THE ONTOGENY AND FUNCTIONAL DISTRIBUTION OF NOVEL,
NEUROCHEMICALLY-DEFINED COLUMNS IN MAMMALIAN VISUAL CORTEX

by

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The synaptic organization of the mammalian visual cortex is susceptible to activity- and experience-dependent modifications during a distinct temporal window of development. In an effort to understand the synaptic mechanisms responsible for initiating and maintaining this period of plasticity, the studies presented in this thesis describe the ontogenic distribution of specific molecular indices of serotonergic and glutamatergic neurotransmission in the developing kitten visual cortex and evaluate their patterns of expression following manipulations of early visual experience.

The distributions of serotonin (5-HT) 1A, 1C, 2 and 3 receptor subtypes and the 5-HT uptake site were assessed in postnatal cat visual cortex using in vitro autoradiographic methods. Each 5-HT receptor subtype exhibited a unique temporal, regional and laminar pattern of expression. The density of 5-HT\textsubscript{1A} receptors was highest within superficial and deep cortical layers around postnatal day (PD) 30, while 5-HT\textsubscript{1C} and 5-HT\textsubscript{2} receptors exhibited peak levels in middle cortical layers at PD50 and PD120, respectively. Between PD30 and PD90, 5-HT\textsubscript{1C} & 5-HT\textsubscript{2} receptors were expressed at high levels within the same columnar compartments, but within different geniculate-recipient layers of area 17. These columns of 5-HT receptors measured about 400 \textmu m wide with a centre-to-centre spacing of approximately 900 \textmu m.

The ontogenic distribution of synaptic zinc (Zn), a vesicular component of a subset of glutamatergic-neuron terminals, was also examined in the cat visual cortex. In the adult, intense Zn-staining was highest in superficial and deep layers. The relative absence of Zn in layer IV conspicuously distinguished visual cortical areas 17 and 18 from adjacent cortical regions. The earliest Zn-positive staining in visual cortex was apparent by PD2 and was restricted to a thin layer at the bottom of the cortical plate. By PD10, and continuing through PD20, synaptic zinc formed a trilaminar pattern of dense staining in areas 17 and 18, which included the top of layer I, and layers III and V. The laminar pattern of synaptic zinc in visual cortex appeared mature by PD30, except that the distribution of zinc in layer IV was not uniform. When examined in the tangential plane at
PD50, Zn-rich patches were found to demarcate columnar compartments in layer IV of area 17. The columnar expression of Zn exhibited similar temporal and spatial characteristics as 5-HT$_{1C/2}$ receptor columns. When compared in adjacent sections at PD50, columns demarcated by 5-HT$_{1C/2}$ receptors and Zn were found to be precisely coaligned.

The input- and activity-dependence of the organization of Zn / 5-HT receptor columns was addressed by constraining cortical visual experience. Lesions produced in early development, which interfered with normal binocular input to visual cortex, had similar detrimental effects on the columnar distributions of 5-HT$_{1C/2}$ receptors and Zn, and indicated that the columnar compartmentalization of these molecules was dependent on normal visual input and activity. Zn / 5-HT receptor-rich columns were found to be precisely complementary to columns rich in cytochrome oxidase (CO) and acetylcholinesterase (AChE), molecules which are known to describe functional columnar compartments in primate visual cortex. Zn and CO were also found to describe an interdigitated columnar mosaic in primate visual cortex, suggesting that these molecules might demarcate functionally homologous columnar compartments in the visual cortex of these phylogenetically distinct species.

The results of these experiments suggest that serotonergic and zinc-containing glutamatergic processes may play important roles in the postnatal development of mammalian visual cortex, particularly with regard to mechanisms of compartmentalization into functional columnar domains.
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FORWARD

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Beaulieu: Electron microscopy represented by Figure 3.1

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Dyck: The remainder.
When you have eliminated the impossible, whatever remains, however improbable, must be the truth.

Sir Arthur Conan Doyle (1890)

GENERAL INTRODUCTION

The development of the nervous system is characterized by an intricate balance of genetic and epigenetic phenomena. The culmination of an early progression of developmental processes, which include cellular proliferation, differentiation, migration, process outgrowth and cell death, is the establishment of selective neural connections which form the basis for information processing in the nervous system. The precision with which synaptic connections are formed is not prespecified, but gradually arises from a diffuse innervation which becomes patterned as a result of the interactions among local and extrinsic elements.

Studies of the mammalian visual system have provided considerable insight into our understanding of the mechanisms involved in establishing specific synaptic arrangements during development. The utility of this system is exemplified by the high degree of orderliness of projections from the two eyes through successive stages of visual information processing, and correspondingly, the relative ease with which one can modify visual input (Sherman and Spear, 1982 for review). Transneuronal labeling of retinogeniculocortical connections following intraocular injection of radiolabeled amino acids, reveals that visual information from the two eyes is conveyed by the axons of retinal ganglion cells to eye-specific layers of the lateral geniculate nucleus (LGN) on both sides of the brain (Hickey and Guillery, 1974; Fig. 1.1, normal). From here, LGN relay cells project to the visual cortex where they terminate in a laminar-specific manner, and are parcelled into alternating vertical compartments dominated by one eye or the other (Hubel and Wiesel, 1972; Shatz et al., 1977; Shatz and Stryker, 1978;
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Wiesel et al., 1974; Fig. 1.1, normal). The existence of so-called "ocular dominance columns", has been verified physiologically and anatomically in a wide range of mammalian species (reviewed in LeVay and Nelson, 1991).

In contrast to the highly ordered arrangement of eye-specific inputs in the mature visual cortex, the initial innervation by LGN afferents to layer IV of visual cortex is characterized by an intermingling of left and right eye-specific terminals (Hubel et al., 1977; LeVay et al., 1978; Rakic, 1976). During early stages of development, geniculocortical inputs gradually become segregated into their respective ocular dominance columns. The process of segregation is species specific, beginning prenatally in monkeys (Rakic, 1976), but not until the third postnatal week in the kitten (LeVay et al., 1978), and appears adult-like in both species by the sixth postnatal week (Hubel et al., 1977; LeVay et al., 1978; LeVay et al., 1980; LeVay et al., 1981 see Fig. 1.1 for example).

