H_3PO_4, reduction of the ester function with $LiAlH_4$ and acetylation with acetic anhydride in pyridine. The product was purified by preparative column chromatography. Synthetic 2-decen-1-yl-acetate was active in the bioassays of *A. dorsata* and *A. florea* but not of *A. mellifera* and *A. cerana*.

The 2nd peak in the gas chromatogram of an unknown substance could be identified as 1-octyl acetate. 1-Octyl acetate is not at all active in all species of genus *Apis*, independent of its concentration or mixed with other substances.

Colony defence of *A. mellifera* and *A. cerana* differs from that of *A. dorsata* and *A. florea* which are free nesting forms. The latter species are able to mark intruders by their stings so that these are pursued over long distances and recognized even after 1 h[4]. Responsible for this behaviour is 2-decen-1-yl-acetate; with its low vapor pressure, it serves as an enemy-marking substance which is effective for much longer than isopentyl acetate as a highly volatile compound can be. By this additional alarm pheromone and by their better developed lancet barbs[8], *A. dorsata* and *A. florea* seem to be well adapted for the defence of their exposed colonies.

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Distribution of monoamine oxidase in hippocampal region of the rat

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Summary. The distribution and development of type A and type B monoamine oxidase (MAO) activities in the hippocampal region of the rat was investigated with biochemical microdetermination. Type A MAO is absolutely dominant and unevenly distributed in the hippocampus. The development of type A MAO in the hippocampus seems to be delayed and reachs adult levels by the 30th day after birth.

The regional distribution of monoamine oxidase (MAO) [EC 1.4.3.4.] activity has been measured in the rat hypothalamus and limbic system[1-3]. Consequently, the difference of distribution and development between type A MAO and type B MAO was noted in hypothalamic nuclei of the rat[4]. In regard to the histochemistry of MAO in the hippocampal region, detailed description was made for the guinea-pig[5-7] and the rat[8]. According to these studies, some differences of the staining pattern of MAO in the hippocampal region were detected between the species. However, regional distribution of MAO activity in hippocampal structure has not been studied biochemically, except for brief comments by Uchimura and others[9]. In the present paper, the distribution and the development of type A and type B MAO activity in the hippocampal region of the rat was investigated with biochemical microdetermination.

Materials and methods. Male Wistar rats of 15 and 30 days of age were used, as well as adult animals of 4 months of age. All animals were killed by decapitation at 09.00 h and the brains were immediately taken out and placed on ice. The blocks including the hippocampus were isolated and frozen quickly in liquid nitrogen. Frontal serial sections of 60 µm thickness were made in a cryostat at −15 °C. The sections were freeze-dried over 12 h at −30 °C and 5×10^{-4} mmHg and stored in evacuated tubes at −20 °C until use. 8 locations in the regio inferior of the hippocampus (figure) were dissected out of the freeze-dried sections with a microknife and a thin steel needle under a stereomicroscope. After being weighed with an electron microbalance (Type 4215, Sartorius Co.), each sample was placed into a pointed microtube. The sensitivity of this balance is 0.1 µg. The weight of each sample was 1.0–2.5 µg.

MAO was assayed by a previously described modification[1] of the method of McCaman et al.[10]. 2 µl of 0.1 M phosphate buffer, pH 7.2, containing BSA and Triton X-100 in each final concentration 0.05% were added to the weighed sample in a microtube. After a 20 min preincubation at 0 °C, 2 µl of ice-cold substrate buffer solution were added. Dopamine (DA) and 5-hydroxytryptamine (5-HT) were used as substrate for type A and B and type A MAO, respectively (final concentration of the substrate: 1.0 mM [2−^{14}C] dopamineethylamine, 60 mCi/mM, 1.1 mM [2-^{14}C] 5-hydroxytryptamine binoxalate, 45 mCi/mM, New England Nuclear Co.). After an incubation at 38 °C for 45 min, the reaction products were extracted in ethyl acetate and the radioactivity was measured by a liquid scintillation spectrometer as described elsewhere[1]. When the assay of type B MAO, 1 µl of 0.1 M phosphate buffer, pH 7.2, and 1 µl of 10^{-5}M clorgyline solution were added in a microtube, and after a preincubation at 0 °C for 20 min, 2 µl of the ice-cold DA-buffer solution (the same solution that was used for the assay of type A and B MAO) was added. In our preliminary experiment under this condition, the inhibition of DA and 5-HT deamination was found to be approximately 40% and 96%, respectively[4]. The inhibition of DA deamination with clorgyline showed plateau at the concentration of clorgyline between 10^{-6}M and 5×10^{-5}M. Accordingly MAO activity for DA in this condition was almost considered to mean type B MAO activity. Moreover, it was ascertained in this experiment that the enzyme reaction towards DA and 5-HT as substrate proceeded linearly with or without the inhibitor with the use of the freeze-dried rat cerebral cortex as enzyme sample between 0.5 µg and 8 µg in weight.

