1. Photograph of transverse section, 15 day embryo.
At "A" are shown the ingrowing central processes from neuroblasts in the vestibular ganglion.
B- The cells of the vestibular anlage as seen at this stage.
C- Spinal tract of the trigeminal nerve.
D- Fibers of the restiform body.
Note the bulge in the floor of the ventricle above the vestibular group, and the deep situation of this group.
E- Neuroblasts of the dorsal cochlear nucleus.
Hemotoxylin eosin stain. Magnification- 140 x.
- Figure # 2. Same section as figure # 1. Magnification- 22 x.
B- Vestibular anlage.
F- Cavity of the fourth ventricle. Note the bulge in floor above B.
Compare with figure # 5.
- Figure # 3. Photograph of saggital section, near the midline, 15 day embryo.
Note the sharply defined pontine flexure at 'y'.
G- Cerebellar plate.
B- The vestibular group of neuroblasts lies lateral to this point.
Hemotoxylin eosin stain. Magnification- 22 x.
- Figure # 4. Same section as figure # 1. Magnification- 1170 x.
A- Neuroblast showing development of axone. No dendrites present at this stage.
- Figure # 5. Transverse section, 17 day specimen.
F- Lateral recess.
D- Restiform body.
B- Vestibular anlage.
Note the line of fusion between the roof and floor plates through which the restiform body extends. Compare with figure # 2.
Photograph. Nissl stain. Magnification- 59 x.
- Figure # 6. 16 day specimen.
A- Neuroblast showing appearance of first dendrites.
A'- Neuroblast showing two dendrites.
Photograph. Nissl stain. Magnification- 450 x.
- Figure # 7. 18 day specimen. Transverse section.
D- Restiform body.
F- Lateral recess.
Compare with figures # 2 and # 5. Note the great increase in width of the restiform body, and the decrease in the aperture of the lateral recess.
Photograph. Nissl stain. Magnification- 59 x.

