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NADPH diaphorase neurones are evenly distributed throughout cat neocortex irrespective of functional specialization of each region

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USING NADPH diaphorase histochemistry as a marker for nitric oxide synthase we investigated the possible sites of nitric oxide synthesis in cat cerebral neocortex. Intensely stained neurones were found mainly in the deep layers of the neocortex and underlying medulla. Virtually all neurones in the cerebral medulla were NADPH diaphorase positive. The density of diaphorase neurones was estimated in the cortex/medulla border zones of each neocortical gyrus. Diaphorase neurones were evenly distributed throughout the neocortex and no significant statistical difference between gyri was observed. These findings indicate that the density of diaphorase neurones is irrespective of functional specialization of each region and are more in line with the hypothesis that NADPH diaphorase neurones are involved in the control of local cortical blood flow.

Key words: NADPH diaphorase; Nitric oxide; NO synthase; Cortical blood flow; Local circulation; Cerebral cortex; Cerebral medulla; Blood vessels; Vascular control; Cat

Introduction

The cerebral cortex shows noticeable heterogeneity of blood flow and metabolism. Changes in the amount of blood flowing in areas of the cerebral cortex are usually taken as a reflection of changes in the local neural functional activity of those areas.[1] Recent evidence indicates that nitric oxide (NO) is a potent vasodilator that plays an important role in the regulation of blood flow in a variety of vascular beds.[2] In the brain, some neurones contain nitric oxide synthase (NOS), enabling them to synthesize NO. NOS-positive processes lie on the wall of the cerebral arterioles and capillaries.[3] These facts suggest the possibility of neural control of cortical blood flow by the NOS neurones, mediated by the action of NO. If NOS neurones are involved in local cerebral neocortical circulation, they must be distributed throughout the neocortex irrespective of the functional specialization of each region.

Nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase is known to be a cofactor of NOS in the mammalian nervous system.[4,5] NADPH diaphorase histochemistry therefore provides a specific histochemical marker for neurones producing NO. We investigated the possible sites of NO synthesis in the cerebral neocortex using NADPH diaphorase histochemistry, and evaluated the mean number of diaphorase cells in each neocortical gyrus in order to estimate the relationship between cell density and neocortical functional localization.

Materials and Methods

Seven adult cats weighing 2.1–3.6 kg were anaesthetized with ketamine hydrochloride (20–40 mg kg^{-1}, i.m.) and pentobarbital (20–30 mg kg^{-1}, i.p.) and perfused transcardially with 800 ml of heparinized saline followed by 2000 ml of freshly prepared 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. Brains were removed, placed in the same fixative for 4 h at 4°C and then placed in 30% sucrosed buffer for 1–2 days. The brains were cut into blocks which were sectioned in the coronal and the sagittal planes, at 50 μm thickness, on a freezing microtome. The free-floating sections were incubated in 0.1 M phosphate buffer (pH 7.4) containing 0.3% Triton X-100, 0.5 mM $MgCl_2$, 0.01 M sodium azide, 0.1% nitroblue tetrazolium and 0.1–0.15% β-NADPH (Sigma) at 37°C for 30–120 min. Following the reaction, the sections were rinsed in phosphate buffer, pH 7.4, and mounted onto chrome–alum coated slides. The slides were air-dried and treated in chloroform for 2 h to remove background staining. Some were then counterstained with neutral red. Using a light microscope at a magnification of 100× equipped with an ocular grid, the number of NADPH diaphorase intensely labelled neurons mm^{-2} was counted at the cortex/medulla border zones of each gyrus (sigmoidal, coronal, ectosylvian, sylvian, lateral and suprasylvian gyri). For comparison, cells were also counted in the mesocortex (cingular gyrus). The mean cell numbers and standard deviations of 688

areas from seven cats were calculated. Comparisons among gyri were statistically evaluated by Ryan's multiple comparison method. The nomenclature of gyri and sulci and boundaries of the areas was based on those of Papez,[7] Sanides and Hoffmann,[8] Hassler and Muhs-Clement[9] and Heath and Jones.[10]

Results

NADPH diaphorase-positive cells were mainly observed in the deep layers of the cortex and underlying white matter (Fig. 1A–C). Virtually all neurones in the white matter were NADPH diaphorase-positive. The network of NADPH diaphorase processes was evenly dispersed throughout the neocortex, and diaphorase neurones were occasionally found in the callosal body (Fig. 1D). The thalamus had extremely few NADPH diaphorase-positive neurones.

