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Brain Tissue Transplanted to the Anterior Chamber of the Eye: 3. Substitution of Lacking Central Noradrenaline Input by Host Iris Sympathetic Fibers in the Isolated Cerebral Cortex Developed *in oculo**

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Summary. Fetal parietal cerebral cortex was homologously transplanted to the anterior chambers of the eyes of adult rats. The transplants got vascularized, proliferated, as measured by *in vivo* stereoscopic inspections, and differentiated into brain tissue similar to cortex cerebri *in situ* and survived for long times, $> 4^1/_2$ months. Fibers from the intact sympathetic adrenergic ground plexus of the iris were able to innervate the transplants in an organotypic way regarding fluorescence morphology, pattern of distribution of the nerve terminals and, to a certain extent, density of innervation, the only variable parameter being density of innervation. Thus, in unpretreated or MAO inhibited transplants only rather few to scattered terminals could be found, while after preincubation in 10^{-5}M α-methyl-noradrenaline the number of visible terminals was normal or slightly less than normal, as compared to cortex cerebri *in situ*.

When superior cervical ganglia (SCG) were transplanted together with fetal cortex tissue to sympathetically denervated eyes the ingrowth in the cortex tissue was similar to that after single cortex transplantation combined with 5 day old SCG, while a marked hyperinnervation was encountered when combined with adult SCG.

It is concluded that the developing cortex cerebri, deprived of its normal CNS source of adrenergic nerves, is able to receive sympathetic adrenergic nerves from the iris in an organotypic way upon transplantation to the anterior chamber of the eye.

Key words: Cortex cerebri — Intraocular transplantation — Sympathetic nerve growth — Heterotopic innervation — Maturation — Embryology.

Introduction

The intraocular transplantation technique has served as a most useful model for studying the isolated development of different brain regions, and their interactions with the vascular and nervous supplies of the iris. A number of fetal CNS areas survive, become vascularized and proliferate without detectable adverse reactions, neither morphologically (Olson and Seiger, 1972, 1975) nor

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electrophysiologically (Hoffer *et al.*, 1974; Olson *et al.*, 1975). Furthermore, monoamine receptor tissues, like developing cerebellar and hippocampal cortices have recently been shown to become adrenergically innervated by peripheral sympathetic adrenergic fibers from the iris upon transplantation to the anterior eye chamber (Hoffer *et al.*, 1975; Freedman *et al.*, 1975; Seiger and Olson, 1975).

It was the purpose of the present study to follow the taking, development and possible innervation of the cortex transplants from the iris sympathetic adrenergic ground plexus. Evidence is presented for a heterotopic innervation by the sympathetic fibres also of developing parietal cortex cerebri. The morphology of the differentiated cortex transplants show similarities with intact cortex cerebri.

Material and Methods

Transplantations. Brains from donor rat fetuses with crown rump lengths (CRL's) ranging from 15–38 mm were used (approx. corresponding to the 16th to the 22nd day of gestation). A square piece, approx. 2×2 mm, was cut out of the parietal region of the cortex anlage on each side. The full depth was taken and the pia peeled off. Each piece was then trimmed to a diameter of approx. 1 to 1.5 mm, and homologously transplanted to the anterior chamber of the eye of recipient female rats (Sprague-Dawley 150–200 g) as previously described (Olson and Seiger 1972, see also Olson and Malmfors, 1970). In a few cases adult or 5 day old superior cervical ganglia (SCG) were homologously transplanted to the anterior chamber together with the cortex anlage. The ganglia were decapsulated and transplanted as one or two pieces. The cortex and ganglion transplants were approximated to each other in the anterior chamber, by gentle pushing from outside the cornea with a pair of forceps. In the double transplantation experiments the host eyes were sympathetically denervated by removal of the normally located SCG's.

The taking and postoperative fate of the transplants was observed regularly using a stereoscopic microscope.

Fluorescence Histochemistry. 44 single cerebral cortex transplants, 6 cerebral cortex plus adult SCG transplants and 9 cerebral cortex plus 5 day old SCG were analyzed by fluorescence microscopy. The *in oculo* times varied from 3 weeks to $4^1/_2$ months. The animals were killed under light ether anaesthesia by neck dislocation. The eyes were rapidly cut out and the irides with their attached transplants dissected free. Some transplants were then incubated in 10^{-5}M (33) or 10^{-6}M (2) α-methyl-noradrenaline (NA) in a modified Krebs-Ringer bicarbonate buffer for 30 min and rinsed in amine-free medium for 10 min (Hamberger, 1967). A few animals (9 cortex transplants) received a monoamine oxidase inhibitor, Pargyline®, Abbott (300 mg/kg) or Nialamide®, Pfizer (500 mg/kg), 4 hrs before sacrifize in order to increase the endogenous neuronal levels of monoamines.

Transplants, often still attached to a piece of their host irides, were then rapidly frozen in liquid propane, cooled by liquid nitrogen, freeze dried (Olson and Ungerstedt, 1970) and further processed for fluorescence histochemical visualization of monoamines according to Falck and Hillarp (Falck *et al.*, 1962, see also Corrodi and Jonsson, 1967). Every second or fifth 6 μ thick section through the whole of each piece was collected for analysis in the fluorescence microscope.

Histology. Some transplants were fixed in buffered 4% formaline solution and further processed for regular histological staining. Serial 6 μ sections were collected and stained with toluidin blue.

Results

In vivo Observations. Transplants from more immature donors gave rise to larger and better taking transplants than the more mature ones did. Blood sinusoids developed initially. They disappeared within approx. 10 days and were replaced

