Mechanism of Neuronal Migration in Human Foetal Cerebrum *In-vitro*

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Active migration of immature neurons occurs when fragments of human foetal cerebral tissues are explanted as organotypic cultures. The sequence of events during neuronal migration is orderly and consistent under different cultural conditions as evidenced by continuous time-lapse cinematographic studies. Migrating neurons utilize astrocytes to anchor neurites, and move in clusters on or along the processes of astrocytes or other neurons. Translocation of neuronal soma is accomplished by nuclear movement within extended neurites. A unique junction develops between neurites and astrocytic membrane during early phases in culture to suggest a special affinity of neurons to astrocytes. It is concluded from these observations that immature neurons have inherent capacity for active migration *in-vitro*; preferentially utilize astrocytes and astrocytic processes for anchoring as well as for directional guidance during migration; and translocate their soma by nuclear movement within extended neurites. It is suggested that similar mechanisms may be at play during migration of postmitotic neurons in developing cerebral cortex in human.

In developing mammalian central nervous system(CNS) neurons are generated exclusively in the germinal zones and young post mitotic neurons migrate to reach final positions (Sidman and Rakic, 1973). Controversy still exists however on the mode of migration of immature neurons. Berry and Rogers(1965) believe that germinal cells retain their attachment to pial and ventricular surfaces and do not undergo complete cytoplasmic division after mitosis. They proposed that only one of the daughter nuclei migrated within cytoplasm of the maternal cell toward pia while the other daughter nucleus remained at ventricular zone to continue generation of new cells. Morest(1970) also suggested that young neurons remain attached to pial and ventricular surfaces and that only nuclei move within cytoplasmic cylinders toward pia. Rakic(1971, 1972) on the other hand proposed that young neurons migrate to distant locations following radial glial guides. One of the arguments against the radial glial guidance theory has been the general notion that neuroglial cells are generated late in the course of ontogenetic development only after a majority of neurons

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have been generated and reached their final destinations. More recently however studies in this laboratory as well as others showed the presence of radial glia in human foetal cerebrum (Choi and Lapham, 1978), cerebellum (Choi and Lapham, 1979), monkey cerebrum (Rakic, 1972) and in rodent foetal cerebrum (Swarz and Oster-Granite, 1976). Thus gliogenesis in developing CNS appears to be much earlier phenomenon than formerly believed and radial glia are available for guidance of neurons during most phases of neuronal migration in cerebrum and cerebellum.

Explant cultures of human foetal cerebral tissues provide a unique opportunity to study sequential growth and development of human foetal brain under living conditions, although results must be interpreted with care, and with an awareness of the limitations of this technique. It has been known for some time that neural tissues are capable of undergoing maturation and differentiation in culture and that many of the events in vitro closely parallel in-vivo phenomenon.

Migration of immature neurons occurs actively within 3 days after explantation of human foetal cerebral tissues. Sequential time-lapse recordings of the cultures were made in an attempt to elucidate the mechanism of neuronal migration in developing cerebrum.

MATERIALS AND METHODS

The materials for cultures were obtained from human foetuses ranging from 12 weeks to 16 weeks of ovulation age. Full thickness (pial surface to ventricular zone) tissue blocks were taken from midportion of the cerebral hemisphere after careful removal of leptomeninges. The fragments were cut into approximately 0.5 to 1.0 mm blocks and were plated in large Falcon T flasks and also in petri dishes with cover slips. The petri dishes with cover slips were prepared by coating with thin film of sterile rat tail collagen (GIECO) immediately before explantation. The T-flasks were used with or without collagen coating. The basic medium was nutrient mixture F-12 supplemented by fetal calf serum, L-glutamine, non-essential amino acids (GIECO), glucose and antibiotics. The cultures were placed in CO₂ incubator (95% air, 5% CO₂) in humid atmosphere. Observations were made in primary cultures during the first 72 hours after explantation and in mechanically dissociated cultures of established organotypic cultures (1 to 5 weeks in-vitro). When established primary cultures were mechanically dissociated and replated, explants were often carried over and gave rise to neuronal migration again.

An Opti Quip time-lapse cinematographic setup was used throughout the experiments. The temperature of the culture flasks was maintained at 37.5°C with air curtain (Sage Instruments) during time-lapse recordings.

