ACTA NEUROBIOL. EXP. 1990, 50: 163-172

Symposium "Recovery from brain damage: behavioral and neurochemical approaches"
4-7 July, 1989, Warsaw, Poland

CHOLINERGIC MARKERS IN THE PLASTICITY OF MURINE BARREL FIELD

S. GŁAŻEWSKI, M. KOSSUT, E. SIUCIŃSKA and J. SKANGIEL-KRAMSKA

Department of Neurophysiology, Nencki Intitute of Experimental Biology,
3 Pasteur St., 02-093 Warsaw, Poland

Key words: muscarinic receptor, AChE, barrel field plasticity, mouse

Abstract. Cholinergic muscarinic receptor binding and acetylcholinesterase (AChE) histochemistry were studied in the barrel cortex of adult, vibrissae deprived and vibrissae denervated mice. In the control barrel field muscarinic receptors labeled with [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) showed a higher density in the granular cortex and a higher accumulation of label in the barrels. AChE staining revealed a punctuated pattern corresponding to the barrels in the upper part of layer IV and a reverse-image pattern of staining showing only the walls of barrels in the lower part of layer IV. Neonatal denervation of rows of vibrissae lowered both binding of [^{3}H]QNB to the tissue in the shrunken rows and AChE activity in the denervated rows of barrels. Deprivation and late denervation produced no effects on either pattern, or intensity, of [^{3}H]QNB labeling and AChE staining. These observations suggest that the changes in cholinergic markers are related to the altered morphological structure and not to the abnormal functioning of the barrel cortex which received reduced sensory input from the vibrissae.

INTRODUCTION

Acetylcholine (ACh) appears to be involved in modulation of cortical excitability in the mammalian cerebral cortex. About 30% of cortical neurons are cholinoceptive and for most of these application of ACh

increases the rate of firing (16, 20). The bulk of the cholinergic innervation of the cortex originates in subcortical sources (18, 19, 43). In the cortex of rats cholinergic interneurons have been identified with ChAT immunocytochemistry (9). Acetylcholine does not seem to be present in the terminals of the primary sensory projections (14), but the studies of Prusky et al. (23) have suggested the presence of nicotinic receptors on the terminals of afferents from the lateral geniculate nucleus to the visual cortex of cats. Ontogenetic studies on muscarinic cholinergic receptors (MChRs) have shown that apart of alteration in number and affinity, these receptors change their laminar distribution in the visual cortex of the cat during the critical period of neuronal plasticity (32, 39). Acetylcholine seems to play a role in the formation and maintenance of memory (4, 8). The decrease in ACh levels in the cortex is also implicated in memory deficits associated with Alzheimer's disease (44). A facilitatory, permissive role of ACh in the processes of developmental plasticity has been suggested by Bear and Singer (1).

Depletion of ACh from the cortex by basal forebrain nuclei lesions interfered with effects of monocular deprivation in kitten visual cortex (5). The same effect was obtained by blocking of the cholinergic muscarinic receptors in the kitten visual cortex by cortical infusion of cholinergic antagonists (7). These data imply the involvement of the cholinergic system in the regulation of developmental processes, and in establishing cortical circuitry.

AChE, apart from being the catabolic enzyme for ACh, is believed to have a function unconnected with neurotransmission (6). It is transiently present in the sensory thalamus and cortex of rodents for the first three postnatal weeks (15, 26). During that period its expression in the visual system of rats seems to be dependent upon functional activity of the visual pathway (27). Robertson (26) suggested a morphogenic role of this enzyme, based on a correlation between the regions of appearance of the AChE staining and its temporal pattern with the regions and time course of thalamocortical axons growth into cerebral cortex.

We decided to investigate the response of some cholinergic markers in the somatosensory cortex of mice to experimentally induced plastic changes in the representation of mystacial vibrissae, the barrel field. The barrel field is a unique region in primary somatosensory cortex (SI) in some rodents because of the special cytoarchitectonics of cortical layer IV, where each vibrissa is represented by a separate aggregation of neurons, called the "barrel" (45). It provides a convenient model system to investigate the effect of manipulation of sensory input on the cortex. Changes in functioning of the barrel neurons can be induced

either with (by neonatal denervation) (38), or without (by denervation in adulthood or deprivation) (42) concomitant changes in the morphology of the barrels.