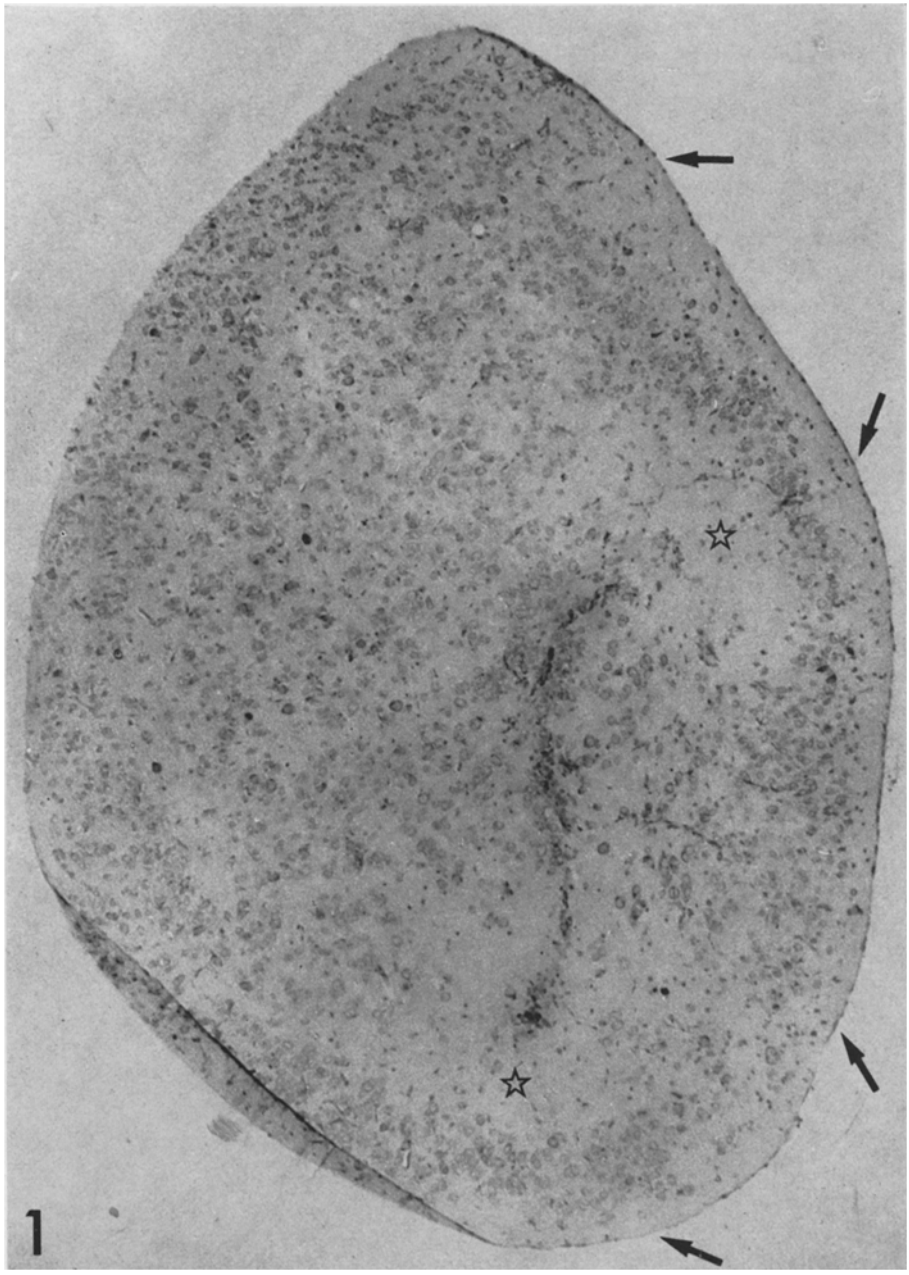

Fig. 1. Overview of a whole cortex transplant. The cell-poor superficial zone is clearly seen as well as the neuron layer beneath it (arrows). The second cell-poor zone is seen in two regions (stars) and the center of the transplant has a diffuse distribution of neurons. Postoperative time: 5 months. CRL of donor fetus: 22–24 mm. Microphotograph of a toluidin blue stained 6 μ section $\times 80$

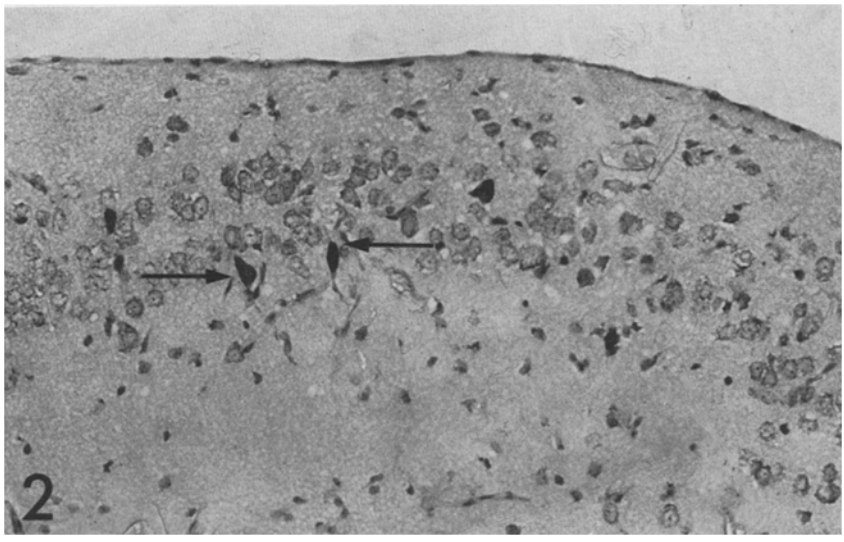

Fig. 2. Detail of the surface of a cortex cerebri transplant. All three "cortex layers" typical of these transplants can be seen, with the "neuron layer" in the middle. Neurons of both types are observed, with the pale type in majority. A few strongly stained neurons with processes partly perpendicular to the surface of the transplant are visible (arrows). Postoperative time: 5 months. CRL of donor fetus: 22–24 mm. Montage of microphotographs of a toluidin blue stained 6 μ section × 185

by regular blood capillaries. There was a correlation between sinusoids initially and good vascularization of the transplants later. The cortex transplants often grew to 3 mm or more in diameter, were rounded and well demarcated and vascularized. A few large transplants however, were flat and thin and lacked visible blood vessels or contained only very few. The vascularized transplants remained in good condition for the longest postoperative times used which was $4^1/_2$ months.

Histology. From the base of most transplants the neuropil and the fine meshed plexus of capillaries radiated towards the periphery giving each transplant a gross organization similar to cortex cerebri *in situ* (Fig. 1). The outermost "layer" was very poor in cells, especially large neurons. This was a constant finding regardless of size and shape of the transplant. The second "layer" was large but with variable thickness and seemed to contain two types of large neurons. The most numerous type (80–90%) had a pale, fairly rounded perikaryon with few visible processes.

The less numerous neuron type had a polygonal, strongly stained perikaryon and distinct processes running at random course from the perikaryon. These cells were evenly spread out in the "neuron layer" (Fig. 2).