Anatomical evidence of ocular dominance segregation is supported by studies utilizing physiological recordings of neuronal responses consequent to visual stimulation. Following a similar time course to that described for the anatomical segregation of geniculate inputs, neurons in layer IV initially respond equally well to stimulation of either eye (Hubel and Wiesel, 1963; LeVay et al., 1980), but gradually become exclusively driven by only one eye or the other (Hubel and Wiesel, 1962; Hubel and Wiesel, 1968). The importance of this process of input-shaping, in the present context is that, shortly after the period of segregation begins, and continuing through a discrete window of development, the functional organization of the visual cortex is modifiable by visual experience.

The pioneering studies of Wiesel and Hubel provided evidence that the segregation of visual cortex into eye-specific compartments was not predetermined, but arose from a competitive interaction between LGN afferents representing the two eyes (Wiesel and Hubel, 1965). They demonstrated that if a kitten was monocularly deprived of vision from birth until 3 months of age, visual cortical neurons responded, almost exclusively, to stimulation of the eye which had not been deprived. In these animals, the cortical territory which normally became devoted to the
Figure 1.1. The retinogeniculocortical pathway for one eye, mapped autoradiographically, in normal and neonatally enucleated kittens. On the left, an intraocular injection of \([^{3}\text{H}]\)proline in the left eye labels retinal ganglion cell terminals in the eye-specific, ipsilateral (A1) and contralateral (A) laminae in the lateral geniculate nucleus (LGN). From these terminals, \([^{3}\text{H}]\)proline is transported transsynaptically to LGN relay neurons, and along their axons, which innervate eye-specific columns in layer IV of the visual cortex. Unlabeled bands represent space occupied by the non-injected eye.

By contrast, on the right, the patterned distribution of eye-specific terminals is not present in the visual cortex of a cat which was monocularly enucleated at birth. Here the geniculate terminals representing the visually-experienced eye diffusely innervate the entire extent of visual cortex of both hemispheres. Note also, the reduction in size of the geniculate laminae representing the deprived eye in the enucleated animal.

In both cases the visual cortex was opened and flattened, and sections were cut tangential to the cortical surface so as to be able to view large portions of visual cortex at once. The large islands lacking label represent cortical layers other than those receiving a direct geniculate input, which dip in and out of the plane of section. Scale bar = 3 mm.
Figure 1.1

Normal Visual Cortex

Monocular Enucleation Visual Cortex

BK378

Lateral Geniculate Nucleus

A

A1

A

[3H] Proline

[3H] Proline

Enucleation PD5
deprived eye, remained invaded by the non-deprived eye (Shatz and Stryker, 1978; Fig. 1.1). The effects of deprivation were not due to changes in the deprived eye itself, nor in the LGN (although neurons within the deprived laminae show morphological changes; Wiesel and Hubel, 1963a; Fig. 1.1), but were specific to the visual cortex, at sites where converging binocular inputs compete for synaptic space (Wiesel and Hubel, 1963a). By contrast, visually evoked responses of visual cortical neurons in animals which had been raised to the same age, but deprived of visual input to both eyes, appeared largely normal (Wiesel and Hubel, 1965). By varying the age of onset, and the duration of monocular deprivation, it has been determined that the period of enhanced sensitivity to the effects of deprivation extends from 4 weeks to 13 weeks postnatally (Hubel and Wiesel, 1970), but lesser effects of deprivation are still evident up to 35 weeks of age (Cynader et al., 1980; Jones et al., 1984). The effect of deprivation during this sensitive period persists for the life of the animal, while monocular deprivation in adult cats, for periods as long as one year, has no effect on the responses of cortical cells to stimulation of one eye or the other.

In addition to providing information regarding the processes involved in determining the functional organisation of visual cortex, these studies had far-reaching implications regarding the etiology of, and therapeutic intervention for, clinical conditions such as amblyopia and strabismus. These disorders, and others which result in reduced visual capability in one eye during childhood (e.g. cataracts, hyperopia or myopia), could result in permanent impairment of vision from the affected eye, if not corrected at an early age.

The underlying bases for many of the processes which influence the plasticity of synaptic function in developing nervous systems, including the stabilization or elimination of synapses, are provided by the levels and patterns of activity of the cells involved. Activity-dependent mechanisms are largely responsible for synapse reorganization during the development of the retinotectal projection in fish and amphibians (Meyer, 1982; Reh and Constantine, 1985), as well as in the innervation of skeletal muscles by motoneurons (Jansen and Fladby, 1990). Similarly, in the visual system of mammals, the successful reorganization of LGN terminals, in their quest to
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appropriate cortical territory, is dependent on the activity of cells in the LGN, as well as those intrinsic to the visual cortex. In blocking the electrical activity of retinal ganglion cells in both eyes of young kittens, by intraocular injections of tetrodotoxin (TTX), Stryker and Harris were able to prevent completely the segregation of LGN axons in visual cortex into ocular dominance columns (Stryker and Harris, 1986). If electrical activity was reinstated in this preparation, by stimulation of the two optic nerves, ocular dominance columns formed normally, but only when the two optic nerves were stimulated asynchronously (Stryker and Strickland, 1984). The shift in ocular dominance normally observed following monocular deprivation was also prevented if neurons in the visual cortex were silenced by chronic infusion of TTX (Reiter et al., 1987), or if inhibitory processes were activated by infusion of the GABA agonist muscimol (Reiter and Stryker, 1988). Moreover, the effect of altering the activity of postsynaptic neurons relative to afferent input, by infusing glutamate into the cortex, also prevented the shift of ocular dominance following monocular deprivation (Shaw and Cynader, 1984). Based on these data it is apparent that the amount of activity in developing systems is less important than the pattern of activity, and furthermore, that the patterned activity of pre- and postsynaptic neurons must be correlated.

