Pergamon

Brain Research Bulletin, Vol. 35, Nos. 5/6, pp. 409–412, 1994

0361-9230/94 $6.00 + .00

0361-9230(94)00157-X

Regeneration of Norepinephrine-containing Fibers in Occipital Cortex of Adult Cats

K. NAKAI,*[1] K. NIIYAMA,† T. KASAMATSU,† Y. NAKA,‡ T. ITAKURA‡ AND N. KOMAI‡

*Department of Neurosurgery, Minami Wakayama National Hospital, Tanabe-shi, Wakayama 646 Japan
†Smith–Kettlewell Eye Research Institute, San Francisco, CA 94115
‡Department of Neurological Surgery Wakayama Medical College, Wakayama 640 Japan

NAKAI, K., K. NIIYAMA, T. KASAMATSU, Y. NAKA, T. ITAKURA AND N. KOMAI. *Regeneration of norepinephrine-containing fibers in occipital cortex of adult cats.* BRAIN RES BULL **35**(5/6) 409–412, 1994.—Regeneration of norepinephrine (NE)-containing nerve fibers in occipital cortex of adult cats was studied using morphological and biochemical methods. Initially, degeneration of cortical NE fibers was induced by direct infusion of 6-hydroxydopamine (6-OHDA) for a week. Cortical reinnervation by NE fibers continuously proceeded throughout 52 weeks, the longest survival period studied, after stopping 6-OHDA infusion. The rate of the reinnervation was slower in mature cortex than that obtained earlier in immature cortex. The present results indicate that the regenerative ability of the central NE neurons is universal, not limited to the immature brain. It is implied that the central NE neurons are equipped with a transmitter-specific repair mechanism throughout life.

Regeneration Norepinephrine Cortex Cat

WE have studied the roles of the central norepinephrine (NE) system in the regulation of neuronal plasticity, which is typically seen in visual cortex of young kittens (5,6). We used physiological changes in ocular dominance of visual cortical cells as an index to measure cortical plasticity. In those studies we consistently found the lack of plasticity, when catecholamine (CA)-containing terminals in kitten visual cortex had been destroyed by continuous infusion of CA-specific neurotoxin, 6-hydroxydopamine (6-OHDA). However, the effect of 6-OHDA was incomplete and transient. This may be due, at least partly, to the reinnervation of a cortical area in which NE terminal fields have been destroyed by 6-OHDA infusion.

CA-containing fibers and terminals in the central nervous system are well known for their remarkable capability to regrow when their somata and proximal axons are kept intact (2,7,8,12,16). Earlier, we studied the regeneration of CA fibers in kitten occipital cortex that had been directly and continuously infused with 6-OHDA for a week. In that study (12) we first confirmed the specific degeneration of CA fibers in the 6-OHDA–infused cortex by the presence of degenerative terminal boutons and by the lack of NE uptake in the 6-OHDA–affected cortex. The time course of the cortical reinnervation was rapid: a) 1 week after the end of 6-OHDA infusion, we found an obvious decrease in the size of the cortical area devoid of CA terminals from that seen a week before. Some fluorescent CA terminals in the regrowing area were considerably swollen up to 10 μ in diameter. b) Two weeks after the end of 6-OHDA infusion, the CA terminal-free area became significantly reduced in size. There was, in fact, no larger any cortical area totally free of CA terminals, and a small number of fluorescent CA fibers were observed even at the edge of the scar caused by the placement of a cannula, through which 6-OHDA had been infused. c) By four weeks after the end of 6-OHDA infusion, CA terminals were seen virtually everywhere including the center of previous cannulation. In an area 2–3 mm from the infusion site, fluorescent CA fibers showed an almost normal distribution. d) In 24 weeks, the longest term studied, many fluorescent fibers were seen at the normal density everywhere, including the scar formation caused by cannulation. Swollen fluorescent fibers, which were prominent in 1-week–survived animals, were no longer found at this stage. In another set of animals, we measured biochemical changes in the endogenous content of CAs in the 6-OHDA–affected cortical area over the survival period up to 24 weeks (10). The results were corroborative with those obtained by the morphological methods.