Beneath the neuron dense layer there was often a region very poor in large neurons where blood vessels and glial elements dominated. Towards the center of the larger transplants the organization was poor and diffusely spread out neurons of both types could be seen. The CNS transplant had fused with the iris so that there was no clear border between the two.

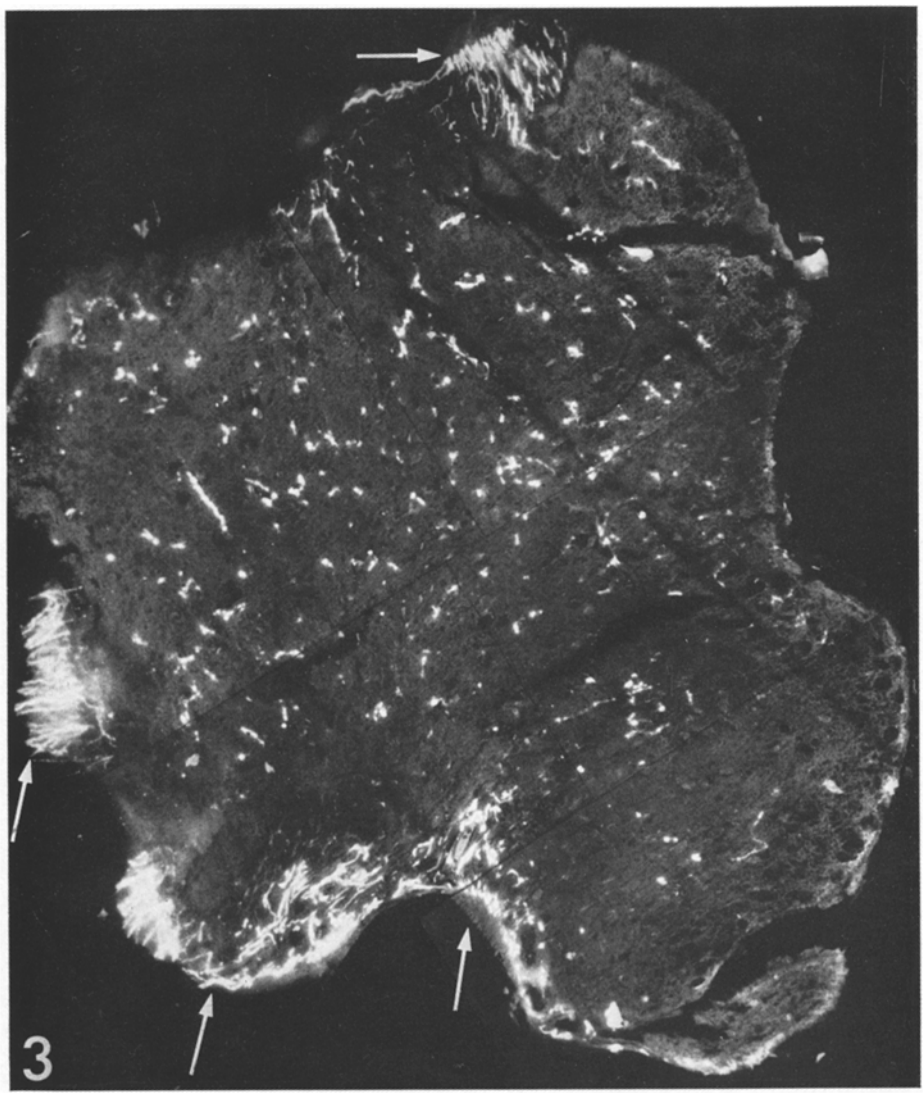

Fig. 3. Survey of a whole cortex transplant attached to a piece of its host iris (arrows). Fluorescent fibers are seen throughout the transplant. Postoperative time: 4 months. Incubated in 10^{-5}M α-m-NA 30 min. CRL of donor fetus: 24 mm. Montage of fluorescence microphotographs of a 6 μ section × 135

Fluorescence Histochemistry. Fibers from the intact sympathetic adrenergic plexus of the iris were able to innervate the developing parietal cortex cerebri transplants in an organotypic way (Figs. 3, 4). While fibers in the iris have large varicosities and clearly visible intervaricose parts and run several together in each strand of the plexus, those innervating the transplants had small rounded varicosities, hardly visible intervaricose parts and run single rather than in bundles,

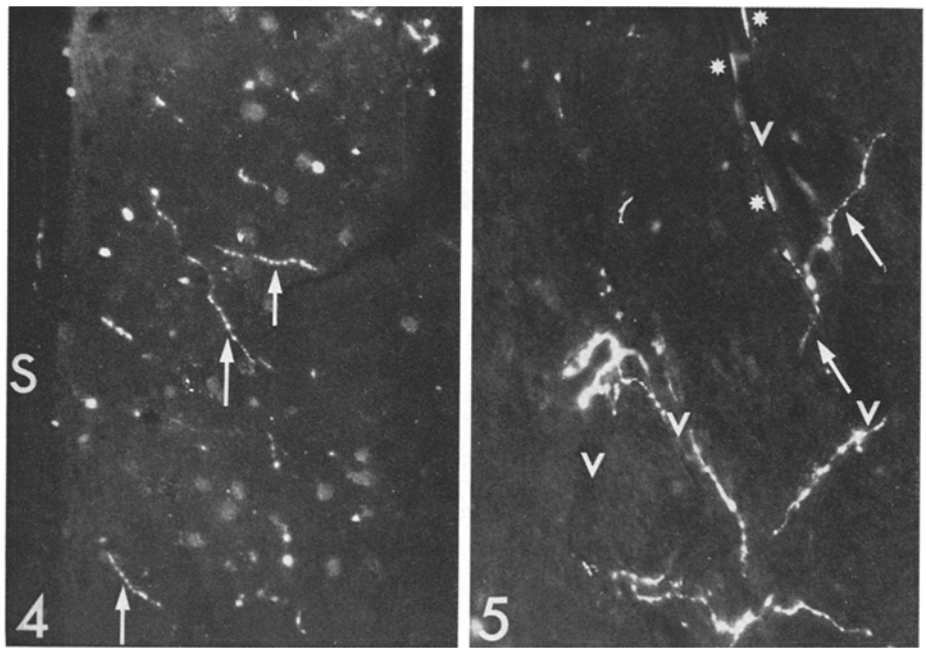

Fig. 4. Close-up of the surface area of a large cortex transplant. Thin varicose "central-looking" fibers (arrows) are seen to run perpendicular to the surface (*S*) through the "neuron layer" of the transplant. Postoperative time: 4 months. CRL of donor fetus: 24 mm. Incubated in 10^{-5}M α-m-NA 30 min. Fluorescence microphotograph of a 6 μ section × 190

