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Cellular Localization of Serotonin 1A, 1B and Uptake Sites in Cingulate Cortex of the Rat[1]

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ABSTRACT

Experimental lesions followed by binding of [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), [^{125}I]cyanopindolol and [^{3}H] paroxetine to cryostat sections and coverslip autoradiography were used to localize 5-HT_{1A}, 5-HT_{1B} and 5-HT uptake sites in rat posterior cingulate cortex. Ablations included: 1) undercutting for removal of all afferent axons; 2) destruction of the raphe nuclei; 3) cortical ibotenic acid injections for removal of neurons and 4) anterior thalamic and caudate nuclei injections of the immunotoxin OX7-saporin which destroys single classes of cortical projection neurons by retrograde axonal transport. Peak paroxetine binding was in layer Ia with low binding in layer Va and moderate amounts in other layers. Undercut lesions reduced binding only in layer Ia by 35%. Major loses were observed after raphe ablations with decreases of 40 to 72% across all layers. Cortical ibotenic acid injections did not alter paroxetine binding. Peak cyanopindolol binding was in layers Ia to Ic. Undercutting decreased binding significantly in layers Ia, Ib, III and IV, whereas after raphe lesions binding was decreased by 34 to 58% in layers Ia to IV. 5,7-Dihydroxytryptamine injection increased binding by 10 to 40% in layers Ib, II, III and IV. Cortical ibotenic acid injections reduced grain density in all layers with a range of 28 to 47%. Peak 8-OH-DPAT binding was in layer Vb. No change was observed after undercut lesions, whereas after cortical ibotenic acid injection, binding reductions of 44 to 75% were observed throughout all nine sublaminae. Thalamic OX7-saporin injections destroyed almost all layer VI neurons, which resulted in a 45% decrease in layer VI 8-OH-DPAT binding. Caudate injections produced a more limited destruction of neurons in layers V and VI and did not decrease binding. This study suggests that 5-HT uptake sites are presynaptic on raphe axons, 5-HT_{1B} receptors are presynaptic on raphe terminals and postsynaptic on cortical neurons and 5-HT_{1A} receptors are only postsynaptic on cortical neurons including corticothalamic projection cells.

Pharmacologic and autoradiographic characterization of the 5-HT_1 receptor (Peroutka and Snyder, 1979) has revealed four distinct subtypes: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_{1D}(Pedigo *et al.*, 1981; Pazos *et al.*, 1985; Heuring and Peroutka, 1987). The 5-HT_{1A} and 5-HT_{1B} sites (Pedigo *et al.*, 1981), which bind the agonist 8-OH-DPAT (Gozlan *et al.*, 1983; Middlemiss and Fozard, 1983) and the antagonist (−)-CYP (Hoyer *et al.*, 1985) with nanomolar affinity, respectively, are abundant in rat brain (Pazos *et al.*, 1985; Pazos and Palacios, 1985). They are distributed heterogeneously throughout cortical regions and are most dense in frontoparietal, hippocampal and entorhinal cortices. Neocortical 8-OH-DPAT binding is highest in layers IV to VI (Pazos and Palacios, 1985), whereas most CYP binding is in layers I to III (Pazos *et al.*, 1985). In the hippocampal formation, 5-HT_{1B} sites are concentrated in the subiculum, whereas the CA sectors contain primarily 5-HT_{1A} sites (Pazos and Palacios, 1985).

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The 5-HT_1 receptor subtypes mediate distinct physiologic effects which may reflect differential cellular localization. Iontophoresis of 5-HT_{1A}-selective compounds such as 8-OH-DPAT (de Montigny *et al.*, 1984) or ipsapirone (Sprouse and Aghajanian, 1987) suppresses spontaneous firing of dorsal raphe and hippocampal pyramidal neurons (Andrade and Nicoll, 1987) *via* an inward potassium current similar to 5-HT itself (Olpe, 1981). In contrast, 5-HT_{1B}-selective compounds have little effect on these cells (Sprouse and Aghajanian, 1987). Conversely, potassium-stimulated release of 5-HT from rat brain slices or synaptosomes (Maura *et al.*, 1986; Engel *et al.*, 1986) is suppressed by RU 24969, a 5-HT_{1B} agonist, but is unaltered by 8-OH-DPAT. Thus, the 5-HT_{1A} site may mediate postsynaptic 5-HT effects as either an auto- or heteroreceptor, whereas one of the functions of the 5-HT_{1B} site is as a presynaptic autoreceptor which regulates 5-HT release (Engel *et al.*, 1986; Peroutka, 1988).

