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Morphological Characteristics of the Developing Human Brain during the Embryonic Period

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Many features of the developing nervous system are visible from external observations of intact human embryos. In this study, a photographic atlas from the 4th to the 7th week after ovulation (Carnegie stages 10-18) is provided. The neural folds began to fuse at stage 10, and the rostral and caudal neuropore were closed during stages 11 and 12, respectively. The three primary divisions of the brain were distinguishable before closing of the neural tube. The five secondary brain vesicles were formed during stages 14-15. The development of the cerebellum and cerebrum were first observed at stages 14 and 15, respectively. The mesencephalic flexure was seen at stage 12, and the cervical flexure and pontine flexure at stage 14. After stages 18-19, it became increasingly difficult to identify detailed features of the brain from the surface. Results from this study will help to correlate the characteristic findings of the developing central nervous system of human embryos from stereomicroscopical and light microscopical observations and to locate the exact parts of the developing human brain for other purposes.

Key Words: Nervous system, human embryo, carnegie stage

After the classical studies of His (1904), Hochstetter (1919, 1923, 1929), Bartelmez and Dekaban (1962), there were few studies on the development of the central nervous system of the human embryo until O'Rahilly's works (O'Rahilly and Müller, 1994). The knowledge of this development is very important in neurological and neurosurgical fields due to the high incidence of congenital anomalies in the central nervous system. To understand the development of the central nervous system in detail, knowledge of gross morphology is necessary. However, external morphological studies of the developing human brain are rare (O'Rahilly *et al.* 1982, 1986).

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The data presented in this study will help to correlate the characteristic findings of the developing central nervous system of human embryos from stereomicroscopical and light microscopical observations and to locate the exact parts of the developing human brain for other purposes.

MATERIALS AND METHODS

We used 31 cases of normal human embryos between 4-to-8 weeks postovulation which had been collected and stored in the Laboratory of Human Embryology and Teratology, Department of Anatomy, Yonsei University College of Medicine. Embryos were classified by Carnegie stage (O'Rahilly and Müller, 1987). According to the Carnegie stage, the 4th week corresponds to stages 10-13, the 5th week to stages 14-15, the 6th week to stages 16-17,

the 7th week to stages 18-19, and the 8th week to stages 20-23. The developmental stage and the number of embyros used in this study were as follows; stage 10 (1), stage 11 (1), stage 12 (3), stage 13 (3), stage 14 (3), stage 15 (3), stage 16 (3), stage 17 (2), stage 18 (2), stage 19 (2), stage 20 (2), stage 21 (2), stage 22 (2), and stage 23 (2). All of the embryos were fixed in 10% formalin.

We observed the characteristic external features, that is, the contour of the brain and ventricle, cranial nerves and other head and neck structures from the surface using a stereomicroscope at ×5 - ×50 magnification. The external and internal structures of the embryos in early development before stage 19 could be observed under fiberoptic illumination, but those in the late stages could not be seen due to the opacity of the tissues in the head and neck region. After external observation, the embryos were dehydrated with graded ethanol and embedded in paraffin by conventional procedures. Serial sections of 7~10 μm in thickness were prepared and stained with hematoxylin and eosin. The light microscopic findings were compared to the stereomicroscopic findings.

RESULTS

Stage 10

The embryo of stage 10 had 9 pairs of somites. The neural folds were closed to form the neural tube at the caudal portion of rhombencephalon, level of occipital somites and rostral portion of cervical somites. The rostral neuropore was wide open and the caudal neuropore was observed as a narrow cleft at the caudal portion of the embryo. In the rostral portion of the embryo, procencephalon, mesencephalon and rhombencephalon could be clearly distinguished (Fig. 1A, B). At the inner surface of the procencephalon, the optic sulcus was observed as a shallow depression. Diencephalon could be divided into the rostral D1 portion, comprising the optic sulcus, and the caudal D2 portion. The rhombomeres or otic disc could not be seen from external observation.

Stage 11

The embryo of stage 11 had 19 pairs of somites.

