

## **Two modes of cell migration in the ventral horn of the spinal cord in the chick embryo. A Golgi study**

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**Summary.** The migration process of the ventral horn in chick embryo spinal cord cells has been studied between 2.5 and 5 days of incubation (HH-17, HH-26), using the Golgi technique.

Two different migratory modes are observed.

Type I.— Migration by nucleus translocation. Most of the ventral horn motor neurons migrate by nucleus translocation within the peripheral cylinder of the cytoplasm (migration by nucleus translocation).

Type II.— Free migration cells. Other cells migrate disconnected from both limiting surfaces (ventricular and pial). On the basis of shape and migratory behaviour they have been identified as smooth cells and multipodial cells.

**Key words:** Spinal cord, Migration, Young motor neurons

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### **Introduction**

Studies on the differentiation of spinal cord cells were initiated by Ramón y Cajal (1909, 1911) in 3-4-day-old chick embryos using argentic impregnation techniques. The development of autoradiographic techniques (Hamburger, 1948; Fujita, 1962, 1963, 1965a,b; Fujita and Fujita, 1964) revealed the time-space sequence of the origin and later localization of the spinal cord cells.

In the last five decades and due to the contribution of the reduced silver staining methods, new information has been acquired regarding the differentiation process of the spinal cord cells in various animal models. These include: the chick (Barron, 1946), rat (Windle and Fitzgerald,

1936), cat (Windle et al., 1934), lamb (Barron, 1945) and human (Windle and Fitzgerald, 1937). The models suggest that the spinal cord cells differentiate following a ventro-dorsal sequential gradient. More recently, the studies of Wentworth and Hinds (1978) and Wentworth (1980, 1984) on mice embryo spinal cord, using Golgi's method, establish 6 stages in the differentiation process of the spinal cord motor cells. Histogenetic analysis in the spinal cord has been principally focused on the differentiation process; while on the contrary, data referring to neuronal migration is scarce.

In this study we describe the morphological sequences that the ventral horn cells of the chick embryo spinal cord follow during their migration process.

### **Materials and methods**

White Leghorn chick embryos were incubated at 37.5° C, and staged, according to Hamburger and Hamilton (1951), at half-day-intervals between the 2.5th and 5th day of incubation (HH-17, HH-26). Whole embryos were directly immersed in Stensaas solution (1967), the fixation time being varied between 1 and 4 days, so as to ensure the staining of a large variety of cells. Fixed embryos were briefly rinsed in tap water, then washed in a 0.75% (w/v) silver nitrate solution, and finally impregnated in a large volume of the same solution for 2 days. This was followed by dehydration, embedding in low viscosity nitrocellulose and clearing of the blocks in cedar wood oil before sectioning. Serial sections (100 µm thick) were collected in the same clearing medium, and mounted with dammar resin, following the procedure published elsewhere (Prada and López-Mascaraque, 1985), to prevent the fading of preparations. The spinal cords of 52 successfully stained embryos were scanned, checking by over- and underfocusing that all relevant cells were wholly included within the section. Drawings were made by use of a camera lucida attachment.

## Results

### Cell identification criteria

Two-and-a-half-day-old chick embryo spinal cords, when stained with Golgi's method, show transitory shapes both of cells in the mitotic cycle and of differentiating neuroblasts. These two cell types are difficult to distinguish by light microscopy, but there are some morphological features that allow their distinction:

*a) Cells in the mitotic cycle.* Are those cells that during the first developmental stages of the spinal cord are round, oval, monopolar and/or bipolar-shaped. Cells that are always attached to the ventricular surface or to both surfaces (ventricular and pial), without any ramification or filopodial expansions, may be cells that are in different phases of the mitotic cycle. These characteristics are in accordance with the classical patterns described for the central nervous system (C.N.S.) by numerous authors (Fujita, 1963; Hinds and Hinds, 1974; Jacobson, 1978) and are very similar to those established by Prada et al. (1987) for the chick retina of 5-5.5 incubation days. Following the above-mentioned data, Fig. 1 and Fig. 19, n, show cells of the spinal cord of a chick embryo of 2.5-3 incubation days in the various stages of the mitotic cycle.

