Morphogenesis of the trigeminal mesencephalic nucleus in the hamster: cytogenesis and neurone death

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SUMMARY

The chronology of cellular development in the trigeminal mesencephalic nucleus of the hamster has been studied by light and electron microscopy. Major developmental features are compressed into a two-week period that spans pre- and postnatal maturation. Special emphasis is focused on the sequence of developmental events, with the following points analyzed in this report: cell proliferation, cytological and axonal development, cell death, and cluster formation.

These centrally located primary sensory neurones seem to arise from a separate germinal population found in the alar plate of the midbrain and pons. The neurones are remarkable for their precocious formation and characteristic appearance. In hamster embryos they are first detected when the neural tube is only a couple of cell layers thick. Prenatally, the neurones grow rapidly and have doubled in diameter by birth. Axons form shortly after the cells are formed. This is marked by a sudden upsurge in cytoplasmic tubules and filaments in one end of the soma. Postnatally, cellular growth continues at a reduced rate. However, shortly after birth many small spines protrude from the cell body, thus increasing the surface area available for nutritive exchange. Myelination of the axon commences 6 days after birth.

Cell counts on a series of pre- and postnatal animals detected a considerable overproduction of neurones in the mesencephalic nucleus. Prior to birth there is a rapid elimination of almost 50% of these cells. Degenerating cells were identified in both light and electron microscopes. The temporal relationship between peripheral innervation, cell death and synaptogenesis suggests a developmental mechanism that provides some flexibility in the formation of synaptic connexions in the mesencephalic nucleus.

Clusters of 2–4 tightly packed neurones are a characteristic feature of the developing and adult mesencephalic nucleus. Specialized junctions connect adjacent neurones and are thought to provide the basis for electrotonic coupling within the nucleus. Macula adhaerens plaques were present in both pre- and postnatal hamsters. However, close appositions were only identified postnatally.

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INTRODUCTION

During the past century the structure of the mesencephalic nucleus of the trigeminal nerve has been intensively studied in a wide variety of vertebrate species. From these investigations it has become evident that this nucleus has many features that are not characteristic of the primary sensory neurones located in either dorsal root or cranial ganglia. Notable among these special aspects are their central location (Weinberg, 1928), the presence of synaptic endings on the soma (Hinrichsen & Larramendi, 1968, 1970; Alley, 1973), and the possible existence of electrotonic coupling between small clusters of these neurones (Hinrichsen & Larramendi, 1968, 1970; Baker & Llinás, 1971).

Functionally the mesencephalic nucleus is believed to mediate proprioceptive input from the jaw apparatus (Corbin & Harrison, 1940; Jerge, 1963). Peripheral processes of these neurones terminate in annulospiral and flower spray endings in the spindle receptors of the masticatory muscles (Szentágothai, 1948; Kawamura, Funakoshi, Tsukamoto & Takata, 1959; Jerge, 1963), and in tension receptors located in the periodontal ligament of the teeth (Corbin & Harrison, 1940; Jerge, 1963; Kidokoro, Kubota, Shuto, Sumino, 1968a, b).

Although both the structure and function of the adult mesencephalic nucleus are well documented, little is known about the development of these neurones. Their large size, lack of dendrites, and characteristic location in the midbrain and pons make them ideal subjects for both quantitative analyses of neuronal number during ontogenesis and electron microscopic observations of cellular maturation. Moreover, the dramatic shift of feeding behavior from the rather stereotyped movements of suckling to the more intricate activity of chewing suggests a greater role for proprioceptive feedback during mastication. In postnatal hamsters the timing of these events seems to be correlated with the maturation of the mesencephalic nucleus.

The purpose of this analysis is twofold: first, it outlines the chronology of cytogenesis, emphasizing cytoplasmic growth and reorganization, the development of somatic spines, and the formation of the axon. Secondly, the role of cell death during morphogenesis of the mammalian mesencephalic nucleus is evaluated by cell counts and histological observation, and the timing of neuronal death is compared with the onset of synaptogenesis. It was hoped that information on the developmental process might provide additional insight into the central location of this nucleus.

A previous report of cell death in the mammalian mesencephalic nucleus was first published in abstract form (Alley & Du Brul, 1972).

MATERIALS AND METHODS

Golden hamsters (Mesocricetus auratus) were procured from Con Olson Animal Supply of Madison, Wisconsin. All animals were housed in a constant
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A rigorous schedule of timed matings was employed and at appropriate intervals from 9 days post coitus (p.c.) to birth the pregnant females were anesthetized with Nembutal and the fetal pups surgically removed. Postnatal pups were also anesthetized with Nembutal and collected at 24 h intervals until 15 days of age. Twenty-five-day postnatal (p.n.) and adult animals were also used. A total of 93 specimens was gathered for analysis in the light and electron microscopes.