The search for mechanisms responsible for the induction and termination of periods of plasticity in the brain, described here for the visual cortex during development, has been elusive, despite the sheer volume of effort which has been devoted to this issue. However, it is obvious from the preceding discussion that, whatever mechanisms are postulated, they must consider processes by which information is communicated, and how this information is modified and refined to produce meaningful neuronal interactions at the synaptic level. Synaptic communication between cells in the nervous system occurs mostly via chemical messengers. The primary goal of the studies which will be described in this thesis, is to cast some light on potential mechanisms of synaptic plasticity in the development of the nervous system, by examining the expression of molecules which are involved in cell-to-cell communication in the developing visual cortex. The bulk of this work entails a detailed study of the anatomical distribution of molecules associated with two important neurotransmitter systems which are
involved in the processing of visual information, namely, glutamatergic and serotonergic pathways. An understanding of the development of these systems, relative to that of the functional organization of visual cortex, will provide much needed information regarding their contribution to visual information processing in general, and their particular role during different periods of visual cortical development.

**Serotonergic Systems**

The participation of specific neurotransmitter systems in the development and plasticity of visual cortical connectivity has been the subject of intensive study for several decades. In fact, noradrenergic (Kasamatsu et al., 1979), cholinergic (Bear and Singer, 1986) and glutamatergic (Fox et al., 1990; Kleinschmidt et al., 1987) systems have all been proposed to be necessary for maintaining visual cortical plasticity in kittens during the critical period. Moreover, transient, region-specific increases in the distribution of neurotransmitter-specific afferents (Bear et al., 1985b; Dyck et al., 1993), and/or their receptors (Aoki et al., 1986; Bode-Greuel and Singer, 1989; Cynader et al., 1991; Cynader et al., 1990; Kasamatsu and Shirokawa, 1985; Prusky and Cynader, 1990) have been described to parallel the time course of the critical period and are thus effectively positioned to direct specific developmental processes (discussed in Cynader et al., 1990; Cynader et al., 1991).

Initial studies assessing the serotonergic innervation of cerebral cortex, using the fluorescent histochemical technique, indicated that it was sparse and restricted primarily to layer I (Fuxe et al., 1968). However, the development of sensitive biochemical and anatomical techniques revealed that the entire neocortical mantle was penetrated by fine, varicose, and highly convoluted serotonin-containing fibers (Descarries et al., 1975; Gaudin-Chazal et al., 1979; Lidov et al., 1980; Morrison et al., 1982; Reader, 1980; Reader, 1981; Reader et al., 1979; Takeuchi and Sano, 1984), leading one researcher to remark that "the density and distribution of these fibers are such that they might innervate every neuron in the neocortex" (Morrison et al.,
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1984). Foremost among these techniques was the development of the serotonin (5-hydroxytryptamine, 5-HT) immunocytochemical technique (Steinbusch et al., 1978), which permitted a more detailed study of the 5-HT innervation of neocortex than had been previously possible. Although serotonergic fibers are distributed throughout the nervous system, their cell bodies are restricted to a group of nuclei in the rostral brainstem collectively known as the raphe nuclei. The majority of ascending serotonergic projections to the forebrain arise from two principle nuclei, referred to as the dorsal raphe and median raphe (Azmitia, 1987; Jacobs and Azmitia, 1992 for reviews). These serotonergic nuclei give rise to morphologically distinct sets of axons that form separate pathways which innervate the neocortex in a highly topographic manner (O'Heahrn and Molliver, 1984). These patterns of innervation are region specific and also laminar specific. The laminar specificity of the serotonergic innervation of neocortex is most striking in the primate visual cortex where the primary geniculate recipient layer (IVCβ) is differentiated by a distinctive decrease of density relative to more superficial and deep laminae (Kosofsky et al., 1984; Morrison et al., 1982). Detailed descriptions of the serotonergic innervation in adult cortex of various mammalian species have been extensively documented (see Jacobs and Azmitia, 1992 for recent review), and are discussed further in Chapter 2.

The possibility that 5-HT has specific trophic or growth related functions in early development, distinctly different from its role in the adult nervous system, has long been indicated (Baker and Quay, 1969; Lauder and Krebs, 1978; Vernadakis and Gibson, 1974). In mammals, serotonergic cells in the raphe nuclei are among the very first to differentiate and innervate subcortical target areas within the brainstem, the thalamus and the tectum (Lauder and Krebs, 1978; Lauder et al., 1982; Lidov and Molliver, 1982a; Lidov and Molliver, 1982b; Wallace and Lauder, 1983). In the neocortex, serotonergic fibers have been observed to arrive before birth and provide their extensive innervation postnatally (Lidov and Molliver, 1982a). Perhaps the most revealing evidence for an important role for serotonergic mechanisms in neocortical development was provided recently in an immunohistochemical and autoradiographic examination of serotonergic fibers in the rat during the early postnatal period (D'Amato et al.,
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1987). In this study D'Amato and his colleagues demonstrated a hyperinnervation of serotonergic fibers in all primary sensory cortices, which was present only during the first two weeks of life, during a discrete period of enhanced synaptogenesis (Blue and Parnavelas, 1983a; Blue and Parnavelas, 1983b). In somatosensory cortex, axon terminals formed dense patches in layer IV demarcating specialized cortical columns, called barrels, which each receive input from one facial vibrissa. In subsequent studies in the rat visual cortex, the transient serotonergic innervation was found to be localized to discrete columnar compartments as well (Nakazawa et al., 1992); however, no functional correlates have yet been elucidated. In two related carnivores, the cat and ferret, the ontogenic innervation of visual cortex by serotonergic fibers exhibits distinct laminar changes in the first few weeks of life, before finally attaining the adult innervation pattern (Gu et al., 1990; Voigt and De Lima, 1991b). Studies such as these, which demonstrate specific temporal and regional patterns of innervation during development, provide evidence for a particular role for serotonin in the ontogeny of visual cortex.