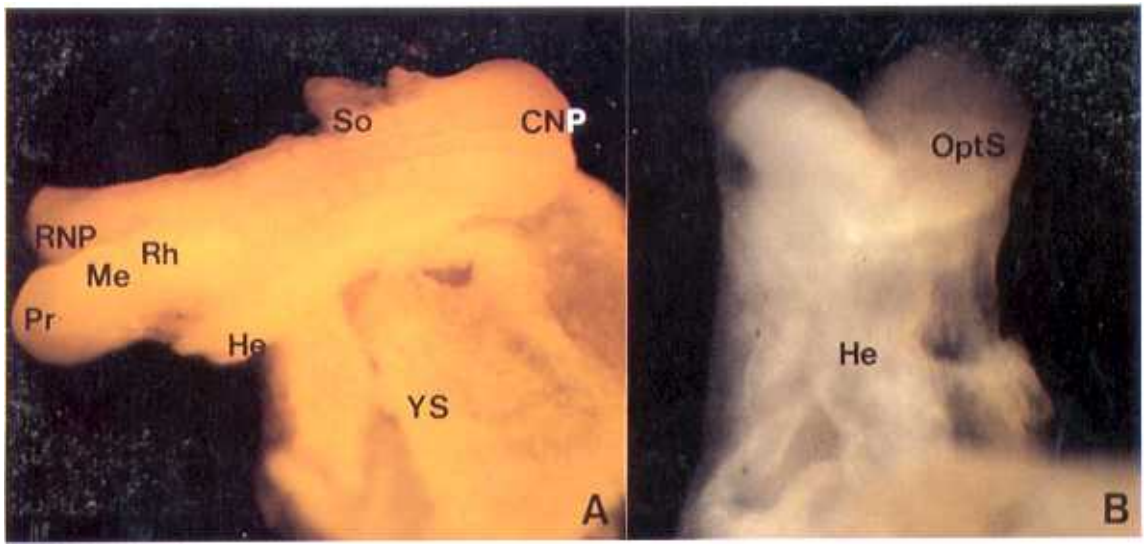

Fig. 1. Stage 10 (9 somites). A. Left superior view (×25). B. Ventral view (×60). CNP; caudal neuropore, He; heart, Me; mesencephalon, OptS; optic sulcus, Pr; prosencephalon, Rh; rhombencephalon, RNP; rostral neuropore, So; somite, YS; yolk sac

The rostral neuropore was closed completely, but the caudal neuropore was still open. The proportion of head to the entire embryo was smaller than that of the stage 10 embryo. The optic vesicles appeared as small sac-like structures and the otic pit could be observed clearly (Fig. 2).

Stage 12-13

The rostral and caudal neuropores were closed completely. The rostral portion of the neural tube was wider than the caudal portion. Although the 4th ventricle was not expanded distinctly, the roof was thinner than the other portions and through it rhombomeres could be observed on the floor of the ventricle. The trigeminal, vestibulocochlear and vagus nerves appeared clearly (Fig. 3A). The mesencephalic flexure was formed at about 90°.

The procencephalon and mesencephalon were clearly demarcated from the ventral view (Fig. 3B). In the area of the procencephalon, the diencephalon comprising the optic vesicles and the telencephalon at the median portion were distinguishable. At the D2 portion, slightly protruded synencephalon was identified. The lumen of the optic vesicles were wide open to the lumen of diencephalon. The infundibulum was closely apposed with the Rathke's pouch bulging from the pharyngeal roof. The mesencephalon was divided into the M1 and M2 portions and a thin marginal layer was found at the wall of mesencephalon. In the lateral portion of rhombencephalon, the ellipsoidal otic vesicle was open to the surface by a narrow pore at stage 12, but appeared as a closed vesicle connected to the surface epithelium by a narrow stalk at stage 13.

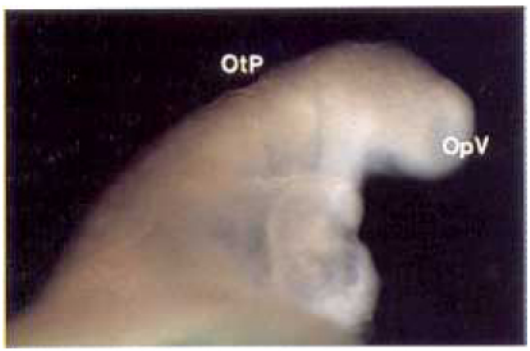

Fig. 2. Stage 11 (19 somites). Right lateral view (×30). OpV; optic vesicle, OtP; otic pore