The above-summarized data from kittens, albeit compelling, have failed to show the presence of genuinely regenerative NE fibers, different from the preprogrammed ingrowth of NE fibers in the rapidly developing brain. Therefore, we wanted to extend our study to the mature brain. We have asked whether central NE fibers reinnervates the 6-OHDA–infused cortex of adult cats and, if so, how rapidly the reinnervation proceeds.

METHOD

Twenty adult cats, 28 weeks to 4 years old, were used. The solution of 4 mM 6-OHDA in saline containing 0.4% of ascorbic acid was continuously infused (1 μl/h) for 1 week in occipital cortex at a stereotaxic site (AP 0 mm, L 2 mm, D 1.5 mm), through a 30 gauge cannula connected to an osmotic minipump

[1] Requests for reprints should be addressed to Dr. K. Nakai, Dept. of Neurological Surgery, Wakayama Medical College, 27, 7-Bancho, Wakayama 640, Japan.

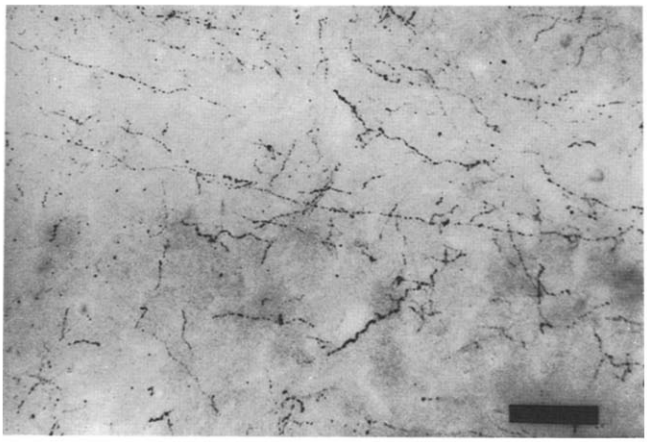

FIG. 1. A photomicrograph of cat occipital cortex processed for anti-dopamine-beta-hydroxylase (DBH) immunohistochemistry. Norepinephrine-containing varicosities are clearly seen under bright-field light microscopy as bluish black staining against the low background. The scale bar, 50 μ.

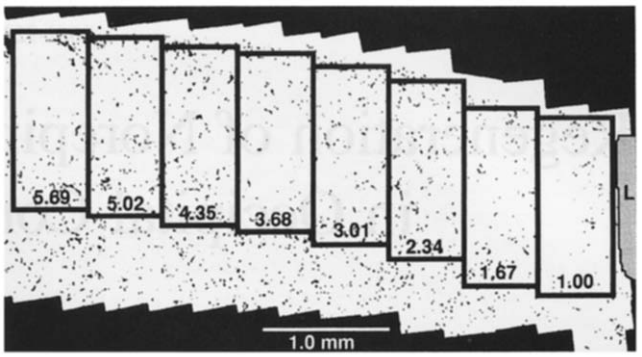

FIG. 2. A parasagittal photomontage of cat occipital cortex was divided into a series of a unit rectangular area (0.67 × 1.5 mm). Numbers at the bottom of each rectangle indicate distances in millimeters from the center of the scar formation (shaded area, L) caused by the placement of a 30 gauge cannula used for 1 week-long 6-OHDA infusion. A case of 45 week's survival.

(Alzet 2001). In all the animals the superior cervical ganglia were bilaterally resected 1 week before the implantation of the cannula-minipump assembly so that the main source of sympathetic NE fibers in the brain was removed. After various survival periods, from 0 to 53 weeks, we processed the 6-OHDA–infused and control occipital cortices using either morphological methods to visualize the NE-containing nerve fibers or biochemical assays of endogenous CAs.

For morphological investigations, we used both glyoxylic acid induced-CA fluorescence histochemistry and immunohistochemistry of antidopamine-beta-hydroxylase (DBH) to visualize the NE-containing varicose fibers. Our standard glyoxylic acid method (3) was partly modified by adding 2% dimethyl sulfoxide to the perfusion solution containing glyoxylic acid. Accordingly, the concentration of glyoxylic acid was lowered to 0.5% from usual 2%. This modification brought stable and even visualization of NE fibers throughout the cortical tissue. The two techniques, modified CA fluorescence histochemistry and anti-DBH immunohistochemistry, showed basically the same staining pattern of NE neurons in the cat brain.