Sister cultures were terminated periodically and cover slips were harvested to stain with May-Grünwald Giemsa, Bodian and Hematoxylin and Eosin. Occasional cover slips were processed for indirect immunofluorescence and unlabeled antibody enzyme technique of Sternberger (1979) for glial fibrillary acidic (GFA) protein (Choi and Lapham, 1976). The petri dishes and falcon flasks were also periodically harvested to process for electron microscopy (Choi and Lapham, 1976). Sections were cut with a diamond knife using LKB ultratome IV, and stained with uranyl acetate and lead citrate.
RESULTS

Neuronal migration occurred in an orderly sequence from organotypic explant cultures of human foetal cerebrum. Within the first 24 hours many processes of unknown nature protruded from the explant proper. These processes showed active filopodial movement on their tips. Indirect immunofluorescent studies using antisera for GFA protein demonstrated many of the processes to be astrocytic while some represented processes of neurons (Fig. 17). At first, large polygonal astrocytes migrated out of the explants (Figs. 1, 2, 17). Their movement was associated with elongation of processes accompanied by ruffled movement of cytoplasmic membrane. As astrocytes proliferated and migrated away from explants young immature neurons with small dark round cell bodies began to migrate out (Figs. 1–6). At first they extended processes with active filopodia continuously palpating adjacent area and anchored securely on astrocyte, the nucleus then moved within its own extended process toward the anchored position. The opposite process followed. The trailing processes also showed active filopodial movement and sometimes made contacts with oncoming neurites. The neurons often followed the same path to anchored positions (Fig. 4).

When three or more neurons reached to anchored position on astrocytic cytoplasm the neurons and astrocytes moved away to distant locations together. The migrating neurons frequently clustered over the cell bodies of astrocytes and gave rise to bundles of neurites which connected with other groups of neurons on astrocytes (Figs. 4, 5). Occasionally astrocytes extended processes to connect with other astrocytic processes so that clusters of neurons would migrate away along the long extended processes. On collagenated surface astrocytes generally extended processes to reach outer rim of fibroblasts which often encircled explants (Figs. 9, 10). Neurons lined up on these processes and moved out following the same sequence of nuclear movement in neurites (Figs. 4, 5, 6). The length of neurites varied from one neuron to the other. Generally however they had short bipolar processes. The nuclear movement within neurites occurred in both directions in some instances.

When explants were carried over to another flask which had no collagen coating astrocytes moved randomly in all directions. However the sequence of neuronal migration remained the same. Regardless of the type of surface (glass, plastic, collagen coated, or uncoated) or the type of cultures (primary or secondary) neuronal migration followed the same orderly sequence. The movement of astrocytes was generally much faster than movement of neuronal nuclei.

After several weeks in-vitro when migration of neurons was completed there remained the skeleton of astroglial processes extending from explant proper to the rim of fibroblasts. They were strongly positive for GFA protein (Figs. 11, 12).

EM examination revealed a unique junction between neurites and astrocytic membranes (Figs. 13, 14). This junction was characterized by the presence of electron-dense material sandwiched between the apposed membranes. On higher magnification the electron-dense material was not homogeneous but showed a segmented appearance as shown in Fig. 14. Although the appearance of the junction resembled gap junction the total width of the membranes at junctional sites measured app-
roximately 35 nm and therefore was different from a classical gap junction which measures generally 15-19 nm. The formation of this type of junction was apparently transient. They were observed in outgrowth areas of explants within a few days to two week in organotypic cultures. In older cultures very few or none of the same junctional complexes were observed.

**DISCUSSION**

Correct identification of cell types in cultures is crucial in the study of this kind. For many years we have utilized a combined technique of cell identification by correlating features observed by phase contrast microscopy of living cultures; stained preparations after conventional histochemical stains; stained preparations after conventional histochemical stains (such as Bodian, phosphotungstic acid hematoxylin, sudan black etc.); one micron sections of Epon embedded material; scanning and transmission EM. Immunofluorescent technique and unlabeled antibody enzyme technique of Sternberger using antiserum to GFA protein were particularly helpful for identification of astroglia. Sections of tissue blocks from which cultures were derived were also compared with the cells observed in cultures. The characteristic features of various cell types particularly of neurons and astrocytes in cultures of human foetal cerebrum were described in detail in our previous publications (Choi and Lapham, 1974; Choi and Lapham, 1976). Oligodendroglial cells were not present in cultures or tissue blocks of early human foetal cerebrum used in this study. The mechanism of neuronal migration has remained speculative and uncertain primarily due to the limitations of static morphological techniques from which many inferences were drawn. Organotypic cultures of human foetal cerebrum, on the other hand, provided a unique opportunity to study sequential movement of immature human brain cells under living conditions. It was particularly revealing when used in conjunction with continuous time-lapse cinematographic studies.

