Transplantation into the mammalian adult spinal cord

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Summary. Embryonic cerebral cortical tissue obtained from rat embryos of 15-day gestation was transplanted into the cervical spinal cord of adult rats. The cortical transplants survived, grew, and established connections with the host animal's spinal cord. In other animals, knife lesions were first made in the host's spinal cord, and then embryonic cortical tissue was transplanted into the site of the lesion. The cortical transplants in these animals were observed to become an integral part of the host animal's spinal cord.

Previous investigators have demonstrated conclusively that embryonic neural tissue could be successfully transplanted into the adult mammalian brain[1-7]. Such transplants not only survived and grew, but also established afferent as well as efferent connections with the host brain[7-10]. In addition, at the interface between transplant and host brain tissue, there was no evidence of an astroglial scar formation[11,12]. Attempts to transplant various types of tissues into the adult mammalian spinal cord have been hindered by the formation of such an astroglial scar. The present report is addressed to the successful transplantation of embryonic cortical tissue into the adult spinal cord of animals which either had hemisections of their spinal cords previous to transplantation or received only the transplant.

Materials and methods. Using the technique of transplantation of Das and Hallas[13], 15-day-old rat embryonic neocortical tissue obtained from pregnant Sprague-Dawley rats was transplanted into the spinal cord, at the level of C_5–C_6, of adult Sprague-Dawley rat hosts. In brief, the technique of transplantation employs a thin-walled glass capillary tube attached to a 0.5 ml glass BD syringe. Thus, the capillary tube serves as a needle for the syringe. Once the embryonic tissue desired for transplantation has been dissected from the embryo and freed of all meningeal membranes, the tissue is then drawn up into the glass capillary tube and is ready for transplantation. This arrangement has proven successful for transplantation into the brain of neonate or adult host animals for the syringe can either be hand-held or inserted into a stereotaxic carrier for more precise localization of the transplants. However, in the present study a slight modification of the technique was employed. The glass capillary tube instead of being straight was bent at a 90° angle. This allowed the embryonic tissue to be easily transplanted into the lesioned area of the spinal cord. The adult host animals were divided into 2 groups. One group, prior to transplantation, received a surgical scalpel hemisection of the spinal cord while the other group of host animals received only the transplant. For transplantation into the spinal cord, the host animals' heads were placed in a Kopf small animal stereotaxic unit, and the upper body of the animal was immobilized by an accompanying spinal unit. Control animals only had a hemisection of their spinal cord. 90 days after transplantation host animals for each group were sacrificed by either transcardial perfusion with 10% formalin or the spinal cords were removed and placed directly into Golgi-Cox solution. Frozen serial sections 20 µm in thickness were cut in the sagittal plane from the perfused material and stained with either cresyl violet or Bodian Fiber stain. Transplants processed for Golgi-Cox impregnation were embedded in Celloidin and sectioned at 100 µm. Additional host animals from each group received scalpel lesions of the spinal cord either rostral or caudal to the transplant to study the afferents to the transplants. Frozen serial sections, 33 µm in thickness, were cut in the sagittal plane of the sections of spinal cord containing the transplants and processed for Fink-Heimer staining[14].

Results. In all host animals the transplanted neocortical tissue was found to have survived, grown, and differentiated (figs 1 and 2). The transplants in animals that had received only the neocortical tissue and no lesion were found to have pushed aside the host spinal cord and in some cases grown along the dorsal surface of the spinal cord (fig. 1). In host animals that had received hemisections of their spinal cord prior to transplantation, the transplants were found to have completely filled the lesioned area. In all host animals, at the interface between transplant and host brain, there was no glial scar formation. The transplants were continuous with the host's spinal cord and the 2 shared common neuropil, however, no intermingling of neural tissue occurred. Fibers from the host spinal cord were observed crossing the interface into the transplant as were axons from neurons contained in the transplant (fig. 3).