Brains of litter-mates were prepared according to the protocol of Nissl, Cajal reduced silver, and Golgi (Valverde, 1970) techniques. Material to be stained with cresylviolet was embedded in paraffin and serially sectioned in either frontal or sagittal planes. These brains were used to calibrate neuronal size and to determine cell number during maturation. Further aspects of somatic and axon developments were examined on the Golgi and reduced silver preparations. However, considerable difficulty was encountered in impregnating neurones of the mesencephalic nucleus in fetuses younger than 12 days p.c.

Fixation for electron microscopy was accomplished either by direct immersion or by vascular perfusion. The fixative consisted of a combination of aldehydes: 2.5% paraformaldehyde, 2.5% glutaraldehyde, 0.001% calcium chloride, and 0.1 M cacodylate buffer at a final pH of 7.4 (Karnovsky, 1965).

Early fetuses were immersed in cold fixative immediately after severing the umbilicus, while older fetuses and all postnatal animals were fixed by vascular perfusion. Each brain was kept overnight in cold fixative. The following day small pieces of tissue were dissected from the caudal region of the nucleus in the pons and postfixed in 1% osmium in 0.1 M cacodylate for two hours. Material was dehydrated in graded acetone and embedded in Epon-Araldite. Each piece was oriented so that the sections would be cut in a coronal plane.

Mallory stained sections, 1 µm thick, were used to localize the mesencephalic nucleus prior to thin sectioning. These thin sections were also of considerable aid in analyzing cell morphology during development. Furthermore, semi-serial series of 1 µm plastic sections through the extent of the mesencephalic nucleus were prepared for the youngest embryos studied.

Thin sections were cut on a Porter-Blum MT-2 ultratome, double stained with uranyl acetate and lead citrate and viewed on a Hitachi 7-S electron microscope.

Serial-sectioned brain stems from 29 animals were used to count the number of neurones in the developing mesencephalic nucleus. Cell counts were done according to the selected-section method (Konigsmark, 1970). Every other section was carefully reviewed and the neurones of the mesencephalic nucleus counted. Only those cells containing a definite nucleus and nucleolus were included in the data. Final estimates of the size of the neuronal population were determined by multiplying the actual cell count by the interval.

Certain difficulties were encountered in identifying accurately the earliest
neurones of the mesencephalic nucleus in the paraffin sections. From 9½ days p.c. to 10 days p.c. cytoplasmic outlines were indistinct and the intensely stained germinal cells tended to mask their appearance. Later in the gestation period care was taken to account for other groups of neurones in the immediate vicinity. Particular attention was paid to the neurones of the locus coeruleus.

Additional quantitative procedures used in the investigation will be described in the appropriate sections.

OBSERVATIONS

Initial appearance of the mesencephalic nucleus

The mesencephalic nucleus is conspicuous by its precocious appearance in the alar plate of the midbrain and pons at 9½ days p.c. At this stage the neural tube is characterized by a mitotically active ventricular zone and a thin intermediate zone. The early cells of the mesencephalic nucleus are sandwiched between these two layers (Fig. 1). On the 1 μm sections these neurones were readily distinguished from the adjacent germinal cells by differences in nuclear and cytoplasmic architecture.

Although the precise primordia of these neurones could not be identified with certainty, it is interesting to note that in embryos from 9½ days p.c. to 12½ days p.c. a few mitotic profiles were encountered at some distance from the ventricu-
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Fig. 2. Histograms of cell diameter in the developing mesencephalic nucleus. Abscissa – neuronal diameter; ordinate – percentage of cells. d.p.c., Days post coitus; d.p.n., days post natal.

lar surface in close proximity to the cells of the mesencephalic nucleus (Figs. 1, 5). Even though additional evidence will be required to pin down the origin of these neurones, the close proximity of the mitotic cells suggests that the mesencephalic nucleus may arise from an independent primordia within the neural tube.

Somatic growth and maturation

The timing and rate of cell growth and development were evaluated for both pre- and postnatal neurones. Indices of growth are portrayed as histograms of cell diameter (Fig. 2) and as estimates of the surface area and volume of the
Table 1. Quantitation of somatic growth during development of the mesencephalic nucleus