It is clear from these observations that immature neurons are capable of active migration *in-vitro* and that they follow an orderly sequence of events. The transperikaryal movement of neuronal nuclei apparently is the principal mode of translocation of neuronal soma. This finding partly supports the thesis that neuronal migration is a translocation of nucleus in the cytoplasmic extension (Berry and Rogers, 1965; Morest, 1970). Therefore it is possible that the same mechanism may be operative in developing cerebrum when the thickness of the cortical plate is still thin enough to have neurons with attachments both in pial and ventricular surfaces.

However, the majority of neurons in migration were bipolar with relatively short processes. This was also true in tissue blocks of human foetal cerebral cortex (Sidman and Rakic, 1973; Choi and Lapham, 1978). At later stages of ontogenesis the cortical plate progressively increases in thickness and the final destinations for immature neurons would become many times the total lengths of neurons. This necessitates additional means or mechanisms for neuronal migration.

Repeated observations in our culture system showed remarkable affinity of neurons and astrocytes. Frequently migrating neurons utilized cell bodies and processes of astrocytes for support and directional guidance.
Rapidly moving filopodia of neurites made contacts invariably with astrocytes. It may be argued that the predominant neurite-astrocytic union may be related to abundance and availability of astrocytes in the immediate vicinity peculiar to the cultural conditions. However, formation of a special junction between neurites and astrocytes as demonstrated by EM, and repeated observations of close contacts between them on phase-contrast microscopy of living cultures suggests that the intimate relationship of neurons and neurites with astrocytes is not a fortuitous one.

The affinity of neurons to astrocytes was more easily visualized in dissociated cultures. Most if not all neurons in the outgrowth area were seen to be attached to astrocytes. These findings correlate well with surface affinity frequently observed between migrating neurons and radial glia in developing CNS (Rakic, 1972; Choi and Lapham, 1978). It has been reported that glial cells produce some “trophic” substance (Varon and Somjec, 1979) for neurons and neurites. However it is not clear at the present time precisely what factor(s) control the specific affinity of neurons and astrocytes.

Locomotion of tissue cells in culture has been the subject of many studies in recent years (Harris, 1973). It has been claimed that nerve cells, in contrast to glial cells, do not migrate in culture (Wessels et al., 1973), and that pulling effects created by tension in the axon were responsible for movement of neuronal soma. However review of many time-lapse films recorded during early phases of explant cultures clearly demonstrated spontaneous motion of neuronal cell bodies in and around margins of explants as well as in outgrowth areas. Frequently neuronal nuclei moved in both directions of extended neurites without apparent movement of underlying astrocytes. It is probable that immature neurons possess an inherent capacity for migration as well as ability for elongation and contraction of neurites and movement of the nucleus. Neurons in long term cultures in our system did not migrate. They became stationary and showed regular pumping action of cell bodies and neurites. In these cultures elongation of neurites sometimes occurred without movement of nuclei. What factor(s) give signal to immature neurons to activate the inherent capacity for migration is not clear; however it is certain that under favorable conditions immature neurons are capable of active spontaneous locomotion in organotypic cultures. Lamont and Vernon (1967) also described the migration of nerve cell bodies for chick dorsal root ganglia in-vitro by contraction of the leading nerve processes.

Admittedly explant culture system is an artificial condition, nonetheless this technique provides the only means to study biological behavior of human foetal neurons in sequential manner under living conditions. Consistent and orderly sequence of events in neuronal migration in-vitro of human foetal cerebrum under variable cultural conditions suggests that the pattern observed may represent general biological properties of immature neurons and astrocytes and that similar mechanisms may be at play during migration of young postmitotic neurons in developing human foetal cerebral cortex.

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REFERENCES


LEGEND FOR FIGURES

Fig. 1. Phase contrast photomicrograph of living culture during the first 24-48 hours after explantation. Note migration of large polygonal astrocytes (A) at the periphery of an explant (E). Small dark neurons (N), with thin bipolar processes begin to migrate out of the explant proper. ×150.

