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Monoamine oxidase activity and distribution in marmoset brain: implications for MPTP toxicity

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This study describes the histochemical localisation of monoamine oxidase (MAO) in marmoset brain. No MAO A staining was observed but MAO B was found in many areas, with medium intensity of staining in the substantia nigra, and high intensity staining in the striatum and nucleus accumbens, among other regions. The high activity in the nigrostriatal tract, compared with the rat, may partially explain the greater sensitivity of the marmoset to MPTP toxicity. As in the rat, high activity was present in the raphe nuclei. However, unlike the rat, no enrichment of MAO B was observed in blood vessels or ventricular linings.

Recent histochemical and immunocytochemical studies have shown the two forms of monoamine oxidase (MAO A and B) to be highly localised in particular cells in the brain [8, 17]. There appear to be certain differences in both the nature and distribution of the two forms in different species [3, 4]. It is of particular interest to compare the nature and distribution of MAO in rat and marmoset brain, because of their different responses to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP is a neurotoxin that causes a parkinsonian-like syndrome in primates [2], including the marmoset [6], with degeneration in the substantia nigra and a permanent behavioural syndrome. Rats [2] are much less sensitive to its action and, at high doses, show only some reversible depletion of dopamine in the striatum. MPTP itself is not toxic but is converted to its toxic metabolite, MPP⁺, by MAO B [7, 11]. It is thus relevant to determine the extent to which differences in the nature or distribution of MAO B could explain the species difference in response to MPTP administration.

In this paper, we report the first study of the histochemical localisation of MAO

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B in marmoset brain. The method [13] employs frozen sections, no fixative, tyramine and benzylamine as substrates and selective concentrations of clorgyline and deprenyl to distinguish A and B enzyme forms. It gives maximal activity, at least for MAO B, but less than optimal cellular resolution. It is considerably more sensitive than immunocytochemical methods but, unlike them, cannot be used conclusively to distinguish localisation in particular cell types but only in brain regions. The method has already been used to map the distribution of MAO A and B in several tissues, including rat brain [18].

Three adult female marmosets were anaesthetised with pentobarbitone (5 mg/100 g b. wt.) and killed by decapitation. The brains were rapidly removed from the skulls and placed on ice cooled plates. Each brain was then divided coronally and frozen on dry ice, to be stored at -20°C . Sections (30 μm) were then prepared from frozen unfixed brain tissue using a cryostat. They were mounted on glass slides and left at room temperature for 30 min prior to incubation. The incubation medium contained 0.05 M sodium phosphate buffer, pH 7.6, 3-amino-9-ethylcarbazole (1 mM), dimethylformamide (5%), peroxidase Type II Sigma (500 U/ml), and either tyramine (as free base, 7 mM) or benzylamine (8 mM). The incubation medium was prepared by dissolving 3-amino-9-ethylcarbazole in dimethylformamide, followed by the addition of sodium phosphate buffer, mixing, filtering and finally adding peroxidase and a substrate. Slides were immersed in this medium (modified for controls by the absence of substrate) and placed in a covered water bath at 37°C for 5 h. Some sections were preincubated in sodium phosphate buffer (0.05 M, pH 7.6) containing either 10^{-7} M clorgyline, 10^{-6} M (–)-deprenyl or both, for 30 min at room temperature. After incubation in the carbazole containing medium, sections were washed in 0.9% (w/v) NaCl solution, postfixed for 2 h in 10% formalin and mounted in glycerol jelly. All sections were compared with adjacent ones stained with Cresyl violet for histological orientation. For areas of especial interest, such as the substantia nigra, adjacent sections were superimposed using a slide projector. Material from one marmoset was stained for tyrosine hydroxylase activity using an antibody supplied by Eugene Tech USA. For anatomical reference, the marmoset atlas of Stephan et al. [15] was used.

For the rat study, animals were killed by cervical dislocation. Otherwise similar procedures to those discussed above, were used to prepare sections from substantia nigra and striatum.

For direct assay of MAO *in vitro*, homogenates (10% w/v) were prepared from the cortex of 4 marmoset brains and 5 rat brains using 100 mM sodium phosphate buffer, pH 7.4. MAO A was assayed using 300 μM [^{14}C]5-hydroxytryptamine and MAO B with 40 μM [^{14}C]phenylethylamine; 700 μM [^{14}C]tyramine was used to measure both MAO A and B. All radiochemicals were from Amersham International, Amersham. The procedures used were as described by Lewinsohn et al. [9]. Protein was assayed by the method of Lowry et al. [10]. Some brain homogenates were preincubated at room temperature for 20 min with clorgyline or deprenyl to inhibit either MAO A or MAO B activity respectively.