Coincidence of AChA stained clusters in layer IV with barrels in SI of rat has been reported by Kristt and Kasper (13). They also registered the alterations of AChE staining pattern after neonatal coagulation of the mystacial vibrissae. The electrophysiological data of Lamour and Dykes (17) (who recorded responses in deafferented somatosensory cortex of rats) show an overall reduction in responsiveness of neurons to iontophoretically applied acetylcholine. This effect could be due to changes in the properties of cholinergic receptors. We report here the results of partial denervation or partial sensory deprivation of the barrel field upon the distribution and level of acetylcholinesterase, and distribution and binding density of cholinergic muscarinic receptors.

MATERIAL AND METHOD

The experiments were performed on twenty six mice. The following groups of animals were studied: (1) mice with two rows of vibrissae removed mechanically at birth, (2) mice with two rows of vibrissae removed at two months of age, (3) mice with two rows of vibrissae deprived of sensory input from birth by plucking out daily. All procedures were performed unilaterally. The experiments were done 2 months after the lesions or after two months of deprivation.

To examine MChRs distribution the *in vitro* binding autoradiography technique was used. Mice (2-4 months old) were anesthetized and killed by vascular perfusion with 0.1% formaldehyde in PBS. After removal from the skull, the majority of the brain cortices were gently flattened according to the method of Strominger and Woolsey (37) and frozen in isopentane with crushed dry ice (−60°C). Serial 20 μm sections were cut on the cryostat. MChRs were labeled with [^{3}H] quinuclidinyl benzilate ([^{3}H]QNB, S.A. Ci/mmol, Amersham) at final concentration 1nM as described previously (35). To evaluate unspecific binding several sections were incubated in the presence of 1 μM atropine. Slide mounted sections were apposed to tritium sensitive film for 9 days.

The distribution of AChE was examined with a histochemical procedure. Animals were anesthetized and sacrificed by vascular perfusion with 1% formaldehyde in 0.1 M phosphate buffer containing 4% sucrose and 1.25% glutaraldehyde. Brains were cut in a coronal plane with a Vibratome at a thickness of 50 μm and directly processed for AChE histochemistry, according to a slightly modified method of Koelle et al. (10).

The exact position of the barrel field was marked out after Nissl staining, succinyl dehydrogenase (SDH) or cytochrome oxidase (CO) histochemistry of adjacent cortical sections. Autoradiograms and AChE stained sections were quantified by a computer assisted image analysis system.

RESULTS

[^{3}H]QNB binding. The laminar distribution of binding sites for the muscarinic radioligand [^{3}H]QNB was examined on coronal sections through the somatosensory cortex, including both the barrel cortex and adjacent face and forepaw representations. A high receptor density was found in cortical layers: II, III, IV and VI (Fig. 1). In the barrel cortex the binding of [^{3}H]QNB was found to be higher, in layers II, III and IV, than in the same layers of the neighboring cortical areas (Fig. 1). Examination of sections cut tangentially revealed that in the normal barrel field the [^{3}H]QNB labeling shows a faint punctuated pattern resembling the pattern of barrels (Fig. 2). This pattern was most clearly visible at the border of layer IV and V probably due to contrast with low labeling of layer V (Fig. 1*C*). The septa and barrel walls between rows had a lower density of labeling than within the rows of barrels. The sides and hollows of the barrels could not be differentiated in autoradiograms.

After neonatal denervation of vibrissae, barrels rows corresponding to denervated whiskers, which were cytoarchitectonically altered, showed a lower binding level of [^{3}H]QNB than in adjacent rows (Fig. 3*B*). Sensory deprivation accomplished by plucking out the vibrissae did not produce any changes in the binding pattern or intensity (Fig. 3*A*). Similarly, denervation of rows of vibrissae performed in two month old animals did not produce changes in the pattern and density of labeling of the barrel field.

AChE histochemistry. In the normal barrel field the AChE staining revealed a punctuated pattern, corresponding to the barrels, in the upper part of layer IV and a reverse-image pattern of staining, showing only the walls of the barrels in the lower part of layer IV (Fig. 4*A* and *C*). An intermediate pattern was also found in between cortical layers IVa and IVb (Fig. 4*B*).

Neonatal denervation lowered AChE staining in the affected rows of barrels (Fig. 5*A*). Removal of rows of vibrissae either at two months of age, or plucking out daily from birth, produced no effects on either pattern or intensity AChE staining (Fig. 5*B*).