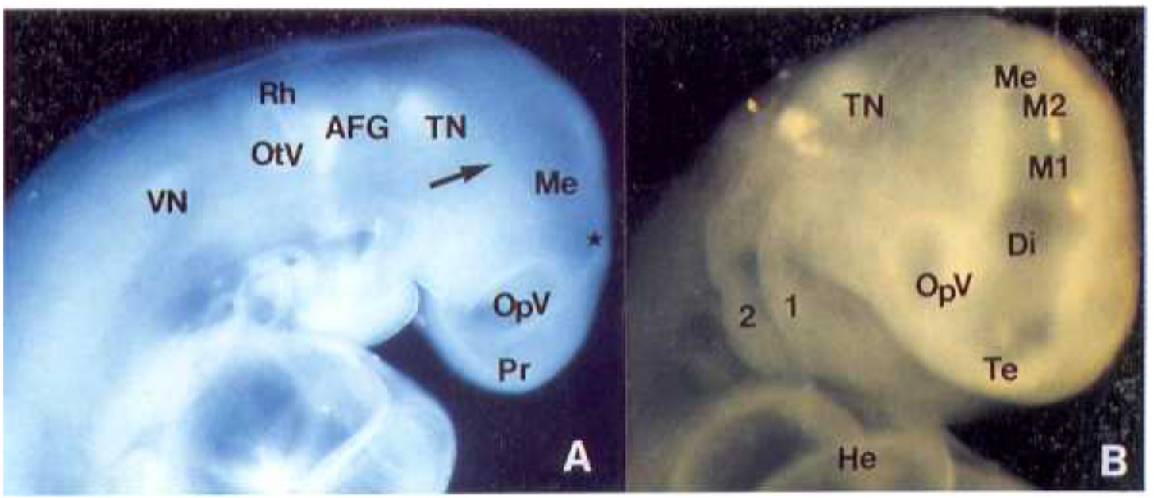

Fig. 3. Stage 12. A. Right lateral view (×30). B. Ventral view (×30). 1; first branchial arch, 2; second branchial arch, →; mesencephalic flexure, ☆; synencephalon, AFG; acousti- cofacial ganglion, Di; diencephalon, He; heart, M1 & M2; segment of mesencephalon, Me; mesencephalon, OtV; otic vesicle, Pr; prosencephalon, Te; telencephalon, TN; trigeminal nerve, VN; vagus nerve

Stage 14

The mesencephalic flexure made a more acute angle than at on earlier stage (Fig. 4A). The lateral wall of the 4th ventricle became wide open to show the expanded lumen. The synencephalon at the D2 portion became more prominent. The caudal portion of the 4th ventricle was wider and longer than the rostral portion (Fig. 4B). The rhombencephalon could be divided into the rostral metencephalon, characterized by developing cerebellum, and the caudal myelencephalon. The rhombomeres were very prominent and the otic vesicles were observed at the position of the 4-5th rhombomeres. From the wall of the brain vesicles, three typical layers, that is, ependymal layer, mantle layer and marginal layer, could be distinguished. The primordium of cerebellum could be identified as the thickening of the ventrolateral lamina at the metencephalon.