Of the seven subnuclei of the raphe system (Taber *et al.*, 1960; Hornung and Fritschy, 1988), the dorsal and median

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine/serotonin; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; CYP, cyanopindolol; 5,7-DHT, 5,7-dihydroxytryptamine.

nuclei project extensively to virtually all regions of neocortex (Moore *et al.*, 1978; Lidov *et al.*, 1980). Serotoninergic axons terminate primarily in superficial cortical layers although the deep layers, especially layer Va, receive projections as well (Lidov *et al.*, 1980; Blue *et al.*, 1988). The 5-HT uptake site which mediates high-affinity uptake of 5-HT is located on 5-HT axon terminals in rat brain (Dawson and Wamsley, 1983; Fuxe *et al.*, 1983) and can serve as a presynaptic marker for 5-HT projections. Paroxetine is a selective inhibitor of 5-HT uptake *in vitro* (Habert *et al.*, 1985; Mellerup and Plenge, 1986) which has been used for autoradiographic mapping of 5-HT uptake sites (De Souza and Kuyatt, 1987), although the laminar profile of binding in the rat has not been described.

Despite extensive pharmacologic characterization, the cellular location of 5-HT_{1A} and 5-HT_{1B} sites, *i.e.*, their pre- and postsynaptic positions, remains to be established in neocortex. In hippocampus, local kainic acid ablations decreased 5-HT_{1A} receptor number (Gozlan *et al.*, 1983; Hall *et al.*, 1985), whereas injections of the presynaptic toxin 5,7-DHT did not affect hippocampal 5-HT_{1A} binding (Verge *et al.*, 1986). Although these data concur with physiologic studies which suggest that 5-HT_{1A} sites are postsynaptic, further localization onto specific neocortical cell types has not been evaluated. There is some controversy over the localization of 5-HT_{1B} receptors. Pharmacologic evidence favors the 5-HT_{1B} site as a presynaptic autoreceptor (Engel *et al.*, 1986; Maura *et al.*, 1986); however, autoradiographic studies have been unable to reduce cortical 5-HT_{1B} binding with intracerebral 5,7-DHT injections (Verge *et al.*, 1986; Offord *et al.*, 1988).

The present study will use a number of different lesion strategies to localize 5-HT_1 receptor subtypes onto particular neurons and axons in neocortex. These include cortical undercutting, raphe nuclei lesion or removal of cortical neurons with the excitotoxin ibotenic acid. In addition, a new approach to transmitter receptor localization was used in which specific cortical projection neurons were destroyed *via* retrograde axonal transport of the immunotoxin OX7-saporin. The synthesis and suicide transport properties of OX7-saporin have been described recently (Thorpe *et al.*, 1985; Wiley *et al.*, 1989). The present study also uses an autoradiographic technique which allows for localization of single grains at a sublaminar level of resolution. Posterior cingulate cortex has been chosen for analysis because it receives heterogeneous serotoninergic projections which terminate primarily in layers I, III and VI (Moore *et al.*, 1978; Lidov *et al.*, 1980) and because our preliminary studies showed that layer Ia had high levels of paroxetine and CYP binding. The goal of this study, therefore, is to account for heterogeneities in 5-HT receptor binding in terms of specific afferent axons and cortical neurons.