Fig. 5. Sympathetic adrenergic fibers along blood vessels (*V*) in a cortex transplant. The vessels are almost lined out with partly varicose fluorescent fibers. Arrows indicate two fluorescent fibers leaving the vessel wall region to penetrate into the cortex tissue proper. A few fluorescent cells in a vessel wall can be seen (stars). Postoperative time: $2^1/_2$ months. CRL of donor fetus: 15–17 mm. Incubated in 10^{-5}M α-m-NA 30 min. Fluorescence microphotograph of a 6 μ section × 330

thereby mimicking the normally present central NA fibers regarding distribution, fluorescence morphology (Fig. 4) and, to a certain extent, density of innervation.

Close to the innervated iris, large vessels were seen arborizing into the CNS tissue. Most of these vessels were well innervated by sympathetic fluorescent fibers that could be followed from the iris or from larger vessels close to the iris. These fibers were sympathetic in appearance, especially close to the iris attachment. Far from the iris some smaller vessels seemed to have more varicose thinner fluorescent fibers of a "central looking" type (Fig. 4). Many of the vessel-associated fibers arborized into CNS tissue and were then always similar to normal central NA fibers (Fig. 5). The NA fibers in the transplants ran in all directions, especially in the center of the larger transplants where the degree of gross organization of the transplant was low (Fig. 3). Closer to the surface of the transplant, *i.e.* in the "neuron layer", long varicose fibers could often be seen perpendicular to the surface just as *in situ* (Fig. 4). At some instances varicose fibers were observed parallel to the surface in the outer most cell-poor layer (Fig. 6).

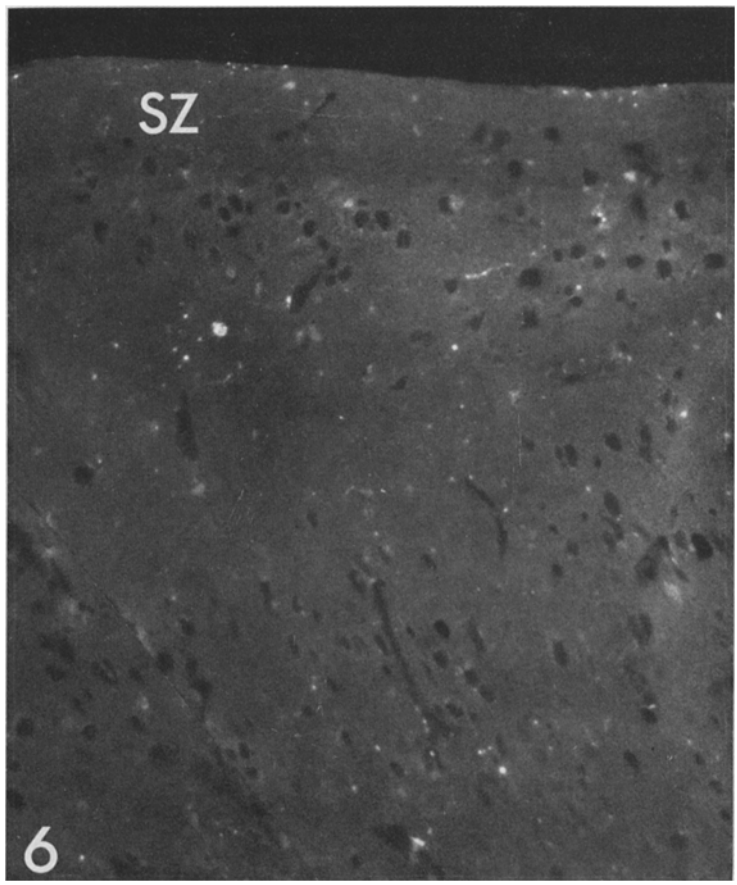

Fig. 6. Cortex transplant with fluorescent fibers from a 5 day old SCG in the same eye. The 6 μ section shows that the cell-poor superficial zone of the cortex transplants (*SZ*) can be seen also in the fluorescence microscope, partly because of different background fluorescence. Postoperative time: $2^1/_2$ months. CRL of donor fetus: 22–24 mm. Incubated in 10^{-5}M α-m-NA 30 min. Fluorescence microphotograph $\times$ 345

Only scattered fibers were observed in most untreated transplants or in transplants pretreated with an MAO inhibitor. Following incubation in 10^{-6}M or 10^{-5}M α-methyl-NA there was a dramatic increase in number of visible fluorescent fibers to normal or slightly less than normal density (cf. Fuxe *et al.*, 1968) (Fig. 3). After incubation in 10^{-5}M α-methyl-NA, uptake of exogenous amine was sometimes observed in cells of the capillary walls in the transplants (Fig. 5).

When transplanting adult SCG together with the cortex anlage the two tissues could be observed close together in most cases although there was a clearcut delineation between the two, formed by large masses of sympathetic fluorescent fibers (Fig. 7). All specimens were incubated in α-methyl-NA 10^{-5}M. The ganglion showed numerous moderately or weakly fluorescent perikarya with strongly fluorescent nerve fiber bundles in between. So called SIF cells could