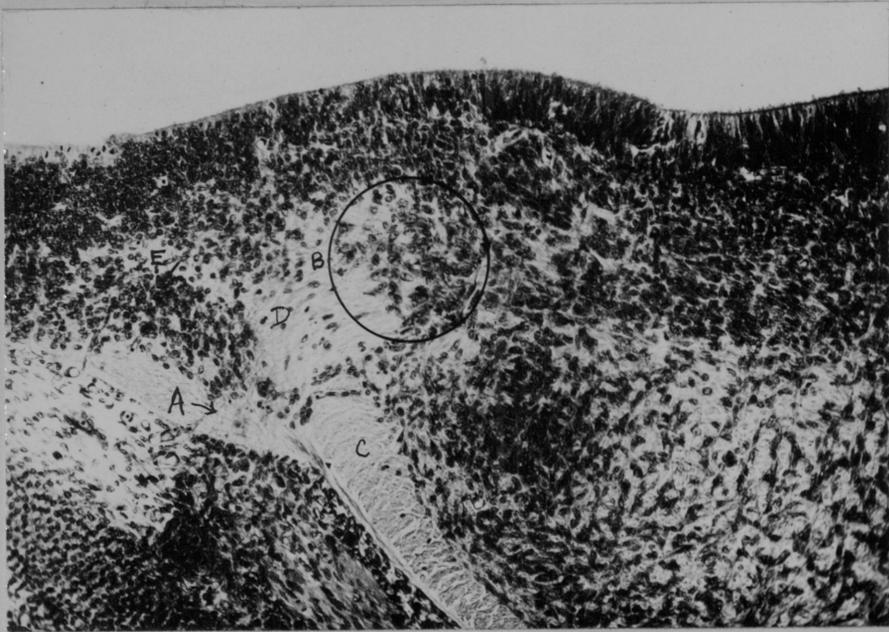


Figure 1



Figure 2

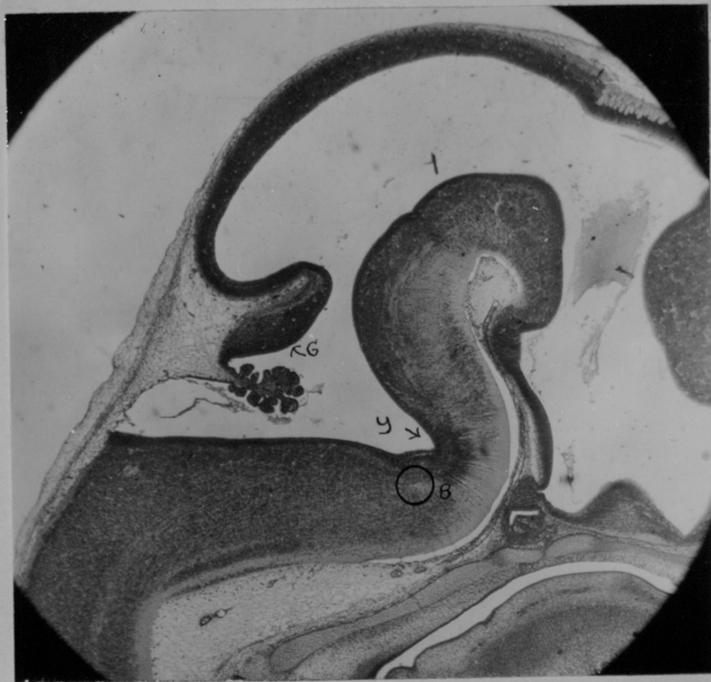


Figure 3

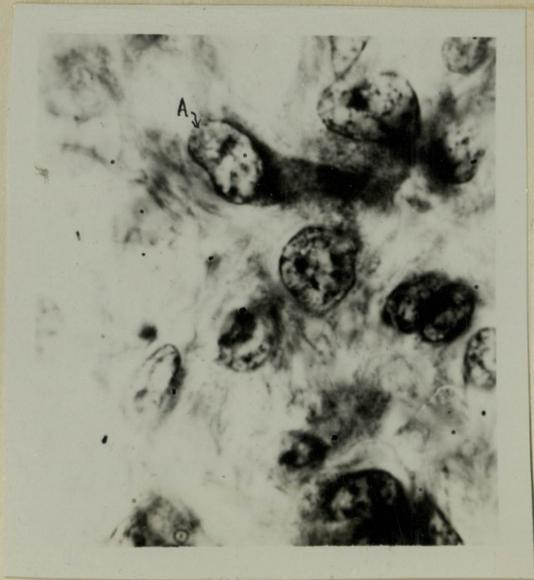


Figure 4.

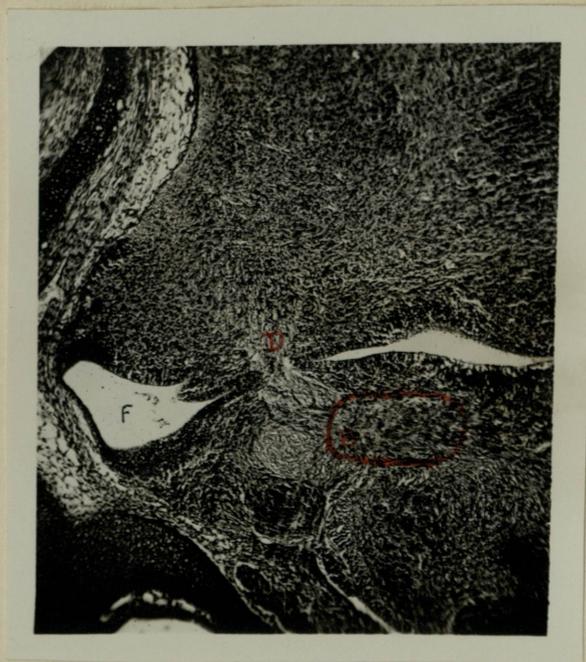


Figure 5.

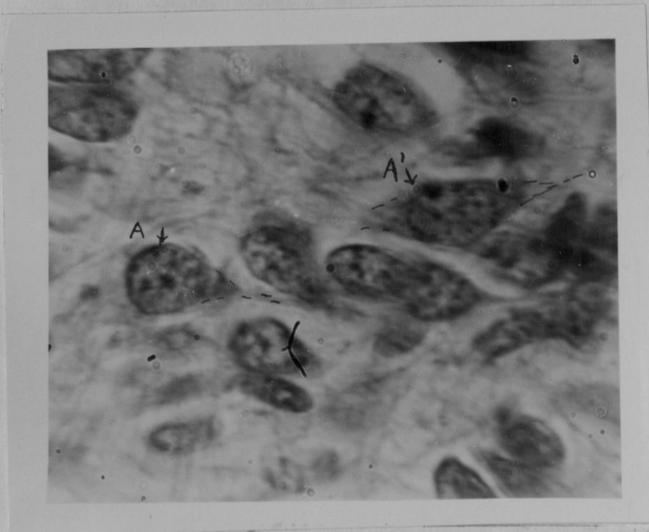


Figure 6.



Figure 7.