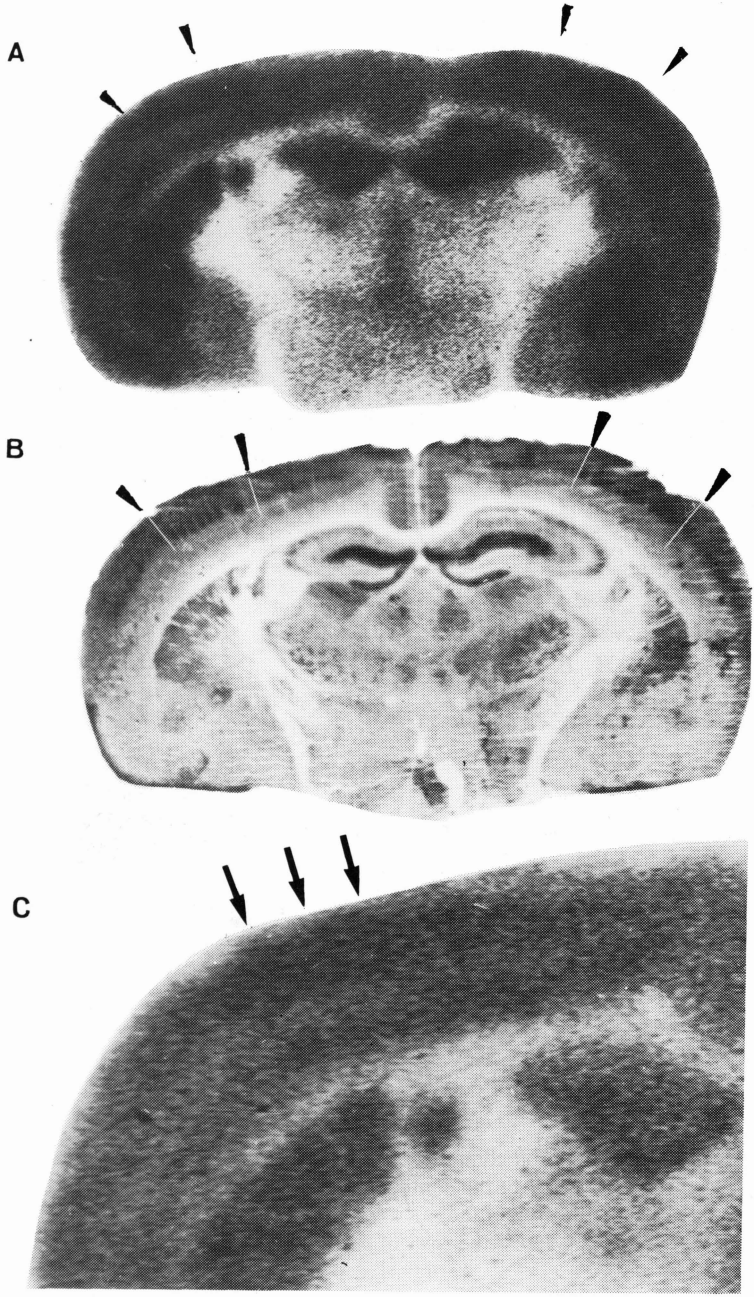

Fig. 1. MChRs distribution on a coronal section of mouse brain. A computer – digitized autoradiogram showing a three-laminar pattern of [^{3}H]QNB binding in the cortex. Arrows indicate the position of the barrel field; small barrels on the left side and large barrels of the postero-medial-barrel-subfield on the right side. Note the high density of labeling in the barrel field. *B*, SDH staining of adjacent section, barrel field indicated by arrows. *C*, enlarged fragment of *A*, barrels indicated by arrows, note patches of higher density in layer VI. Scale bar, 1000 μm.