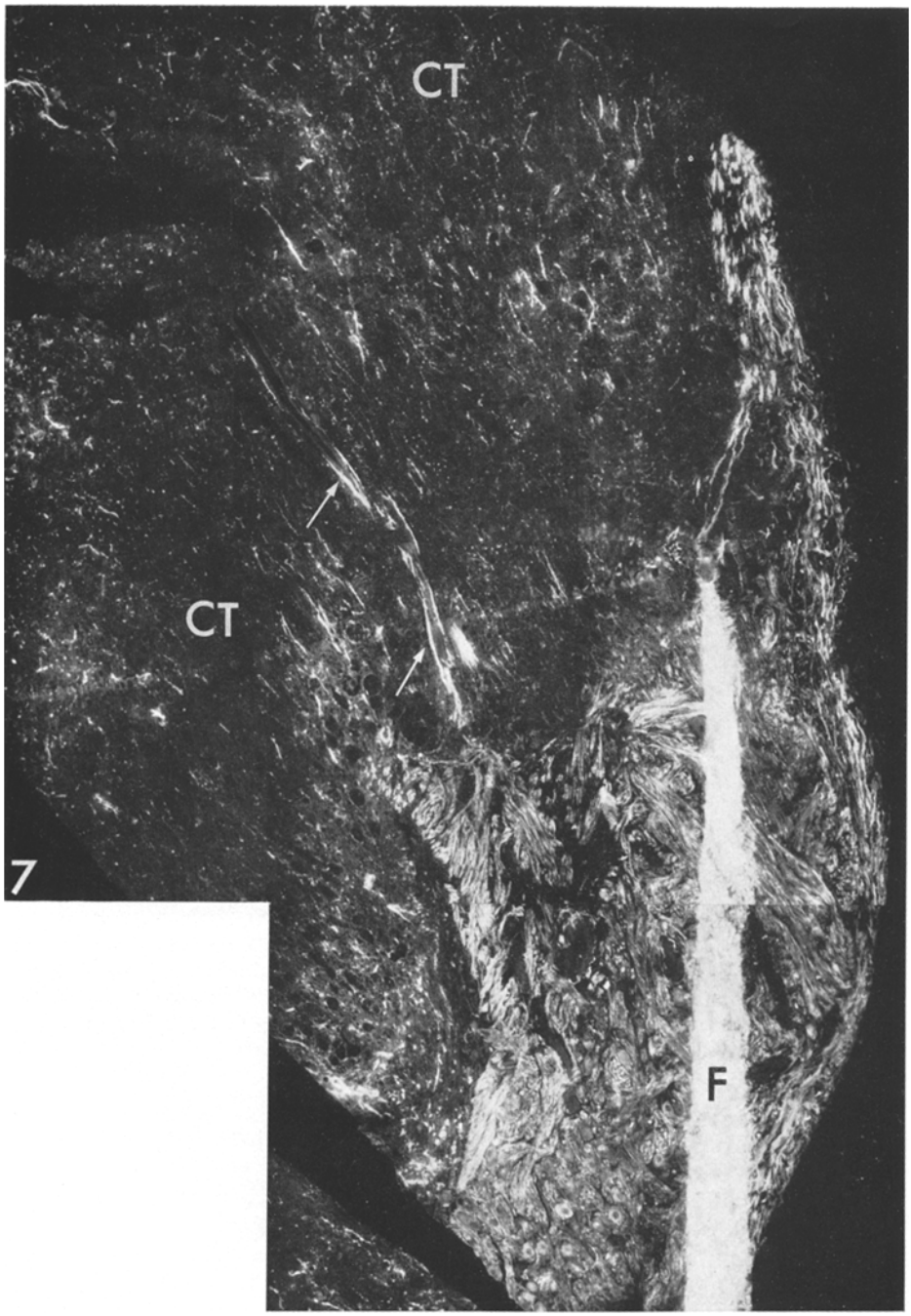

Fig. 7. An adult SCG attached to a cortex transplant. Numerous moderately fluorescent sympathetic ganglion cells are observed in the ganglion (below), intermingled with a large amount of grown out sympathetic fibers. Bundles of fluorescent axons follow large vessels (arrows) into the cortex tissue where they diverge. The cortex transplant (*CT*) is hyperinnervated but the fluorescence morphology of the fibers is organotypic. *F* fold of the section. Note clear-cut border between ganglion and cortex (cf. also Fig. 8). Incubated in 10^{-5}M α-m-NA 30 min. Postoperative time: 2 months. CRL of donor fetus: 24–25 mm. Montage of fluorescence microphotographs of a 6 μ section × 135

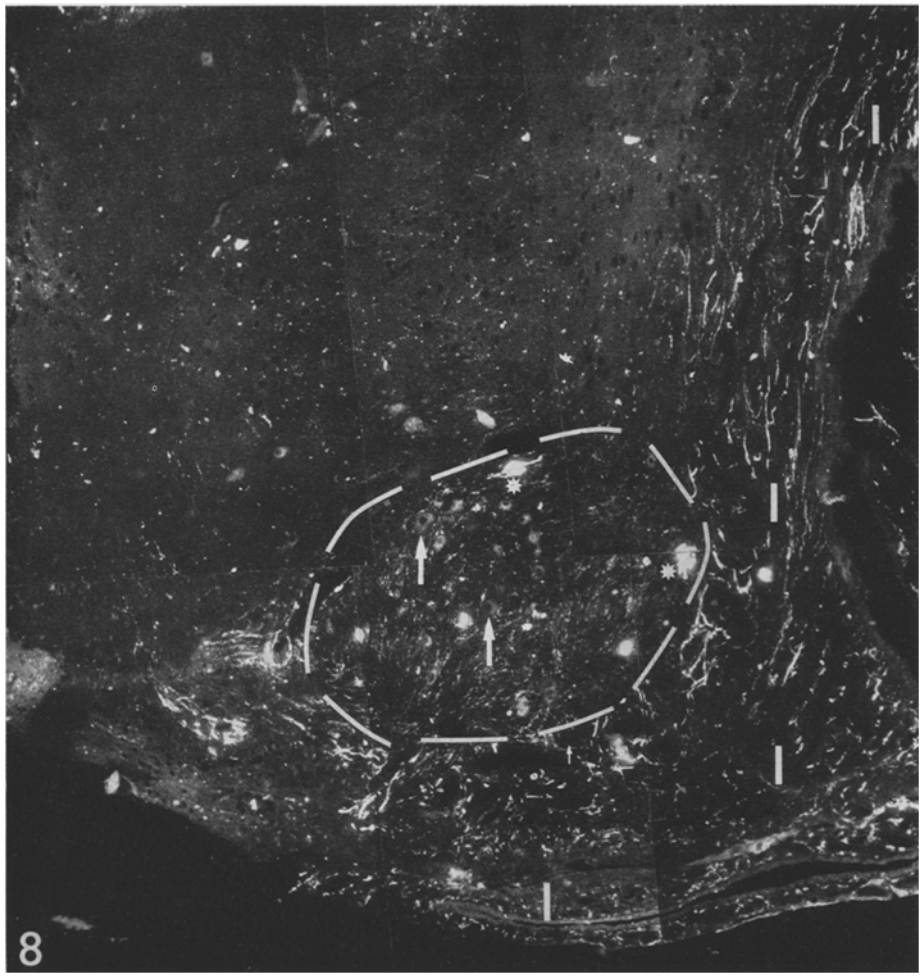

Fig. 8. A cortex transplant and a SCG, 5 days old at transplantation. A number of weakly fluorescent sympathetic ganglion cells (arrows) can be seen in the ganglion (outer border indicated by dotted line) intermingled with nerve fibers and a couple of SIF cells (stars). The iris (*I*) partly surrounding the ganglion is richly innervated. The cortex transplant (above and left) is also innervated and in a cortex-like manner. Note the absence of a marked border between the SCG and the cortex tissue (cf. also Fig. 7). Incubated in 10^{-5}M α-m-NA 30 min. Postoperative time: $2^1/_2$ months. CRL of fonor detus: 22–24 mm. Montage of fluorescence microphotographs of a 6 μ section × 135