Fig. 2. Phase contrast photomicrograph of living culture 72 hours after explantation. Astrocytes (A) proliferated and migrated further. Note an astrocyte which underwent a cell division at the left lower part the picture (dark oblong images). Small dark neurons (N) are visible along the processes of astrocytes. The neurons extend short thin processes (thin arrowhead). E: Explant. ×50.

Fig. 3. Phase contrast photomicrograph of living culture 72 hours after explantation. Note large numbers of small dark neurons (N) migrating out of the explant proper (E) on the bed of astrocytes (A) which have migrated out further. A rim of leptomeningeal cells (LMC) encircle the explant at the periphery. ×150.

Fig. 4. Phase contrast photomicrograph of living culture one week after explantation. Proliferating astrocytes (A) and their processes provide support under the migrating neurons (N). Neurons are clustered on the same path. E: Explant. ×150.

Fig. 5. Phase contrast photomicrograph of living culture one week after explantation. Note bundles of neuronal processes (Np) reaching out to connect with astrocytes (A) at the periphery of explant. ×150.

Fig. 6. Phase contrast photomicrograph of living culture one week after explantation. Note groups of neurons (N) with short processes migrating on top of astrocytic cytoplasm (A). E: Explant. ×150.

Fig. 7. Phase contrast photomicrograph of living culture 10 days after explantation. Note large numbers of neurons (N) migrating out of the explant (E). Astrocytes (A) are present underneath the clusters of neurons and touch the leptomeningeal cells (LMC) at the periphery. ×150.

Fig. 8. Phase contrast photomicrograph of living culture 12 days after explantation. Note a ring of leptomeningeal cells (LMC) surrounding the explant. Astrocytes extend long processes (Ap) to attach at the rim of leptomeningeal cells. Neurons (N) migrate along the extended astrocytic processes. ×150.

Fig. 9. Phase contrast photomicrograph of living culture 3 weeks after explantation. Note many astrocytic processes (Ap) attached to leptomeningeal cells (LMC). There are scattered small dark neurons along the astrocytic processes. ×150.

Fig. 10. Phase contrast photomicrograph of living culture 3 weeks after explantation. Higher magnification of the features seen at the margin of leptomeningeal cells (LMC) where astrocytic processes (Ap) are attached with conical swellings. ×300.

Fig. 11. Photomicrograph of indirect immunofluorescence for GFA protein of an explant at the outer zone. Note immunofluorescence of fibers which are shown as in Fig. 9. ×150.

Fig. 12. Photomicrograph of indirect immunofluorescence for GFA protein on higher magnification. Strongly immunofluorescent fibers extend from explant toward the ring of leptomeningeal cells. ×300.

Fig. 13. Electron micrograph of formation of junctions (thick short arrows) between neurites containing parallel arrays of neurotubules (dark arrows) and astrocytes containing bundles of glial filaments (GF) as pointed by arrows with white heads in the center. Electron dense material is present between the membranes of neurite and astrocyte. ×54,000.
Fig. 14. Electron micrograph of a junction (short thick arrow) between neuronal process (Np) and astrocyte. nt: neurotubule. GF: glial filament. The material sandwiched between the membrane appears to be segmented and shows an interruption. ×63,000.

Fig. 15. Scanning electron micrograph of an astrocyte (A) and a neuron (N) with thin process. Note intimate association of neuronal cell body and a process of an astrocyte. ×6,200.

Fig. 16. Electron micrograph of astrocyte (A) and neurons (N) in migration. Note bundles of glial filaments (gf) in the cytoplasm of astrocyte. Neurons make close apposition with formation of density (j) at contact points. ×7,650.

Fig. 17. Photomicrograph of indirect immunofluorescence for GFA protein during early phase of neuronal migration. Note large polygonal cell showing immunofluorescence for GFA protein indicating that they are astrocytes (A). Many processes extended from explant proper (white area at the lower margin) also represent astrocytic processes. Neurons (n) are non-immunofluorescent and their cell bodies are closely apposed to astrocytic processes. ×150.

Fig. 18. Photomicrograph of Bodian stained preparation of explant culture. Note dark strongly silver positive neuronal processes (Np) extending from round to oval dark small cell bodies. Pale stained nuclei of astrocytes are visible in the background. ×400.

Fig. 19. Photomicrograph of astrocytes demonstrated by unlabeled antibody enzyme technique of Sternberger. Note dark immune product in the cytoplasm and processes of astrocytes. ×670.