The histochemical study of the marmoset brain showed low activity of MAO B fairly evenly distributed throughout the brain, together with several areas of more

TABLE I

SUMMARY OF MAO B STAINING IN MARMOSET BRAIN

Staining intensity: + + + +, high; + + +, medium; + +, low to medium; +, low; -, no staining above even MAO B background. N, nucleus.

Area	Intensity	Area	Intensity
Caudate	+ + + +	N. paraventricularis thalami	+ + / + + +
Putamen	+ + + +	N. medialis dorsalis thalami	+
N. accumbens	+ + + +	N. centralis amygdalae	+ + / + + +
N. interpeduncularis	+ + + +	N. medialis amygdalae	+ + / + + +
N. interpeduncularis paramedianus	+ + + +	N. ventromedialis hypothalami	+ + / + + +
N. supratrochlearis	+ + + / + + + +	N. periventricularis hypothalami	+ / + + / + + +
N. raphe	+ + +	N. paraventricularis hypothalami	+ / + +
Substantia nigra compacta	+ + +	N. perifornicalis hypothalami	+
Substantia nigra reticulata	+ + +	Area dorsalis hypothalami	+
N. reticularis tegmenti pontis	+ + +	Cortex	-
N. centralis superior	+ + +	Lining of ventricles	-
N. ventralis tegmenti	+ +	Locus coeruleus	-
Substantia grisea centralis	+ + / + + +		

intense MAO B staining, shown in Table I. All the areas staining intensely with benzylamine also stained with tyramine but more faintly; all staining with both substrates was prevented by preincubation with 10^{-6} M (-)-deprenyl. Thus, all the staining observed was solely due to MAO B and no staining due to MAO A or other enzymes such as benzylamine oxidase or peroxidase was discernible.

Table I shows that the striatum (caudate and putamen) was one of the most intensely staining areas. The ventral tegmental area at the level of the third nerve and substantia nigra stained with medium intensity whilst the nucleus accumbens showed high activity. Staining in all these regions was diffuse. In other areas, notably the raphe nuclei, the interpeduncular nuclei, the nucleus supratrochlearis and the nucleus reticularis tegmenti pontis, the staining was in intense spots.

MAO B staining in the substantia nigra (shown in Fig. 1) was present in the pars compacta and reticulata. The pattern of staining (shown in Fig. 1A) did not correlate well with the density of neuronal populations within the substantia nigra, shown with tyrosine hydroxylase in Fig. 1C; indeed, some of the highest staining intensity was observed in the substantia nigra pars reticulata, an area with a low population of neuronal cell bodies. In general the staining was most intense close to the third nerve and decreased laterally. The high overall staining for MAO B in the marmoset nigrostriatal tract is in marked contrast to that of the rat. Fig. 2 shows that there was no staining above background for MAO B in the rat substantia nigra or striatum.

In order to try to understand the absence of MAO A staining with tyramine in the marmoset, both MAO A and B were measured in homogenates from rat and marmoset brain (Table II). It is of interest that the tyramine/PEA activity ratio for MAO