Methods

Histological procedures. Male Long-Evans rats (350–400 g) were anesthetized with Chloropent (0.3 ml/100 g, b.wt. i.p.) and one of the following lesions was stereotaxically placed: 1) Unilateral undercut deafferentation of area 29 was according to Vogt (1984). A scalpel blade was passed 1.0-mm lateral to the midline, 3.5-mm ventral to the dura extending from 2.5 to 5.5-mm posterior to bregma. Coronal knife cuts 1.0- to 1.5-mm lateral to the midline were made at the same points posterior to bregma, $N = 10$. 2) Two microinjections of the excitotoxin ibotenic acid (10 μg/μl 0.9% saline) were placed 0.7-mm apart unilaterally into area 29, $N = 10$, or midsagitally into the dorsal raphe nucleus, $N = 6$. 3) A single microinjection of the neurotoxin 5,7-DHT (200 μg/10 μl 0.2% ascorbic acid in 0.9% saline) was placed into the right lateral ventricle to destroy serotoninergic neurons. Thirty minutes before injection, these animals were pretreated with desipramine (25 mg/kg i.p.) to prevent uptake of 5,7-DHT into noradrenergic neurons, $N = 5$. 4) Unilateral injections of OX7-saporin were made into the anterior thalamic nuclei (1.0 μg/0.4 μl of phosphate buffer solution) or into the caudate nuclei (1.25–2.5 μg/0.5–1.0 μl of phosphate buffer), $N = 10$. Unablated hemispheres from undercut cases served as controls for these studies.

After a 2 week postoperative survival period, animals were sacrificed with CO_2 and perfused intracardially with 50 ml of cold Krebs-Henseleit buffer, pH 7.4. Brains were removed and blocks from posterior cingulate cortex were dissected in an ice cooled chamber. Blocks were then frozen rapidly in hexane (−75°C) for 2 min and stored at −80°C. Coronal 16-μm thick cryostat sections were cut at −30°C and thaw-mounted on chrome-alum coated microscope slides. To confirm pharmacologic or mechanical ablation, Nissl-stained sections of each case were screened histologically before receptor binding analysis was undertaken. A total of 300 slides with 3 to 4 sections per slide were prepared and analyzed for these studies.

Lesion strategy. Because sections were counterstained with thionin, direct comparisons of receptor binding density in cortical sublaminae were possible. Specific binding profiles of each ligand were heterogeneous in area 29 and were consistently observed in 16 control hemispheres. Nonspecific binding of each ligand was homogeneous bilaterally in control and experimental hemispheres, represented 10 to 20% of total binding and was unaffected by any experimental manipulation.

The effects of each ablation method were distinct. Cortical injections of ibotenic acid selectively destroyed cortical neurons and their associated dendrites, axons and receptors but did not damage afferent axon terminals which contact cortical neurons (Schwarcz *et al.*, 1979). Decreased grain density after ibotenic acid injection reflected perikaryal/dendritic damage and suggested a postsynaptic receptor locus. Ibotenic acid lesions extended through all layers with little involvement of the contralateral hemisphere.

Selective removal of cortical projection neurons was accomplished by injecting the immunotoxin OX7-saporin into cingulate cortical projection targets. Layer VI neurons in posterior cingulate cortex project to the anterior thalamus (Kaitz and Robertson, 1981), whereas those in layers III, V and VI project to the caudate nucleus (Schwab *et al.*, 1977; Royce, 1982). OX7-Saporin injected into anterior thalamus or caudate nuclei is endocytosed by axon terminals because OX7 is an antibody to the abundant neuronal membrane glycoprotein Thy 1.1. OX7-saporin is then transported retrogradely to the cells of origin where the endonuclease activity of saporin inhibits protein synthesis by enzymatically inactivating ribosomes resulting in cell death (Wiley *et al.*, 1989). Thus, in the present study, suicide transport of OX7-saporin produced selective destruction of cortical projection neurons and was the basis for more refined postsynaptic localization.

In contrast, undercutting of area 29 removed afferent axons to this region but did not affect intrinsic cortical neurons (Vogt, 1984). Receptor binding changes after undercut ablations reflected loss of afferent axons and suggested a presynaptic receptor locus. Histologic assessment of undercut lesions revealed consistent lesion placement at the junction of layer VI and the white matter.

Both 5,7-DHT and dorsal raphe ibotenic acid injections destroyed serotoninergic terminals in area 29, the former by direct toxicity to the axon terminal and the latter by somal toxicity and subsequent anterograde degeneration. Of the six animals prepared with dorsal raphe ablation, four were processed with midline lesions which did not extend beyond the ventral or lateral margins of the nucleus.