The cellular actions of 5-HT in the central nervous system are mediated by specific, high-affinity receptors. In the last decade, the number of different 5-HT receptor subtypes has proliferated from 2, to at least 7 members, belonging to 3 major families (for recent reviews see Peroutka, 1990; Radja et al., 1991; Zifa and Fillion, 1992). The 5-HT1 and 5-HT2 families belong to the G-protein-coupled receptor superfamily (Hartig, 1989) and were originally identified on the basis of their differential affinities for $[^3H]5$-HT and $[^3H]$spiperone (Pedigo et al., 1981; Peroutka and Snyder, 1979). The 5-HT1 and 5-HT2 families are further subdivided into specific subtypes. The 5-HT1 family consists of 5-HT1A, 5-HT1B (Pedigo et al., 1981), 5-HT1D (Heuring and Peroutka, 1987) and 5-HT1E (Leonhardt et al., 1989) receptor subtypes. The 5-HT1B and 5-HT1D subtypes are considered to be homologous receptors in different species (Heuring and Peroutka, 1987). The 5-HT1C receptor subtype (Pazos et al., 1984a; Yagaloff and Hartig, 1985), initially classified as a 5-HT1 receptor, is now considered to belong together with the classical 5-HT2 receptor subtype, based on their similar molecular structure, second-messenger coupling, and pharmacological properties (Hartig, 1989; Hoyer, 1988;
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Sanders-Bush, 1988a), as members of the 5-HT2 family. The last major family of serotonin receptors, called 5-HT3, belong to the ligand-gated ion channel superfamily (Derkach et al., 1989; Kilpatrick et al., 1987), and preliminary reports indicate that multiple subtypes may exist (Richardson and Engel, 1986), but their characterization has not yet been detailed.

Although limited information regarding the distribution of some of the 5-HT receptor subtypes is available for the visual cortex of the adult mammalian visual cortex (rat: Pazos et al., 1985; Pazos and Palacios, 1985; human: Hoyer et al., 1986a; Hoyer et al., 1986b; Pazos et al., 1987a; Pazos et al., 1987b; and non-human primate: Lidow et al., 1989; Parkinson et al., 1989; Rakic et al., 1988), surprisingly little is known about the distribution of the different receptors responsible for the transduction of the 5-HT signal during development.

The studies described in Chapter 2 address the contribution by serotonergic neurotransmission, to the development of visual cortex, with the first examination of the comparative distribution of the different receptors subtypes responsible for 5-HT signal transduction in the visual cortex of the cat during postnatal development.

Zinc-Containing Systems

The possibility that glutamatergic neurotransmission plays an important role in mechanisms of visual information processing is obvious, considering that communication from LGN neurons to the visual cortex is conveyed by an excitatory neurotransmitter, which is thought to be glutamate (Hagihara et al., 1988; Hicks et al., 1985; Tamura et al., 1990; Tsumoto et al., 1986), and that glutamatergic receptors are developmentally regulated and differentially expressed in visual cortex (Bode-Greuel and Singer, 1989; Cynader et al., 1991; Fox et al., 1991; Fox et al., 1989; Fox et al., 1990).

The mammalian telencephalon contains a class of neurons which can be histochemically differentiated by virtue of a chelatable pool of zinc (Zn\textsuperscript{2+}; Danscher et al., 1985; Frederickson and Danscher, 1988). Although the methodology for selectively staining this pool of zinc had
been reported four decades ago (Mager et al., 1953; Timm, 1958), the significance of a stain which only detected 10% of zinc in the brain was questioned by biochemists and was therefore, largely ignored. The subsequent demonstration that the "Timm-stain" was able to demarcate hippocampal subfields, clearly and beautifully, stimulated renewed interest in its application in the brain (Danscher and Zimmer, 1978; Haug, 1967). The histochemically detectable pool of zinc has since been found to be restricted to presynaptic vesicles within the axon terminals of zinc-containing neurons (Friedman and Price, 1984; Haug, 1967; Ibata and Otsuka, 1969; Pérez-Clausell and Danscher, 1985b). These zinc-containing neurons are considered, on anatomical bases, to represent a specific subclass of glutamatergic-aspartergic neurons (Storm-Mathisen et al., 1983); namely they contain clear, round vesicles, form asymmetric (Gray's Type I) synaptic contacts (Haug, 1967), and are highly enriched with glutamate (Beaulieu et al., 1992; Martinez-Guijarro et al., 1991). Potassium-evoked and spontaneous release of zinc (Aniksztejn et al., 1987; Assaf and Chung, 1984; Charton et al., 1985; Howell et al., 1984) into the extracellular space (Pérez-Clausell and Danscher, 1986) has been clearly demonstrated in the hippocampus, and, at high rates of neuronal firing, the extracellular concentration of zinc has been estimated to attain 300μM (Assaf and Chung, 1984; Chung et al., 1986).

Recent ultrastructural evidence indicates that synaptic zinc is enhanced within a subpopulation of glutamatergic terminals in the cat and kitten visual cortex (Beaulieu et al., 1991; Beaulieu et al., 1992). However, the specific physiological function of released zinc has not been established. There is increasing evidence that zinc is co-released with glutamate and may regulate neurotransmission by modulating receptor-mediated events. Exogenous zinc has been demonstrated to affect binding of specific ligands to opiate (Stengaard-Pedersen et al., 1981b) and glutamate receptors (Slevin et al., 1986; Wolf and Schmidt, 1983) and it affects neuronal activity by modulating GABAergic (Westbrook and Mayer, 1987) and both NMDA- and non NMDA-activated glutamatergic neurotransmission (Christine and Choi, 1990; Mayer and Vyklucky, 1989; Peters et al., 1987; Westbrook and Mayer, 1987), indicating that zinc could participate in a modulatory capacity in neural communication.
A particular role for glutamatergic neurotransmission in the plasticity of developing visual cortex has recently been hypothesized (see Constantine-Paton et al., 1990; Rauschecker, 1991 for reviews). As mentioned earlier in this discussion, the processes involved in developmental plasticity of synaptic connections in the visual cortex are experience-dependent and, furthermore, require the correlated participation of afferent inputs and intrinsic cortical neurons. The assumption that correlated activity of both presynaptic and postsynaptic neurons was necessary for the activity-dependent stabilization of synaptic connections, and that asynchronous activity between pre- and postsynaptic cells weakened their connections, was first postulated by D. O. Hebb (1949). Much of the data gathered during the succeeding years, which establish potential mechanisms for the Hebbian rule, have been derived from studies of long term potentiation (LTP), first described in the hippocampus (see Bliss and Lynch, 1988 for review). However, the mechanisms involved in LTP induction could be similar for other activity-dependent synapse stabilization mechanisms, particularly those occurring during development of the visual system. In hippocampal LTP, an increase in synaptic efficacy which is induced following a strong activation of convergent inputs (Bliss and Lynch, 1988), has been demonstrated to depend on the presynaptic release of glutamate and, in most circumstances, the activation of postsynaptic NMDA receptors (see Bliss and Lynch, 1988 for review). Zinc has recently been speculated as contributing to the induction of long-term potentiation (LTP), by virtue of its morphological and physiological relationship with NMDA-activated glutamatergic neurotransmission (Weiss et al., 1989), however, direct evidence is not available. More recently, the modulation of NMDA-activated processes has been demonstrated to alter the processes of experience-dependent synaptic modifications in visual systems as diverse as kittens (Bear et al., 1990) and frogs (for review see Constantine-Paton et al., 1990).