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Av. diameter of soma ($\mu$m)</th>
<th>Surface area of soma ($\mu$m$^2$)</th>
<th>Cytoplasmic volume of soma ($\mu$m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 p.c.</td>
<td>8.4</td>
<td>222</td>
<td>310</td>
</tr>
<tr>
<td>10½ p.c.</td>
<td>10.6</td>
<td>353</td>
<td>623</td>
</tr>
<tr>
<td>11 p.c.</td>
<td>11.4</td>
<td>408</td>
<td>778</td>
</tr>
<tr>
<td>12 p.c.</td>
<td>11.9</td>
<td>445</td>
<td>858</td>
</tr>
<tr>
<td>13 p.c.</td>
<td>15.6</td>
<td>764</td>
<td>1985</td>
</tr>
<tr>
<td>14 p.c.</td>
<td>15.8</td>
<td>784</td>
<td>2030</td>
</tr>
<tr>
<td>Newborn</td>
<td>17.5</td>
<td>962</td>
<td>2757</td>
</tr>
<tr>
<td>1 p.n.</td>
<td>18.5</td>
<td>1075</td>
<td>3135</td>
</tr>
<tr>
<td>2 p.n.</td>
<td>19.1</td>
<td>1145</td>
<td>3580</td>
</tr>
<tr>
<td>3 p.n.</td>
<td>21.0</td>
<td>1385</td>
<td>4836</td>
</tr>
<tr>
<td>5 p.n.</td>
<td>21.2</td>
<td>1411</td>
<td>4990</td>
</tr>
<tr>
<td>7 p.n.</td>
<td>21.4</td>
<td>1438</td>
<td>5128</td>
</tr>
<tr>
<td>9 p.n.</td>
<td>20.8</td>
<td>1384</td>
<td>4187</td>
</tr>
<tr>
<td>11 p.n.</td>
<td>23.3</td>
<td>1704</td>
<td>8180</td>
</tr>
<tr>
<td>13 p.n.</td>
<td>25.0</td>
<td>1962</td>
<td>8567</td>
</tr>
<tr>
<td>15 p.n.</td>
<td>26.8</td>
<td>2255</td>
<td>10176</td>
</tr>
<tr>
<td>25 p.n.</td>
<td>27.7</td>
<td>2409</td>
<td>11243</td>
</tr>
<tr>
<td>Adult</td>
<td>34.1</td>
<td>3651</td>
<td>20569</td>
</tr>
</tbody>
</table>

Soma (Table 1). All neuronal measurements were done with a filar micrometer on the Nissl-stained paraffin sections. The size recorded was the largest diameter of each profile. At least 100 neurones along the length of the nucleus were measured at each stage. Data from either frontal or sagittal planes gave comparable distributions of cell size. It was assumed that this was indicative of a spheroidal shape for their soma. However, it might also suggest that the neurones lack a preferential axis of orientation.

Before birth the cells expand very rapidly. At 10 days p.c. each cell averages less than 9 $\mu$m in diameter. Six days later the diameter has doubled as a result of a tenfold volumetric increase. Postnatally the cell body grows at a much reduced rate. However, during this period many other factors of importance to the final maturation of these neurones take place.

Simultaneously with cellular growth there are marked changes in the organization of the cytoplasm which are apparent in both light and electron microscopes. Early neurones, 9½ days p.c., are irregularly round, oval, or elongated but few cytoplasmic processes are present. The large nucleus is eccentrically positioned in the soma and nucleoli are usually flattened against the nuclear envelope. The remainder of the karyoplasm is granular in appearance and more electron dense than in the adult (Fig. 3). Many free ribosomes or small polysomal clusters are evenly distributed in the cytoplasm. In addition, a few strips of granular endoplasmic reticulum are present, but distinct Nissl bodies were not found.
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Figures 3-5

Fig. 3. Electron micrograph of an immature neurone in the mesencephalic nucleus, 9½ days p.c. × 15000.

Fig. 4. Mesencephalic neurone and process 10½ days p.c. × 8000.

Fig. 5. A cluster of three neurones in the mesencephalic nucleus of an 11 days p.c. fetus. Note the mitotic cell (asterisk) surrounded by these neurones. × 4300.
Mitochondria are also distributed throughout the cytoplasm, while stacks of Golgi cisternae and vesicles are confined to the perinuclear area. Very few filaments or tubules could be identified at this stage.

By 10½ days p.c. cell processes are frequently found in the neighborhood of the mesencephalic nucleus (Fig. 4). The eccentric orientation of the nucleus confines the cytoplasm to one pole of the cell, and the cell processes arise from this side of the soma. Ribosomes exist free, as rosettes or polysomal chains, but there is also an increase in the number of membrane-bound particles. The most remarkable change is the tremendous upsurge in the number of neurotubules and filaments at one pole of the neurone.

A major re-ordering of the cytoplasmic organelles begins about 12 days p.c. (Fig. 5). Most prominent is the increasing basophilia of the peripheral cytoplasm as a result of the condensation of ribosomes (Fig. 5).

Postnatally, free and membrane-bound ribosomes coalesce into distinct Nissl granules that form a peripheral basophilic ring. Small patches of Golgi apparatus also migrate into this region, and by 4 days p.n. the cytoplasmic features of these neurones are generally complete.