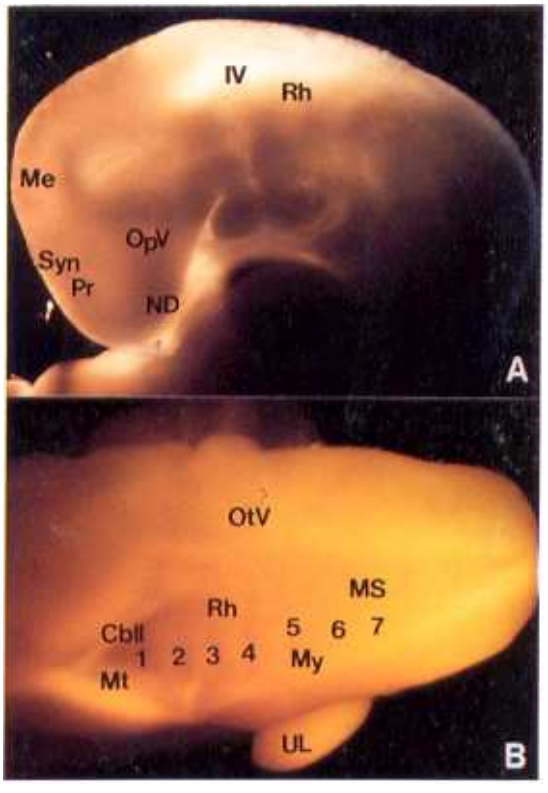

Fig. 4. Stage 14. A. Left lateral view (×20). B. dorsal view (×30). 1-7; rhombomeres, Cbll; cerebellum, IV; fourth ventricle, Me; mesencephalon, MS; median sulcus of fourth ventricle, Mt; metencephalon, My; myelencephalon, ND; nasal disc, Opv: optic vesicle, Otv; otic vesicle, Pr; prosencephalon, My; myelencephalon, ND; nasal disc, Opv; optic vesicle, OtV; otic vesicle, Pr; prosencephalon, Rh; rhombencephalon, Rhm; rhombomeres 1-7, Syn; synencephalon, UL; upper limb bud

Stage 15-16

The primordium of cerebrum and the lateral ventricle could be observed lateral to the procencephalon (Fig. 5A, B, C), and the di-telencephalic sulcus became prominent. At the caudal portion of the brain, the cervical flexure could be distinguished. The 4th ventricle expanded more than the previous stage, and at the floor of the 4th ventricle the pontine flexure could be observed. The rhombencephalic isthmus between mesencephalon and metencephalon appeared clearly.

The primordial meninges enveloping the brain could be seen in histological observation. At the rhombencephalon and mesencephalon, the alar and basal plates could be demarcated by sulcus limitans. The cerebellar primordium was enlarged. The cerebral hemispheres were formed by evagination of the lateral wall of the telencephalon. The diencephalon could be divided into the ventral thalamus and the epithalamus by the ventral sulcus. The optic chiasm, preoptic area and primordium of the neurohypophysis appeared at this stage. At stage 16, the cerebral hemispheres enlarged rostro-ventrally to make the prominent and deep longitudinal cerebral fissure and the interventricular foramen.

At stages 17 (Fig. 6A), 18 and 19 (Fig. 6B), the developing central nervous system became enlarged dramatically, but stage specific characteristics were not found.

DISCUSSION

The menstrual age or the length of the embryos usually does not correspond to the real developmental age. Therefore, the concept of developmental stage is needed to standardize the embryos. In human embryos, Carnegie stage (O'Rahilly and