Developmental changes in the distribution of synaptic zinc have been demonstrated in the rat olfactory cortex (Friedman and Price, 1984), striatum (Vincent and Semba, 1989) and amygdala (Mizukawa et al., 1989). However, the distribution and ultrastructural localization of zinc in adult and developing cerebral neocortices of the rat, or other species, have not been
Introduction

reported. Several groups have demonstrated general increases in the total amount and concentration of zinc per gram of wet weight brain tissue during the life span of humans (Völk et al., 1974) and rats (Crawford and Connor, 1972), but these do not differentiate synaptic from non-synaptic sources. The hippocampal mossy fibers, which contain the highest density of synaptic zinc in the brain, are the most well characterized of the zinc-containing neuronal systems. In the rat (Zimmer and Haug, 1978) and cat (Frederickson et al., 1981) developmental gradients in the distribution of histochemically localizable zinc in the hippocampal mossy-fiber region have been reported to reflect developmental synaptogenetic gradients and may be related to synaptic maturity.

In order to provide support for a particular role for synaptic zinc in neocortical development and function, the results of the first ontogenic study of the ultrastructural and regional distributions of synaptic zinc in the cat visual cortex during postnatal development are described in Chapter 3.
Rationale

There is significant evidence indicating that serotonergic and zinc-containing neuromodulatory systems are intricately involved in specific roles during development of the nervous system. However, specific indices of their expression in neocortex during development, have not been described. The primary goal of the studies presented in this thesis was to provide a detailed anatomical description of the postnatal distributions of serotonergic receptors and of zinc-containing fibers. Second, their contribution to mechanisms of activity-dependent plasticity in the developing visual cortex was assessed. Finally, the novel compartmentalized distributions of serotonin receptors and synaptic zinc are compared to known indices of the functional organization of the visual cortex in cats and primates.
ONTOGENIC EXPRESSION OF SEROTONIN RECEPTORS IN THE CAT VISUAL CORTEX

The synaptic organization of the visual cortex of mammals is particularly susceptible to experience-dependent modifications during a distinct window of postnatal development. Although the mechanisms underlying the formation and stabilization of synapses are not known, it is generally believed that the production of chemical messengers, at specific times during development, might influence the synaptic organization of visual cortex. Indeed, many neurotransmitter systems have been proposed to function in a unique capacity, during the development of the nervous system, in regulating neuronal differentiation, growth and synaptic plasticity (for reviews see Lipton and Kater, 1989; Mattson, 1988; Whitaker-Azmitia, 1991).

In this chapter are described the results of an autoradiographic study in cats, that compares the ontogenic distributions of 5-HT$_{1A}$, 5-HT$_{1C}$, 5-HT$_2$ and 5-HT$_3$ receptors as well as the high affinity 5-HT uptake site in visual cortical areas 17, 18, 19, and lateral suprasylvian cortex. The unavailability of specific ligands and radioligands for 5-HT$_{1B/D}$ and 5-HT$_{1E}$ sites precluded an autoradiographic evaluation of their distribution at this time. The temporal and regional expression patterns of the 5-HT receptor subtypes that we did examine, strongly indicate that 5-HT receptors are effectively positioned to mediate important functional processes at critical stages of visual cortical development.
Materials and Methods

Animals

The normal distribution of 5-HT receptors was assessed in 24 cats at 10 ages between postnatal day 0 (PD0, day of birth) and adulthood (> PD360). At least two animals were used at every age described in the results, along with 4 adults and 8 kittens studied at PD50. The cats were anaesthetized with an overdose of sodium pentobarbital and perfused through the ascending aorta with 50-200 ml 0.1M phosphate buffer (pH 7.4) containing 0.9% NaCl. The brain was immediately removed, frozen in isopentane at -50°C and then stored at -30°C prior to sectioning. In two PD50 animals, the visual cortex from one hemisphere was opened and flattened, prior to freezing, to assess the overall distribution of 5-HT receptors in the tangential plane. The other hemisphere was cut in the parasagittal plane. Serial sections were cut on a cryostat at -20°C at a thickness of 20 µm, thaw-mounted onto gelatin-coated glass slides and stored at -20°C for not longer than 4 weeks before processing for autoradiography.