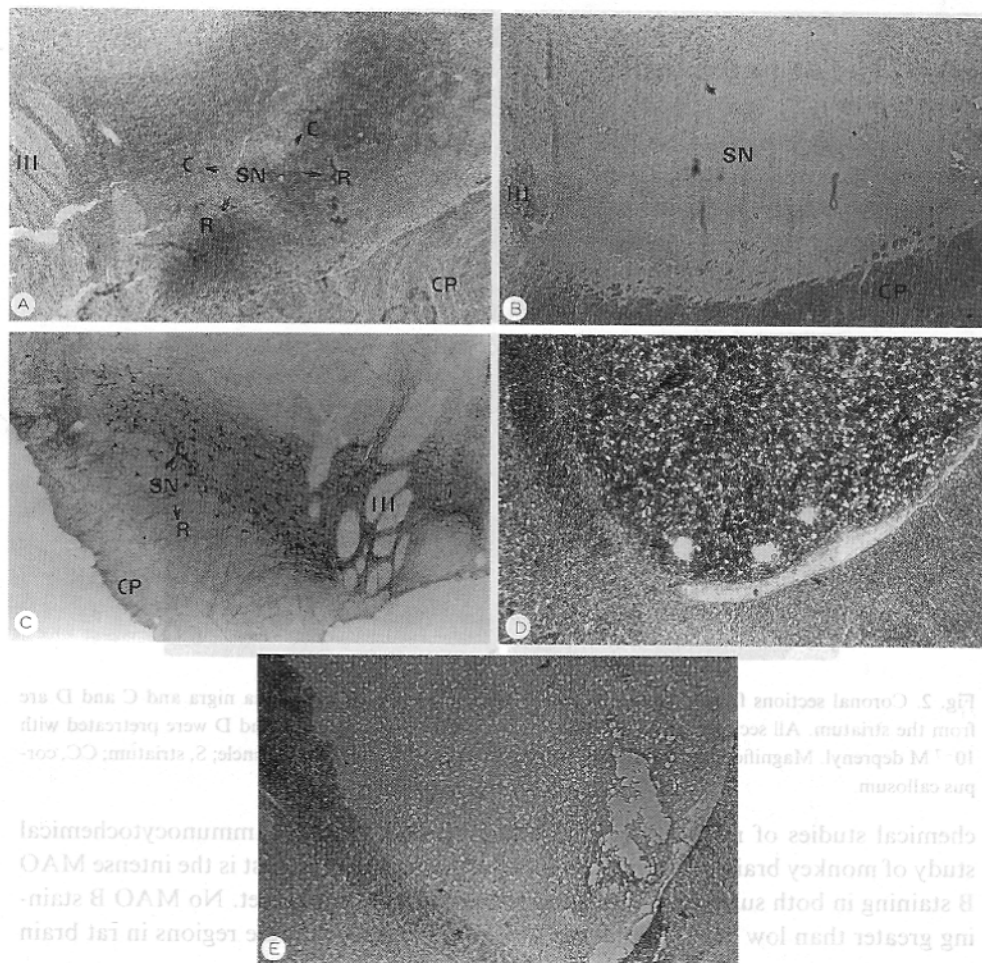


Fig. 1. Coronal sections from marmoset brain. A-D show substantia nigra at level of third nerve (III) and ventral tegmental area above the cerebral peduncle. A: substrate benzylamine. B: substrate, benzylamine but pretreated with 10^{-7} M deprenyl. C: stained for tyrosine hydroxylase. D and E: sections from the striatum with benzylamine as substrate. E had been pretreated with 10^{-7} M deprenyl. Magnification 1A, B, E and F = $\times 18.8$; 1C and D = $\times 12.4$. SN, substantia nigra; C, pars compacta; R, pars reticulata; CP, cerebral peduncle.

B appears greater for the marmoset than the rat, presumably indicating a species difference in the nature of the active site. Table II also shows that MAO A in the marmoset preparation measured with either tyramine or 5-hydroxytryptamine, had only about one quarter of the specific activity of that in the rat. Thus the activity in general was probably below the threshold of detection of the histochemical method. It is still notable, however, that no high spots of MAO A activity, such as in the locus coeruleus, were observed.

Using our current method then, we are only able to discuss the distribution of MAO B in the marmoset brain. However, several interesting differences from histo-