Results and discussion. The results are summarized in the table. As compared with the results on the hypothalamus[4], the hippocampus exhibited slightly higher MAO activity than the hypothalamus when DA was used as substrate. On the other hand, the hippocampus exhibited much lower activity of MAO towards 5-HT than the hypothalamus. According to MAO measurement with 5-HT, the hippocampus showed activity as low as the cerebral cortex[3]. In

Distribution of MAO activity in hippocampal region of the rat

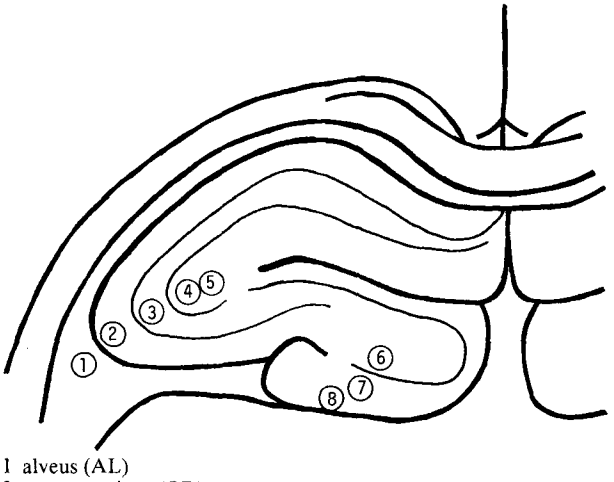
Areas	MAO type A and B*			MAO type B**	MAO type A***
	Adult (6)	30-day-old (4)	15-day-old (5)	Adult (6)	Adult (6)
AL	63.30± 6.54	48.58±13.88	6.24±1.36	4.98±1.11	13.27±1.18
OR	92.98±15.08	132.65±21.59	9.22±2.57	2.20±0.27	16.68±1.51
PL	115.38±17.05	131.97±14.10	8.52±2.57	7.31±2.53	25.39±3.06
RAO	74.05± 7.73	99.35±13.01	7.18±1.52	2.64±0.25	18.01±1.69
RAI	95.52±13.50	97.10±10.64	7.80±1.23	4.42±1.29	18.16±0.89
GL	90.64±15.55	104.36± 9.23	7.26±1.54	3.98±0.60	17.66±2.21
MOI	139.34±10.10	160.15±24.08	8.54±1.84	2.92±0.41	20.76±1.13
MOO	136.42±26.13	133.13± 7.39	10.20±2.66	2.35±0.69	19.94±2.03

The results represent the means ± SEM of the number of animals given in parentheses. *Type A and B; μmoles DA oxidized/g dry wt/h. **Type B (DA as substrate, treated with clorgyline); μmoles DA oxidized/g dry wt/h. ***Type A: μmoles 5-HT oxidized/g dry wt/h.

that study, however, detailed regional examination was not performed within the hippocampal region. DA is known as a substrate towards both type A and type B MAO[11]. In the present study, activity of type B MAO was recognized in minimum in each area of the adult rat hippocampus after treatment with the specific inhibitor (clorgyline) of type A MAO. Also DA MAO activity was found to be parallel with that of 5-HT MAO (type A MAO) within the hippocampus. The result suggests that type A MAO is absolutely dominant in the rat hippocampus, while type A and type B MAO are distributed differently in the hypothalamus[4]. As for type B MAO, however, comparison with the activity shown with typical substrate for the type, such as phenylethylamine, is necessary, due to the indirect measurement of type B MAO in the present study.

With regard to distribution of type A MAO in the adult rat hippocampus, the enzyme was found to be unevenly distributed and present in a stratified pattern. An almost 2fold difference in activity was detected in the areas examined. The highest enzyme activity was detected in the molecular layer of the area dentata and the lowest in the alveus. It has been noted in histochemical investigations that MAO content in the hippocampal region may be associated with extrinsic afferent fibres, since no stained cell bodies could be observed[12]. In the present study, the layers of pyramidal cells and granular cells exhibited together no little activity as compared with the molecular layer. With reference to the pyramidal cell layer, the possibility that the samples dissected as the pyramidal cell layer included the mossy fibre layer cannot be negated, due to a technical limitation of micro-dissection out of unstained freeze-dried sections. There was some distinction of MAO activity towards DA between outer and inner parts of the stratum radiatum, but no difference between 2 parts of the molecular layer. While it was shown in the guinea-pig dentate molecular layer that the MAO histochemical activity was present in 3 distinct laminae embracing the granular cell layer[7], a homogeneously staining pattern was recognized in the rat molecular layer[8].

MAO activity on the 15th day after birth was exhibited in trace. The development of type A MAO in the hippocampus seems to be more delayed as compared with that in the hypothalamus. The disparity of the maturation among each area within the hippocampus was not apparent. According to our previous study on the hypothalamus, type A MAO activity reached to adult levels by the 15th day after birth, and type B MAO only by the 30th day[4]. In the hippocampus, the maturation course of type A MAO simulated that of type B MAO in the hypothalamus. In general, the development of the exzyme either type A or type B may not be uniform in various regions of the brain. Though the content of biogenic amines such as DA, norepinephrine or 5-HT is very low in the hippocampal region, despite high enzyme activity of MAO towards DA in the present study, the detailed distribution of these amines in this region is little known. Further investigations are necessary to understand functional involvement of the catabolic enzyme of biogenic amines in the hippocampus.



1 alveus (AL)
2 stratum oriens (OR)
3 pyramidal cell layer (PL)
4 stratum radiatum outer layer (RAO)
5 stratum radiatum inner layer (RAI)
6 granular cell layer (GL)
7 molecular layer inner layer (MOI)
8 molecular layer outer layer (MOO)

Schematic drawing of the dissected areas of the hippocampus. 1, alveus (AL); 2, stratum oriens (OR); 3, pyramidal cell layer (PL); 4, stratum radiatum outer layer (RAO); 5, stratum radiatum inner layer (RAI); 6, granular cell layer (GL); 7, molecular layer inner layer (MOI); 8, molecular layer outer layer (MOO).

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