Histologically, the transplants contained fully differentiated pyramidal and stellate neurons. Such neurons mimicked their in vivo counterparts and appeared normal in size and shape (figs 2 and 4). Analysis of serial sections from the Golgi-Cox impregnated material revealed a laminar arrangement of cortical neurons reminiscent of the normal cerebral cortex. Dendrites of both pyramidal and stellate cells contained spines, while through the use of serial reconstruction, the axons were observed to extend approximately 5–6 mm and in some cases formed bundles that either coursed throughout the transplant or crossed the interface between transplant and host brain and ended in the host's spinal cord. Fiber bundles that entered the host's spinal cord were traced using serial section and found to have either ascended 1–3 segments or descended into lower spinal cord segments.

In the transplants from host animals that had received lesions of their spinal cords either rostral or caudal to the transplants and processed for Fink-Heimer staining, degenerating fibers were observed in the transplants. Such fibers were providing afferents to the transplants and provided additional evidence that the transplants and host spinal cord were anatomically integrated and were not isolated islands of neurons.

Discussions and conclusions. The findings presented here suggest it is possible not only to transplant embryonic neocortical tissue into the adult spinal cord, but also that the transplant will fill lesioned areas of spinal cord and provide anatomical continuity between the 2 ends of the spinal cord. The neocortical transplants in the spinal cord, like those in the cerebellum or forebrain, are anatomically integrated with the host's neural tissue. This is supported by the absence of any glial scar formation between transplant and host brain, the sharing of common neuropil, the observance of fibers crossing the interface between transplant and host brain, and from Fink-Heimer stained material the evidence that the host spinal cord is providing afferents to the transplant. In host animals that received a hemisection of their spinal cord prior to transplantation, recovery of gross motor movements of the limbs was

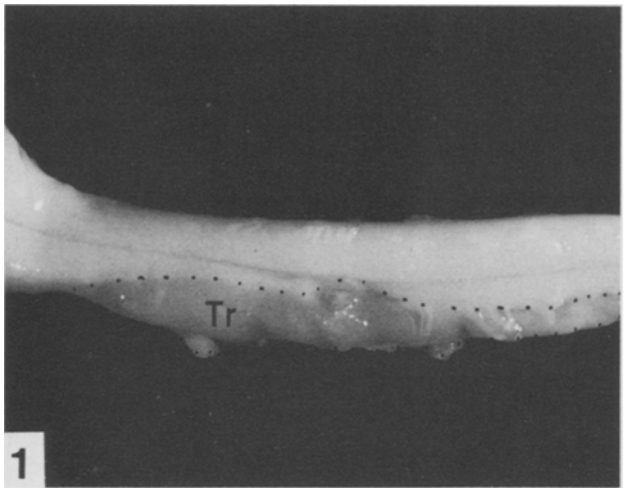

Figure 1. Low power view of ventral spinal cord. Dotted line indicates area of transplant (Tr). 15-day embryonic cortical transplant, adult host. × 3.5.

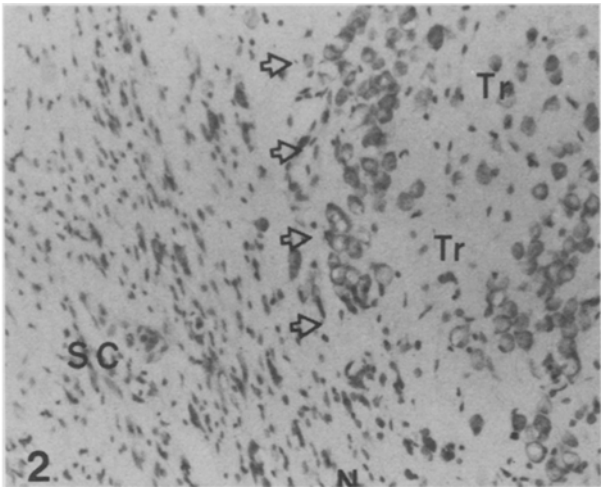

Figure 2. 15-day embryonic neocortical transplant (Tr) in the spinal cord (SC) of an adult host. Arrows indicate area of interface between transplant and host spinal cord. Note absence of glial scar formation at interface. Cresylviolet stain, × 86.