*b) Glial cells.* The principal morphological features that characterize the ventral horn glial cells in the spinal cord of a chick of 2.5-3 incubation days are: 1.- They are always attached to the ventricular and pial surfaces of the spinal cord, showing in their outer attachment a characteristic triangular shaped or flat foot, (Fig. 2 and Fig. 19, G arrow heads); 2.- The nucleus of these cells is located in the periventricular layer and shows thickenings and spines at different levels of its outer prolongation from the first differentiation stages (Fig. 2 and Fig. 19, G thin arrows). This prolongation sometimes ramifies, forming an arch that also ends in the pial surface in a triangular foot (Fig. 19).

*c) Young motor neurons.* Our criteria for the identification of young motor neurons is mostly based on the sprouting of the axon. Following this, and independently of the location of the nucleus, all of the cells attached to both limitant surfaces, or only to the pial surface, and which project a prolongation out of the incipient ventral horn of the spinal cord, are considered young motor neurons (Fig. 19).

### Spinal cord motor cell migration (Type I)

The migration of most of the chick cervical spinal cord motor cells occurs between the 2.5 and 4th differentiation days (HH-17, HH-23).

The young motor neurons are disconnected from the ventricular surface when their perikaryon is still located in periventricular zones (Fig. 9 and Fig. 19), and they already have a subpial attachment. In later phases, the cell bodies are radially disposed and can be found at different levels within the spinal cord (Figs. 3 to 8 and

Fig. 19, 1 to 7). During translocation of the nucleus, most of the young motor neurons preserve a little cytoplasmic storage in the inner pole of the perikaryon (Figs. 3, 4, 7, 8 and 19, 2, 3, 6 arrows), which progressively retracts. Others show a rounded perikaryon (Fig. 6).

The morphology of the young migratory motor neuron's outer prolongation depends on the cell's origin zone at the level of the ventricular layers. Therefore, those cells which originate in the most anterior and posterior zones of the ventral horn show, in their trajectory, an inflection with opposite direction that allows the above-mentioned prolongation to project to the radix ventral root (compare Figs. 5 and 6 or 7 and 8, and Fig. 19, 3 and 7). Those neurons that originate in a determined zone of the ventricular layer, which is associated with the origin of the radix ventral root, show a rectilinear outer prolongation (Fig. 9 and Fig. 19, 4 and 5).

About the 4th day of incubation, most of the cell bodies occupy definitive positions in the prospective ventral horn of the spinal cord.

### Free migration cells (Type II)

Between the 3rd and the 5th days of incubation the ventral horn of the chick embryo spinal cord has cells that are disconnected from the ventricular and pial surfaces (Figs. 10-18). These cells are located in different zones of the spinal cord's thickness and, according to their shapes and outlines, can be classified into two different groups: smooth cells and multipodial cells.

*Smooth cells.*— These cells show a bipolar or monopolar shape and a radial arrangement. Their perikaryon is oval shaped and located in the innermost part of the cell (Figs. 10-14 and Fig. 19, s1, s2 and s3). A single prolongation arises from the outer pole of the perikaryon, ending in a

**Figs. 1 to 18.** Partial aspects of the cervical spinal cord of the chick embryo during 2½ to 4½ days of incubation.

**Fig. 1.** Cells in the mitotic cycle at 2½ days of incubation. × 400

**Fig. 2.** Glial cell at 3 days of incubation. The arrow head shows a flat foot attached to the pial surface. × 400

**Figs. 3, 4, 6 and 7.** Young motor neurons located in the most anterior zone of the ventral horn at 3-3½ days of incubation. The arrows show the inner prolongation in retraction. × 400

**Figs. 5 and 8.** Young motor neurons located in the most posterior zone of the ventral horn at 3-3½ days of incubation (Figure 5) and 2½ and 3 days of incubation (Figure 8). The arrow shows the last stage of retraction of the inner prolongation. × 400