**Somatic spines**

Small thorns protrude from the neurones of the adult mesencephalic nucleus (Fig. 6). Hinrichsen (1968) suggested that these may be vestiges of the larger processes sometimes observed during cellular development. However, other observations on the cerebellum have implicated spine formation with synapto-
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Table 2. Quantitative features of somatic spines on mesencephalic neurones

<table>
<thead>
<tr>
<th>Age (days p.n.)</th>
<th>Perimeter surveyed (µm)</th>
<th>Spines counted</th>
<th>Density (spines/100 µm)</th>
<th>Diameter* of spine base (µm)</th>
<th>Perimeter* of spine (µm)</th>
<th>Calculated number of spines/cell</th>
<th>Perimeter increase due to spines (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1672</td>
<td>182</td>
<td>10.9</td>
<td>0.28</td>
<td>2.44</td>
<td>540</td>
<td>21</td>
</tr>
<tr>
<td>7</td>
<td>1152</td>
<td>128</td>
<td>11.1</td>
<td>0.26</td>
<td>2.51</td>
<td>615</td>
<td>19</td>
</tr>
<tr>
<td>10</td>
<td>755</td>
<td>85</td>
<td>11.3</td>
<td>0.27</td>
<td>2.24</td>
<td>690</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>1014</td>
<td>106</td>
<td>10.5</td>
<td>0.26</td>
<td>2.30</td>
<td>720</td>
<td>18</td>
</tr>
<tr>
<td>25</td>
<td>965</td>
<td>76</td>
<td>7.9</td>
<td>0.26</td>
<td>2.32</td>
<td>740</td>
<td>16</td>
</tr>
<tr>
<td>Adult</td>
<td>1727</td>
<td>118</td>
<td>6.8</td>
<td>0.27</td>
<td>2.35</td>
<td>920</td>
<td>14</td>
</tr>
</tbody>
</table>

* Average measurement of 20 spines.

genesis (Larramendi, 1969). In order to determine if either of these was applicable to the mesencephalic nucleus a developmental analysis of these spines was undertaken.

Prenatally the cell membrane is smooth and devoid of any outpocketings (Fig. 3, 4, 5). Shortly after birth the cell outline becomes more undulating and true spines can be recognized 3 or 4 days later, and by 4 or 5 days p.n. the cell surface forms a smooth boundary punctuated by definite projections (Fig. 7). The density of spines increases at 5, 7, and 10 days p.n. when the actual surface density is greater than in the adult (Table 2). Subsequently, the number of spines per 100 µm of perimeter decreases slightly at 15 days p.n., and by 25 days p.n. the density is approximately equivalent to the adult figure of 6.8 spines per 100 µm of perimeter. This sort of density analysis does not take into account the continued expansion of the surface membrane, thus a decrease in surface density could reflect either the gradual growth of the cell surface or the regression of some spines during maturation. Estimates of the number of thorns projecting from an average neurone were determined for the mesencephalic nucleus at 5, 7, 10, 12, 25 days p.n. and in the adult (Table 2). The method employed was previously used to analyze synaptogenesis in this nucleus and is described in detail elsewhere (Alley, 1973). Briefly, the calculations include the following. First, the percentage of the cell perimeter giving off spines was determined from random cell profiles (spine density x base diameter of spine ÷ 100). Subsequently, this percentage was used to compute the actual area of the surface emitting spines (total surface x percentage of perimeter covered). Finally, the number of spines per soma was estimated by dividing this portion of the total surface area by the average cross-sectional area of the spine base (spinous surface area ÷ cross-sectional area of base). According to these calculations, even though spine density decreases dramatically in the period from 15 days p.n. to adulthood, the actual number of projections continues to grow (Table 2). Additional computations that account for the surface added by
the spines indicate that the contribution they make to the total area of the soma declines with age (Table 2).

Although the timing of spine formation and synaptogenesis coincide, they are only rarely associated in direct synaptic contact. This situation is true for both adult and developing hamsters. However, it should be noted that terminals frequently lie near the base of a spine, but the adhesion is on the soma (Figs. 6, 7).

**Development of neuronal polarity**

Cajal (1929) and Tennyson (1965) have outlined the morphologic transformations that occur during cellular maturation in spinal and cranial ganglia. These cells begin as simple apolar neurones, pass through a phase of bipolarity, and eventually form a stalk from which the central and peripheral processes are directed.

Neurones of the mesencephalic nucleus emit a single, large process that passes into the mesencephalic root. Cajal (1911) indicated that in contrast to adult
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Fig. 9. Golgi section and drawing of somatic and axonal structure in the mesencephalic nucleus 14½ days p.c. × 400.

ganglionic neurones, this axon does not bifurcate in the region of the cell body (Fig. 8), but passes into the pons where many collaterals are given off in the region of the trigeminal motor nucleus. These provide the basis for the mono-synaptic activation of the motoneurones (Szentagothai, 1948; Jerge, 1963; Kidokoro et al. 1968 a).