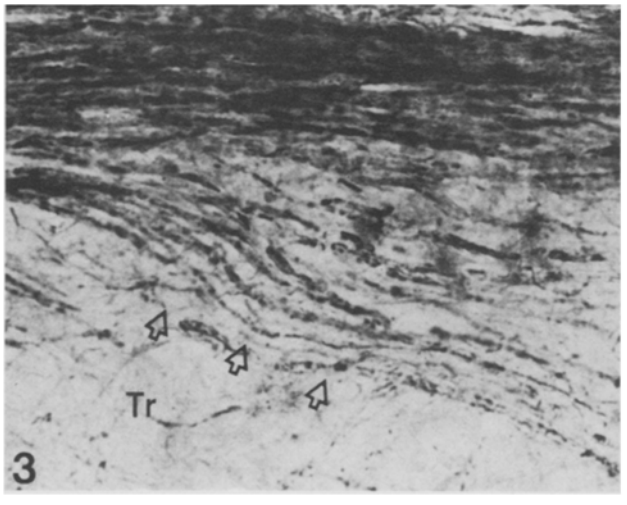

Figure 3. Interface of cortical transplant (Tr) and host animal's spinal cord. Arrows indicate fibers from host animal's spinal cord crossing the interface and entering the transplant. Host animal initially had a hemisection of spinal cord made prior to transplantation. Bodian fiber stain, × 104.

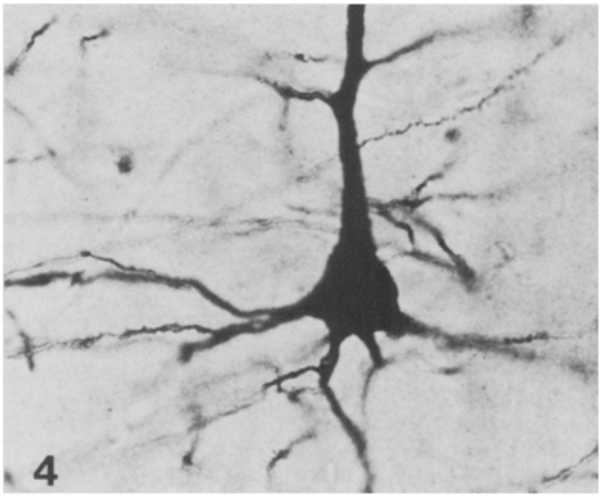

Figure 4. Golgi-Cox preparation showing typical pyramidal cell located in the cortical transplants in the spinal cord. × 278.

observed. This was not observed in any of the control animals that had received only a hemisection of the spinal cord. Histological examination of the lesioned areas of the spinal cords of these animals revealed large glial scars surrounding the entire lesioned area. This, along with the histological evidence, suggests that the transplants are not only able to fill the space created by the lesion but are also able to establish an anatomical continuity between the 2 severed portions of the spinal cord. It appears then that the transplant provides a 'bridge' between the ends of the spinal cord, for it receives afferents from both rostral and caudal portions of the spinal cord.

The establishment of the 'bridge' seems to have a critical time element. Initial pilot observations have suggested that the longer the interval between lesion and transplantation the less likely functional and anatomical integration will be established. Transplantation performed immediately after the lesion had the greatest success while those performed 5 days after lesion had the least amount of success. Transplantations performed between these 2 times after lesion followed a linear success pattern. In other words, the closer to the time of lesion that transplantation can be performed the greater the chances of success will be in the establishment of anatomical continuity between transplant and host brain. Hypothetically, this may be due to the extremely rapid growth of the 15-day embryonic transplants. It may be possible that this growth is so rapid that it initially establishes contact with the host spinal cord before a glial scar can form and thus blocks the formation of such a scar. Studies are in progress to determine this possibility.

In this study both donor embryos and host animals were Sprague-Dawley. Other studies using various combinations of donor and host animals from different strains of rats have shown that inter- and intrastrain transplantation are equally successful.

In conclusion, this study demonstrated that it is possible to successfully transplant embryonic neocortical tissue into the adult spinal cord. The absence of any neuroglial scar at the interface of transplant and host spinal cord and the recovery of gross motor limb function suggest that the

transplants are capable of providing a bridge through which anatomical continuity of the spinal cord is re-established.

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Growth of trophoblast in mouse lung

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Summary. When blastocysts are introduced into the lungs of mice via the circulation, trophoblast cells grow, as they do in other extrauterine sites.