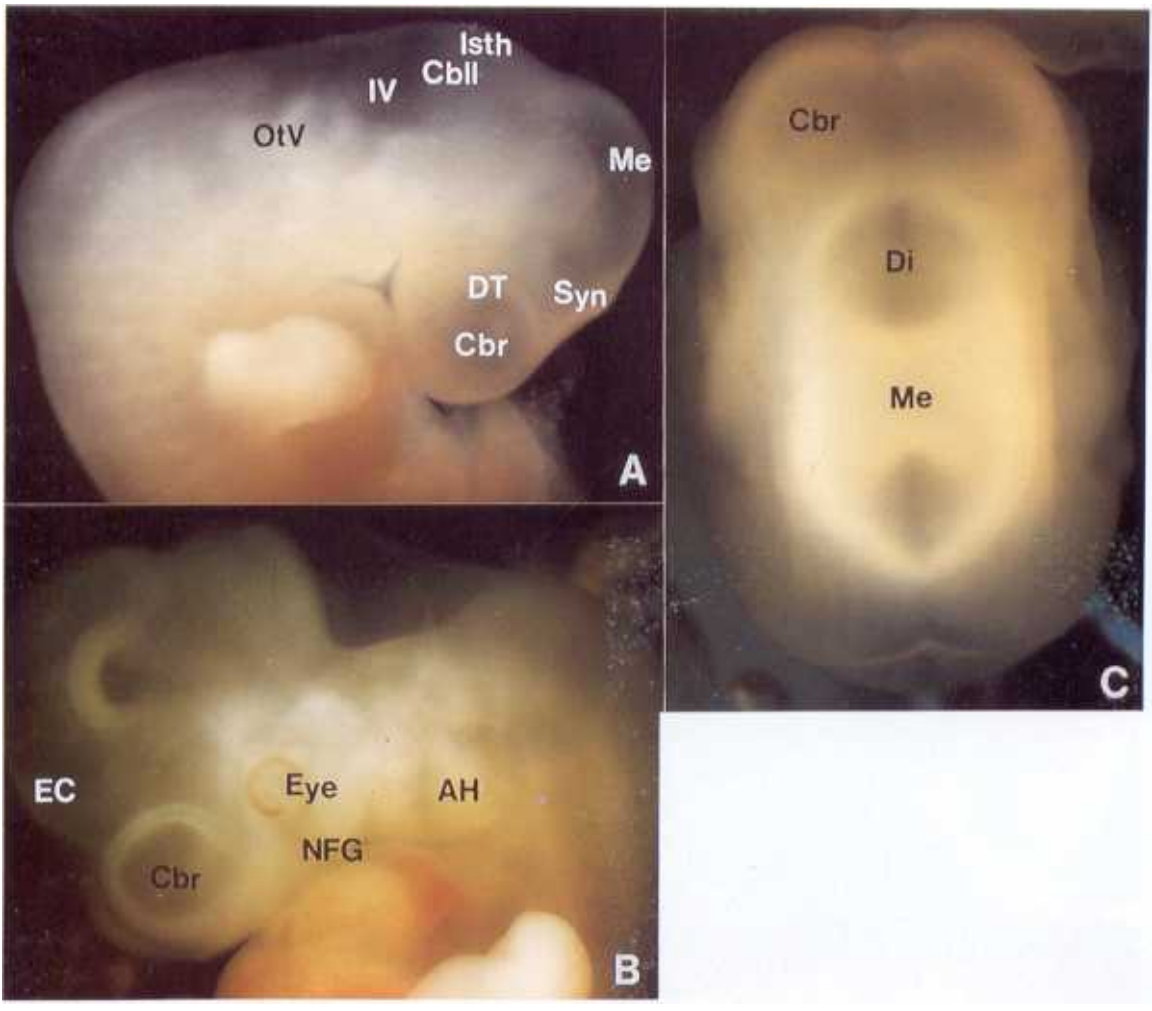

Fig. 5. A. Right lateral view of stage 15 (×15). B. Left lateral view of stage 16 (×15). AH; auricular hillocks, Cbll; cerebellum, Cbr; cerebral vesicle, Di; diencephalon, DT; di-telencephalic sulcus, EC; epiphysis cerebri, Isth; rhombencephalic isthmus, IV; fourth ventricle, NFG; nasofrontal groove, Me; mesencephalon, OtV; otic vesicle, Syn; synencephalon

Müller, 1987) is widely used. In this study, we have correlated the gross and microscopic findings of the developing central nervous system in human embryos classified by Carnegie's stage. External morphological characteristics observed at each stage have been approved to be valid and useful by previous studies (Park *et al.* 1992a, 1992b).

The appearance of neural folds at stage 8 is the first morphological evidence of the formation of the central nervous system in humans (O'Rahilly and Müller, 1981). The neural folds at this stage are composed of pseudostratified columnar epithelium. At stage 9, the neural folds become prominent and the neural groove becomes deepened. At this stage, Müller and O'Rahilly (1983) could distinguish the procencephalon, mesencephalon and rhombencephalon in the neural folds, although the neural tube did not form completely.

The neural tube begins to close at stage 10, and closes completely at stage 12 (O'Rahilly and Müller, 1994). We could observe that the rostral neuropore was wide open at stage 10, and closed at stage 11.