As a reflexion of this adult configuration young neurones in the mesencephalic nucleus appear to bypass the true bipolar phases of development. Instead, each apolar cell may sprout one or more exploratory processes. Eventually one of these becomes dominant while the others appear to be redundant and are usually lost later in development. These findings account for the earlier reports of a decreasing number of multipolar neurones found with advancing maturation of these cells (Pearson, 1949 a, b).

In the hamster this metamorphosis takes place between 9·5 and 14 days p.c. Neurites first appear at 9·5 or 10 days p.c. when cells with multiple short projections are present. By 12 days p.c. the polarity of most neurones is established, with the axon emanating from the pole of the cell opposite the nucleus. These axons have been traced for long distances in 13-, 14- and 15-day fetuses with no evidence of bifurcation (Fig. 9).

After birth the axon increases greatly in diameter as the axoplasm matures. Myelin wrappings first appear around the fibers of the mesencephalic root at 6 days p.n.
Table 3. *Cell counts in the developing mesencephalic nucleus of the hamster*

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Neuronal count (right side)</th>
<th>Neuronal count (left side)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>794</td>
<td>868</td>
<td>1662</td>
</tr>
<tr>
<td>Adult</td>
<td>984</td>
<td>920</td>
<td>1904</td>
</tr>
<tr>
<td>25 p.n.</td>
<td>850</td>
<td>824</td>
<td>1674</td>
</tr>
<tr>
<td>25 p.n.</td>
<td>904</td>
<td>918</td>
<td>1822</td>
</tr>
<tr>
<td>15 p.n.</td>
<td>704</td>
<td>738</td>
<td>1442</td>
</tr>
<tr>
<td>13 p.n.</td>
<td>916</td>
<td>916</td>
<td>1832</td>
</tr>
<tr>
<td>11 p.n.</td>
<td>840</td>
<td>808</td>
<td>1648</td>
</tr>
<tr>
<td>9 p.n.</td>
<td>820</td>
<td>800</td>
<td>1620</td>
</tr>
<tr>
<td>8 p.n.</td>
<td>950</td>
<td>860</td>
<td>1910</td>
</tr>
<tr>
<td>7 p.n.</td>
<td>984</td>
<td>946</td>
<td>1930</td>
</tr>
<tr>
<td>6 p.n.</td>
<td>804</td>
<td>824</td>
<td>1628</td>
</tr>
<tr>
<td>5 p.n.</td>
<td>832</td>
<td>840</td>
<td>1672</td>
</tr>
<tr>
<td>4 p.n.</td>
<td>836</td>
<td>848</td>
<td>1684</td>
</tr>
<tr>
<td>3 p.n.</td>
<td>852</td>
<td>894</td>
<td>1746</td>
</tr>
<tr>
<td>2 p.n.</td>
<td>974</td>
<td>1072</td>
<td>2046</td>
</tr>
<tr>
<td>1 p.n.</td>
<td>794</td>
<td>802</td>
<td>1596</td>
</tr>
<tr>
<td>1 p.n.</td>
<td>864</td>
<td>846</td>
<td>1710</td>
</tr>
<tr>
<td>Newborn</td>
<td>844</td>
<td>856</td>
<td>1700</td>
</tr>
<tr>
<td>Newborn</td>
<td>896</td>
<td>884</td>
<td>1790</td>
</tr>
<tr>
<td>15 p.c.</td>
<td>1024</td>
<td>1056</td>
<td>2080</td>
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<tr>
<td>14 p.c.</td>
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<td>1128</td>
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<td>2412</td>
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<tr>
<td>13 p.c.</td>
<td>1190</td>
<td>1134</td>
<td>2324</td>
</tr>
<tr>
<td>12½ p.c.</td>
<td>1454</td>
<td>1508</td>
<td>2962</td>
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<td>1422</td>
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<tr>
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<td>1448</td>
<td>1596</td>
<td>3044</td>
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<td>1592</td>
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<td>3016</td>
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<tr>
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<td>1404</td>
<td>1436</td>
<td>2840</td>
</tr>
<tr>
<td>10 p.c.</td>
<td>1166</td>
<td>1076</td>
<td>2242</td>
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</table>

*Cell death in the developing mesencephalic nucleus*

Preliminary data from a small series of pure-bred dogs suggested that many more neurones are produced than ultimately reach maturity. Since these initial observations were confined to a limited number of animals, additional quantitative data were gained from the more extensive series of developing hamsters. Furthermore, it was thought that the period of cell death would be compacted into a short span which might provide ample opportunity to observe the degenerative sequence.

The first age for which reliable quantitative data are available is 10 days p.c. At this stage a bilateral total of 2242 neurones was counted. The definable population increases over the next 36 h, reaching an ultimate of over 3000 neurones at 12 days p.c., which is almost twice the adult number (Table 3). During the final days before birth a precipitous decline in cell number occurs. By birth the nucleus is almost numerically equivalent to the adult. A comprehensive
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A search for degenerating neurones was carried out on both pre- and postnatal animals. A few degenerating cells were first identified histologically at 13 days p.c. Although only a limited number of dying neurones was observed at any stage, they were most numerous just before birth (Fig. 11). In addition, a couple of instances of dying cells were noted in postnatal animals.