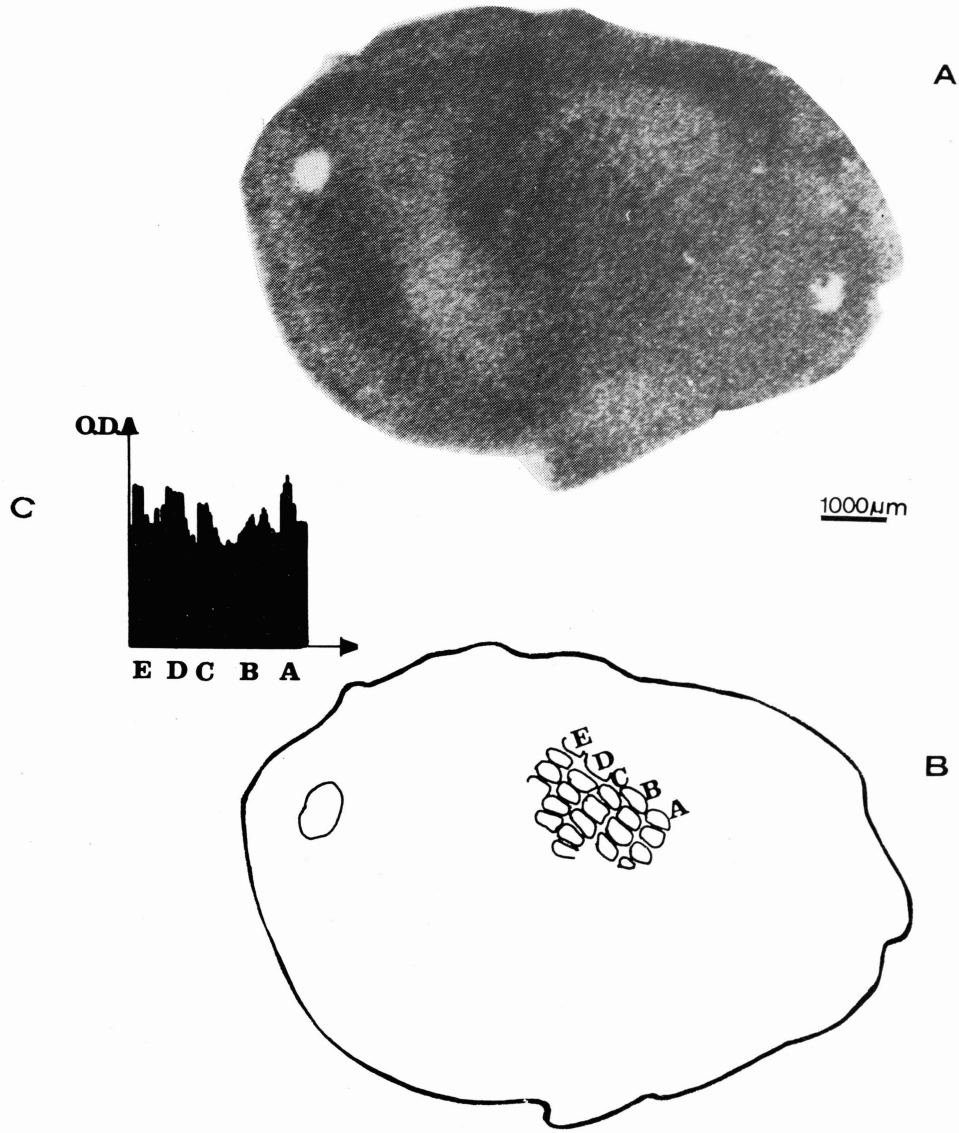

Fig. 2. *A*, MChRs distribution on computer – digitized autoradiogram of a section tangential to the barrel field through layer IV of the control hemisphere. Rows of barrels A–E marked on the picture. *B*, Camera lucida drawing of the same section stained with cresyl violet. *C*, densitometric profile of [^{3}H]QNB labeling across the rows of barrels, documenting the periodic arrangement of optical density.