**Fig. 9.** Panoramic view of the ventral middle of the spinal cord at 4-4½ days of incubation. × 200

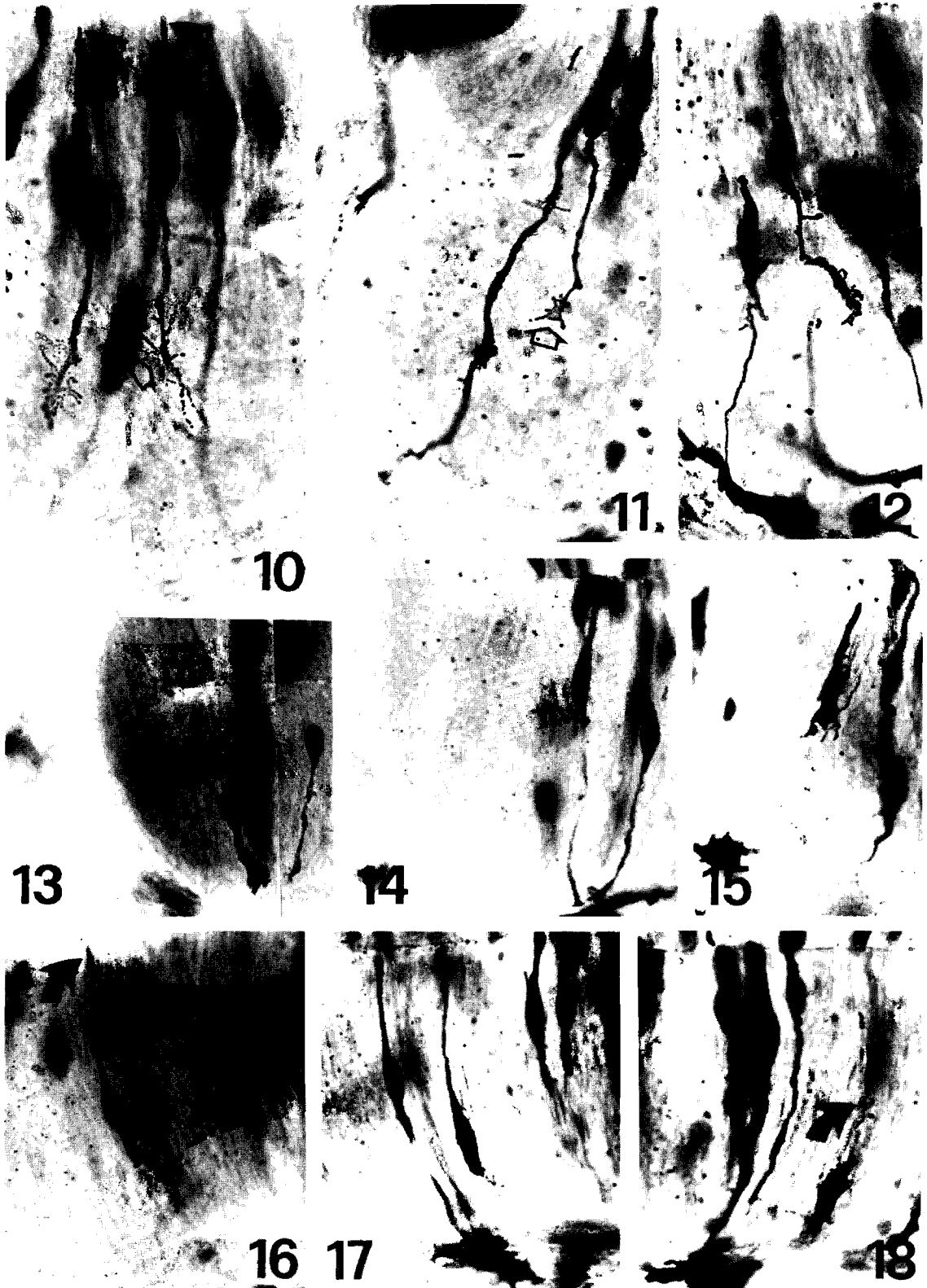
**Figs. 10 to 12.** Bipolar (Figure 10) and Monopolar (Figures 11 and 12) smooth cells at 4-4½ days of incubation. The arrows show the posterior of the inner prolongation, which seems damaged. × 400

**Figs. 13 and 14.** Monopolar smooth cells at 3-3½ days of incubation. × 400

**Figs. 15 to 18.** Different multipodial cells located in the most anterior (Figure 15) and posterior (Figures 16 to 18) zones of the ventral horn at 3-3½ days of incubation. The arrows show the portion of the inner prolongation, which seems damaged. × 400