In the light microscope moribund cells were characterized by both nuclear and cytoplasmic changes. The nucleus becomes irregular in shape and stains more intensely. The cytoplasm exhibits some swelling and premature peripheral packing of its ribosomes. Later it becomes very basophilic and disrupted.

Attempts to localize degenerating neurones with the electron microscope were only partially successful. A couple of examples of early nuclear and cytoplasmic changes were recorded (Fig. 12). The nucleus is more electron dense and its boundary irregularly involuted. The cytoplasm is full of ribosomes.

Although the initial phases of cell degeneration occur rapidly; later events must proceed at a more leisurely pace. Postnatal animals show an accumulation of cellular debris around the viable neurones. The most usual appearance is a large, round, electron-opaque core surrounded by a granular halo. Frequently these particles had been engulfed by glial processes (Fig. 13).

Neuronal clusters

Tightly grouped aggregates of neurones are a normal occurrence along the length of the mesencephalic nucleus. Recently, Hinrichsen & Larramendi (1968, 1970) identified specialized zona adhaerens and zona occludens junctions
between these closely applied cells in the mouse. Similar junctions were identified in the adult hamster (Fig. 16, 17).

Individual clusters in developing hamsters have been examined under the electron microscope in order to determine the time of formation of these specializations. At 9½, 10½ and 11 days p.c. cell membranes of adjacent neurones may parallel each other for a considerable distance without any definite contacts. Small macula adhaerens junctions were identified 12½ days p.c. when single or multiple adhesions dot the surface (Fig. 14). Similar kinds of appositions were also found in newborn and postnatal animals of 5, 7, 12, 15 and 25 days p.n. (Fig. 15). The overall length of junctions is longer in older animals. Observations on the formation and development of the zona occludens junctions were not clear. A suitable plane of section through these junctions is only rarely encountered.
Morphogenesis of the trigeminal mesencephalic nucleus in the hamster occurs during a 2-week period that spans pre- and postnatal development. The outstanding events of ontogeny are schematically illustrated in Fig. 18. As illustrated these include: A, the formation and initial cytodifferentiation of neurones; B, development of cellular polarity and cell loss; C, formation of intranuclear contacts; D, outpocketing of somatic spines; E, synaptogenesis; and F, myelination of the mesencephalic root. In the following sections the first four of these topics will be analyzed in terms of associated developmental mechanisms.

Neuronal formation and cytological maturation

It has been assumed that neurones of the mesencephalic nucleus develop from neural crest elements that are either retained in or migrate into the neural tube after closure (Johnston, 1909; Herrick, 1948; Piatt, 1945; Rogers & Cowan, 1973). Indirect evidence presented here also suggests that these cells arise from a germinal population other than the ventricular zone of the alar plate. Mitotic cells were frequently found some distance from the luminal surface in close proximity to the mesencephalic nucleus. In addition, their presence corresponds to the time when the number of neurones in the mesencephalic nucleus is increasing.
Fig. 14. Two small macula adhaerens junctions (arrows) at the interface of clustered neurones in the mesencephalic nucleus 12½ days p.c. × 60000.

Fig. 15. Multiple attachment plaques anchor two neurones together in 5-day p.n. hamster. × 26000.

Fig. 16. High power electron micrograph of zona adhaerens junction in the adult mesencephalic nucleus. × 140000.

Fig. 17. Region of close apposition between adjacent neurones in the adult nucleus. × 90000.

By 9½ days p.c. some neurones of the mesencephalic nucleus are apparent in the alar plate of the midbrain and pons. Their conversion from germinal cells into immature apolar neurones is characterized by cytoplasmic changes identical to those described for other systems (Lyser, 1964; Tennyson, 1965; Fisher & Jacobson, 1970). Most notable of these are the rapid increase in cytoplasmic volume and the accumulation of ribosomes and membranous
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A number of authors have implicated proliferation of neurotubules with the initiation of axonal development (Tennyson, 1965; Lyser, 1968; Fisher & Jacobson, 1970). In the mesencephalic nucleus proliferation of neurotubules and the formation of the neurites take place soon after the initial morpho-differentiation of the apolar neurones. By 10½ days p.c. cell processes are abundant, and in those instances when continuity between soma and neurite could be determined, a rich meshwork of tubules and filaments was present in the soma.

Neurones in the mesencephalic nucleus do not pass through a true phase of bipolarity. Instead, each cell sends out a number of processes. Most are very short and penetrate only a short distance from the cell body, giving the cell a multipolar appearance (Pearson, 1949a, b; Hinrichsen & Larramendi, 1969). Later in development many of the shorter neurites are lost, leaving one main process to enter the mesencephalic root. Around 13 or 14 days p.c. most neurones have attained their adult configuration. However, a few neurones maintain these short processes even into adulthood.