be observed scattered and in small groups throughout the ganglion. Outside the ganglion, axons in heavy bundles radiated towards the cortex transplant and into it along large blood vessels (Fig. 7).

The iris was always hyperinnervated close to the attachment of the ganglion transplant and had a fluorescent ground plexus of normal density far from the ganglion. The density of innervation of the cortex transplant from the ganglion was dependent on the actual distance between the two during the postoperative

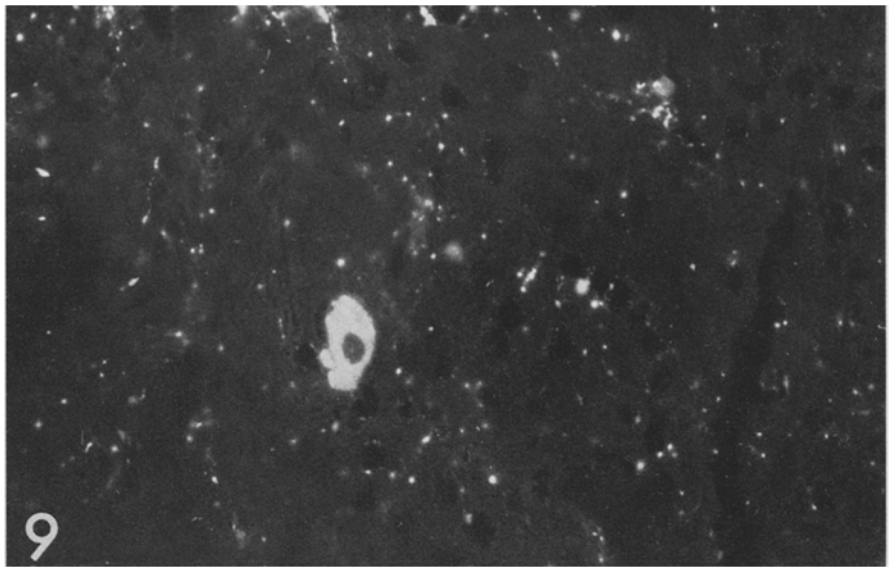

Fig. 9. Cortex transplant tissue with one strongly fluorescent sympathetic ganglion cell from a SCG, 5 days old at transplantation, to show the total integration of the young ganglia in the cortex tissue. This fluorescent cell has probably migrated out into the CNS tissue. Same transplant and treatment as in Fig. 8. Fluorescence microphotograph of a 6 μ section × 330

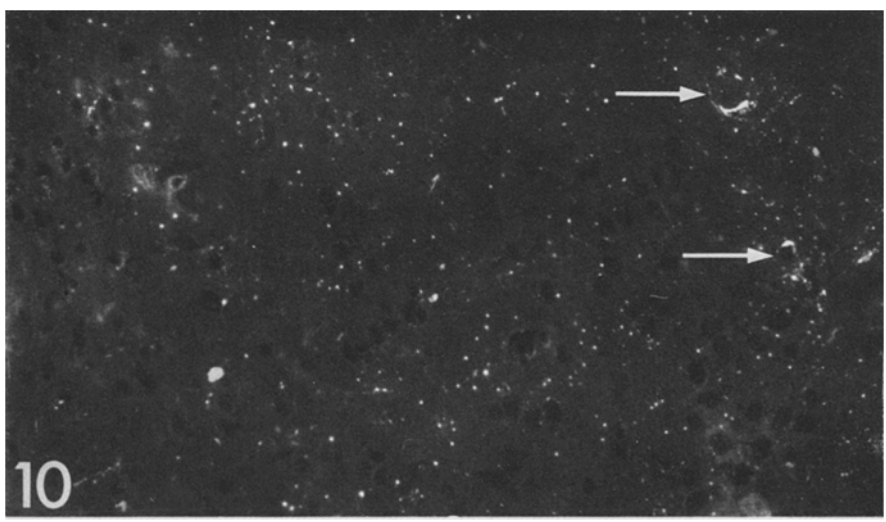

Fig. 10. A highly organotypic sympathetic innervation of a cortex transplant from a SCG, 5 days old at transplantation. Two cross sectioned vessels (arrows) can be seen with sympathetic innervation of their walls. Same transplant and treatment as in Figs. 8 and 9. Fluorescence microphotograph × 130

time. Thus, a variation from heavily hyperinnervated cortex transplant when the ganglion was in close apposition with it (Fig. 7) to scattered fibers when the ganglion was found at long distance from it was encountered. In the cortex tissue some fluorescent terminals lined out nonfluorescent perikarya but most terminals were diffusely spread out in the neuropil. Also here, smaller vessels were often accompanied by "central-looking" varicose fluorescent fibers. In all transplants the NA terminals were indistinguishable from those normally seen in cortex cerebri.

Upon transplantation of 5 day old SCG with the cortex anlage, the outgrowth of fluorescent fibers into the CNS tissue resembled that seen with adult ganglia, but a few differences were noted. Firstly, the younger ganglia were integrated in the CNS piece remarkably well (Fig. 8). Fluorescent ganglion cells and SIF cells could sometimes be seen to lie freely in the cortex tissue (Fig. 9). There was never a clear border between the two transplants.

Secondly, all cortex transplants were richly innervated with fluorescent terminals (approx. as *in situ*), but no marked hyperinnervation could be observed (Fig. 10), although the attachment of the two transplants was intimate.

Thirdly, the number of adrenergic nerve cell bodies recovered in SCG's transplanted at the age of 5 days was much lower than that found in the SCG's transplanted from adult donors (Fig. 8).

Fourth, in most "young" ganglia numerous mast cells could be seen.