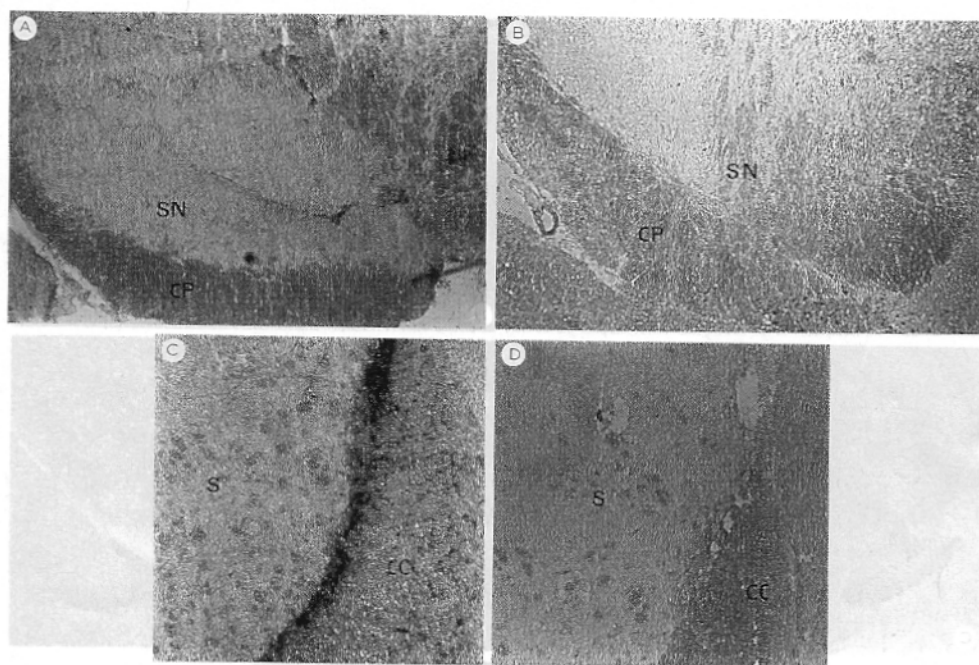


Fig. 2. Coronal sections from rat brain A and B are sections of the substantia nigra and C and D are from the striatum. All sections are stained with benzylamine as substrate; B and D were pretreated with 10^{-7} M deprenyl. Magnification $\times 18.8$. SN, substantia nigra; CP, cerebral peduncle; S, striatum; CC, corpus callosum.

chemical studies of rat brain (Fig. 2 and [18]) and from the immunocytochemical study of monkey brain performed by others emerge [17]. The first is the intense MAO B staining in both substantia nigra and striatum in the marmoset. No MAO B staining greater than low background was observed in either of these regions in rat brain

TABLE II
COMPARISON OF MONOAMINE OXIDASE (MAO) A AND B ACTIVITIES IN RAT AND MARMOSSET BRAIN

MAO activity was determined from the cortical homogenates of 5 rat brains and 4 marmoset brains. MAO substrates were [14 C]5-hydroxytryptamine (5-HT), [14 C]phenylethylamine (PEA) and [14 C]tyramine at 300 μ M, 40 μ M and 700 μ M respectively. Clorgyline and (-)-deprenyl were used at 10^{-7} M selectively to inhibit MAO A and MAO B respectively using tyramine as substrate.

MAO activity (nmol/min/mg protein) $\pm$ S.E.M.

Substrate:	5-HT		PEA		Tyramine	
	MAO A	MAO B	A/B	MAO A	MAO B	A/B
Rat	1.21 ± 0.06	0.59 ± 0.04	2/1	1.37 ± 0.11	0.73 ± 0.09	2/1
Marmoset	0.29 ± 0.06	0.35 ± 0.03	1/1	0.36 ± 0.06	1.04 ± 0.3	1/3

using either the present histochemical method or other methods, pointing to a real species difference [1, 8, 12, 18].

Thus, in the marmoset, where MPTP causes nigrostriatal degeneration, more MAO B is available in the general vicinity of where the toxic damage occurs. It remains a puzzle as to why the lateral cells are more susceptible to toxic damage than those adjacent to the third nerve. The marmoset ventral tegmental area and nucleus accumbens also contained high MAO B activity; whilst these regions are less affected by MPTP, they may show some degeneration [5, 14]. MAO B staining in all the dopamine-rich areas was diffuse. This finding probably indicates that the enzyme is located in fibres or glia, rather than neuronal cell bodies, which appear to stain as spots. If MAO B is extraneuronal, it suggests that, in the marmoset as in the rodent, MPP⁺ is formed outside the dopaminergic cell bodies, which then take it up.

The spotty distribution of staining in the raphe nuclei was similar to that observed in the rat and suggests that, here again, MAO B is present in 5-hydroxytryptamine-containing cell bodies. This is an observation that has also been made in monkey [17] and human brain [16] using immunocytochemical techniques.

Two further differences in MAO B distribution in rat and marmoset concern the lining of the ventricles and the corpus callosum. In the rat, the ventricular lining was one of the most intensely staining MAO B areas; but no staining greater than low background was found in the marmoset. In addition, the corpus callosum of the rat showed dense MAO B staining resembling the pattern of blood vessels. No such staining was observed in the marmoset. It is thus possible that MAO B plays a larger part in the blood-brain and brain-CSF barrier in the rat than in the marmoset.