Autoradiography. [^{3}H]8-OH-DPAT (specific activity = 143 Ci/mmol), [^{3}H]paroxetine (specific activity = 23 Ci/mmol) and [^{125}I]CYP (specific activity = 2200 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Incubation conditions for each radioligand were according to previously published procedures which are summarized in table 1: [^{3}H]8-OH-DPAT (Pazos and Palacios, 1985), [^{3}H]paroxetine

TABLE 1
Incubation conditions

Receptor: Ligand	Buffer	Preincubation	Incubation	Wash
		min:°C	*min:°C:Lig*	
5-HT_{1A}: 8-OH-DPAT	0.17 M Tris 4 mM $CaCl_2$	30:27	60:27:2 nM	0.17 M Tris pH 7.6
	0.1% ascorbate pH 7.6			2 × 5 min:5°C
5-HT_{1B}: CYP	0.17 M Tris 150 mM NaCl pH 7.4	10:27	120:27:20 pM	Buffer 2 × 5 min:4°C
Uptake: Paroxetine	50 mM Tris 120 mM NaCl 5 mM KCl pH 7.7	None	120:27:100 pM	Buffer 2 × 60 min:27°C dH_2O rinse: 0°C

(De Souza and Kuyatt, 1987) and [^{125}I]CYP (Pazos *et al.*, 1985). CYP binding was carried out in the presence of 30 μM isoproterenol to prevent binding of CYP to *beta* adrenergic receptors. Nonspecific binding was generated in a parallel series of sections by incubation of [^{3}H]8-OH-DPAT or [^{125}I]CYP in the presence of 10 μM 5-HT and [^{3}H] paroxetine with 10 μM fluoxetine (a gift of Eli Lilly Co.).

After final washing, all sections were rapidly air dried, apposed to Kodak NTB-2 emulsion-coated coverslips and exposed for 3 ([^{125}I] CYP) or 60 ([^{3}H]8-OH-DPAT and [^{3}H]paroxetine) days in a freezer at −20°C. Autoradiographs were developed in Kodak D-19 at 18 to 20°C, fixed with Kodak rapid fixer without hardener and counterstained with thionin to allow simultaneous histologic and autoradiographic analysis.

Kinetic analysis of 5-HT receptor binding was not undertaken in this study because autoradiographs were prepared with emulsion-coated coverslips instead of tritium-sensitive film. However, previous work with these ligands has demonstrated that after various ablation methods including 5,7-DHT, the K_d was unaltered despite reductions in the B_{max} (Habert *et al.*, 1985; Verge *et al.*, 1986; Offord *et al.*, 1988).

Data analysis. The cytoarchitectural divisions of area 29 were according to Vogt and Peters (1981) and area 29c was chosen for analysis because of its many laminar heterogeneities in serotoninergic connections and receptor patterns. The distribution of neurons in this area is shown in figure 4a. Binding of 5-HT receptor ligands was never homogeneous in layer I. Because there are three divisions in layer I and each receives different proportions of thalamic and callosal afferents (Vogt *et al.*, 1981), binding was quantified in each of these sublayers.

Grain density was quantified by a computerized image-analysis system (Image Technology model 1000, DonSanto Corp., Natick, MA). Radiolabeled grains viewed *via* darkfield microscopy were counted per 2500 μm^2 of a cortical layer and then corrected visually for overlapping grains. Specific binding was determined bilaterally in 9 sublaminae of area 29c, 2 to 3 sections per brain, by subtracting grain number in sections coincubated with unlabeled 5-HT or fluoxetine and radiolabeled grains from sections incubated with radioligand alone. The mean ± S.E.M. was calculated. When comparing two groups differences between means were evaluated with two-tailed *t* tests and $P < .05$ values were accepted as significant. The use of multiple *t* tests was avoided by applying one-way analysis of variance for each layer and for each ligand followed by the Scheffe multi-comparison procedure and acceptance of the same P values. Specific binding from both hemispheres was combined and quantitated in animals receiving dorsal raphe nucleus ablation or 5,7-DHT injection because the raphe nuclei project bilaterally to area 29 (Lidov *et al.*, 1980) and because these treatments resulted in equally extensive damage bilaterally such that left and right hemisphere binding did not differ after either procedure.

When ibotenic acid was injected into cortex, neuron losses and gliosis obliterated laminar architecture. Intact layers from adjacent cortex and depth measurements below the pial surface were used to position grain counts. In OX7-saporin-injected cases losses of neurons resulted in shrinkage of the cortex. The width of layers I and VI were reduced by 20% and 20 to 27%, respectively. Because binding was expressed per unit area, grain counts were reduced by these percentages in these layers to account for shrinkage.