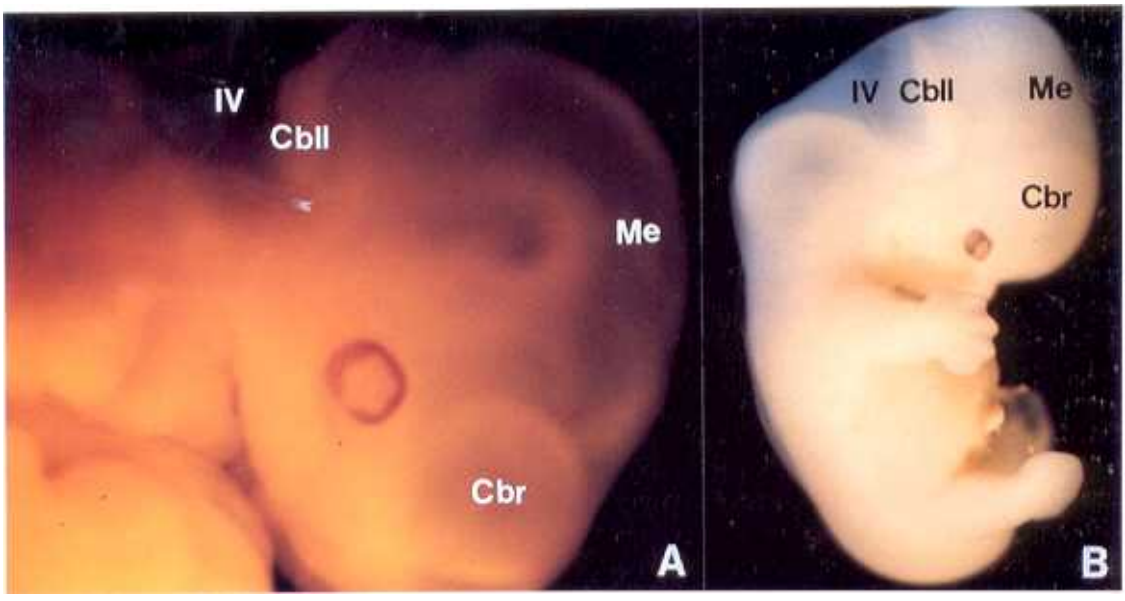

Fig. 6. A. Right lateral view of stage 17 (×10). B. Right lateral view of stage 19. CbII; cerebellum, Cbr; cerebral vesicle, IV; fourth ventricle, Me; mesencephalon

It is believed that the primary brain vesicles, which are composed of prosencephalon, mesencephalon, and rhombencephalon, appear at stage 10 (Müller and O'Rahilly, 1985) and we could identify it clearly by stereomicroscopical observatioin at this stage. The secondary brain vesicles are known to be formed at the 5th week of development. In this study, we could observe that the prosencephalon was divided into the telencephalon and the diencephalon at stage 12, and that the rhombencephalon was divided into the metencephalon and the myelencephalon at stage 14 with the appearance of cerebellar primordium. When the optic sulcus appeared at stage 10, a portion of the prosencephalon rostral to the sulcus could be regarded as the telencephalon. So the secondary brain vesicles formed during a rather longer period.

The neuroaxis is straight at stages 8-9, but curves gradually at the head portion probably due to a different growth rate of the wall of the neural tube (O'Rahilly and Müller, 1994). The mesencephalic flexure is known to be appear at stage 9, the cervical flexure at stage 13, and the pontine flexure at stage 14 (O'Rahilly and Müller, 1994). In this study, we could observe the mesencephalic flexure at stage 12, and the cervical and pontine flexures at stage 15 from external obeservation.

Like the spinal cord, the developing brain is known to be composed of segmental structures (Bergquist, 1952), but the information on the segmental structures were scanty in human embryos. We could identify the D1 and D2 portions in diencephalon, the M1 and M2 portions in mesencephalon, and 8 rhombomeres in rhombencephalon. In embryos earlier than stage 13, the rhombomeres could not be seen because the 4th ventricle was not expanded to show it's floor clearly. The development of the cranial nerves and brain vesicles are believed to be related to each segmental structures but it's significance is not well understood.

The primordium of cerebellum is known to appear at stage 13 (Müller and O'Rahilly, 1988a), but we could observe it as the proliferation of the ventrolateral lamina of the first rhombomere at stage 14. The rhombencephalic isthmus, which eventually develops into a part of the cerebellum, could be identified at stage 15, which is about a week later than other reports (Müller and O'Rahilly, 1988a). Cerebrum is known to arise from the rostral portion of the telencephalon at stage 14 (Müller and O'Rahilly,

1988b). In this study, the cerebral hemispheres could be identified clearly at stage 15, and at stage 16 the longitudinal cerebral fissure and the interventricular foramina became apparent due to the rostroventral growth of the hemispheres.

During stages 17-19, we could not find any stage specific characteristics from external observations except for the remarkable growth of parts of the central nervous system. After stage 19, the brain showed similar conformation to adult structures, but the central nervous system could not be observed from the surface due to the increasing opacity of the surface structures of the enlarged head.

In this study, we correlated the characteristic findings of the developing central nervous system of human embryos from stereomicroscopical and light microscopical observations. These results may contribute to locate exactly the part of the developing human brain for other purposes.

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