5-HT\(_1\) receptors in the structures of visual pathway of normal and monocularly deprived kittens

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Abstract. The density and pattern of distribution of 5-HT\(_1\) receptor sites was examined using quantitative in vitro autoradiography with \([3H]5-HT\) as a ligand in the visual structures of 5 weeks old kittens. One group of animals had normal binocular vision, the other was monocularly deprived during the last three days of life. The density of 5-HT\(_1\) receptor sites and the pattern of their distribution in the visual structures showed distinct regional, areal and laminar differences. In the primary visual cortex (area 17) a high labelling density was found as compared with other cortical areas investigated. Three bands of high binding density were observed corresponding to cortical layers II-III, IV c and VI. This pattern distinguished area 17 from other cortical areas investigated. In subcortical visual structures very high labelling was present in the superficial visual layers of superior colliculus, but LGN showed rather weak labelling although the lamination of LGN was also seen in the pattern of distribution of 5-HT\(_1\) sites. Neither density nor pattern of 5-HT\(_1\) sites in the visual cortex and superior colliculus were affected by 3 days of monocular deprivation. High density of labelling and the distinct pattern of 5-HT\(_1\) receptor sites in the primary visual cortex suggest the important role of serotonergic transmission in the modulation of visual afferent input activity.

Key words: cat, serotonin receptors, visual cortex, superior colliculus, monocular deprivation
INTRODUCTION

Serotonin (5-HT) is produced by neurones of the raphe nuclei of the midbrain, whose axons innervate structures throughout the brain (Azmitia 1978, Kosofski and Molliver 1987). 5-HT elicits or modulates a wide variety of behaviours (for review see Jacobs et al. 1990) and its role is implicated in the development and plasticity of the nervous system (Haydon et al. 1987). It the cerebral cortex a large proportion of serotonergic afferents do not establish classic synaptic contacts but rather form "nonsynaptic" varicosities (Descarriers et al. 1975, 1991). It has been suggested that released 5-HT might diffuse in the extracellular environment and reach a relatively distant target. It has been proposed that 5-HT is involved in the modulation of cortical neuronal activity (Descarriers et al. 1975, 1991, Reader et al. 1979, Nedegaard et al. 1987). Serotonin receptive sites are heterogeneous; currently four major classes are recognized (for review see Peroutka 1988, also Hen 1992). The 5-HT1 class can be further subdivided into four receptor subtypes: 5-HT1A, 5-HT1B, 5-HT1C and 5-HT1D showing distinct pharmacological profiles and being coupled to different second messenger systems (Hamon et al. 1990, Hen 1992). 5-HT1 receptors were identified as inhibitory autoreceptors on soma and dendrites of raphe serotonergic cells and also on their nerve endings as presynaptic autoreceptors; they were also identified as postsynaptic receptors in brain structures that receive serotonergic innervation (Hall et al. 1985). Distribution of 5-HT1 mRNA, which is similar to that of 5-HT1 receptor sites, supports both a presynaptic autoregulatory and postsynaptic modulatory role for this receptor in serotonergic transmission (Chalmers and Watson 1991). The regional and cortical laminar variations in the pattern of distribution and density of 5-HT1 receptors has been described in the rat (Biegon et al. 1982, Pazos and Palacios 1985; Zilles et al. 1985), monkey (Rakic et al. 1988) and human (Pazos et al. 1987) brains.

In the visual pathways, serotonergic fibres innervate sparsely the A, A1 and C laminae of LGN in the cat, and more densely the parvocellular C layer and the medial intralaminar nucleus (Mize and Payne 1987). Visual layers of the superior colliculus receive very dense serotonergic innervation (Biegon et al. 1982). In the visual cortex of the cat layer IV is sparsely innervated (Gu et al. 1990, Mower 1991), while in the monkey layer IV receives the most of serotonergic fibres; within layer IV denser bands of innervation in sublayers IVa and IVc can be distinguished (Morrison et al. 1982). In the cat the majority of 5-HT immunoreactive fibres is found in layers I-III (Gu et al. 1990, Mower 1991).

Involvement of serotonin in plastic changes of the visual cortex in cats was noted for the level of neurotransmitter, which was found to rise after 3 h of monocular stimulation and fall below the control values after 14 h (Kossut et al. 1981). Gu and Singer (1991) reported that blocking the serotonergic receptors by intracortical infusion of methysergide and ketanserine interfered with ocular dominance shift induced by monocular deprivation in kittens. Mower (1991) found that dark rearing increases the binding level of 5-HT1 receptors in the visual cortex of cats.