Results

[^{3}H]Paroxetine binding. The laminar distribution of [^{3}H] paroxetine-labeled sites is shown in figure 1. Peak grain density occurred in layer I, whereas moderate binding was in layers II to VI.

After dorsal raphe nucleus ablation, significant binding reductions of 64, 54 and 51% were observed in layers Ia, Ic and IV, respectively; reductions from 40 to 50% occurred in the remaining layers (fig. 1). The overall postlesion laminar profile was virtually homogeneous. Similarly, after 5,7-DHT injection, grain density was decreased by 72, 66 and 68% in layers Ia, Ib and Ic, respectively; 48 to 65% reductions were observed in layers II to VI. The effect of 5,7-DHT was consistent with that after dorsal raphe nucleus ablation in that layer I was most affected and deeper layers less so. In addition, the laminar binding profile was reduced to homogeneity because 5,7-DHT removes all serotoninergic afferents and, thus, total specific binding was decreased to a greater extent than removal of only dorsal raphe afferents.

Undercut ablations moderately decreased paroxetine binding in all nine sublaminae analyzed; however, significant reduction occurred only in layer Ia, which was decreased by 35%. Specific paroxetine binding was not affected by ibotenic acid ablations (data not shown).

[^{125}I]CYP binding. The pattern of CYP binding was heterogeneous. Peak grain density occurred in layers Ia to c, whereas moderate binding was in layers II to IV (fig. 2). After undercut ablation CYP binding was decreased significantly in layers Ia, Ib, III and IV by 54, 43, 39 and 25%, respectively. Although greater binding decreases were observed in layers Ia and b than in deeper layers, these laminae remained enriched in CYP binding sites when compared to deeper layers and, thus, the laminar profile of CYP binding was not reduced to homogeneity.

After dorsal raphe nucleus ablation, CYP binding was significantly reduced by 34 to 58% in layers Ia to IV. There was no significant change in layers Va, Vb or VI binding. The postlesion binding profile was virtually homogeneous, although a small peak remained in layer Ia.

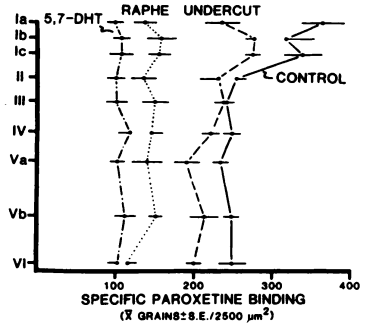

Fig. 1. Laminar pattern of specific paroxetine binding. Peak binding (mean ± S.E.; $N = 4$) was in layer I with moderate amounts in deeper layers. Both 5,7-DHT and dorsal raphe nucleus ibotenic acid ablations reduced paroxetine binding in all layers, whereas undercut lesions primarily affected layer I.

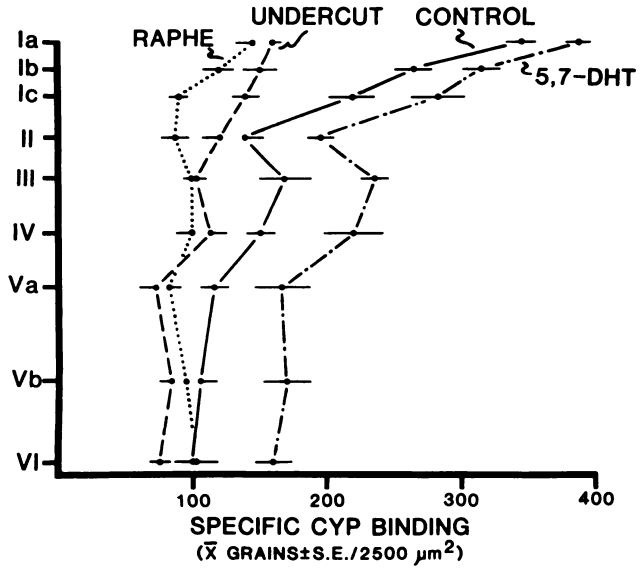

Fig. 2. The laminar distribution of specific CYP binding was similar to that of paroxetine. Although binding was increased by 5,7-DHT injections, undercut and ibotenic acid injections into the dorsal raphe nucleus significantly reduced CYP binding ($N = 4$ for each group).