*Migration in the ventral horn*



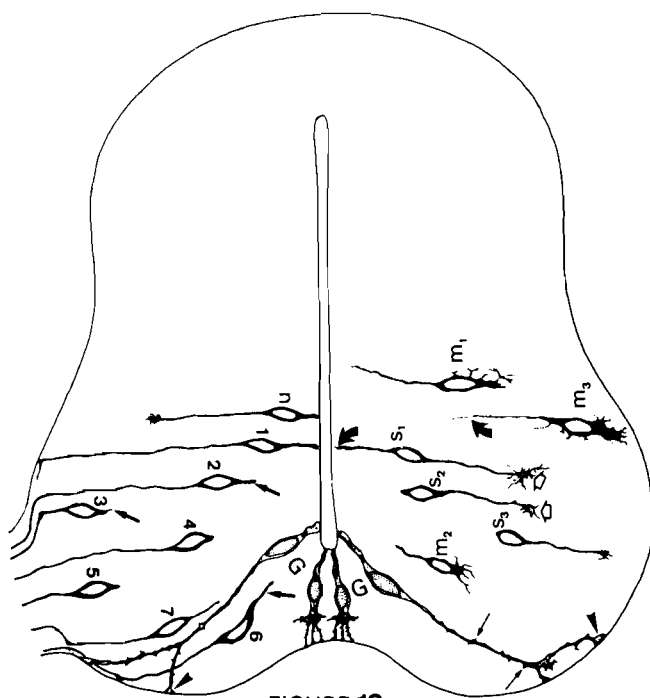


FIGURE 19

**Fig. 19.** Camera lucida drawings of two representative modes of cell migration in the ventral horn of the cervical spinal cord in the chick embryo, during 2½ to 4½ days of incubation.

**Abbreviations:** n, cell in mitotic cycle; 1 to 7, young motor neurons; G, glial cells, s1, s2 and s3, smooth cells; m1, m2 and m3, multipodial cells.

lamellipodia (Fig. 11 and Fig. 19, s2 frames arrow) or a filopodial (Fig. 10 and Fig. 19, s1 frames arrow) growth cone.

**Multipodial cells.** – The second group of free migration cells shows a high plasticity. These cells show great variability in shape and have multiple cytoplasmic processes (Figs. 10-18 and 19, m1, m2 and m3), which are variable in number and orientation. Most of these cell's expansions are located in the outer pole of the perikaryon and they are generally filiform; although some cells also present lamelliform processes. The inner pole of the smooth bipolar shaped cells and of the multipodial cells is continued in a prolongation, which, in most cases, seems damaged (Figs. 10, 16, 18 and 19, s1, m3 curved arrows). This could be the prolongation by which the cell was attached to the ventricular surface.

## Discussion

Most of the knowledge concerning the spinal cord's histogenesis is based on the studies of Ramón y Cajal (1909, 1911, 1929). This author considered that the differentiation of the spinal cord's ventral horn in the chick embryo occurs following a morphological sequence which was divided into five stages.

Wentworth (1980, 1984), basing himself on Ramón y Cajal's studies, established 6 stages in the differentiation process of the spinal cord's ventral horn in the mouse

embryo.

These authors describe, from a morphological point of view, partial aspects of the neuronal migration process in the spinal cord's ventral horn.

At present there is no agreement on the way in which young postmitotic neurons reach their eventual destination in the layer of the mantle in the CNS. The first researchers that took up this problem, His (1890) and Ramón y Cajal (1909, 1911), assumed that the neuroblasts migrated as free amoeboid elements. But Berry and Rogers (1965) and Morest (1970) state that the young neurons do not migrate wholly, only the nucleus and its surrounding cytoplasm migrates, but within the limits of its cytoplasmic prolongation. On the other hand, the studies of Rakic (1971, 1972) in the monkey's telecephalic cortex, and the studies of Rakic and Sidman (1973) in humans, seem to show the existence of free migration cells.