We counted the number of varicosities in thin sections processed not for CA histofluorescence but with anti-DBH antisera (polyclonal, Eugene Tech. Lot no. 2012, dilution 1:1,000), because the latter (30 μm thick) produced less intense background staining than the former (16 μm thick) in the adult brain, which usually has many nonspecific fluorophores under fluorescence microscopy. The parasagittal section was divided into a series of rectangles whose unit dimensions were 1.5 mm in height (from the cortical surface) and 0.67 mm in width (anteroposteriorly) (Fig. 2). The number of varicosities was counted per unit rectangle under light microscopy at ×400 magnification.

For biochemical assays, we used a standard electrochemical detection method of CAs with high pressure liquid chromatography. The parasagittal slab was cut out from the posterolateral gyrus of the experimental hemisphere, and included the cannulation site of 6-OHDA infusion at its posterior end that was clearly seen as a black dot (oxidation product of 6-OHDA) on the cortical surface. The control slab was carved from the opposite hemisphere at the corresponding location. Then, the 10 mm-long cortical slabs were divided into eight coronal slices (average wet weight 34.4 mg). Slice number 1 contained the infusion site and slice number 8 an area 10 mm anterior to it. Endogenous CA contents in each slice were measured and expressed in relation to the distance from the infusion site.

RESULTS

Morphology

NE-containing terminal fields in cat occipital cortex were identified by CA fluorescence histochemistry as a rich network of varicose fibers having a green fluorescent appearance (data not shown). In anti-DBH immunohistochemistry, they appeared to be bluish-black varicosities (Fig. 1).

Immediately after stopping the continuous 6-OHDA infusion, practically no NE-containing fibers were found in an area up to 5 mm from the center of infusion. To quantify this finding, we counted the number of varicosities per unit rectangle, while its position was successively shifted anteriorly starting from the cannulation site (Table 1). Only a small number of anti-DBH immunoreactive varicosities, less than 50, were found within a unit rectangle up to an area 4 mm from the infusion site. At an area 5 mm from the infusion site, the number was 260. These are compared with data from control occipital cortex infused with the vehicle solution alone, the average number of varicosities immunoreactive to anti-DBH antisera was 2,628 ± 298 per unit rectangle.

By scanning the cortex of cats that survived for 25 weeks, the varicosity number increased up to more than 300 and 1,471 per rectangle at 3.0 mm and 5.0 mm from the infusion site, respectively. In a 52-week-survived animal, the number was 1,003 and 1,797 at 3.0 mm and 5.0 mm, respectively. Thus, the number of

TABLE 1

NUMBER OF VARICOSITIES PER UNIT AREA IN HISTOLOGY SECTIONS

	Survival Period		
Distance	0 week	25 weeks	52 weeks
Control	2,628 ± 298	—	—
3 mm	<50	>300	1,003
5 mm	260	1,471	1,797

varicosities in the 6-OHDA-affected area was found to increase when the survival term was extended, though the maximum at the boundary of the initially denervated area (about 5 mm from the infusion site) remained still substantially lower than the varicosity density in control.

Biochemistry

Immediately after the end of 6-OHDA infusion, the NE content was significantly depleted in a large area up to 10 mm away from the infusion site. It remained remarkably low, down to a value near 0 within a radius of 5 mm from the infusion site. This corroborates the morphological data mentioned above (Table 2).

The NE content showed a steady recovery in time: in animals with 25-week survival it was 4.1 ± 4.0 ng/100 mg wet weight at 5 mm. This value is still less than the half that of control value of about 9 ng/100 mg wet weight. In the cortex of a 53-week-survived cat, the NE content 5 mm away from the infusion site was 6.5 ± 3.0 ng/100 mg wet weight, approaching the control. In the same animal, the NE content at an area 10 mm was 12.0 ± 4.5 ng/100 mg wet weight, the value being not different from that of control at the same distance. Meanwhile, the dopamine (DA) content in the same 6-OHDA–affected area also showed the recovery, at a rate faster than that of NE. The same trend of the DA recovery was found earlier in kitten visual cortex (11).

