

Estrogen Receptors are Present in Neocortical Transplants

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SUMMARY

Fetal neocortical tissue was grafted into neocortical lesion cavities made in newborn rats. After two weeks survival, *in vitro* binding of [³H]-estradiol to cytosolic preparations provided evidence of estrogen receptors within the transplants. The observed high levels correspond to previous work demonstrating elevated estrogen receptor levels during the first postnatal week in the rat cerebral cortex.

KEY WORDS

neural graft, newborn, cortex lesion, rat

Fetal, neocortical tissue grafted into cortical lesion cavities in newborn rats will survive and form extensive afferent and efferent connections with the host brain /3, 4, 6-9, 12, 13, 22, 24, 25/. These findings combined with other studies of transplant electrophysiology /5, 17, 21/, neuropeptide content /11/ and blood-brain barrier for-

mation /26/ demonstrate that such transplants develop and retain many features of normal cortex. In order to examine transplant characteristics further, the present study was initiated to examine the estrogen receptor (ER) content of neocortical grafts. These results are derived from a larger ongoing study concerning the ontogeny of ER in the developing cerebral cortex as well as in intracerebral transplants of neocortical, hippocampal and hypothalamic tissue.

Thirty-two Sprague-Dawley rats with neocortical transplants were used in this study. They were derived from a continuing series of experiments demonstrating a 90% neocortical transplant survival rate. According to routine methods described previously /3, 7/, blocks of fetal (embryonic day 14-15) presumptive sensorimotor cortex were grafted into corresponding neocortical lesion cavities in newborn (postnatal day 0-1) rats. Lesion cavities were made by aspiration immediately before grafting. Dams for donor animals were anesthetized with sodium pentobarbital (50 mg/kg) and newborn pups to be used as graft recipients were anesthetized by hypothermia. Fourteen days after transplant surgery host animals were killed by decapitation and their brains removed immediately, placed on an ice cold brass plate and sliced coronally on either side of the transplant with the aid of a surgical microscope. Being careful not to include host tissue, a wedge of transplant tissue was dissected free. One mm punch samples of the contralateral homotypic cortex were also taken. The samples from 2 animals were pooled and the ER content of these pooled

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samples ($n=14$) was quantified according to a modification of MacLusky *et al.* /16/ as previously described /18/. Four animals with transplants were killed by anesthetic overdose and perfused with 4% paraformaldehyde. The brains from these animals were cut at $40\ \mu\text{m}$ and stained with cresyl violet for routine histology.

Measurable ER levels were consistently found in the transplants. These levels (8.84 ± 2.8 fmol/mg protein) were significantly higher ($p < 0.01$) than those found in the contralateral homotopic cortex (1.63 ± 0.30 fmol/mg protein) (Fig. 1). Saturation analysis of [^3H]-estradiol binding revealed an apparent dissociation constant (K_d) of 6.55×10^{-10} M ($n=2$) for the neocortical grafts. These values correspond to those previously reported for the normal neocortex /18/. Histologically, the transplants appeared well integrated within the host cortex and displayed the characteristic transplant organization with whorls and bands of cells found in previous studies.

The high ER levels found in the cortical transplants do not appear to represent a deviation from normal values. When considered in terms of their 1 week theoretical age (i.e., transplanted at E14-15 and sacrificed 2 weeks later), the observed ER values correspond to the high levels found in normal cerebral cortex at this postnatal age /16, 18/. Related studies of perinatal neocortical tissue grafted into the cerebral cortex of young adult rats demonstrated normal concentrations of muscarinic receptors at 6-8 weeks post grafting /15/. In contrast, abnormally high levels of bombesin and vasoactive intestinal peptide (VIP) receptors were found in long-term surviving neocortical grafts placed into the third ventricle of adult rats /14/. These high values were interpreted in terms of a possible relationship to transplant growth and vascularization.