About 800–1200 cells were counted on the slides for each animal. Estimation of the number of NADPH diaphorase-positive neurones per unit area showed that all the neocortical gyri had more positive cells than the mesocortex (cingular gyrus; Fig. 2). However, no significant differences in the number of neurones were observed.

The anterior and lateral walls of the crucial sulcus (anterior and lateral sigmoid gyri) contain a motor area, while the somatosensory area is located in the posterior wall (posterior sigmoid gyrus).[9] Cell density in the motor and somatosensory areas was 12.0 ± 1.9 mm^{-2} and 12.8 ± 2.6 mm^{-2}, respectively. The somatosensory area in the anterior portion of the lateral gyrus and the visual association area[10] in the posterior portion had a cell density of 10.6 ± 1.9 mm^{-2} and 10.8 ± 2.2 mm^{-2}, respectively. The cell density of the somatosensory association area at the rostral level of the suprasylvian gyrus and the more caudal visual association area[10] was 10.6 ± 2.0 mm^{-2} and 10.7 ± 2.3 mm^{-2}, respectively. No statistically significant differences in the number of neurones were found between the two functional cortical areas within each gyrus.

Discussion

The present study shows that NADPH diaphorase-positive neurones are evenly distributed throughout

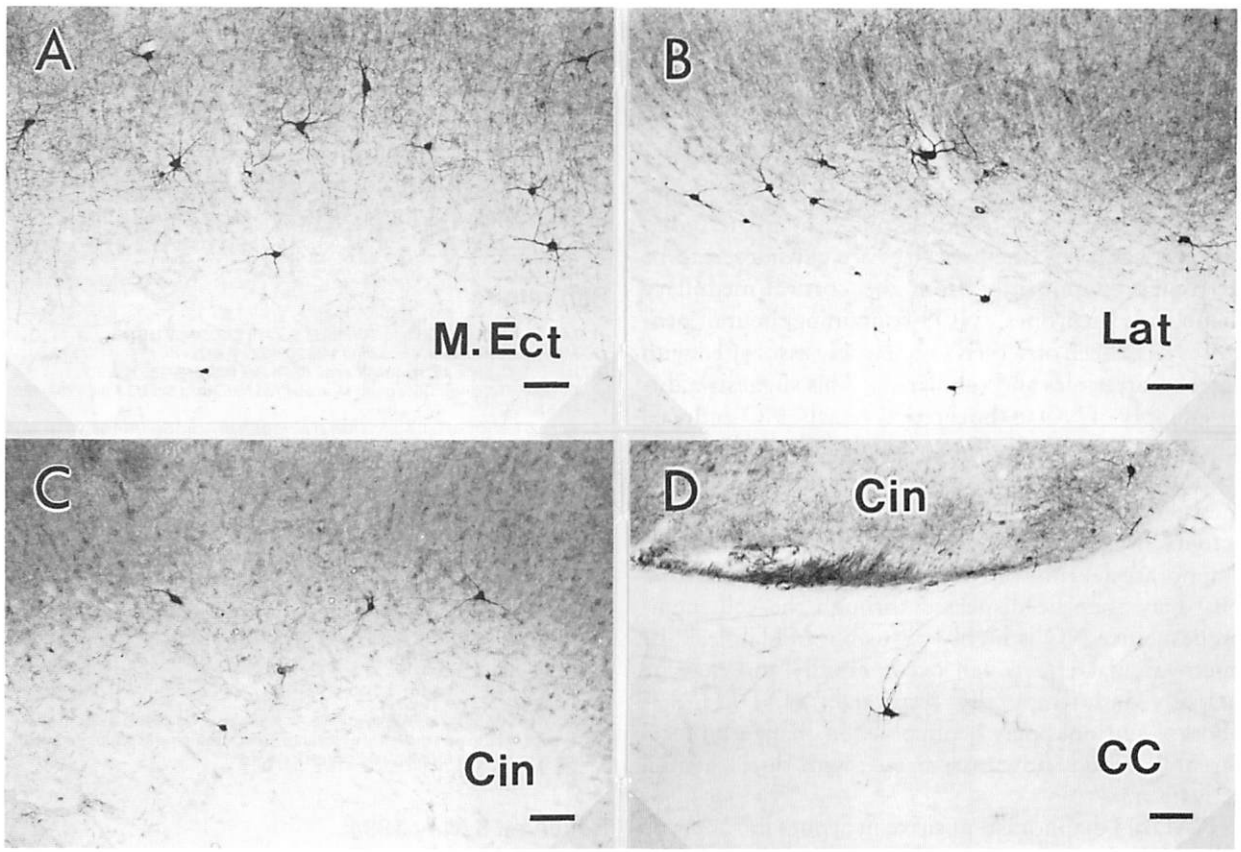

FIG. 1. (**A–C**) photomicrograph showing the distribution of neurones intensely labelled for NADPH diaphorase in the middle ectosylvian gyrus (M.Ect: A), lateral gyrus (Lat: B) and cingular gyrus (Cin: C). Note that NADPH-positive cells are located mainly in the cortex/medulla border zones and that cell density in the cingular gyrus is lower than in the neocortices. (**D**) NADPH diaphorase neurones in the corpus callosum (CC) underlying the cingular gyrus (Cin). Bars = 100 μm.