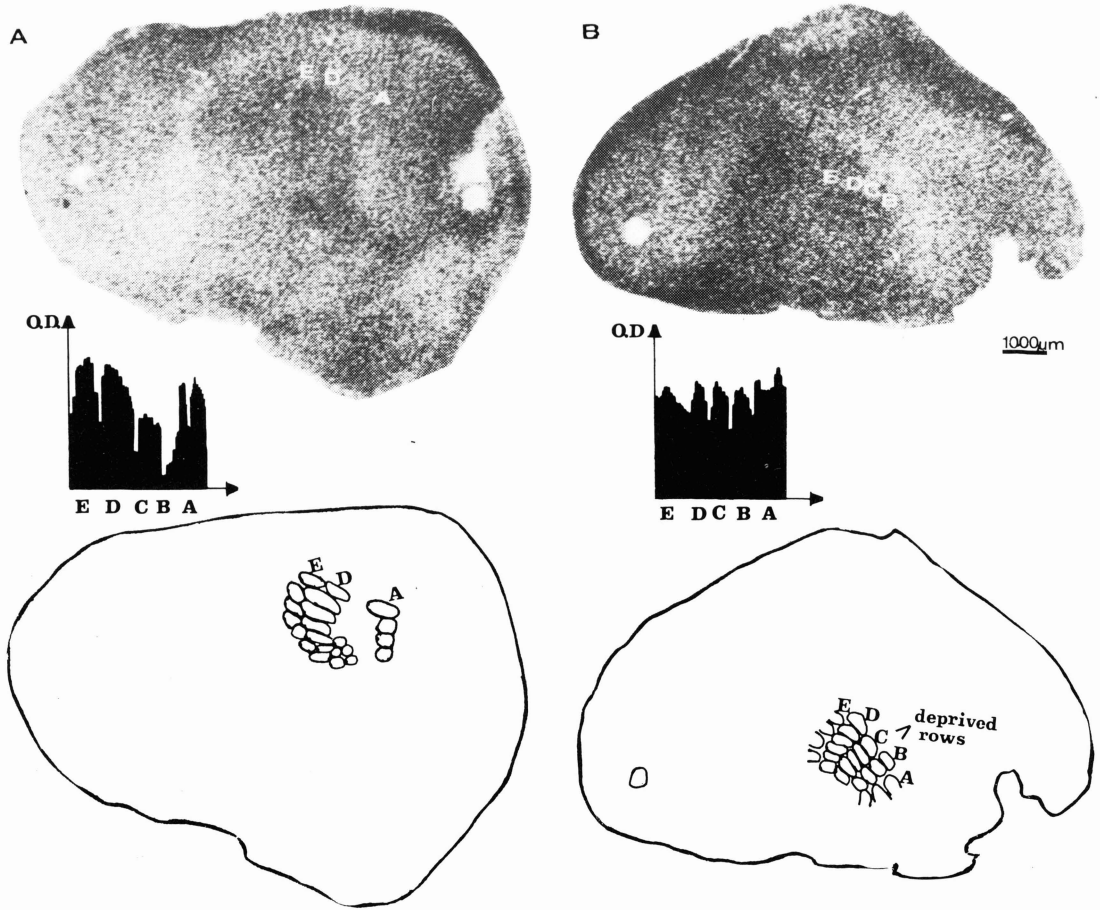

Fig. 3. Comparison of [^{3}H] QNB binding to tangential sections through layer IV of denervated and deprived barrel field; computer – digitized autoradiograms, camera lucida drawings of the same sections stained with cresyl violet and optical density profiles across the rows of barrels. *A*, effects of sensory deprivation and *B*, of neonatal denervation of rows *B* and *C*.

DISCUSSION

The pattern of distribution of [^{3}H]QNB binding and AChE staining suggests a strong connection with arrangement of the barrels. The density of MChRs distribution appeared to vary among the different cortical areas (25). The pattern of [^{3}H]QNB binding usually shows a three-laminar pattern with the lowest density in the middle lamina. In the visual cortex of kittens the low density band of labeling corresponds to layer IV and V (35) and in monkey to layer IVca (25). In the rat cortex the lowest [^{3}H]QNB binding was found in layer IV (21). In the somatosensory cortex of mice it was found to be situated in layer V. Interestingly, we observed that in rodents (21, 36) the [^{3}H]QNB labelel in layer VI is well defined in comparison with that observed in primates (25). These differences may reflect variations in the distribution of intracortical and afferent connectivity.

In the barrel field of rodents the distribution of a number of different biochemical markers mimicks the cytoarchitectonic pattern (3, 22, 30). For example [^{3}H] muscimol, an agonist of the GABA A receptor sites, binds preferentially to the barrel hollows in mice (2). The pattern of [^{3}H]QNB binding observed in the present study seems to be less pronounced than the [^{3}H] muscimol binding. It is more difficult to delineate individual barrels and it was not possible to distinguish between sides and hollows. We made similar observation while studying GABA receptors and cholinergic muscarinic receptors in barrel field of the rat (36). Possibly, GABA receptive elements are concentrated within the area of termination of the ventrobasal nucleus projection (barrel centre), while the cholinoceptive ones are more dispersed. The pattern of high labeling density in the centers of the barrels is not due to the quenching of tritium emission by the septa and barrel walls, as shown in the studies of quenching in the barrel field by Chmielowska et al. (2).

We found decreases in the binding density of [^{3}H]QNB only following neonatal denervation, when the plastic changes involved alterations of cytoachitecture (33). Denervation performed in adult animals, which is known to produce changes in metabolic activity (12), and sensory deprivation which causes changes in neuronal responsiveness (34) and metabolic activity (11), neither result in cytoarchitectonic changes, nor do they affect the level of QNB binding. Other studies of changes of MChR after manipulation of sensory input showed only transient changes of [^{3}H]QNB binding in the visual cortex of the rat (28, 31), or found no change at all in these receptor sites (23). In the somatosensory cortex Sampson et al. (29) reported that amputation of a digit in opossum did not alter [^{3}H]QNB binding in the forepaw projection cortex.