As previously noted, Hinrichsen (1968) suggested that the adult somatic spines might represent vestiges of the lost neurites. However, two facts argue against this interpretation. First, there is a time gap of almost one week between the loss of neurites and the onset of spine formation, and during the interim the cell surface is smooth. Second, the actual number of spines is far in excess of the number of early processes.

Perhaps a more adequate explanation of spine function can be gleaned from...
an analysis of cell growth. It has been well established that the perikaryon is the metabolic hub of the neurone. The enzyme systems for the production of cytoplasmic proteins are concentrated in the cell body. Materials are manufactured here and shipped to the far reaches of the axon (Weiss & Hiscoe, 1948; Droz & Leblond, 1963). Thus it seems crucial that a sufficient surface must be available for nutrient exchange. Since the surface area of the soma increases as a square function while the volume expands as a cube function of the cell diameter, perhaps the tremendous increase of cytoplasmic and axoplasmic volume during development may outstrip the surface available for exchange. The accumulation of spines provides a significant increase in surface area both during postnatal maturation and in the adult. Although their total effect dwindles with time, they still provide a substantial (14 %) increase of somatic membrane in the adult.

In other systems the presence of spines has been extensively used as an index of the presence of synapses. Particular attention has been devoted to the Purkinje cell (Hillman, 1969; Larramendi, 1969) and the pyramidal cells of the cerebral cortex (Pappas & Purpura, 1961; Jacobson, 1967; Peters & Kaiserman-Abramof, 1970). In contrast to these dendritic spines, somal thorns of the mesencephalic neurones show no special affinity for synaptic endings.

A deeper understanding of spine function has been provided by analyzing the development of the climbing fiber–Purkinje cell association (Larramendi & Victor, 1966; Kornguth, Anderson & Scott, 1968). Climbing fibers first contact spine-like processes on the soma. As these fibers migrate over the soma and on to the main dendrites the spines regress. Possibly the transitory spines provide a guidance system for the wandering climbing fibers. The close timing of spine formation and synaptogenesis in the mesencephalic nucleus initially suggested that a similar mechanism may be operant here. However, closer inspection of these two events did not substantiate this notion. From their first appearance the spines are totally independent of the developing synapses.

Neuronal death as a morphogenetic mechanism

Genetically planned patterns of cell degeneration are an important morphogenetic mechanism in vertebrate and invertebrate development (Glücksmann, 1951; Lockshin & Williams, 1965; Saunders, 1966; Saunders & Fallon, 1966). Definite localized arrays of moribund cells occur at selected instances throughout ontogenesis. They help to determine the final size and mold the shape of organs and organ complexes.

Cell death has also been implicated as a morphogenetic mechanism in the developing nervous system. Dying cells free the early neural tube from the overlying ectoderm (Glücksmann, 1951), help shape the optic cup (Silver, 1972), and determine the final size of various neural centers in the spinal cord and brain stem (Hughes, 1961; Prestige, 1965, 1967a, b; Cowan & Wenger, 1967; Rogers & Cowan, 1973). These authors have indicated that the normal matura-
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ion of central–peripheral connectivity is not based on a 1 to 1 relationship, but in fact many more young neurones are produced than eventually connect with the periphery. Ultimately, the size of a mature neuronal pool is determined by an intricate balance between cellular proliferation and degeneration; and the fate of each neurone is directly dependent on its ability to establish effective contacts in the periphery. Those unable to do so cannot maintain themselves, and quickly regress. To date, quantitative analyses on the magnitude of this spontaneous regression have concentrated on submammalian vertebrates. Hughes (1961) and Prestige (1965, 1967a, b) counted viable and dying cells in the ventral horn and spinal ganglia of *Xenopus laevis* and found that there is a continuous production and turnover of neurones in these larvae. From a total population of 10000 neurones only about 1200 reach maturity. In other words, 9 out of 10 cells end up in death. Similar investigations of chick trochlear motoneurone (Cowan & Wenger, 1967) and mesencephalic neurones (Rogers & Cowan, 1973) have depicted a less severe reduction, averaging 50 and 70 % respectively.

Overproduction of neurones in the mesencephalic nucleus of the hamster indicates that neuronal death may also play an important role in the ontogenesis of mammalian brains. Although the degree of cell loss in the hamster is much less exhaustive than in the amphibian spinal cord, it is in general accord with the decreases noted in the chick. These differences are at least partially accounted for by the apparent lack of continuous neuronal production and turnover in the nervous system of higher vertebrates. In contrast, the proliferative and cell-death phases of development occur sequentially. By 12 days p.c. the mesencephalic nucleus is numerically complete. These quantitative data along with the apparent lack of degenerating cells prior to this time suggest that no new neurones are added once cell death commences. Perhaps this is an adaptive modification to limit unnecessary cell wastage, and thus increases the efficiency of neurogenesis in the bigger-brained higher vertebrates.