Autoradiography

At each age 5-HT1A, 5-HT1C, 5-HT2 receptors and the 5-HT uptake site (5-HTUp) were labelled in near adjacent sections using [3H]8-hydroxy-2(di-n-propyl-amino)tetralin ([3H]8-OH-DPAT; 169.9 Ci / mmol; New England Nuclear), [3H]mesulergine (78 Ci / mmol; Amersham), (±)-1-(2,5,-dimethoxy-4-[125I] iodophenyl)-2-aminopropane ([125I]DOI; 2200 Ci / mmol; NEN) and [3H]cyanoimipramine ([3H]CN-IMI; 83.6 Ci / mmol; New England Nuclear). In preliminary studies we also assessed the utility of [3H]2,5-dimethoxy-4-bromoamphetamine ([3H]DOB; NEN) to label 5-HT2 receptors, and [3H]citalopram (NEN) to label 5-HTUp. Although all ligands demonstrated similar regional and temporal patterns of expression, [125I]DOI and [3H]CN-IMI proved superior, in terms of specificity and specific activity, in labeling 5-HT2 receptors and 5-HTUp, respectively, and the results presented in this study are based on the binding of these ligands. We also used [3H]BRL43694, [3H]GR65630, or [3H]quipazine in binding studies to assess the distribution of 5-HT3 receptors during visual cortical development.
Table 1. Summary of ligands and procedures used to label $5\text{-HT}_{1A}$, $5\text{-HT}_{1C}$, $5\text{-HT}_{2}$ and $5\text{-HT}_{3}$ receptors and the $5\text{-HT}_{Up}$ site in kitten visual cortex.
Table 1. Summary of Incubation Parameters

<table>
<thead>
<tr>
<th>Receptor Subtype</th>
<th>5-HT&lt;sub&gt;1A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1C&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;3&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;up&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radioligand concentration</strong></td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]8-OH-DPAT 2.0 nM</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]Mesulergine 4.5 nM</td>
<td>[&lt;sup&gt;125&lt;/sup&gt;I]DOI 0.5 nM</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]BRL43694 2.0 nM</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]CN-IMI 0.3 nM</td>
</tr>
<tr>
<td><strong>Cold Displacer</strong></td>
<td>10 μM 5-HT</td>
<td>10 μM 5-HT</td>
<td>1 μM DOB</td>
<td>10 μM 5-HT</td>
<td>1 μM citalopram</td>
</tr>
<tr>
<td><strong>Wash and Incubation Buffer</strong></td>
<td>170 mM Tris-HCl (pH 7.6); 4 mM CaCl&lt;sub&gt;2&lt;/sub&gt;; 10 μM pargylline; 0.01% ascorbate</td>
<td>170 mM Tris-HCl (pH 7.6); 2 μM spiperone; 0.01% ascorbate</td>
<td>50 mM Tris-HCl (pH 7.4); 0.1% BSA; 0.01% ascorbate</td>
<td>50 mM HEPES (pH 7.4)</td>
<td>50 mM Tris-HCl (pH 7.4); 5 mM KCl; 120 mM NaCl</td>
</tr>
<tr>
<td><strong>Pre-Incubation Wash</strong></td>
<td>30 min</td>
<td>30 min</td>
<td>30 min</td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td><strong>Incubation Time</strong></td>
<td>60 min</td>
<td>120 min</td>
<td>60 min</td>
<td>60 min</td>
<td>24 hour</td>
</tr>
<tr>
<td><strong>Post-Incubation Wash</strong></td>
<td>2 X 5 min</td>
<td>2 X 10 min</td>
<td>3 X 10 min</td>
<td>2 X 3 min</td>
<td>1 hour</td>
</tr>
<tr>
<td><strong>Film Exposure</strong></td>
<td>8 weeks</td>
<td>10 - 12 weeks</td>
<td>3 days</td>
<td>6 - 8 months</td>
<td>2 weeks</td>
</tr>
</tbody>
</table>

DOB: 2,5-dimethoxy-4-bromoamphetamine; BSA: bovine serum albumin.
The binding procedures which were used, and the specificity of each radioligand for individual 5-HT receptor subtypes, are based on ligand characterizations which have been described previously in rat (Kilpatrick et al., 1987; Kilpatrick et al., 1988; Kovachich et al., 1988; McKenna et al., 1989; Nelson and Thomas, 1989; Pazos et al., 1985; Pazos and Palacios, 1985; Titeler et al., 1987; Titeler et al., 1985), primate (Lidow et al., 1989; Parkinson et al., 1989), and human brain (Hoyer et al., 1986a; Hoyer et al., 1986b; Pazos et al., 1987a; Pazos et al., 1987b). Any deviations from the published protocols are indicated by the specific incubation parameters used (buffers, incubation times, and ligand concentrations), outlined in Table 1, and briefly described here. The frozen sections were thawed, washed for 30 min in buffer and then incubated, in the dark, in buffer containing the appropriate radioligand. Nonspecific binding was assessed in near adjacent sections by including an excess of non-labeled displacer in the incubation solution. Following incubation, sections were washed in buffer to remove unbound radioligand, briefly dipped in ice-cold distilled water, dried under a stream of cool air and then exposed against Hyperfilm-3H along with either 3H- or 125I - standards (Amersham). After developing the Hyperfilm, selected sections were stained with cresyl violet to facilitate the identification of cytoarchitectonic areas and cortical layers in relation to radioligand binding patterns.

Quantitative Densitometry

Autoradiographic images were captured digitally using a Macintosh IIfx-based image analysis system (Cohu 4915 CCD camera; Data Translation DT-2255 quick capture board) running Image software (NIH, v 1.45). This software allowed radioligand binding to be measured in calibrated units of isotope concentration or moles of ligand (nCi / mg tissue; fmol / mg tissue), within individual cortical layers. Nissl stained sections were digitally superimposed upon autoradiographic images and individual regions and cortical layers were identified and measured densitometrically.
In the youngest animals (PD0), density measurements were obtained from 100 µm-wide regions, drawn within, and corresponding to, layers I, IV, V and VI as well as superficial (CPs), deep (CPd) cortical plate and white matter (Wm) from area 17, 18, 19 and lateral suprasylvian cortex (LS). In animals older than PD0, the average density of 200 µm wide regions drawn from layers I, II, III, superficial IV (IVs), deep IV together with superficial V (IVd), VI, and Wm was measured in each region. In all cases, four sections from each brain were used for the determination of specific ligand binding. Specific binding within an individual lamina or region was obtained by subtracting non-specific binding, measured in near-adjacent sections incubated with unlabeled displacer, from the total bound radioligand measured in each of the four sections.