We examined the distribution of 5-HT1 receptors in the visual system of kittens and investigated the effects of short, 3 days lasting monocular deprivation, where the changes in ocular dominance were well visible. For this purpose we applied in vitro quantitative autoradiography using [3H]5-HT as a ligand.

METHODS

Animals

Six 35 days old kittens from the Nencki Institute colony were used: three normal controls (N), and three monocularly deprived (MD) from visual input
for 3 last days of life. MD kittens had one eye covered with an occluder (Skangiel-Kramska and Kossut 1984).

**Tissue preparation**

Animals were anaesthetized with Nembutal (50mg/kg i.m.) and perfused with 0.1% formaldehyde in phosphate buffered saline (PBS), pH 7.4. The brains were quickly removed, and blocks from the caudal poles of the brains were transected coronally at the level of the ectosylvian sulcus. The tissue was immersed in isopentane cooled on dry ice (-60°C) and was stored in dry ice until sectioning.

The tissue was cut coronally on a cryostat (20 μm) and collected onto gelatine-coated slides, dried at room temperature and then stored for up to 5 days in a freezer at -20°C.

**[3H]5-HT1 receptor binding autoradiography**

The autoradiography procedure was done according to the method described by Biegon et al. (1982), slightly modified. Slide-mounted sections were preincubated at room temperature in 0.17 M Tris-HCl buffer (pH 7.6) containing 4 mM CaCl2 for 30 min. They were then transferred to the incubation medium containing 2nM [3H]5-HT (S.A. 15.1 Ci/mmol, Amersham) to obtain total binding. The incubation solution contained 0.17M Tris-HCl, pH 7.6; 4 mM CaCl2, 10 μM pargyline, 0.01% ascorbate and 5 μM chlorimipramine (to block 5-HT reuptake). An adjacent set of slides was incubated in the same solution containing 1 μM nonradioactive 5-HT to obtain nonspecific binding. After 60 min of incubation at 25°C, the sections were washed twice in ice-cold buffer (for 5 min each), dipped in distilled water and rapidly dried in a stream of cold air. The dried slides were apposed against LKB Ultrofilm at 4°C together with calibrated brain paste standards for 2 months. After the film was developed with Kodak D19, the tissue sections were stained with cresyl violet for histological reference.

The quantitative analysis of autoradiographic studies was performed using computerized image analyzing system (CCD camera, Panasonic and frame-grabber, Visionetics (256 grey levels). The counterstained (Nissl) sections, from which the autoradiograms were obtained, were filmed by CCD camera and the section outline and the the position of cortical laminae were marked on the video screen. Then the appropriate autoradiograms were located into the section outlines and the labelling of visual structures investigated as well as the labelling of particular cortical layers were analyzed with respect to the marked contours. In the incubation conditions applied, non-specific binding was very low (below 3% of total binding). The results (grey level of films) were expressed in arbitrary units.

Four readings were taken from each section in each examined area and the binding values were calculated from at least 3 consecutive brain sections from each animal and the average from one animal was considered as one observation.

Quenching of the tritium β emission by myelin and tissue density were checked by placing 20 μm brain section on isotope coated slides and observing variations in tritium transmission across the sections on Ultrofilm.

**RESULTS**

The density of 5-HT1 binding sites and the pattern of their distribution in the visual cortex exhibited distinct areal and laminar differences. The control experiment to test for quenching inhomogeneities revealed no local differences in tritium absorption throughout cortical regions and within cortical laminae of 5 weeks old kittens. Therefore it was not necessary to correct our results for tritium quenching, and the obtained autoradiograms show real regional differences in labelling density. Area 17 and its monocular segment showed the highest binding density (Figs. 1, 2 and 3) and a very well defined laminar pattern of 5-HT1 sites distribution. Three very well expressed bands of high activity were present throughout area 17 (Figs. 1, 2, 3, 4).
Fig. 1. Color-coded images showing the distribution of [3H]5-HT binding sites in coronal (20 μm) sections from visual regions of normal 5 weeks old kittens. The cortical layers indicated were determined from Nissl stained sections. Color scale shows the density of labelling, the higher values are up. A: cross-section through the entire hemisphere, examined areas are indicated; Cg, cingulate cortex; 17,18,19, visual area 17,18,19; SS, suprasylvian gyrus; Pmls, postero-medial lateral suprasylvian visual area; Plls, postero-lateral lateral suprasylvian visual area; Au, auditory cortex; Lgm, lateral geniculate nucleus. Scale bar = 2 mm. B: high magnification of the image showing the change of pattern of [3H]5-HT binding sites between area 17 and 18 and 19. Scale bar = 500 μm. C: pattern of 5-HT1 sites labelling in LGN, A, A1,C, C1, C3, laminae of LGN. Scale bar = 500 μm. D: high magnification of the section showing the sharp transition of labelling between area 17 and 18. Scale bar = 500 μm. E: sc, superior colliculus; pag, periaqueductal central grey. Note the extremely high labelling in the superficial, visual layers. Scale bar = 500 μm. F: high magnification of the section showing the border between monocular segment of area 17 and cingulate cortex -Cg. Scale bar =0.500 μm.

