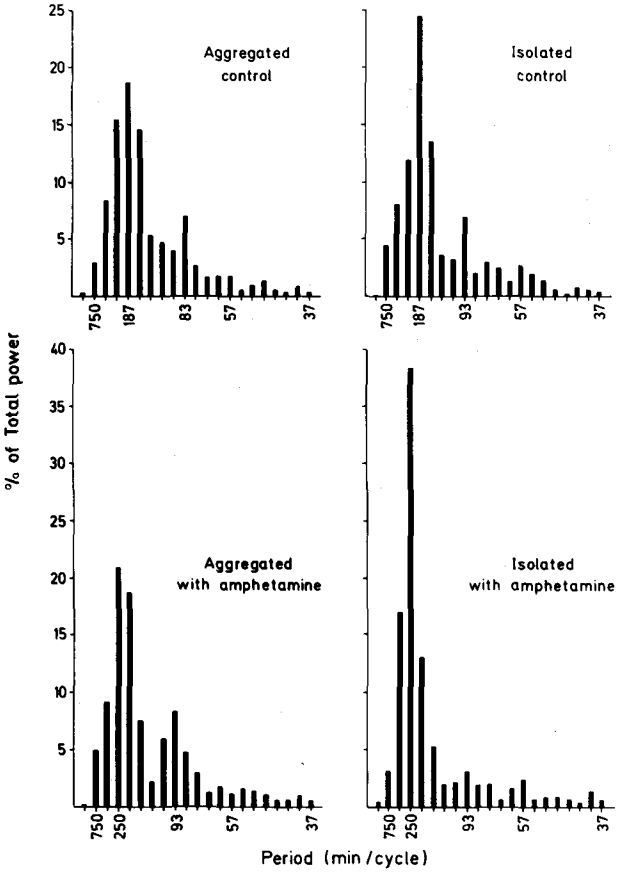

Fig. 4. Power spectra depicting predominant periods of ultradian oscillations in isolated and aggregated mice and the effects of injections of d-amphetamine-SO_4 (2 mg/kg). Power spectra were calculated via autocorrelation function with a data point-autocorrelation lag ratio of 6.7, which gave a quadratic error of the spectral estimate of $< 15\%$. Sampled raw data (sampling interval = 5 min) during the 23-h experiments were linked to improve resolution, and a temporal hamming window with 8% pedestal was applied to the autocorrelation function to avoid side lobe leakage. Low pass filtering was also used to remove low components (3 db, cut-off period 300 min). The period of each bar can be calculated by dividing 750 min by the order of the bar (1st bar has order zero, and is the dc component). Spectral power is represented as percentage of total power. Note that the major periodicity (187 min) has greater power in isolated mice. d-Amphetamine injections produced qualitative and quantitative changes in these ultradian rhythms (6–8 mice in all cases).

injected mice revealed both quantitative and qualitative differences (figure 4). For aggregated mice, the main power peak was shifted from 187 min to 250 min and increased by about 20% and the peak at 83 min was shifted to 93 min after drug treatment. For isolated mice, the main peak was also shifted from 187 min to 250 min and its power was increased by about 60%, and the peak at 93 min was reduced to about half its power by the drug.

These results corroborated earlier findings obtained with long-term differentially-housed mice; i.e., isolated mice displayed higher locomotor activity than aggregated mice and d-amphetamine exerted a greater effect on isolated mice[2–7]. The effects of d-amphetamine of prolonging the duration of the initial peak of motor activity, of producing a phase shift in motor activity, and of affecting the power spectra of motor activity, indicated that cerebral mechanisms regulating this activity are subject to modification by exogenous stimuli.

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Transplantation of brain tissue in the brain of adult rats[1]

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Summary. Brain tissues obtained from rat embryos were transplanted in the forebrain and/or cerebellum of the adult rats. The transplants survived, grew and achieved normal cellular and cytoarchitectural differentiation. They had become anatomically integrated with the host brain. The animals did not show any obviously detectable abnormal behavior or pathology of the brain. The transplants survived as long as the animals did suggesting that they had become a part and parcel of the host brain.