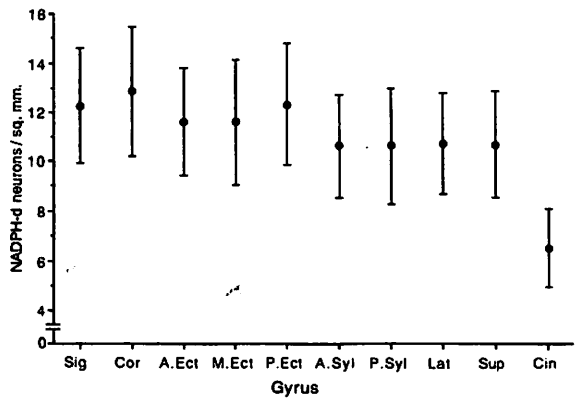

FIG. 2. The density of neurones intensely stained for NADPH diaphorase in each gyrus. The number of NADPH diaphorase neurones mm^{-2} in each gyrus is expressed as the mean ± s.d. averaged from sections from seven cats. Sig, sigmoid gyrus; Cor, coronal gyrus; A.Ect, anterior ectosylvian gyrus; M.Ect, middle ectosylvian gyrus; P.Ect, posterior ectosylvian gyrus; A.Syl, anterior sylvian gyrus; P.Syl, posterior sylvian gyrus; Lat, lateral gyrus; Sup, suprasylvian gyrus; Cin, cingular gyrus. Note that the density of neurones per unit area is higher in the neocortical gyri than in the mesocortical gyrus (cingular gyrus).

the neocortical gyri and functional areas. There was no correlation between cell density and functional localization of neocortical areas, suggesting that the diaphorase neurones are not involved in cortical functions such as visual, auditory, somatosensory, motor, or associative functions. The diaphorase neurones in the callosal body also seem to be unrelated to such functions.

This study also showed that network of NADPH diaphorase processes is evenly dispersed throughout the neocortex. Since the thalamus contains few diaphorase neurones, the processes are considered to be derived predominantly from the cortical/medullary diaphorase neurones. NOS-containing neural processes have been observed to be closely associated with cerebral arterioles and capillaries.[3] This suggests a diffuse supply of NO to the cerebral vessels. NO-induced elevations in cyclic GMP may produce smooth muscle relaxation[11] and may influence endothelial permeability.[12] It is conceivable that changes in local activity in areas of cerebral neocortex may influence diaphorase neurone activity within those areas and that NO may then be dispersed through the cell membranes. Since NO is highly diffusible and labile,[2,13] its microvascular effects can occur rapidly and may be spatially and temporally restricted.[3] NADPH diaphorase neurones may be involved in changes in local cerebral blood flow associated with local neural activation.

NADPH diaphorase-positive neurones in subcortical white matter have already been described in the cat.[14] Little attention has been directed to these neurones in the rat, a species in which they are very few in number.[6] In higher mammalian species such as the monkey, the density of the diaphorase neurones in the white matter is much higher,[15] as in the cat. The present study showed that the cell density in the cat mesocortex is slightly lower than in the neocortices. It is tempting to speculate that NADPH diaphorase neurones may increase in number as the development of neocortical functional localization proceeds.

The present study indicates that virtually all neurones in the subcortical white matter are NADPH diaphorase positive. The significance of such locations in the white matter remains obscure.

Conclusion

Neurones intensely stained for NADPH diaphorase were observed predominantly in the deep layers of the cat cerebral neocortex and in the underlying white matter. Virtually all neurones in the white matter were NADPH diaphorase positive. NADPH diaphorase-positive processes were evenly dispersed throughout the neocortical layers and seemed to be chiefly derived from these diaphorase neurones. The present study also indicates that NADPH diaphorase neurones are distributed evenly throughout the cerebral neocortex. This indicates that there is no correlation between cell density and function of neocortical areas, suggesting that the diaphorase neurones are unrelated to the cortical functional specialization of each region. It is suggested that NADPH diaphorase neurones are involved in the control of local cortical blood flow.

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