The experiments of Prusky et al. (24) on the rat visual cortex showed that the elimination of afferent input affected nicotinic rather than muscarinic cholinergic receptors, possibly because of their presumed localization on the thalamocortical terminations. Electrophysiological studies of Lamour and Dykes (17) found that following deafferentation of the hindlimb in rat, the proportion of cells affected by ACh is not markedly different from normal, although the reactivity to ACh is reduced in layers V and VI, but enhanced in the upper cortical layers.

Acetylcholinesterase staining is known to produce a distinct barrel-like pattern in the cortex of immature mice and rats (13, 30). In animals older than 21 days this pattern fades, but is still apparent to some extent. Kristt (13) described the staining of a lattice corresponding to the periphery of barrels in deep aspects of layer IV, and preservation of some staining in the centers of the barrels in the upper part of layer IV. Here AChE staining of the centers of the barrels was found in the lower part of layer IV. We did not observe the lighter than the surrounding cortex areas corresponding to the barrel centers, like those shown by Sandell (30).

Neonatal deprivation and denervation of vibrissae of adult animals did not produce changes in the pattern and intensity of AChE staining. Neonatal denervation, however, altered the pattern of staining in a manner corresponding to the cytoarchitectonic alteration induced in the barrel field. The deafferented rows of barrels showed lower AChE staining than the adjacent, expanded rows of barrels, and no barrel-like pattern was discernible in the affected rows. In immature animals Kristt (14) reported a loss of the punctuate pattern of AChE staining over a row of barrels altered by neonatal vibrissal removal, although he did not comment on the intensity of the staining over that row. In the visual cortex of rats, neonatal enucleation or blocking of the optic nerve activity by tetrodotoxin lowered the AChA staining in the monocular part of the visual cortex (27). In both of the above described cases the brains were examined in young animals, during the period of transiently high AChE activity; it is not known if these effects persist untill adulthood. Another neurotransmission linked enzyme, glutamic acid decarboxylase (GAD) reacted differently to deafferentation of SI cortex. Welker et al. (41) reported an increase of GAD immunoreactivity after denervation of rows of vibrissal follicles in adult mice. These changes were, however, observed only for a few days after denervation. It is possible that with longer survival times the denervated cortex is taken over by sensory projections from adjacent hairs and skin (22, 40), and the initial imbalance in transmitters linked with sensory information processing

is compensated for. We shall presently examine the changes in muscarinic receptor binding immediately after denervation.

In conclusion, we obtained lasting changes in the two cholinergic markers examined only when the neonatal lesion of vibrissal receptors produced reorganization of cytoarchitectonic structure of layer IV. These results show clearly the close relationship between distribution of cholinergic markers, and the morphological structure of the cortex. Partial denervation of an adult barrel field, or sensory deprivation, did not produce long lasting effects, showing abnormal afferent activity does not automatically affect MChRs or AChE. Further experimentation is needed to ensure that this lack of effect is not due to functional reinnervation of the cortex by the adjacent intact sensory inputs.

The authors wish to thank professor Wolf Singer for [^{3}H]QNB and Ultrofilm and dr Michael Stewart for ethopropazine and corrections of the manuscript. This investigation was supported by the Project CPBP 04.01 of the Polish Academy of Sciences.

REFERENCES

1. BEAR, M. F. and SINGER, W. 1986. Modulation of visual cortical plasticity by acetylcholine and noradrenaline. Nature. 320: 172-176.
2. CHMIELOWSKA, J., STEWART, M. G. and BOURNE, R. C. 1987. Autoradiographic localization of aminobutyric acid receptors in mouse barrel field. Brain Res. 425: 283-289.
3. DIETRICH, W. D., DURHAM, D., LOWRY, O. H. and WOOLSEY, T. A. 1981. Quantitative histochemical effects of whisker damage on single identified cortical barrels in the adult. J. Neurosci. 9: 929-935.
4. FLICKER, C., DEAN, R. L., FISCHER, S. K. and BARTUS, R. T. 1983. Behavioral and neurochemical effects following neurotoxic lesions of a major cholinergic input to the cerebral cortex in the rat. Pharmacol. Biochem. Behav. 18: 973-981.
5. GEIGER, H. and SINGER, W. 1986. A possible role of calcium currents. *In* U. Heihemann, M. Klee, E. Neher and W. Singer (ed.), Developmental plasticity. Springer-Verlag, Berlin, p. 256-270.
6. GREENFIELD, S. 1984. Acetylcholinesterase may have novel functions in the brain. Trends Neurosci. 7: 364-368.
7. GU, Q. and SINGER, W. 1988. Blockade of muscarinic receptors prevents ocular dominance plasticity of kitten striate cortex. ENA (Abstr.) Vol. 11, 73: 3.
8. HEPLER, D. J., WENK, G. L., CRIBBS, B. L., OLTON, D. S. and COYLE, J. T. 1985. Memory impairments following basal forebrain lesions. Brain Res. 346: 8-14.
9. HOUSER, C. R., CRAWFORD, G. D., SALVATERRA, P. M. and VAUGHN, J. E. 1985. Immunocytochemical localization of choline acetyltransferase in rat cerebral cortex: a study of cholinergic neurons and synapses. J. Comp. Neurol. 234: 17-34.