Unlike the effect of dorsal raphe nucleus ablation, CYP binding was increased in all layers of area 29c after 5,7-DHT injection. Grain density was increased significantly by 10 to 40% in layers Ib, II, III and IV.

Ibotenic acid injection significantly reduced CYP binding in all layers. The following values are for control and experimental cases, respectively: layer Ia, 150 ± 15.5/105 ± 13.0; Ib, 121 ± 10.5/86 ± 11.5; Ic, 112 ± 12.5/79 ± 8.2; II, 98 ± 11.1/56 ± 4.2; III, 101 ± 8.9/55 ± 4.9; IV, 113 ± 11.8/68 ± 8.8; Va, 71 ± 3.3/51 ± 8.1; Vb, 100 ± 9.2/53 ± 3.6 and VI, 86 ± 6.6/61 ± 1.2. Though reduced in density, peak binding remained in layer Ia. Interestingly, binding reductions were most prominent in layers exhibiting low to moderate grain density in control hemispheres, whereas little effect was observed in layers which normally exhibit peak binding.

[³H]8-OH-DPAT binding. Specific 8-OH-DPAT binding was dense in layers Vb and Ic, moderate in layers Va and VI and low in layers Ia, Ib, II, III and IV (fig. 3). After undercut ablation (fig. 3) and 5,7-DHT (data not shown), there was no change in the 8-OH-DPAT binding pattern or density. In contrast, after cortical ibotenic acid injections, 8-OH-DPAT binding was significantly decreased by 44 to 75% throughout all 9 sublaminae (fig. 3). Because more substantial binding decreases occurred in layers which exhibited peak binding in control hemispheres, the laminar profile of 8-OH-DPAT binding was reduced to homogeneity.

Injection of OX7-saporin into the anterior thalamic or caudate nuclei produced two patterns of neuron degeneration in area 29c (fig. 4). Anterior thalamic nuclei injections removed virtually all layer VI neurons and resulted in decreased thickness of layers I and VI. Layer I shrinkage was likely due to destruction of thalamic afferents which project to layer I in addition to apical dendrites of layer VI cells. Injection into the caudate nucleus produced limited cell death in layer V and shrinkage of some of the large pyramidal cells in this layer. Because these latter injections also spread into the anterior thalamus, there was an additional loss of neurons in layer VI.

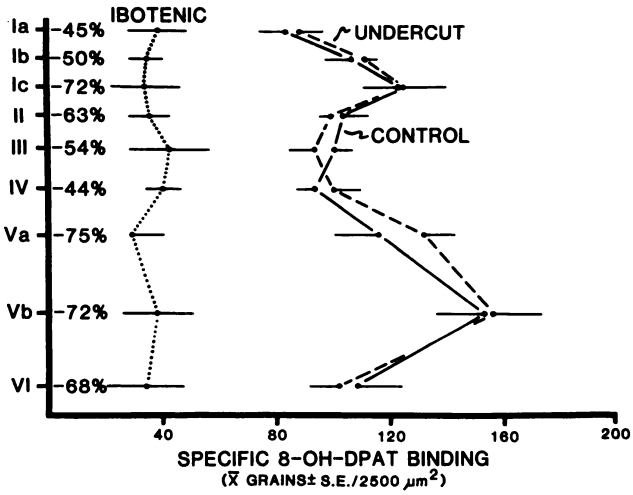

Fig. 3. Laminar distribution of specific 8-OH-DPAT binding in normal area 29c and after undercut and ibotenic acid lesions. Although no alterations occurred after complete removal of afferent axons by undercut ablation, there were substantial reductions after ibotenic acid-induced destruction of cortical neurons as noted by the percentage reductions for each layer ($N = 4$ for each group).