In short, like the density of anti-DBH immunoreactive varicosities, the NE content also showed the steady recovery throughout a year-long survival period in the 6-OHDA–affected area. The NE content, indeed, regained the control level with over 50-week survival at the periphery of the initial denervation area (> 5 mm from the 6-OHDA infusion site).

DISCUSSION

Why NE Neurons?

Aminergic neurons have a remarkable capability to regrow in general. We sought an area in the brain suitable for further examination of the specificity of the regenerative process, using a relatively small chemical lesion confined to the pure terminal field. We chose occipital cortex because: a) It is innervated by the remote terminal field of the ascending NE fiber projection from the locus coeruleus. b) Cat occipital cortex receives the dense innervation of the NE fibers (4) and has a substantial amount of endogenous NE (4). c) The contribution of NE fibers originating from peripheral source (13), such as the superior cervical ganglion, is limited to blood vessels in the pia mater and the most superficial gray matter, layer I, and d) cat visual cortex has been used as a model for studying the NE system-dependent regulation of neural plasticity (see the Introductory paragraphs).

TABLE 2

ENDOGENOUS NOREPINEPHRINE CONTENT
(ng PER 100 mg WET TISSUE)

	Survival Period		
Distance	0 week	25 weeks	53 weeks
Control	—	9	—
3 mm	~0	—	—
5 mm	~0	4.1 ± 4.0	6.5 ± 3.0
10 mm	—	—	12.0 ± 4.5

Evidence for Regeneration in Adult Cortex

To ascertain that reappearing NE terminals we found in the 6-OHDA–infused occipital cortex of adult cats were genuinely regenerative, first of all, we have to be sure that NE terminals had been specifically destroyed by 6-OHDA infusion. In our previous studies in kitten occipital cortex, the presence of specific degeneration of NE terminals after continuous infusion of 6-OHDA was proven by the combination of in vitro study on desipramine-sensitive NE uptake, quantitative β-adrenoreceptor autoradiography, and electron microscopy (10,11). In the present study we did not rigorously assess the degree of the initial degeneration as done earlier for kitten cortex. Nevertheless, it was obvious that 6-OHDA infusion in adult cortex caused morphological and biochemical changes very similar to those obtained in kitten cortex (10,11). Accordingly, we interpreted that reappearing anti-DBH immunoreactive fibers and green fluorescent fibers in the 6-OHDA–infused area were regenerative in nature. The present data have, thus, provided further support to the concept that central nerve fibers in the mammalian brain are able to regrow under appropriate conditions (1,14,15) albeit a reversal of Ramon y Cajal's view (13).

Mechanism Underlying Regeneration

Our previous data on kitten occipital cortex showed the remarkably rapid reinnervation of 6-OHDA–denervated cortex with central NE fibers from the locus coeruleus (10,11). In those studies, we argued that the ability of central NE fibers to reinnervate the previously denervated cortical area might vary with the age of animals. The present results from the adult brain have clearly demonstrated a regenerative capability of the central NE fibers. However, the rate of the NE reinnervation was much slower in adult cortex than that in kitten cortex. Our unpublished morphological observations on the normal development of NE fibers in cat occipital cortex, from 6 days to 10 years old, have shown a steady increase in the density of NE fibers and terminals, and this is consistent with our earlier biochemical data that the endogenous content of NE monotonously increases throughout life of the animal (4). We think that this normal ingrowth of central NE fibers significantly contributes to the faster rate of reinnervation by NE fibers in younger animals. At the same time, the present findings on mature cortex strongly suggest the existence of another mechanism for the regeneration of central NE fibers, independent of the developmentally regulated ingrowth of the central NE fibers in neocortex.

We described previously that substance P administered into the fourth ventricle could accelerate the regrowth of central NE fibers in 6-OHDA–infused occipital cortex of kittens (9). The finding has raised a possibility that substance P-stimulated regrowth of NE fibers works in the brain to maintain the integrity of vast terminal fields of the central NE neurons as a part of built-in repair or protective mechanisms against injury of NE axons. At the moment, however, we do not know whether substance P or other neurochemical factors promote the regeneration of central NE fibers in the adult brain, as well.