Trophoblast is naturally deported to the lungs during pregnancy in only 2 species, man and chinchilla[1,2]. In neither species does the tissue proliferate. Trophoblast has been introduced experimentally into the circulation of mice and rabbits using placental tissue from donor animals in the 2nd half of pregnancy[3,4]. This disappears from the lungs without trophoblast growth and, usually, without reaction. We have previously described experiments in which 3.5-day blastocysts or 7.5-day ectoplacental cones have been introduced into the lungs of mice[5,6]. Trophoblasts grew whether they were introduced directly, intravascularly or intrabronchially with 1 exception; trophoblast never showed sustained growth from blastocysts introduced into the circulation in adult mice. There appeared to be an initial growth of trophoblast when mice were killed 3 days after introducing blastocysts[5], but in animals killed on the 4th day, the trophoblast was seen to be dying[6]. Those killed more than 4 days after injecting blastocysts into the lung via the femoral vein showed a reaction involving hyperplasia of all elements of the lung. These results were observed in 27 adult, syngeneic mice whether male or female recipients were used. A series of experiments were designed to investigate the reason for this failure of blastocysts to produce trophoblast in adult lungs. However, during the 2 years in which these experiments were conducted, the results changed and we began to see healthy trophoblast in the lungs of these animals. The re-investigation of this process is the subject of the present report.

Methods. All the experiments were carried out in syngeneic C3H/HeJ mice (Jackson Laboratories) which were housed under standard conditions. Operative procedures were conducted under ether anesthesia and sterile conditions were maintained. The animals were 12–14 weeks old and donor blastocysts were flushed from the uterus with Hanks solution at 3.5 days gestation. The blastocysts were introduced into the lungs of recipient mice via the femoral vein and the animals killed with ether 4–10 days later.

Results and discussion. The results of earlier, published, experiments and of the present study are summarized in the table. In the earlier experiments, up to 3 blastocysts were instilled into the lungs and in animals killed on the 4th day, degenerating trophoblast cells were found in 1 or 2 areas of the lung (fig. A). In animals killed 5–10 days after the experiment no trophoblast remains were found but extensive hyperplastic reaction of the lung was seen[5,6].

In the 2nd series of experiments, reported here, 3–9 blastocysts were instilled into the lungs via the femoral veins of 20 animals. These were killed 7–10 days later and 15 showed 1–6 areas of trophoblast proliferation in the lungs (fig. B). Giant trophoblast nuclei were seen at the periphery of the mass and small trophoblast nuclei in the core. Both appeared healthy in animals killed up to 10 days after blastocyst injection. Mice were not studied later than 10 days after instillation of blastocysts because this generally represents the day of maximum trophoblast growth in extra-uterine sites[7]. Extensive invasion of the lungs with tissue destruction and bleeding which is frequently observed when trophoblast grows in other organs[7] was not seen in the lungs in these experiments. The 5 mice that were negative for trophoblast growth showed no reaction of the type noted in the earlier experiments. The lungs were normal in these animals and the failures appeared to be technical.

The reason for the different results between the earlier and present studies is not definitely known. The strain of mice and their source remained the same. Blastocyst donors and recipients were syngeneic and the experimental method remained unchanged. The formula of the Hanks solution had not changed but in order to eliminate minor changes, in trace elements for instance, dry Hanks solution from the period covering the 1st experiments was obtained[8] and used for most of the current investigations.

We had been aware that, during the period of the earlier work, mice tended to die after weeks or months in stock, although those used in the actual experiments always appeared healthy. During the later experiments, this high mortality of stock animals had ceased. In retrospect, this 1st period had been marked by endemic outbreaks of murine virus pneumonia. If this was the cause of the change in results, it only affected the growth of trophoblast from 3.5 day blastocysts with the zona pellucida intact. It is possible

Growth of trophoblast in lungs of mice killed 4–10 days after injecting blastocysts via circulation

	No. of animals	Degenerating trophoblast	Lung reaction	Normal trophoblast	Normal lungs
Earlier reports[5,6]	39	5	22	0	12
Present study	20	0	0	15	5