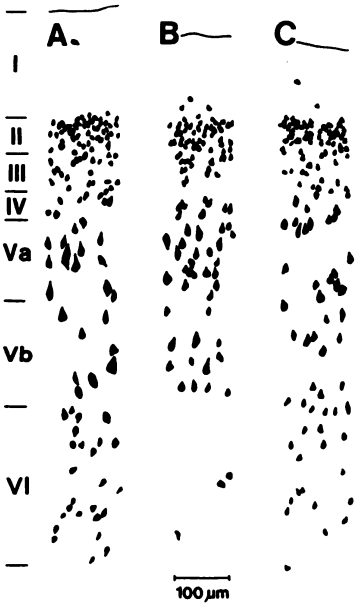

Fig. 4. Drawing of neuronal perikarya through the full thickness of area 29c in a normal case (A) and in cases which received OX7-saporin injections into the anterior thalamus (B) or caudate nucleus with some spread into the anterior thalamus (C). Only perikarya which contained nucleoli indicating viable cells at the time of fixation were drawn. After thalamic injections there was almost complete removal of layer VI neurons, whereas the effects of caudate injections on layer V projection neurons were less dramatic.

Injection of OX7-saporin into the anterior thalamic nuclei significantly reduced grain density in layer VI by 45%; binding reductions in layers Ia, Ib and Ic of 22, 27 and 37%, respectively, approached but did not reach significance (fig. 5). After OX7-saporin injection into the caudate nucleus, there were no statistically significant changes in 8-OH-DPAT binding.

Discussion

The present study demonstrates that the heterogeneous distribution of 8-OH-DPAT, CYP and paroxetine binding in

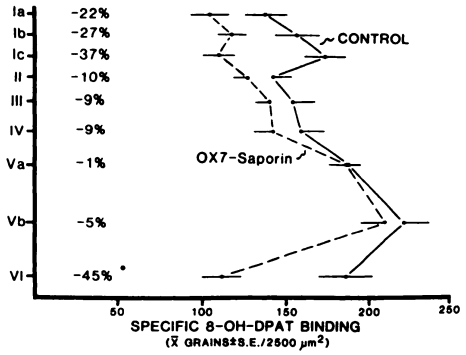

Fig. 5. Distribution of 8-OH-DPAT binding after injection of OX7-saporin into the anterior thalamus ($N = 4$). The only layer in which there was a significant reduction in binding was in layer VI (*), the site of most neuron degeneration. Reduced 8-OH-DPAT binding in layer VI, therefore, is due to massive degeneration of corticothalamic projection neurons.

posterior cingulate cortex of the rat reflects differential cellular localization of 5-HT_{1A}, 5-HT_{1B} and 5-HT uptake sites, respectively. The 5-HT uptake site is presynaptic on raphe axon terminals, 5-HT_{1B} receptors are both presynaptic on raphe terminals and postsynaptic on cortical neurons and 5-HT_{1A} receptors are only postsynaptic on cortical neurons including corticothalamic projection cells.

Heterogeneities in the laminar profile of paroxetine binding likely represent the distribution of 5-HT terminals inasmuch as peak binding in layers Ia to c with moderate amounts in deeper layers corresponded to the pattern of 5-HT terminations in area 29 (Moore *et al.*, 1978; Bobillier *et al.*, 1979; Lidov *et al.*, 1980). This study confirmed previous reports (Habert *et al.*, 1985; De Souza and Kuyatt, 1987) of the presynaptic locus for this site because 5,7-DHT, dorsal raphe and undercut ablations reduced paroxetine binding in posterior cingulate cortex. In contrast, there was no evidence for postsynaptic localization because cortical ibotenic acid injection did not alter paroxetine binding. It is interesting that paroxetine binding was not removed completely by either 5,7-DHT or undercut ablation. Conceivably, some sites were present on fibers of passage through area 29 which were not affected by these lesions. Alternatively, incomplete destruction of raphe afferents to area 29 may have left some uptake sites intact. Finally, a small proportion of 5-HT uptake sites are present on glial cells (Kimelberg and Katz, 1985) which may have been up-regulated in response to 5-HT release secondary to axon terminal damage and thus compensated for binding reductions due to ablation.