La Vail and Cowan (1971), using autoradiographic techniques, clearly show the existence of an abundant cell migration towards the cortex plate. Its pattern is variable depending on the birth-date of the migratory cells. Evidently, the nuclear branding of the autoradiographic techniques is not sufficient to establish how this migration occurs. When Domesick and Morest (1977a,b) studied the development of multipolar and arch-shaped cells of the optic tectum using the Golgi method, they thought that nerve cells migrated exclusively by nucleus translocation and were doubtful about the free migration model proposed by Rakic and Sidman (1973). Later, Puelles and Bendala (1978) demonstrated, using Golgi's technic, that in the optic tectum of the chick embryo, both migratory modes existed.

In the chick embryo retina, Genis Gálvez (1977), Genis Gálvez et al. (1978) and Prada et al. (1984) and more recently Prada et al. (1987) have demonstrated, using the Golgi and autoradiographic techniques, the free migration of young horizontal and amacrine neurons.

Regarding the spinal cord, Ramón y Cajal (1909, 1911, 1929) and Wentworth (1984) implicitly support, though they do not show in their results, that the young neurons of the spinal cord migrate following the migration by soma translocation model; the young neuron always being attached at least to the pial surface.

Our results support that the ventral horn cells of the chick embryo spinal cord follow two different modes of migration:

1.– The ventral horn's motor neurons of the spinal cord mostly migrate translocating their cell bodies through the cell cytoplasm, which does not disconnect from the pial surface.

2.– On the other hand, we have observed, in the chick spinal cord, smooth or multipodial shaped free migration cells which could originate association neurons as occurs in other parts of the C.N.S. such as in the cerebellum (Rakic, 1971b), cortex (Rakic, 1972), optic tectum (Puelles and Bendala, 1978) and in the retina (Genis et al., 1977; Prada et al., 1984, 1987).

The mechanisms responsible for the free migration of the smooth and multipodial cells are also likely to be different. While the multipodial type of cells would

associated to amoeboid exploratory movements, the smooth cells would suggest the existence of contact guidance cues, such as glial guides.

Therefore, we can conclude than in the chick embryo spinal cord two neuronal migration modes exist similar to those proposed by several authors for other parts of the Central Nervous System: soma translocation and free migration.

Several procedures are used at present in our laboratory to study the nature of the smooth and multipodial cells.