These features differentiated the primary visual cortex from other cortical areas. The band of highest binding corresponded to cortical layers I, II and III (Fig. 5). The remaining two bands corresponded to layer IVc and to layer VI, respectively. In the ventral part of the splenial gyrus (splenial visual area) -the superficial band of labelling remained in layers I/II, layer IV was not heavily labelled, and a thin band appeared over layer VI.

This pattern was virtually absent more ventrally in the neighbouring cingulate cortex (Cg) (Figs. 1, 2 and 3). In this region the labelling was much lower (about 15 arbitrary O.D.units, that is 50% of value estimated in area 17) (Figs. 1 and 7), and the laminar pattern of labelling was different. The band of higher labelling was situated over the layer V (Fig. 6). Laterally from area 17, area 18 showed also the different pattern of 5-HT1 sites.

The tri-laminar pattern abruptly disappeared at the border of area 17 and 18 (Figs. 1B and D, Figs. 2, 3 and 4). The binding density of area 18 was lower (by about 20%) than in area 17 (Fig. 7). The superficial band of dense labelling continued in area 18 and 19, although it was lighter than in area 17. Bands corresponding to layers IV and VI in the striate cortex, became diffuse and seemed to merge in area 18. In area 19 dense labelling appeared over layer V. This pattern of labelling (bands in layers I/II and V) continued in more laterally situated cortical areas (Fig. 6). The labelling in layer VI was weak and diffuse (Figs. 2 and 3).

In the LGN the labelling was about 40% lower than in the striate cortex (Figs. 1 and 7). The lamination of LGN was visible on the autoradiograms (Fig. 1C). Layer C and especially C1-3 showed heavier labelling than layers A and A1. The heaviest labelling was seen in the ventral LGN.

The superior colliculus had the highest binding of all structures examined (Figs. 1E and 7). The superficial visual layers showed the highest binding density of 5-HT1 sites (17% higher than area 17). The medial parts of layers V/VI also showed high binding density. Heavy labelled central grey matter stands out from other midbrain structures.

Three days of monocular eye occlusion had no effect upon 5-HT1 sites density in area 17 and the monocular segment deprived of visual input (Table I). The binding values in the stimulated and deprived sides were of the same magnitude and did not differ from the control values estimated in left and right hemispheres of normal kittens. Similarly, we could not detect any effect.

Fig. 2. Representative autoradiogram of 5-HT1 binding sites from visual cortical areas of normal kitten. Arrows indicated the position where the optic density across cortical depth were taken. EC, ectosylvian gyrus; MS, monocular segment of area 17. Other explanations see Fig. 1. Scale bar - 2 mm.
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Fig. 3. Representative optical density profiles showing variations in pattern of laminar distribution of [3H]5-HT labelling in the cortical area examined. Ordinate: grey level in arbitrary units, abscissa: distance from cortical surface in mm. The measurement were taken across cortical depth. The densitometric window was 170 μm wide and was positioned perpendicular to the cortical surface.
Fig. 4. 5-HT1 receptors in visual cortex - examples of laminar distribution of receptors. A, B and D: normal kittens; C: monocularly deprived kitten.

Fig. 5. Area 17, localization of labelling into cortical layers, a montage of Nissl stained section and autoradiogram of adjacent fragment of tissue, with the position of layers indicated. Small autoradiogram below shows the region from which high magnification picture was taken.

Table I

Lack of the effect of 3 days monocular deprivation on [3H]-HT binding density in the visual structures of kitten brain; S- side with normal vision; D- side deprived of vision. Density binding in arbitrary units

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of 3 days of monocular vision upon 5-HT$_1$ binding sites in the superior colliculus and LGN.

**DISCUSSION**

From the present study it appeared that the primary visual cortex of the kitten is singled out from visual cortical areas with respect to binding density of 5-HT$_1$ receptor sites and the special pattern of their laminar distribution. We did not observe such phenomena for other neurotransmitter receptor sites studied in the visual cortical areas of kittens at the same age, i.e. the muscarinic cholinergic and GABA$_\text{A}$ sites (Skangiel-Kramska et al. 1986). A similar phenomenon was found by Rakic et al. (1988) in the area 17 of the monkey, where the labelling of 5-HT$_1$ sites was heavy and pronounced variations in laminar distribution were present.

