Conditions for Adrenergic Hyperinnervation in Hippocampus: I. Histochemical Evidence from Intraocular Double Grafts

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Summary. The developing hippocampal anlage of rats was homologously grafted to the anterior chamber of the eye of adult recipients. After intraocular maturation of the hippocampal graft and removal of the sympathetic innervation of the eye by ipsilateral superior cervical ganglionectomy, four types of monoamine neurons were transplanted to the eye chamber: Peripheral sympathetic neurons, central adrenergic neurons of locus coeruleus, central dopaminergic neurons of substantia nigra, and central 5-hydroxytryptamine neurons of the lower raphé nuclei. All four classes of monoamine neurons were able to reinnervate the hippocampal graft, but the fiber ingrowth differed markedly. Although peripheral sympathetic neurons produced a pattern of adrenergic innervation in the hippocampal graft which resembled innervation of the hippocampus by the locus coeruleus in the brain, locus coeruleus neurons themselves produced an extremely dense plexus of fibers within the graft. This hyperinnervation remained intact for up to 9 months, the longest period of time studied. The locus coeruleus graft itself received fibers from the hippocampal graft, as demonstrated by the retrograde transport of horseradish peroxidase. We conclude that the hippocampal graft exerts a much stronger growth stimulation on the locus coeruleus than on the peripheral sympathetic neurons. The difference between innervation patterns suggest that both presynaptic and postsynaptic influences determine fiber ingrowth in the central nervous system.

Key words: Brain grafts – Noradrenergic fibers

Recent evidence suggests that there are important differences in growth regulatory mechanisms governing central and peripheral adrenergic neurons (Seiger and Olson 1977a, c, 1978; Olson et al. 1978; Olson et al. 1979a). Studies on growth regulation are complicated by the differences in target tissue and surrounding substratum associated with these two types of norepinephrine-containing nerve cells. We have attempted to resolve these complexities by studying adrenergic fiber ingrowth into brain target tissue grafts in oculo.

When the fetal hippocampal formation is grafted to the anterior eye chamber, it develops into an organotypic structure with histological and electrophysiological characteristics similar to its in situ counterpart, including the ability to develop sustained epileptiform activity (Olson et al. 1977; Hoffer et al. 1977a, b; Woodward et al. 1977; Taylor et al. 1978, 1979). Interestingly, the hippocampal graft, deprived of its normal afferents, becomes functionally innervated by adrenergic and cholinergic nerves from the autonomic ground plexus of the host iris (Hoffer et al. 1977b; Taylor et al. 1978; Freedman et al. 1979). Using sequential double brain tissue grafts, it is also possible to innervate an intraocular hippocampal graft with central adrenergic fibers from a locus coeruleus transplant (Olson et al. 1979b).

Since both the locus coeruleus and superior cervical ganglion can innervate the hippocampal graft, and the target tissue and surrounding milieu are equivalent in both cases, this preparation would seem ideal for studying the differences in growth of central and peripheral fibers. In the present experiments, we have compared innervation patterns of fibers derived from different types of monoamine neurons innervating hippocampal grafts. It was found that locus coeruleus, in contrast to the superior cervical ganglion, can give rise to an extreme hyperinnervation of all parts of the hippocampal graft. The electrophysiological correlates of this hyperinnervation are described in the succeeding report (Taylor et al. 1980).
Fig. 1. Border between fused locus coeruleus transplant (upper right) and hippocampus transplant (lower left). The adrenergic neurons of locus coeruleus form a dense cluster of cells with some scattered somas reminiscent of the subcoeruleus area outside the main group. One end of the locus coeruleus is in contact with the hippocampal graft, the rest of the border between the two grafts is sharply outlined by the differing nerve density in the two areas. The neuropil surrounding locus coeruleus has a sparse network of varicose terminals; hippocampus has an extreme hyperinnervation. Surface of the two joined grafts in upper left corner. Fluorescence microphotograph; magnification × 175

Materials and Methods

Intraocular grafts of fetal hippocampus were sequentially (1-7 months) combined with grafts of fetal brain stem areas containing locus coeruleus (LC grafts), substantia nigra (SN grafts), caudal raphé nuclei (raphé grafts), or the adult superior cervical ganglion. Methods for grafting these various tissues and their intraocular developmental patterns have been described (Olson and Malmfors 1970; Seiger and Olson 1977b; Olson et al. 1977, 1979b). The peripheral sympathetic nerves to the double-grafted eyes were removed by extirpation of the superior cervical ganglion. Control eyes contained only hippocampal grafts and had an intact peripheral sympathetic innervation.

Fluorescence Histochemistry

At various times after addition of the second graft, animals were killed by cervical dislocation under ether anesthesia, and the grafts and their attached host irides were frozen, freeze-dried (Olson and Ungerstedt 1970), and further processed for Falck-Hillarp fluorescence histochemistry for visualization of monoamines. Pretreatment with nialamide (500 mg/kg, 4 h) was sometimes used (Falck et al. 1962; Corrodi and Jonsson 1967). Cell counts were made in a series of locus coeruleus transplants using the formula given by Abercrombie (1946). Nerve terminal densities were estimated on a blind basis using a semiquantitative scale (Nygren et al. 1974; Seiger and Olson 1977b).

Horseradish Peroxidase Histochemistry

Injections of horseradish peroxidase (HRP) were performed to determine, via retrograde transport, the cell bodies of origin of fibers innervating various portions of the transplant. HRP (0.005 ul) in a saturated solution in saline was injected into either the hippocampus or locus coeruleus portion of the transplant. After 2 h, the animal was killed and the transplant prepared for HRP histochemistry by the method of LaVail et al. (1973).

Results

A. Locus Coeruleus Part of the Double Grafts

Usually, the majority of the LC cell somas were located in a dense cluster, with only a few cells lying scattered outside this cluster. The main group was normally close to one surface of the graft. This made the entire arrangement cytoarchitectonically similar to the normal location of LC in the floor of the fourth ventricle, with scattered larger cells of the subcoeruleus area deep to it (Fig. 1). Several important differences between grafts and the normal LC were, however, noted. Most strikingly, the LC cell somata,