10. KOELLE, G. B. and FRIEDENWALD, J. S. 1949. A histochemical method for localizing cholinesterase activity. Proc. Soc. Exp. Biol. Med. 70: 617-622.
11. KOSSUT, M. 1985. Effects of sensory denervation and deprivation on a single cortical vibrissal column studied with 2-deoxyglucose. Physiol. Bohemoslov. 34: 70-83.
12. KOSSUT, M., HAND, P. J., GREENBERG, J. and HAND, C. 1988. Single vibrissal cortical column in SI cortex of rat and its alterations in neonatal and adult vibrissae deafferented animals — a quantitative 2 DG study. J. Neurophysiol. 60: 829-852.
13. KRISTT, D. A. and KASPER, E. K. 1983. High density of cholinergic muscarinic receptors accompanies high intensity acetylcholinesterase-staining in layer IV of infant rat somatosensory cortex. Dev. Brain Res. 8: 373-376.
14. KRISTT, D. A. 1987. Acetylcholinesterase in the cortex. *In* E. G. Jones and A. Peters (ed.), Cerebral cortex. Vol. 6, p. 187-236.
15. KRISTT, D. A. 1989. Acetylcholinesterase in immature thalamic neurons: relation to afferentation, development, regulation and cellular distribution. Neurosci. 29: 22-44.
16. LAMOUR, Y., DUTAR, P. and JOBERT, A. 1982. Excitatory effect of acetylcholine on different types of neurons in the first somatosensory neocortex of the rat: laminar distribution and pharmacological characteristics. Neurosci. 7: 1483-1494.
17. LAMOUR, Y. and DYKES, R. W. 1988. Somatosensory neurons in partially deafferented rat hindlimb granular cortex subsequent to transection of the sciatic nerve: effects of glutamate and acetylcholine. Brain Res. 449: 18-33.
18. LEVAY, A. I., HALLANGER, A. E. and WAINER, B. H. 1987. Cholinergic nucleus basalis neurons may influence the cortex via the thalamus. Neurosci. Lett. 74: 7-13.
19. MESULAM, M. M., MUFSON, E. J., WAINER, B. H. and LEVAY, A. I. 1983. Central cholinergic pathways in the rat: An overview based on an alternative nomenclature (Ch 1-Ch 6). Neuroscience 10: 1185-1201.
20. METHERATE, R., TREMBLAY, N. and DYKES, R. W. 1988. The effects of acetylcholine on response properties of cat somatosensory cortical neurons. J. Neurophysiol. 59: 1231-1252.
21. NONAKA, R. and MOROJI T. 1984. Quantitative autoradiography of muscarinic cholinergic receptors in the rat brain. Brain Res. 296: 295-303.
22. PIDOUX, B., DIEBLER, M. F., SAVY, Cl., FARKAS, E. and VERLEY, R. 1980. Cortical organization of the postero-medial barrel-subfield in mice and its reorganization after destruction of vibrissal follicles after birth. Neuropathol. Appl. Neurobiol. 6: 93-107.
23. PRUSKY, G. T., SHAW, C. and CYNADER, M. S. 1987. Nicotine receptors are located on lateral geniculate nucleus terminals in cat visual cortex. Brain Res. 412: 131-138.
24. PRUSKY, G. T., ARBUCKLE, J. M. and CYNADER, M. S. 1988. Transient concordant distributions of nicotinic receptors and acetylcholinesterase activity in infant rat visual cortex. Dev. Brain Res. 39: 154-159.
25. RAKIC, P., GOLDMAN-RAKIC, P. S. and GALLAGER, D. 1988. Quantitative autoradiography of major neurotransmitter receptors in the monkey striate and extrastriate cortex. J. Neurosci. 8 (10): 3670-3690.