Transplantation of brain tissue in the central nervous system of mammals has been attempted by early investigators who employed spinal ganglia, primarily, or pieces of brain tissue as the transplants[2–7]. They were successful to the extent that the transplants did survive and grow, but eventually these transplants degenerated. During past few years it has been shown that mitotically active precursors of neurons from the cerebellum of neonatal animals and neural tissues obtained from rat embryos can be successfully transplanted in the brain of the neonatal hosts[8,9]. These

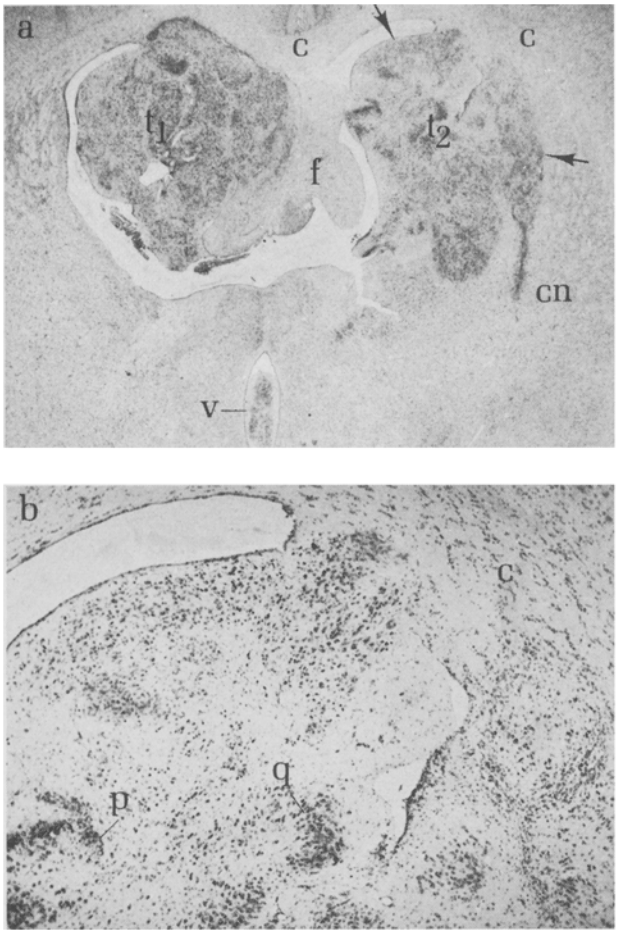

Fig. 1. *a* Neocortical transplants in the forebrain. The neocortical tissue was obtained from a 15-day-old rat embryo, and transplanted bilaterally in the forebrain of a 6-month-old animal. The host was sacrificed 3 months after transplantation. Both the transplants (t_1 and t_2) have grown extensively along the antero-posterior axis, thereby pushing hippocampus farther caudally. Note some portions of transplant have grown in the third ventricle (c). Transplant t_1 is continuous with column of fornix (f) and corpus callosum (c), and transplant t_2 with corpus callosum (v) and caudate nucleus (cn) surrounding it. Caudally both these transplants are continuous with hippocampus. Portion of transplant indicated by arrows is shown at a higher magnification in figure 1, b. Nissl stain. ×7.5. *b* Cellular and cytoarchitectural differentiation of the transplant. The nerve cells appear differentiated and organized in bits of layers (p, q). In other regions also layering pattern can be discerned in serial sections. Note neuropil between different neuronal clusters within the transplant. The transplant is continuous with corpus callosum (c), indicating the possibility of receiving fibres from it. Nissl stain. ×30.