Experimental investigations of limb development in submammalian vertebrates have begun to unravel the complex central–peripheral relationships that occur during neurogenesis (Hamburger & Levi-Montalcini, 1949; Prestige, 1967a, b; Cowan, Martin & Wenger, 1968). In essence, three periods are evident and these correspond to an ever-increasing reliance of the nerve cell on the periphery:

1. A proliferative, pre-axonal stage when the neurone is relatively free from peripheral influence,
2. A saturation period, as the over-abundance of cells send their axons into the target organ, and
3. A consolidative phase when definite connexions are formed and the survival of selected neurones is guaranteed by virtue of these peripheral contacts.

To date no information is available on either the growth of axons from the mesencephalic root or the ontogenesis of muscle spindles in the jaw muscles. However, the chronology of developmental events within the mesencephalic nucleus suggests that a similar time table may be applicable. The temporal
relationship of axon development and cell death hints that many cell processes reach the jaw muscles about 12 days p.c.

Of added importance is the timing between cell death and the onset of synaptogenesis. It would seem to be a futile exercise to form synapses on neurones that will not survive. In the mesencephalic nucleus cellular degeneration is generally finished at birth, while the early events indicative of synaptogenesis were first found four days later (Alley, 1973). This assures that synaptic terminals will contact only neurones that have already made connexions in the periphery and are sure to survive. Aside from the added economy of this sequence, it also introduces a certain level of plasticity into the development of this system. For example, any modification of the periphery, such as an increase or decrease in the muscle mass, will modify the level of cellular degeneration in the mesencephalic nucleus (Piatt, 1946; Rogers & Cowan, 1973). If more muscle is available to the ingrowing nerve fibers one would expect fewer neurones to die. Furthermore, the effect of peripheral modification would not be limited to the primary sensory neurones alone. Changes in neuronal number would also effect the synaptic relationships of those fibers making contact with the cells.

The importance of this sequence and the flexibility it imparts can be best explained in terms of adaptive evolutionary change, since any modification of the periphery must be accompanied by concomitant alterations of the associated neural subsystems. Du Brul (1960) has shown that cell number in the adult mesencephalic nucleus of a wide variety of vertebrates is correlated with the size and complexity of the jaw muscles. In a later report (Du Brul, 1967) he proposed that the target site for evolutionary modification is in the periphery, and that adaptive changes occurring here somehow impress themselves on the appropriate neural centers. Evidence presented here on the chronological sequence of cellular proliferation, axon formation, cell death and synaptogenesis provides a plausible developmental mechanism whereby peripheral changes can alter the size and synaptic patterns in the relevant neural centers.

**Intraneural cell contacts in the mesencephalic nucleus**

Cellular interactions are of fundamental importance in the developing as well as the mature nervous system. One aspect of membrane adhesions receiving much attention is their role in morphogenesis. Tight junction and low-resistance coupling have been found in a number of embryonic systems (Sheridan, 1968; Trelstad, Revel & Hay, 1966). Loewenstein (1967) and Furshpan & Potter (1968) have implicated these junctions in the passage of regulatory molecules directly between cells, which could coordinate the growth and differentiation of cell groups. Pannese (1968) found macula adhaerens and occludens junctions between early primary sensory neurones in the dorsal root ganglia of chicks. However, these appositions are only temporary in nature and are later pried apart by the infiltration of glial cells. No trace of these junctions is left in the adult fowl. Identical macula adhaerens junctions were first identified between
adjacent mesencephalic neurones at 12 days p.c. From this time on they were frequently found. The earliest adhesions are small, single junctions. Later in development the occurrence of multiple densities becomes more prevalent and some of these may extend 7 μm or more in length. Sheffield & Fischman (1970) have suggested that these junctions may serve to stabilize intercellular relationships in rapidly growing systems. However, their increasing size with age and persistence in the adult seem to indicate that they do more than simply anchor mesencephalic neurones together. Maybe they encircle patches of the cell surface, isolating these from the surrounding extracellular space and creating a specialized intercellular environment. This area could be conducive to the formation of low-resistance electrotonic synapses. A search for zonula occludens junctions in the prenatal hamster was unsuccessful. This may reflect their small size or labile nature, making identification extremely tedious.

It is interesting to speculate that the membrane specializations could serve a dual role in the ontogeny of the mesencephalic nucleus. Initially they may be similar to those observed in other embryonic systems, serving to coordinate maturation of the nucleus. However, in contrast to spinal ganglia neurones the junctions are not separated by glia. These persist into adulthood and provide the basis for electrical coupling in the adult nucleus (Hinrichsen & Larramendi, 1968, 1970; Baker & Llinás, 1971).

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