26. ROBERTSON, R. T. 1987. A morphogenic role for transiently expressed acetylcholinesterase in developing thalamocortical systems? Neurosci. Lett. 75: 259-264.
27. ROBERTSON, R. T., AMBE, R. K. and YU, J. 1989. Intraocular injections of tetrodotoxin reduce transiently expressed acetylcholinesterase activity in the visual cortex. Dev. Brain. Res. 46: 69-84.
28. ROSE, S. P. R. and STEWART, M. G. 1978. Transient increase of muscarinic acetylcholine receptor and acetylcholinesterase in visual cortex on first exposure of dark reared rats to light. Nature 271: 169-170.
29. SAMPSON, S. M., SHAW, C., WILKINSON, M. and RASMUSSON, D. D. 1988. Sensory deafferentation fails to modify muscarinic receptor binding in raccoon somatosensory cortex. Brain Res. Bull. 20: 597-602.
30. SANDELL, J. H. 1983. The distribution of hexokinase compared to cytochrome oxidase and acetylcholinesterase in the somatosensory cortex and the superior colliculus of the rat. Brain Res. 290: 384-389.
31. SCHLIEBS, R., BIGL, V. and BIESOLD, D. 1982. Development of muscarinic cholinergic receptor binding in the visual system of monocularly deprived dark reared rats. Neurochem. Res. 7: 1181-1197.
32. SHAW, C. J., NEEDLER, M. C. and CYNADER, M. 1984. Ontogenesis of muscarinic acetylcholine binding sites in cat visual cortex: reversal of laminar specific distribution during the critical period. Dev. Brain. Res. 14: 295-299.
33. SIMONS, D. J., DURHAM, D. and WOOLSEY, T. A. 1984. Functional organization of mouse and rat SmI barrel cortex following vibrissal damage on different postnatal days. Somatosensory Res. 1: 207-245.
34. SIMONS, D. J. and LAND, P. W. 1987. Early experience of tactile stimulation influences organisation of somatic sensory cortex. Nature 326: 694-697.
35. SKANGIEL-KRAMSKA, J., CYMERMAN, U. and KOSSUT, M. 1986. Autoradiographic localization of GABAergic and muscarinic cholinergic receptor sites in the visual system of the kitten. Acta Neurobiol. Exp. 46: 119-130.
36. SKANGIEL-KRAMSKA, J. and KOSSUT, M. 1989. The distribution of $GABA_A$, muscarinic and $5\text{-}HT_1$ receptor sites in the barrel field of rat somatosensory cortex. J. Neurochem. 52: (Suppl.) S 144.
37. STROMINGER, R. M. and WOOLSEY, T. A. 1987. Templates for locating the whisker area in fresh flattened mouse and rat cortex. J. Neurosci. Meth. 22: 113-118.
38. Van der LOOS, H. and WOOLSEY, T. A. 1973. Somatosensory cortex: structural alterations following early injury to sense organs. Science 179: 395-398.
39. Van HUIZEN, F., STROSBERG, A. D. and CYNADER, M. S. 1988. Cellular and subcellular localisation of muscarinic acetylcholine receptors during postnatal development of cat visual cortex using immunocytochemical procedures. Dev. Brain Res. 44: 296-301.
40. WAITE, P. M. E. and TAYLOR, P. K. 1978. Removal of whiskers in young animals causes functional changes in cerebral cortex. Nature 274: 600-604.
41. WELKER, E., SORIANO, E. and Van der LOOS, H. 1989. Plasticity in the barrel cortex of the adult mouse: effects of peripheral deprivation on GAD-immunoreactivity. Exp. Brain Res. 74: 441-452.

42. WELLER, W. L. and JOHNSON, J. I. 1975. Barrels in cerebral cortex altered by receptor disruption in newborn, but not in five-day-old mice (Cricetidae and Muridae). Brain Res. 83: 504-508.
43. WENK, H., BIGL, V. and MEYER, U. 1980. Cholinergic projection from magnocellular projections nuclei of the basal forebrain to cortical areas in rats. Brain Res. Rev. 2: 295-316.
44. WHITEHOUSE, P. J., PRICE, D. L., STRUBLE, R. G., CLARK, A. W., COYLE, J. T. and De LONG, M. R. 1982. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science 215: 1237-1239.
45. WOOLSEY, T. A. and Van der LOOS, H. 1970. Structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res. 17: 205-242.