ACKNOWLEDGEMENTS

We are grateful to Ms. M. Mejia for her skillful assistance in fluorescence histochemistry and immunohistochemistry, and Ms. R. Sallaberry for photo reproduction. We wish to thank Dr. S. Hayashi for his encouragement throughout the study and Ms. Tada for her assistance in biochemical assays of catecholamines at Wakayama Medical College. Supported by USPHS RO1 grant EY 06733 and Core grant EY 06883 and partly by a fund from the Science and Technology Agency of Japan.

REFERENCES

1. Aguayo, A. J.; Rasminsky, M.; Bray, G. M.; Carbonetto, S.; McKerracher, L.; Villegas-Peres, M. P.; Vidal-Sanz, M.; Carter, D. A. Degenerative and regenerative responses of injured neurons in the central nervous system of adult mammals. Philos. Trans. R. Soc. [Lond.] 331:337–343; 1991.
2. Björklund, A.; Katzman, R.; Stenevi, U.; West, K. A. Development and growth of axonal sprouts from noradrenaline and 5-hydroxytryptamine neurons in the rat spinal cord. Brain Res. 31:21–33; 1971.
3. Itakura, T.; Kasamatsu, T.; Pettigrew, J. D. Norepinephrine-containing terminals in kitten visual cortex: Laminar distribution and ultrastructure. Neuroscience 6:159–175; 1981.
4. Jonsson, G.; Kasamatsu, T. Maturation of monoamine neurotransmitters and receptors in cat occipital cortex during postnatal critical period. Exp. Brain Res. 50:449–458; 1983.
5. Kasamatsu, T.; Pettigrew, J. D. Preservation of binocularity after monocular deprivation in the striate cortex of kittens treated with 6-OHDA. J. Comp. Neurol. 185:139–161; 1979.
6. Kasamatsu, T.; Pettigrew, J. D.; Ary, M. Restoration of visual cortical plasticity by local microperfusion of norepinephrine. J. Comp. Neurol. 185:163–182; 1979.
7. Katzman, R.; Björklund, A.; Stenevi, U.; West, K. A. Evidence for regenerative axon sprouting of central catecholamine neurons in the rat mesencephalon following electrolytic lesion. Brain Res. 25:579–596; 1971.
8. Moore, R. Y.; Björklund, A.; Stenevi, U. Plastic changes in the adrenergic innervation of the rat septal area in response to deprivation. Brain Res. 33:13–35; 1971.
9. Nakai, K.; Kasamatsu, T. Accelerated regeneration of central catecholamine fibers in cat occipital cortex: Effects of substance P. Brain Res. 323:374–379; 1984.
10. Nakai, K.; Jonsson, G.; Kasamatsu, T. Norepinephrinergic re-innervation of cat occipital cortex following localized lesions with 6-hydroxydopamine. Neurosci. Res. 4:433–453; 1987.
11. Nakai, K. Regenerative catecholamine-containing terminals in kitten visual cortex: An ultrastructural study. Neurosci. Res. 4:475–485; 1987.
12. Nygren, L.-G.; Olson, L.; Seiger, Å. Regeneration of monoamine-containing axons in the developing and adult spinal cord of the rat following intraspinal 6-OH-dopamine injections or transections. Histochemie 28:1–15; 1971.
13. Ramon y Cajal, S. Degeneration and regeneration of the nervous system. New York, 1928 [edited and translated by May, R. M., Hafner Publishing].
14. Saporta, S. How plastic is the central nervous system? J. Fla. Med. Assoc. 79:645–647; 1992.
15. Schwab, M. E. Myelin-associated inhibitors of neurite growth and regeneration in the CNS. Trends Neurosci. 13:452–456; 1990.
16. Stenevi, U.; Björklund, A.; Moore, R. Y. Growth of intact central adrenergic axons in the denervated lateral geniculate body. Exp. Neurol. 35:290–299; 1972.