Discussion

Embryonic brain tissue has a remarkable capacity to survive trans- or explantation, both as intact tissue and in the form of dispersed cells. *In vitro*, prenatal brain tissue can proliferate and differentiate to form complex neural connections that functions very much as *in situ* (see Crain and Peterson, 1974). Even dispersed cells can reaggregate and then mimic at least some basic organizational features of *e.g.* hippocampal and cerebral cortex (DeLong, 1970, 1972) indicating a largely autonomous intrinsic regulation of the gross organization in these brain areas. Furthermore, Berry and Hollingworth (1973) could show that chronically isolated neocortex of the rat *in situ* was capable of "normal migration and subsequent lamination of neuroblasts into the 6 layers of the neocortex, in the absence of thalamocortical fibres, favouring the thesis that cortical histogenesis and the movements of neuroblasts are organized by intrinsic rather than extrinsic factors". Hence, it is not surprising that the well proliferated and vascularized transplants on the iris show a gross organization reminescent of cortex cerebri *in situ* (cf. Krieg, 1946). The thinner appearance of the "cortex" layers in the transplants support Berrys and Hollingworths (1973) notion that especially the subventricular and intermediate zones are reduced upon isolation and that in addition the cortical plate gets thinner.

In the rat cortex cerebri, most of the differentiation events occur between birth and 10 days postnatally (Bass *et al.*, 1970). Therefore, since the shortest postoperative time was 3 weeks, our transplants had probably all reached a mature state. This is further supported by the observation of a very short develop-

mental lag in the cerebellar cortex following transplantation to the eye (Hoffer *et al.*, 1974).

Almost all cortex transplants became innervated by the sympathetic adrenergic nerves from the iris or from a sympathetic ganglion respectively. This is a heterotopic but organotypic innervation when compared to the normal pattern of innervation in the cerebral cortex and its nerve fiber source (Fuxe *et al.*, 1968; Ungerstedt, 1971). The adrenergic innervation develops during late prenatal and postnatal ontogeny but the knowledge of NA nerve density and distribution during this time is poor. Loizou (1972) reported that NA nerve terminals can first be seen in the untreated rat brain one week *post partum*, and that the amount of visible terminals would then hardly increase. The newborn cortex was negative. Our recent report on the late prenatal ontogeny of central monoamine neurons (Seiger and Olson, 1973) shows that NA nerves can be seen in the MAO-inhibited cortex anlage already several days before birth. Details on the time-table for innervation densities of different cortical regions during ontogeny awaits further analysis. It follows that it is not possible to say at present if and to what extent the cortex anlage gets adrenergically denervated at dissection.

The sympathetic NA nerve fibers were seen to change their fluorescence morphology on growing into the cortex tissue, so that they became thinner, with smaller terminals and almost invisible intervaricose parts far from the iris, whereas they still retained their "sympathetic" appearance close to the iris, *e.g.* in the walls of larger blood vessels where the varicosities were larger and the intervaricose parts visible. This morphological transformation has also been seen when cerebellar cortex (Hoffer *et al.*, 1975) and hippocampal cortex (Seiger and Olson, in preparation) were studied in this model. These results indicate that developing cortices can modulate the fluorescence morphology of ingrowing peripheral sympathetic adrenergic nerves from the iris. Possibly, the assumption that the receptor tissue regulates the morphology of the ingrowing nerves can be extended also to other neuron systems, since fetal central monoamine neurons have been shown to achieve a fluorescence morphology and pattern of distribution similar to that of the peripheral sympathetic NA nerves of the iris (Olson and Seiger, 1972) and the vas deferens (Olson and Seiger, 1975) upon transplantation to the eye.

SCG's that were 5 days old at transplantation contained much fewer fluorescent ganglion cells and the total volume was thus much smaller than that of the adult ganglion at the time of sacrifice. In spite of this the degree of reinnervation of the host iris was similar for the two ganglion types. The innervation of the cortex transplant also had equal densities, the only exception being adult ganglin closely attached to the cortex transplant, which gave rise to extensive hyperinnervation of the latter. This adaption of the cell number by immature ganglia to a reduced receptive field upon transplantation to the anterior chamber of the eye has been reported before (Olson and Malmfors, 1970).

Most cortex transplants got vascularized within a few days after transplantation. *In situ* the developing cortex cerebri of the rat gets its first vascular supply on day 12–14 of gestation in the form of fenestrated sinusoids. Subsequently sinusoidal sprouts, and later immature capillaries develop. Mature capillaries are encountered from the third week on (Bär and Wolff, 1972). At transplantation

the vascular supply of the dissected pieces were thus probably in the stage of sprouting sinusoids. Blood filled sinusoids developed in the transplants and disappeared progressively within 10 days leaving a capillary plexus in the transplant. This disappearance of the sinusoids in the transplants coincided in time with development of immature capillaries *in situ* from the sinusoidal sprouts suggesting that the fine-meshed capillary plexus of the mature cortex transplants would be derived from cortical immature vascular sinusoids and not from the vascular bed of the iris. This hypothesis is supported by the observation of α-m-NA accumulating cells in the capillary walls of the transplant. Such amine accumulating cells, pericytes and/or endothelial cells, are normally found in the cortex but not in the iris (Hamberger, 1967). The close association between small vessels of the transplant and NA fibers observed in the present experiments is not at variance with the hypothesis above, since recent studies have indicated a closer association between central adrenergic fibers and blood vessels in the normal brain than was earlier realized (Hartman *et al.*, 1972; Edvinsson *et al.*, 1973). The blood supply of the transplant is of course from the iris, but at what level the iris vessels connect with the CNS capillary plexus is not known.

Chronic isolation of the adult cortex cerebri gives progressive hyperexcitability (see Krnjevic *et al.*, 1970). In cortical slabs, EEG activity ceases in the acute phase, but is reestablished with time (see *e.g.* Vazquez and Krip, 1973). Our recent studies dealing with cerebellar cortex transplants (Hoffer *et al.*, 1974, 1975) or hippocampal cortex transplants (Olson *et al.*, 1975; Freedman *et al.*, 1975) show that not only histologically but also electrophysiologically do the transplants mimic adult brain tissue from the corresponding regions. It is therefore possible that cortex cerebri transplants also possess a series of normal or near-normal electrophysiological features.