The presence of functional estrogen receptors in hypothalamus-preoptic area grafts /19/ suggests that such receptors found in our developing neocortical grafts may also be functional. Estrogen receptors in the developing nervous system, especially those located in areas mediating reproductive functions, are clearly involved in morphological and functional sexual differentiation. Cerebral cortical ER may also be involved in this process given the evidence for sex differ-

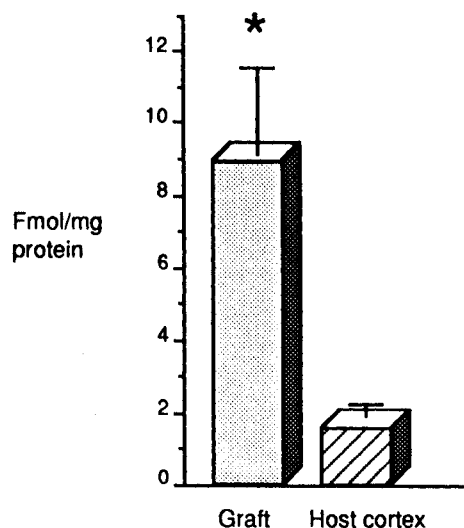


Fig. 1: Estrogen receptor levels found in neocortical grafts and in the contralateral homotopic host cortex. (* $p < 0.01$; Student's t-test)

ences in certain cognitive functions /1/. Estrogen administration at birth has been shown to increase cortical myelination /10/ as well as enhance neurite outgrowth from cerebral cortical and hippocampal /2, 27/ neurons *in vitro*. These data support a role for estrogen as a trophic factor underlying sex differences in cerebral cortical functions. The rather widespread distribution of ER mRNA in the adult brain /23/ suggests its participation in many other neural activities.

As estrogen appears to play an important role in several basic neural functions, our finding of estrogen receptors in neocortical grafts adds to a growing body of literature suggesting that grafted fetal neocortex will continue to develop and assume the characteristics of normal cortical tissue and become functionally integrated with the host nervous system. In this regard, electrophysiological studies demonstrated the integration of cortical grafts into the host afferent system by showing transplant unit activity evoked by peripheral stimulation /5, 17/. These results correspond to anatomical findings showing transplant afferents from the thalamus and several other areas of the host brain /4, 6, 8, 9, 12/. Transplant efferents to the contralateral neocortex, thalamus, striatum, pons and spinal cord have also been reported /3, 7, 13, 22, 24, 25/. Electrophysiological studies confirm

that transplants project to the thalamus and medullary pyramid /17, 21/. Although less dense than normal, these connections generally resembled normal connectivity patterns. Behavioral studies demonstrating the recovery of cortical lesion induced motor deficits by neocortical grafts reflect this host-transplant integration /20/.

In conclusion, our findings of high ER content in developing neocortical transplants correspond to the transiently high levels found in the intact newborn cortex. These findings support previous anatomical, electrophysiological and behavioral studies which suggest the presence of normal tissue specific characteristics in neural transplants.

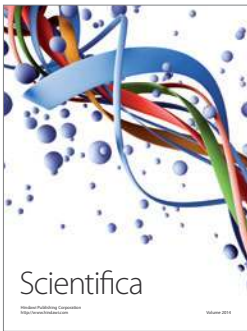
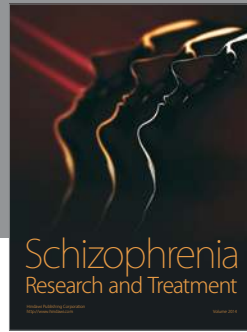
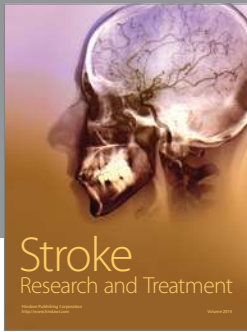
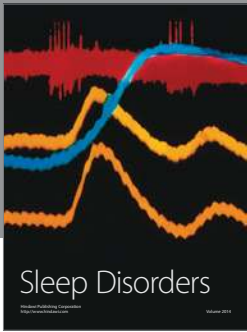
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