Several approaches were utilized to relate the pattern of ligand binding to laminar and regional borders. Selected sections were counterstained with cresyl violet which were then used to establish laminar borders in the various visual cortical areas based on cytoarchitectonic criteria established by Otsuka and Hassler (1962) in adult cats and Shatz and Luskin (1986) for early postnatal development. Some sections were histochemically stained for cytochrome oxidase to demarcate layer IV within areas 17 and 18 (Wong-Riley, 1979). The borders of visual cortical areas were also determined according to the electrophysiologically defined maps of Tusa et al. (1978, 1979, 1980, 1981) which describe the relationships of regional borders and gyral patterns. Additional information was derived from comparisons with previous studies using autoradiographic markers having laminar-specificity in area 17 / 18 of developing cat visual cortex (Prusky et al., 1987; Shaw et al., 1984; Shaw et al., 1986).

**Results**

The photographs in Figure 2.1 illustrate the changing distributions of 5-HT$_1$A (A-I), 5-HT$_1$C (A'-I') and 5-HT$_2$ (A"-I") receptors in near-adjacent sections through visual cortical areas 17, 18, and 19 between birth and adulthood. Autoradiographic images showing the binding to these receptors in lateral suprasylvian cortex (LS) during development are shown in Figure 2.2.
Photographs of representative sections which illustrate the binding of $[^3\text{H}]$-CN-IMI to 5-HT$_{1p}$ sites, in PD20 (A), PD75 (B), and adult cat visual cortex (C), are presented in Figure 2.3. All sections for each ligand were processed simultaneously, exposed against the same sheet of film, and were photographed and printed under identical conditions. Within these figures, the observable changes in density across different regions and laminae reflect real changes in total bound radioligand.

Regardless of the radioligand used, and even with 6 months film exposure, we were unable to detect specific binding to 5-HT$_3$ receptors in the visual cortex at any age. However, very high concentrations of 5-HT$_3$ receptors (best labeled using $[^3\text{H}]$BRL43694) were found in sections through the striatum and hippocampal formation of the same brains, indicating that this 5-HT receptor subtype is not likely to be found in the visual cortex at detectable levels. The observation that the density of 5-HT$_3$ receptor binding sites is significantly less than that of any other 5-HT receptor class, has been previously reported, and the authors also indicated that the utility of tritiated radioligands to visualize 5-HT$_3$ sites in the brain would be limited (Radja et al., 1991).

The photographic images generated from the autoradiograms represent the total binding (specific + nonspecific) of each radioligand. In the quantitative analyses described below, specific binding was assessed densitometrically by subtracting nonspecific (ns) from total binding. Across all ages used in this study, specific binding accounted for greater than 88.6% of total binding of $[^3\text{H}]$8-OH-DPAT ($8.2\% < \text{ns} < 11.4\%$), > 75.5% of binding of $[^3\text{H}]$mesulergine ($11.1\% < \text{ns} < 24.5\%$), > 88.0% binding of $[^{125}\text{I}]$DOI ($8.9\% < \text{ns} < 12.6\%$), and > 84.0% of $[^3\text{H}]$CN-IMI ($8.6\% < \text{ns} < 16.0\%$). In the following sections, the binding data are presented in units of fmol / mg tissue (wet weight) ± standard error of the mean.

It is apparent from the autoradiograms in Figures 2.1, 2.2, and 2.3, that the specific expression of each 5-HT receptor subtype is manifested by complex temporal, regional and laminar patterns. In order to appreciate more fully this complexity, the autoradiographic data are broken down in the following sections beginning with general temporal, regional and laminar analyses and ending with an examination of specific laminar and intra-laminar distributions.
**Figure 2.1.** Autoradiographic images demonstrating the total binding of $[^3\text{H}]8$-OH-DPAT (A-I), $[^3\text{H}]$mesulergine (A'-I'), and $[^{125}\text{I}]$DOI (A"-I"), to 5-HT 1A, 1C and 2 receptors, respectively, in near-adjacent sections through visual cortical areas 17, 18, 19 and lateral suprasylvian cortex (LS) during postnatal development (age, in postnatal days is indicated to left of each row). All the sections for each ligand were processed simultaneously, apposed to the same film and photographed under identical conditions. Regional and temporal changes in density thus reflect actual changes in level of binding for each radioligand. Regional boundaries are indicated by dotted lines in the left-hand panels.
Figure 2.2. Autoradiographic images demonstrating differential binding patterns of $[^3H]8$-OHDPAT, $[^3H]$mesulergine and $[^{125}I]$DOI to 5-HT$_{1A}$, 5-HT$_{1C}$ and 5-HT$_2$ in lateral suprasylvian cortex (LS) at postnatal day 0, 10, 90 and in adult cat. The expression of 5-HT$_{1A}$, and 5-HT$_2$ receptors was very high in LS at ages beyond PD10 but 5-HT$_{1C}$ receptors were present only at very low abundance at all ages.
Figure 2.3. Autoradiographic images illustrating total binding of $[^3\text{H}]\text{CN-IMI}$ to $5\text{-HT}_{\text{Up}}$ sites in visual cortex of PD20 (A), PD75 (B) and adult (C) cats. $5\text{-HT}_{\text{Up}}$ sites exhibited a general increase in density with age but levels were essentially homogeneous across all cortical regions and laminae throughout early development but exhibited a slight preference over superficial layers in the adult.
Figure 2.3
Figure 2.4. Age-related changes in the binding of \[^3\text{H}]8\text{-OH-DPAT}, \[^3\text{H}]\text{mesulergine}, \[^{125}\text{I}]\text{DOI}\text{ and }[^3\text{H}]\text{CN-IMI} to 5-HT\text{ 1A, 1C, 2 and Up receptors, respectively, in cat visual cortex are plotted as an average for all cortical regions and layers combined. The average density of receptor binding for each ligand, expressed as a proportion of maximal binding, was typically low at birth, increased to a maximum, at a different age for each binding site, and then declined to adult levels. The peak binding levels of individual receptor subtypes exhibited surprisingly complementary temporal distributions with 5-HT\text{ 1A} receptors expressed earliest in development (PD10 - PD40) followed by 1C receptors (PD40 - PD75), and the 5-HT\text{ 2 and 5-HT}\text{ Up} later in development (PD75 - PD120).
Ontogeny of Serotonin Receptors

