The Morphological Study of Host Nerve Fibers Projecting into the Hippocampal Transplants in Rats
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Hippocampi are frequently used as a model in brain tissue transplantation research. Many valuable discoveries have been obtained due to the efficient work of participating scientists and investigators [1, 2, 3, 4]. Unfortunately, so far little progress has been made as to how the hippocampal transplants are innervated or controlled by the host brain.

In the experiments, our studies are concentrated in the survival and development of embryonic hippocampal nerve cells implanted in the host brain, and especially, the growth patterns of host nerve fiber projections in the hippocampal grafts. In operations, ear-like embryo hippocampi (17 ± 1 days) were carefully isolated from the remaining nerve tissue, transplanted into male healthy Wistar rats at approximate 200g. Afterwards, the rats were allowed to survive for at least 3 months; consequently, they were sacrificed for various studies. Our results are as follows:

1] Brain sections stained by Neutral Red showed that hippocampal transplants survived and grew very well in the host hippocampi closely in touch of the lateral ventricles. In addition, employing the improved Golgi-Cox methods, we studies the similarities and dissimilarities of cellular architectures and other structural features between host and transplanted pyramidal or granular cells. In the control hippocampus, a mossy fibre and 1-2 main dendrites covered by spines were extended from oval or spindle-like granular somas with a variable diameter from 14 to 18 um. While the pyramidal somas possessed larger diameters ranging from 19 to 27 um and more branches arborized thickly. Similarly, the transplanted pyramidal and granular cells were well developed and clearly stained in the transplants, resembling host neurons in size and shape. Nevertheless, it was revealed in grafts that the cellular laminar fashion was less orderly (some neurons were even discretely distributed) and that dendrites were less branched, covered by fewer spines.

2] The experimental rats were transcardially perfused. Brain sections were cut on a vibratome at a thickness of 50 um and immuno-stained by anti-ChAT monoclonal antibodies, following a routine immunocytochemical method (ABC). Tissue sections were divided into
two groups, one for light microscopical studies and the other for electron microscopical studies. In each group, the opposite host hippocampi served as control. It demonstrated that immuno-positive neuron (ChAT+) were distributed both in the host hippocampi, i.e. the hippo-hilus, the stratum moleculare and the hippo-fissure, and in the hippocampal transplants. A majority of these dark-brown cells were star-like, with a diameter of 12-18 um, emitting 3-5 branches, projecting into as far as the stratum pyramidae. From our statistic analysis, there was no obvious differences between the host and the implanted ChAT+ neurons. For the second group these 50 um thick sections were dehydrated, embedded and cut for further ultrastructural observations. Examined with the OPTON EM902 electron microscope at a high voltage of 80kv, ChAT+ buttons were exhibited to contact with the ChAT (negative) dendrites or axon processes to form symmetrical or asymmetrical synapses. On the other hand, synaptogenesis also occurred between ChAT somas or dendrites and ChAT- axon fibres. Compared with the host hippocampal ChAT+ neurons, the characteristic ultrastructural features were also rather similar.

3] We have commonly acknowledged that the somas of noradrenergic (NA) and serotoninergic(5-HT) neurons are chiefly located in the Locus Coeruleus and Mesencephalon Raphes respectively and their fibre projections are extended into the hippocampi in rats. In the experiments, sections were prepared either on a Cryostat or on a Vibratome to be examined by the 5-HT and Tyrosine Hydroxylase(TH) immunocytochemical methods(ABC). The results were that discrete TH+ fibres, 0.5-1 um in diameter, were extensively distributed throughout the stratum pyramidale, stratum granulosum, stratum moleculare and the dentate hilus of host hippocampi. Simultaneously, even thinner(0.5 um in diameter) 5-HT+ fibres were detected to be mainly distributed in stratum moleculare and inferior areas of dentate granular cells of the host. While in the hippocampal transplants, the immuno-positive stained processes were more sparsely and thickly distributed and their fibre terminals were either in an individual pattern or densely arboized. Such kind of terminal fashion has not yet been observed in the host hippocampi. In addition, selected areas containing relatively rich immuno-positive fibres were dissected from the grafts, embedded in Epon 812 and cut on a LKB-5 Ultrotome. Ultrathin sections were examined with the OPTON EM902 electron microscope. We observed that TH+ and 5-HT+ buttons made synaptic contacts primarily with immuno-negative shafts or spines of dendrites. Except
for a minor proportion, such synapses were largely asymmetrical types, with obviously thickened post-synaptic membranes.

We conclude that implanted embryonic hippocampal cells are able to survive and develop normally in the hosts and to be innervated by the host monoaminergic fibre projections. Since it has been viewed synaptic contacts among the implanted cells or between the implanted and host brain nerve cells, transmitter or modulator release must also occur in this area. We have discovered that the transplants receive projections and innervation from the host brain, therefore, we may further deduce that cells in the transplants play certain physiological and biochemical roles under the control of the host brain. Such results promise a future application of brain tissue transplantation to ultimately cure patients of many central nervous system (CNS) diseases horrifying people with mental dysfunction and health destruction. However, our results also indicate that though 3 months' growth and development may be enough for certain implanted immature cells to enlarge and finally differentiate more or less like the adult lost hippocampal neurons, in a changed environment, it is by no means a simple repetition of genuine embryonic development. Thus, real efficacious clinical practice is still remote, needing continuing and arduous studies.

References