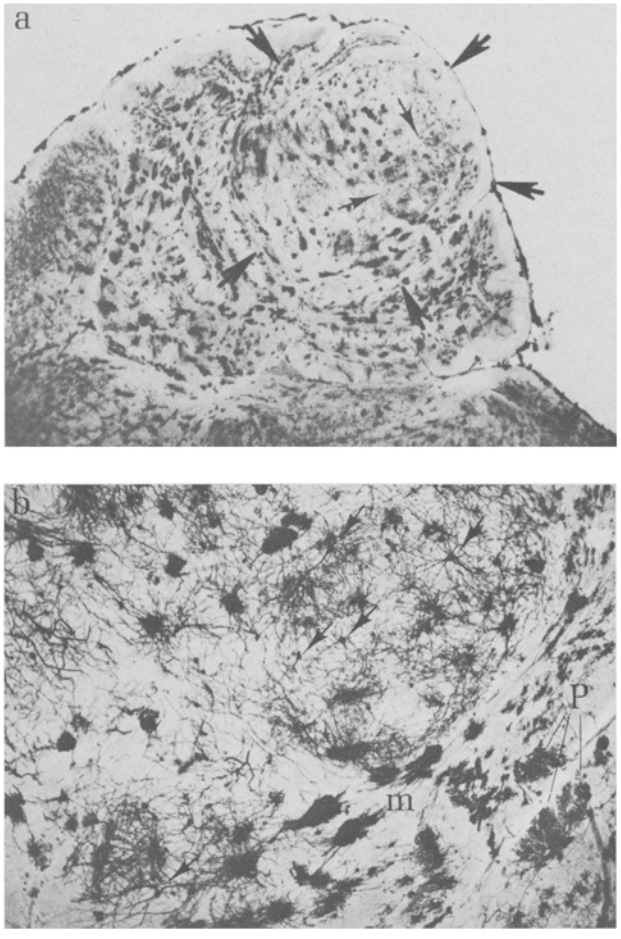

Fig. 2. *a* Neocortical transplant in the cerebellum of a 10-month-old animal (large arrows). In this case neocortical tissue for transplantation was obtained from an 18-day-old rat embryo. The host was sacrificed 8 months after transplantation. The portion of transplant indicated by small arrows is shown at a higher magnification in figure 2, b. Golgi-Cox. ×7.5. *b* Cellular differentiation of the transplant. Arrows indicate some of the well-differentiated pyramidal cells of the neocortical transplant. Other cell types also can be identified. Note the Purkinje cells (P) of the host cerebellum. The transplant is continuous with the medullary layer (m) of the cerebellum, indicating the nature of anatomical integration that the 2 have established. Golgi-Cox, ×30.

homotopic as well as heterotopic transplants grew and differentiated, and survived for the life of the host animals. The success in these studies could be attributed to the facts that the brain of the neonatal host animals itself is developing and therefore has a high degree of plasticity to accept the transplants, and that these immature hosts are immunologically incompetent and therefore do not reject the transplants. If such transplantations of brain tissues can be successfully achieved in adult host animals a crucial test for the significance of these researches will have been passed. Wenzel and Bärlehner[10], and Stenevi et al.[11] have achieved some success in this direction, and recently our studies[12] have shown success in these researches.

Materials and methods. Laboratory-bred adult Wistar albino as well as Long-Evans hooded rats, males and females 2–18-month-old, were used as host animals. They received cortical tissue as the transplant from 15- or 18-day-old embryos employing the technique identical to that used in previous studies on the neonatal animals[13]. 10 animals received transplants from 18-day-old embryos, and 28 from 15-day-old embryos. In each group half the animals received transplants in the cerebellum only, and the other half in the cerebellum as well as forebrain. From each group the animals were sacrificed at different intervals ranging from 15 days to 8 months following the transplantation. 2 animals were allowed to live to their old age, and were sacrificed when they were 2 years old. Brains obtained from these animals were used for cresyl violet stain, Holme's neurofibrillar stain and Golgi-Cox impregnation.

Results. In all the preparations the transplants had survived, grown, and differentiated. The transplants from 15-day-old embryos had grown more extensively than those from 18-day-old embryos, although the amount of brain tissue

transplanted in all the cases was relatively constant. This property of extensive growth by the transplants from the embryos of younger ages has also been observed when neonate animals were used as the hosts. Histological analysis revealed that this was due to the fact the transplants of the neocortical tissue from the embryos of younger ages, i.e. 15 days, contained more mitotically active neuroepithelial cells than the postmitotic neuroblasts. Whereas the transplants from the embryos of older ages, i.e. 18 days, contained a small number of neuroepithelial cells and a large number of postmitotic neuroblasts. This indicated that the mitotically active precursor cells of neurons could survive and proliferate to give rise to neurons in large numbers.