A very striking aspect of the distribution of 5-HT$_1$ receptors in the kittens' cortex is the presence of three bands of strong labelling in area 17. Again, a similar sharp transition between area 17 and area 18 in the distribution of 5-HT$_1$ and $\alpha2$ noradrenergic receptors was found by Rakic et al. (1988) in the monkey visual cortex. They found that the distribution of 5-HT$_1$ receptors in area 17 reflects the pattern of primary thalamic input. In the cat, the pattern of LGN input to area 17 and 18 is similar (LeVay and Gilbert 1986), except that area 18 receives more of $Y$ cell axons and no $X$ axons. According to Ferster and LeVay (1978), $X$ cells terminate in area 17 in layer IV$_c$, which is in the region where a band of labelling is observed, that is not present in area 18. It may be that the $X$ cells terminals are under a direct modulatory 5HT influence, or that their input is modulated on layer IV$_c$ neurones via serotonergic receptors. In the adult cat no such differentiation between area 17 and 18 was reported (Mower 1991). Unfortunately, we had no material from the adult animals to make sure that the observed pattern is not specific to the young, plastic visual cortex. The study of Jonsson and Kasamatsu (1983) suggested a transient overproduction of both serotonin and its receptors in the visual cortex during the critical period of visual system plasticity in cats (Hubel and Wiesel 1970). This result was not confirmed by developmental study of serotonergic innervation of the visual cortex of cats (Gu et al. 1990). However, for the monkey this phenomenon was observed in an adult animal (Rakic et al. 1988). We found a high density of 5-HT$_1$ sites also in the auditory cortex, but it was not as high as in area 17. Nonvisual cortical areas (Cg and SS), included in the plane of our sections, showed lower la-
belling also than the visual cortex. The rat cingulate cortex also showed a low value of 5-HT$_1$ binding sites (Biegon et al. 1982). From the study of Pazos et al. (1987) it appeared that the human visual cortex also exhibits pronounced differences in the laminar pattern of [3H]5-HT binding sites.

The distribution of 5-HT$_1$ binding sites did not reflect precisely the pattern of serotonergic innervation of the visual cortex in the cat. The layer I/II receives a strong serotonergic input and shows a high density of 5-HT$_1$ sites, but layer IV and VI are only sparsely innervated but show a high density of 5-HT$_1$ receptor sites. This mismatch between 5-HT containing terminals and their receptive sites in the cerebral cortex, as well in other brain regions, have been previously reported (Herkenham 1987, 1991). A certain disparity between the pattern of 5-HT innervation in cat visual cortical areas and the pattern of laminar distribution of 5-HT$_1$ receptor sites suggest that in addition to synaptic transmission 5-HT mediates a "volume transmission" (Herkenham 1991, also Hen 1992).

The distribution of 5-HT$_1$ sites in the superior colliculus and LGN found in the present studies corresponded well to the results obtained in the rat (Biegon et al. 1982, Segu et al. 1986). Also, the relative density of labelling agreed with their data. LGN layer C, which showed the highest binding values of this structure, also receives the densest serotonergic innervation (Mize and Payne 1987).

The relatively high density of 5-HT$_1$ receptor sites in the striate cortex of 5 weeks old kitten might suggest the possibility of the involvement of serotonergic transmission in the visual cortical plasticity. 3 days of monocular deprivation of 5 weeks old kittens did not change neither the intensity nor the pattern of [3H]5-HT binding in the visual cortex and superior colliculus. The changes observed by us previously in the level of serotonin after the first short monocular experience in binocularly deprived kittens were transient, and disappeared after 3 days (Kossut et al. 1981). A slightly different deprivation paradigm did not evoke changes in binding density of 5-HT$_1$ receptor sites. The same experimental procedure, however, produced the increase of GABA$_A_3$ receptor sites in the visual cortex of kittens (Skangiel-Kramska and Kossut 1984). Other forms of visual deprivation were found to affect the serotonergic system. Mower (1991) found an increase of 5-HT$_1$ receptor sites in area 17 of the cat brain after prolonged dark-rearing. Segu et al. (1986) observed the decrease of 5-HT$_1$ binding sites in the superior colliculus 3 days after enucleation of rat. Enucleation also increased 5-HT turnover in the LGN (Vizuete et al. 1992). Short monocular deprivation used in our studies, which did not cause degeneration of afferents from the retina, did not affect [3H]5-HT binding in the superior colliculus.

The high labelling of 5-HT$_1$ receptors in the primary visual cortex of young kittens and their distinct laminar pattern of distribution suggest the importance of serotonergic transmission in the processing of the afferent visual input.

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