Adrenergic nerves in the cortex cerebri seem to have an inhibitory effect on the majority of cortical neurons (Nelson *et al.*, 1973; Stone, 1973). Having an isocortex transplant in the eye with ingrown peripheral sympathetic adrenergic nerves originating in the SCG, is a unique opportunity to study the influence on an isolated well defined cortical area of adrenergic input, without interference from other known and unknown extracortical regions and connections. Similar selective sympathetic trunk stimulations have been performed with cerebellar cortex transplants (Hoffer *et al.*, 1975) and are under study with hippocampal cortex transplants (Freedman *et al.*, 1975). It should be possible with our techniques to further elucidate factors regulating the central noradrenergic synaptic mechanisms also in the cortex cerebri.

References

Bär, Th., Wolff, J. R.: The formation of capillary basement membranes during internal vascularization of the rat's cerebral cortex. Z. Zellforsch. **133**, 231–248 (1972)

Bass, N. H., Netsky, M. G., Young, E.: Effect of neonatal malnutrition on developing cerebrum. Arch. Neurol. (Chic.) **23**, 289–302 (1970)

Berry, M., Hollingworth, T.: Development of isolated neocortex. Experientia (Basel) **29**, 204–207 (1973)

Corrodi, H., Jonsson, G.: The formaldehyde fluorescence method for the histochemical demonstration of biogenic monoamines. A review on the methodology. J. Histochem. Cytochem. **15**, 65—78 (1967)

Crain, S. M., Peterson, E. R.: Development of neural connections in culture. Ann. N.Y. Acad. Sci **228**, 6–34 (1974)

DeLong, G. R.: Histogenesis of fetal mouse isocortex and hippocampus in reaggregating cell cultures Develop. Biol. **22**, 563–583 (1970)

DeLong, G. R.: Long-term organotypic patterning of nerve cell aggregates. In: NRP Bulletin **10**, 535–539 (1972)

Edvinsson, L., Lindvall, M., Nielsen, K. C., Owman, Ch.: Are brain vessels innervated also by central (non-sympathetic) adrenergic neurones? Brain Res. **63**, 496–499 (1973)

Falck, B., Hillarp, N.- Å., Thieme, G., Torp, A.: Fluorescence of catechol amines and related compounds condensed with formaldehyde. J. Histochem. Cytochem. **10**, 348–354 (1962)

Freedman, R., Hoffer, B., Olson, L., Seiger, Å.: Electrophysiology and cytology of hippocampal formation transplants in the anterior chamber of the eye. III. Adrenergic and cholinergic influence on normal and epileptogenic activity. (1975) (to be publ.)

Freedman, R., Seiger, Å., Hoffer, B.: Electrophysiology and cytology of hippocampal formation transplants in the anterior chamber of the eye. I. Intrinsic organization. (1975) (to be publ.)

Fuxe, K., Hamberger, B., Hökfelt, T.: Distribution of noradrenaline nerve terminals in cortical areas of the rat. Brain Res. **8**, 125–131 (1968)

Hamberger, B.: Reserpine-resistant uptake of catecholamines in isolated tissues of the rat. A histochemical study. Acta physiol. scand., Suppl. **295**, 1–56 (1967)

Hartman, B. K., Zide, D., Udenfriend, S.: The use of dopamine β-hydroxylase as a marker for the central noradrenergic nervous system in the brain. Proc. nat. Acad. Sci. (Wash.) **69**, 2722–2726 (1972)

Hoffer, B., Olson, L., Seiger, Å,, Bloom, F.: Formation of a functional adrenergic input to intraocular cerebellar grafts: ingrowth of sympathetic fibers and inhibition of Purkinje cell activity by adrenergic input. J. Neurobiol. (1975) (in press)

Hoffer, B., Seiger, Å., Ljungberg, T., Olson, L.: Electrophysiological and cytological studies of brain homografts in the anterior chamber of the eye: maturation of cerebellar cortex *in oculo*. Brain Res. **79**, 165–184 (1974)

Krieg, W. J. S.: Connections of the cerebral cortex. I. The albino rat. B. Structure of the cortical areas. J. comp. Neurol. **84**, 277–323 (1946)

Krnjevic, K., Reiffenstein, R. J., Silver, A.: Chemical sensitivity of neurons in long isolated slabs of rat cerebral cortex. Electroenceph. clin. Neurophysiol. **29**, 269–282 (1970)

Loizou, L.: The postnatal ontogeny of monoamine-containing neurones in the central nervous system of the albino rat. Brain Res. **40**, 395–418 (1972)

Nelson, C. N., Hoffer, B. J., Chu, N.-S., Bloom, F. E.: Cytochemical and pharmacological studies on polysensory neurons in the primate frontal cortex. Brain Res. **62**, 1–20 (1973)

Olson, L., Malmfors, T.: Growth characteristics of adrenergic nerves in the adult rat. Fluorescence histochemical and ^{3}H-noradrenaline uptake studies using tissue transplantations to the anterior chamber of the eye. Acta physiol. scand., Suppl. **348**, 1–112 (1970)

Olson, L., Seiger, Å.: Brain tissue transplanted to the anterior chamber of the eye. 1. Fluorescence histochemistry of immature catecholamine and 5-hydroxytryptamine neurons reinnervating the rat iris. Z. Zellforsch. **135**, 175–194 (1972)

Olson, L., Seiger, Å.: Brain tissue transplanted to the anterior chamber of the eye: 2. Fluorescence histochemistry of immature catecholamine- and 5-hydroxytryptamine neurons innervating the rat vas deferens. Cell. Tiss. Res. (1975) (in press)

Olson, L., Ungerstedt, U.: A simple high capacity freeze-drier for histochemical use. Histochemie **22**, 8–19 (1970)

Seiger, Å., Olson, L.: Late prenatal ontogeny of central monoamine neurons in the rat: fluorescence histochemical observations. Z. Anat. Entwickl.-Gesch. **140**, 281–318 (1973)

Stone, T. W.: Pharmacology of pyramidal tract cells in the cerebral cortex. Naunyn-Schmiedeberg's Arch. Pharmacol. **278**, 333–346 (1973)

Ungerstedt, U.: Stereotaxic mapping of the monoamine pathways in the rat brain. Acta physiol. scand., Suppl. **367**, 1–48 (1971)

Vazquez, A. J., Krip, G.: Evidence for an inhibitory role for acetylcholine, catecholamines, and serotonin on the cerebral cortex, p. 137–159. New York: Raven Press 1973