Histologically the transplants contained fully differentiated neurons that were organized in the typical laminar pattern. The laminar cytoarchitecture, was more readily identifiable in serial sections than in any single section. In Golgi-Cox preparations dendritic pattern of various neurons in different layers was identical to that of their counterparts in a normal brain. The axons extended long distances and formed compact bundles. Such bundles of axons could also be observed in the Holme's preparations. The bundles of axons demarcated transplants into various regions, and also were seen to course between the transplants and the host brain. The latter provided for anatomical integration of the transplants, in terms of both afferents and efferents, with the host brain. In addition to this the transplants were continuous with the host neural tissue, where the cellular masses of the 2 met. The cellular masses of the transplants and the host brain did not mix freely; each maintained its own characteristic cytoarchitecture. But they shared a common neuropil, and one blended into the other without any intervention by the pia mater. The animals that were sacrificed when they were 2 years old still showed presence of normal-looking transplants. These observations suggested that from morphological viewpoint the brain transplants, whether heterotopic or homotopic, can grow, differentiate, become an integral part of the host brain and survive as long as the animals do. It is important to stress that these transplants in no manner resembled brain tumors. And, no transplant, which was cytologically and cytoarchitecturally fully differentiated, contained any degenerating cellular elements or neoplastic cell mass (figures 1 and 2).

The extensively growing transplants were seen to push aside the neural structures of the host brain, but there were no obvious signs of gross degeneration. It is possible that there may have been some degeneration at cellular and ultrastructural levels, particularly of the synapses, in the host neural structures. Nevertheless, behaviorally the animals were normal. The behavior of these animals was studied in their cages as well as in the openfield test situations. No abnormalities related to their feeding, drinking, locomotion, mating, nursing, and social behavior were observed. For instance the host animals receiving transplants in the cerebellum showed no motor abnormalities even though the cerebellum was pushed aside and somewhat distorted by the transplant. In the animals receiving the transplants in the forebrain, the basal ganglia and the hippocampus were pushed aside by the transplants but no behavioral abnormalities could be detected. Even the animals that received multiple transplants, 2 in the forebrain and 1 in the cerebellum, did not show any abnormal behavior. Some of the females were mated with the males, and they gave birth to and nursed their pups in a normal manner.

Discussion and conclusions. These findings suggest that transplantation of embryonic brain tissue can be successfully achieved in the fully-differentiated brain of the adult rat. It is possible that such successful transplantations of brain tissue may also be achieved in other adult mammals. Comparatively a transplant grows more extensively in the brain of the neonatal animals than in that of the adult animals. Very likely this is due to the fact that both brain and cranium in the neonatal animals are growing in size during the development, and, thus, provide a large room for the transplants to grow. Nonetheless the neural transplants in the adult brain do survive, grow, differentiate, and establish anatomical connections with the host brain.

The neural transplants in the adult brain, just like in the brains of the neonatal animals, are anatomically integrated with the host brain. This is indicated by sharing of common neuropil by the transplant and the host brain structure surrounding it and existence of fibre bundles coursing between the 2. Although exact source of origin and termination of these fibre bundles is not yet known, it is likely they are the afferent and efferent fibres of the transplants. Such connections between the transplants and the host brain may provide trophic influences on the transplants thereby contributing to their survival for the life of the animals. On this basis neural transplants in vivo, like these, become distinguishable from explants in the anterior eye chamber or in tissue culture preparations[14].

The success of transplantation of brain tissue, as demonstrated here, depends not only upon the employment of the embryonic neural tissues as the transplants but also upon the purity of the transplants. In these studies the neural tissues obtained from the embryonic nervous system were freed from the meningeal membranes as completely as possible. If any meningeal membranes were inadvertantly transplanted along with the neural tissues, they grew rapidly in the host brain and isolated the transplants from achieving the appropriate anatomical integration with the host brain. As a consequence such transplants failed to grow and differentiate, and eventually degenerated. It is likely that lack of success in transplantation of brain tissues by other investigators, quoted earlier, may have been partly due to the use of impure neural transplants.

There is no evidence to establish that these neural transplants having been anatomically integrated with the host brain are also functionally integrated. But the presence of synaptic profiles in the transplants, as established in other ultrastructural studies in our laboratory, lifelong survival of these neural transplants, and normal behavior of the host animals even when the transplants had displaced most of the neural structure of the host brain strongly indicate that they may have some functional role to play in the host brain.

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