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## References

- Barron D.H. (1945). The role of sensory fibers in the differentiation of the spinal cord in sheep. *J. Exp. Zool.* 100, 431-443.
- Barron D.H. (1946). Observations of the early differentiation of the motor neuroblasts in the spinal cord of the chick. *J. Comp. Neurol.* 85, 149-169.
- Berry M. and Rogers A.W. (1965). The migration of neuroblasts in the developing cerebral cortex. *J. Anat.* 99, 691-709.
- Domesick V.B. and Morest D.K. (1977a). Migration and differentiation of ganglion cells in the optic tectum of the chick embryo. *Neuroscience* 2, 459-475.
- Domesick V.B. and Morest D.K. (1977b). Migration and differentiation of shepherd's crook cells in the optic tectum of the chick embryo. *Neuroscience* 2, 477-491.
- Fujita S. (1962). Kinetics of the cell proliferation. *Exp. Cell. Res.* 28, 56-60.
- Fujita S. (1963). The matrix cell and cytogenesis in the developing central nervous system. *J. Comp. Neurol.* 120, 37-42.
- Fujita S. (1965a). The matrix cell and histogenesis of the nervous system. *Laval Med.* 36, 125-130.
- Fujita S. (1965b). An autoradiographic study on the origin and fate of the subpial glioblasts in the embryonic chick spinal cord. *J. Comp. Neurol.* 124, 51-60.
- Fujita H. and Fujita S. (1964). Electron microscopic studies on the differentiation of the ependymal cells and the glioblast in the spinal cord of domestic fowl. *Z. Zellforsch. Mikrosk. Anat.* 64, 262-272.
- Genis-Gálvez J.M., Puelles L. and Prada C. (1977). Displaced retinal amacrine cells in the chick. *Exp. Neurol.* 56, 151-157.
- Hamburger V. (1948). The mitotic patterns in the spinal cord of the chick embryo and their relation to histogenetic processes. *J. Comp. Neurol.* 88, 221-283.
- Hamburger V. and Hamilton H.L. (1951). A series of normal stages in the development of the chick embryo. *J. Morphol.* 88, 49-92.
- Hinds J.W. and Hinds P.L. (1974). Early ganglion cell differentiation in the mouse retina: An electron microscopic analysis utilizing serial sections. *Dev. Biol.* 37, 381-416.
- His W. (1890). Histogenese und Zusammenhang der Nervenelemente. *Arch. Anat. Physiol. Leipzig. Anat. Abt. Suppl.* 95, 95-119.
- Jacobson M. (1978). *Developmental Neurobiology*. Second Edition. Plenum Press. New York, London.
- La Vail J.H. and Cowan W.M. (1971). The development of the chick optic tectum II. Autoradiographic studies. *Brain Res.* 28, 421-441.
- Morest D.K. (1970). A study of neurogenesis in the forebrain of opossum pouch young. *Z. Anat. Entwicklungsgesch.* 130, 265-305.
- Prada C. and López-Mascaraque L. (1985). Dammar resin prevents the fading of celloidin sections of Golgi impregnated embryonic central nervous system. *Mikroskopie* 42, 146-147.
- Prada F.A., Armengol J.A. and Genis-Gálvez J.M. (1984). Displaced horizontal cells in the chick retina. *J. Morphol.* 182, 221-225.
- Prada C., Puelles L., Genis-Gálvez J.M. and Ramírez G. (1987). Two modes of the free migration of amacrine cell neuroblasts in the chick retina. *Anat. Embryol.* 175, 281-287.
- Puelles L. and Bendala M.C. (1978). Differentiation of neuroblasts in the chick optic tectum up to eight days incubation: A Golgi study. *Neuroscience* 3, 307-325.
- Rakic P. (1971a). Guidance of neurons migrating to the fetal monkey neocortex. *Brain Res.* 33, 471-476.
- Rakic P. (1971b). Neuron-glia relationship during granule cell migration in developing cerebellar cortex: A Golgi and electron microscopic study in *Macacus rhesus*. *J. Comp. Neurol.* 145, 283-312.
- Rakic P. (1972). Mode of cell migration to the superficial layers of fetal monkey neocortex. *J. Comp. Neurol.* 145, 61-84.
- Rakic P. and Sidman R.L. (1973). Neuronal migration with special reference to developing human brain: A review. *Brain Res.* 62, 1-35.
- Ramón y Cajal S. (1909). *Histologie du Systeme Nerveux de l'Homme et des Vertebres*. Vol I (1952 reprint). Instituto Ramón y Cajal. Madrid.
- Ramón y Cajal S. (1911). *Histologie du Systeme Nerveux de l'Homme et des Vertebres*. Vol II (1955 reprint). Instituto Ramón y Cajal. Madrid.
- Ramón y Cajal S. (1929). *Studies in vertebrate neurogenesis*. L. Guth, transl (1960), Charles C Thomas, Springfield, Ill.
- Stensaas L.J. (1967). The development of hippocampal and dorsolateral pallial regions of the cerebral hemisphere in fetal rabbits. II. Twenty millimeter stage neuroblast morphology. *J. Comp. Neurol.* 129, 71-84.
- Wentworth L.E. and Hinds J.W. (1978). Early motoneuron formation in the cervical spinal cord of the mouse. An electron microscopic serial section analysis. *J. Comp. Neurol.* 177, 611-634.
- Wentworth L.E. (1980). Golgi analysis of neuronal relationships in the spinal cord of the mouse embryo. *Neurosci. Abstr.* 8, 287.
- Wentworth L.E. (1984). The development of the cervical spinal cord of the mouse embryo I. A Golgi analysis of ventral root neuron differentiation. *J. Comp. Neurol.* 222, 81-95.
- Windle W.F., Orr D.W. and Minear W.L. (1934). The origin and development of reflexes in the cat during the third fetal week. *Physiol. Zool.* 7, 600-617.
- Windle W.F. and Baxter R.E. (1936). Development of reflex mechanisms in the spinal cord of albino rat embryos. Correlations between structure and function and comparison with the cat and the chick. *J. Comp. Neurol.* 63, 189-209.
- Windle W.F. and Fitzgerald J.E. (1937). Development of the spinal reflex mechanism in human embryos. *J. Comp. Neurol.* 67, 493-509.