The 5-HT_{1B} receptor is believed to be located on 5-HT axon terminals in cortex and to function as an autoreceptor which regulates 5-HT release (Engel *et al.*, 1986; Maura *et al.*, 1986). In the present study, CYP binding was highest in layers Ia to c and lower in the deeper layers. This pattern was similar to paroxetine binding and also 5-HT terminals. Both undercut and dorsal raphe nucleus ablations reduced CYP binding in the superficial laminae, suggesting a presynaptic locus for 5-HT_{1B} receptors in layers I to IV. However, because peak binding in layer I was not abolished completely by these ablations it was likely that some of the remaining binding was to postsynaptic sites. In support of this hypothesis, cortical ibotenic acid injections decreased CYP binding in all layers although peak binding in layer I remained, *i.e.*, a presynaptic component. These results suggest that 5-HT_{1B} receptors are located both pre- and postsynaptically in all 9 sublaminae of area 29. In layer I, presynaptic sites predominate because dorsal raphe and undercut ablation resulted in a larger binding reduction than ibotenic acid injection. Although presynaptic 5-HT_{1B} receptors are most likely on serotoninergic raphe afferents, they may also be present on nonserotoninergic raphe projections which terminate in cortex (Kohler *et al.*, 1981; Kohler and Steinbusch, 1982).

In layers II to IV, CYP binding was reduced to a similar extent by dorsal raphe, undercut and ibotenic acid ablation suggesting an equal proportion of pre- and postsynaptic 5-HT_{1B} receptors. The presence of postsynaptic 5-HT_{1B} receptors in these laminae is supported by increased CYP binding after 5,7-DHT lesions, suggesting up-regulation of 5-HT_{1B} receptors. This effect has been described previously (Nelson *et al.*, 1978; Offord *et al.*, 1988) and may reflect a unique cellular response to pharmacologic ablation with 5,7-DHT. In layers V and VI, binding was moderately reduced by ibotenic acid injection and not altered by undercut or dorsal raphe lesions, indicating that a larger percentage of postsynaptic 5-HT_{1B} receptors was present in these laminae.

It is generally presumed that postsynaptic receptors occupy somatic and proximal dendritic positions. However, although layer I contains few cell bodies and proximal dendrites, there were many postsynaptic 5-HT_{1B} receptors in this layer inasmuch as peak binding was reduced after ibotenic acid ablation. Moreover, after undercut and raphe lesions, some of the peak layer I binding remained. To account for these sites, it is likely that postsynaptic 5-HT_{1B} receptors are located on apical dendritic tufts of deep layer pyramidal neurons which arborize in layer I (Vogt and Peters, 1981). These observations raise questions as to the functional role of 5-HT_{1B} receptors on apical tuft dendrites because physiologic studies have not demonstrated a 5-HT_{1B} receptor-mediated effect on neuron firing in cortex.

There was a heterogeneous profile of 5-HT_{1A} receptors in area 29c with peak binding in layer Vb which is similar to reports by other investigators (Pazos and Palacios, 1985). Previous studies have demonstrated that after i.c.v. 5,7-DHT injection, 8-OH-DPAT binding was decreased in the raphe nuclei but was unaltered in cortical regions, suggesting postsynaptic 5-HT_{1A} receptor localization on cortical neurons (Gozlan *et al.*, 1983; Hall *et al.*, 1985; Verge *et al.*, 1986). Physiologic and biochemical studies indicate that the 5-HT_{1A} site mediates an inward, hyperpolarizing potassium current and may be coupled negatively to adenylate cyclase (DeVivo and Maayani, 1986). In the present study, we have demonstrated that 8-OH-DPAT binding is located postsynaptically on cortical neurons of area 29c because ibotenic acid treatment, which destroys cortical neurons, removed a significant proportion of 5-HT_{1A} receptors. In contrast, undercut and 5,7-DHT ablations did not alter binding in this region.

Postsynaptic localization of 5-HT_{1A} receptors was further defined with the immunotoxin OX7-saporin. Binding reductions after OX7-saporin injection into the anterior thalamic nuclei revealed that a large percentage of 5-HT_{1A} sites were localized to the perikaryal part of layer VI projection neurons which have been shown to project to the thalamus (Kaitz and Robertson, 1981). Although binding reductions in layers Ia to c of 22 to 37% were not quite statistically significant, it is likely that the trend suggests a lesion effect. Thus reductions in layers Ia to c may reflect localization of 5-HT_{1A} receptors on the apical tuft dendrites of layer VI corticothalamic projection neurons

which preferentially arborize in layers Ia to c (Vogt and Peters, 1981). It is clear that OX7-saporin is a potent compound for localization of receptors to specific classes of cortical projection neurons.

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