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- 1 Arai, R., Kimura, H. and Maeda, T., Topographic atlas of monoamine oxidase-containing neurons in the rat brain studied by an improved histochemical method, *Neuroscience*, 19 (1986) 905-925.
- 2 Chiueh, C.C., Markey, S.P., Burns, R.S., Johannessen, J.N., Jacobowitz, D.M. and Kopin, I.J., Neurochemical and behavioral effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rat, guinea pig and monkey, *Psychopharmacol. Bull.*, 20 (1984) 548-553.
- 3 Ekstedt, B. and Orelund, L., Heterogeneity of pig liver and pig brain mitochondrial monoamine oxidase, *Arch. Int. Pharmacodyn.*, 222 (1976) 157-165.
- 4 Glover, V., Sandler, M., Owen, F. and Riley, G.J., Dopamine is a monoamine oxidase B substrate in man, *Nature (Lond.)*, 265 (1977) 80-81.
- 5 Jacobowitz, D.M., Burns, R.S., Chiueh, C.C. and Kopin, I.J., *N*-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes destruction of the nigrostriatal but not the mesolimbic dopamine systems in the monkey, *Psychopharmacol. Bull.*, 20 (1984) 416-422.
- 6 Jenner, P., Rupniak, N.M.J., Rose, S., Kelley, E., Kilpatrick, G., Lees, A. and Marsden, C.D., 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the common marmoset, *Neurosci. Lett.*, 50 (1984) 85-90.
- 7 Langston, J.W., Irwin, I., Langston, E.B. and Forno, L.S., The importance of the '4-5' double bond for neurotoxicity in primates of the pyridine derivative MPTP, *Neurosci. Lett.*, 50 (1984) 289-294.
- 8 Levitt, P., Pintar, J.E. and Breakfield, X.O., Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons, *Proc. Natl. Acad. Sci. U.S.A.*, 79 (1982) 6385-6389.
- 9 Lewinsohn, R., Glover, V. and Sandler, M., Development of benzylamine oxidase and monoamine oxidase A and B in man, *Biochem. Pharmacol.*, 29 (1980) 1221-1230.

- 10 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the Folin phenol reagent, *J. Biol. Chem.*, 193 (1951) 265–275.
- 11 Markey, S.P., Johannessen, J.N., Chiueh, C.C., Burns, R.S. and Herkenham, M.A., Intraneuronal generation of a pyridinium metabolite may cause drug-induced parkinsonism, *Nature (Lond.)*, 311 (1984) 464–467.
- 12 Nakamura, S. and Vincent, S.R., Histochemistry of MPTP oxidation in the rat brain: site of synthesis of the parkinsonism-inducing toxin MPP⁺, *Neurosci. Lett.*, 65 (1986) 321–325.
- 13 Ryder, T.A., MacKenzie, M.L., Pryse-Davies, J., Glover, V., Lewinsohn, R. and Sandler, M., A coupled peroxidatic oxidation technique for the histochemical localisation of monoamine oxidase A and B and benzylamine oxidase, *Histochemistry*, 62 (1979) 93–100.
- 14 Schneider, J.S., Yuwiler, A., and Markham, C.H., Selective loss of subpopulations of ventral mesencephalic dopaminergic neurons in the monkey following exposure to MPTP, *Brain Res.*, 411 (1987) 144–150.
- 15 Stephan, H., Baron, G. and Schwerdtfeger, W.K., *The Brain of the Common Marmoset*, Springer, Berlin, 1980.
- 16 Thorpe, L.W., Westlund, K.N., Kochersperger, L.M., Abell, C.W. and Denny, R.M., Immunocytochemical localisation of monoamine oxidases A and B in human peripheral tissues and brain, *J. Histochem. Cytochem.*, 35 (1987) 23–32.
- 17 Westlund, K.N., Derry, R.M., Kochersperger, L.M., Rose, R.M. and Abell, C.W., Distinct monoamine oxidase A and B populations in primate brain, *Science*, 230 (1985) 181–183.
- 18 Willoughby, J., Glover, V. and Sandler, M., Histochemical localisation of monoamine oxidase A and B in rat brain, *J. Neural Transmission*, in press.