Figure 2.4
Temporal Patterns of Expression

Figure 2.4 demonstrates the effect of age on the binding of $[^3\text{H}]8\text{-OH-DPAT}$, $[^3\text{H}]\text{mesulergine}$, $[^{125}\text{I}]\text{DOI}$, and $[^3\text{H}]\text{CN-IMI}$ to 5-HT$_{1A}$, 5-HT$_{1C}$, 5-HT$_2$ receptors and 5-HT uptake sites, respectively in postnatal cat visual cortex. These data represent the binding density of each receptor subtype averaged across areas 17, 18, 19 and LS collectively, and are plotted as a proportion of peak binding. In general, all receptor subtypes exhibited their lowest levels of expression at birth, increased with a different time course to temporally unique peak levels of expression, and then declined to adult levels. The 5-HT$_{1A}$ receptors exhibited maximal levels of expression between PD20 and PD30 but dropped to near-adult levels by PD75. 5-HT$_{1C}$ receptors also exhibited a transient peak in expression, which occurred slightly later, between PD40 and PD75, but then declined, by adulthood, to levels similar to that seen in early postnatal development. The expression of 5-HT$_2$ receptors and 5-HT$_{Up}$ sites increased gradually through early postnatal development, with a very similar time course, to exhibit their highest levels between PD40 and PD75. In both cases the highest levels of expression were maintained beyond PD120, but the reduction to adult levels was much greater for the 5-HT$_2$ subtype than for 5-HT$_{Up}$ sites.

Regional and Laminar Patterns

In addition to displaying complementary temporal patterns of expression, individual 5-HT receptor subtypes demonstrated striking regional and laminar complementarity in their distributions. Developmental changes in the levels of expression of each 5-HT receptor subtype within individual regions, averaged across laminae, are expressed in the three-dimensional plots in Figure 2.5. The binding data for each radioligand, within individual laminae of each visual cortical region, are presented in Figure 2.6. Quantitative changes in regional and laminar expression described in the following sections in terms of fmol / mg tissue (wet weight), can also be followed qualitatively in the representative micrographs presented in Figures 2.1, 2.2 and 2.3.
Figure 2.5. Three-dimensional plots demonstrating regional patterns in the distribution of 5-HT_{1A} (A), 5-HT_{1C} (B), 5-HT_{2} (C) 5-HT_{Up} (D) receptors in postnatal cat visual cortical areas 17, 18, 19 and lateral suprasylvian cortex (LS). A. The temporal pattern in the distribution of 5HT_{1A} receptors was similar across all visual cortical areas. From lowest levels at birth (PDO), the density of 5-HT_{1A} receptors peaked between PD10-PD30 and subsequently decreased to adult levels. B. Receptor binding specific for 5-HT_{1C} receptors was highest at PD40 and was essentially restricted to area 17. C. 5-HT_{2} receptors demonstrated highest levels of expression in LS at all postnatal ages beyond PD10 except for a striking decrease in level of expression at P40. In areas 17, 18 and 19 peak levels were displayed between PD75 and PD120. D. Little regional disparity was seen in the distribution of 5-HT_{Up} sites. 5-HT_{Up} sites demonstrated a gradual increase from PDO to PD40 and then maintained this high level to adulthood. Note that the left abscissa in plots C and D are reversed to make the relative binding in LS more visible than it would be otherwise.
Figure 2.5
Figure 2.6. The bar graphs illustrate temporal changes in specific binding of \(^3\text{H}\)8-OH-DPAT (A), \(^3\text{H}\)mesulergine (B), \(^{125}\text{I}\)DOI (C) and \(^3\text{H}\)CN-IMI (D) in individual laminae within visual cortical areas 17, 18, 19 and LS. A detailed description of their expression levels and patterns can be found in the text.
Ontogeny of Serotonin Receptors

Figure 2.6
Ontogeny of Serotonin Receptors

5-HT\textsubscript{1A}

The binding of \textsuperscript{3}H-8-OH-DPAT to 5-HT\textsubscript{1A} receptors was lowest at birth (PD0) with equally low levels distributed across all visual cortical areas (Fig. 2.5A, Fig. 2.6A; area 17, $\bar{x} = 27.68 \pm 2.85$; area 18, $\bar{x} = 29.06 \pm 2.67$; area 19, $\bar{x} = 31.17 \pm 2.70$; LS, $\bar{x} = 28.39 \pm 2.66$). The majority of 5-HT\textsubscript{1A} receptors in all four regions were localized to a narrow band below the cortical plate (CP) which included layers V and VI and extended, only superficially, into the subplate (Fig. 2.6A; Fig. 2.7A). The superficial layers, including the CP and layer 1, were equally labeled, at lower levels. A sharp increase in the level of binding was realized in all four regions between PD10 and PD30 (Fig. 2.5A; Fig. 2.6A). This increase in binding density was greater in areas 17 and LS ($\bar{x} = 105.89 \pm 6.96$; 108.29 ± 7.26) than in areas 18 and 19 ($\bar{x} = 95.67 \pm 6.13$; 98.76 ± 6.22) and was manifested by increases in binding levels predominantly in supragranular (I-III) and infragranular (V, VI) layers, although a significant increase was present in layer IV as well (Fig. 6A). The increase in binding was clearly visual cortex specific, distinguishing these areas from directly adjacent cortical regions (Fig. 2.1; Fig. 2.2). The level of 5-HT\textsubscript{1A} binding peaked at PD30 with higher levels than those found at any other stage in development. The subsequent decline in expression seen beyond PD40 followed a similar time course across the different cortical regions (Fig. 2.5A, Fig. 2.6A). By adulthood, in contrast, the visual cortex was conspicuously unlabeled relative to adjacent non-visual areas. This regional disparity was due, primarily, to reduced numbers of 5-HT\textsubscript{1A} receptors in cortical layers III, IV, V and VI in areas 17 / 18 ($\bar{x} = 37.40 \pm 2.38$; 30.44 ± 2.67), which distinguished them from the laterally adjacent area 19 ($\bar{x} = 50.10 \pm 4.28$) and the ventro-medial cingulate cortex (Fig. 2.1; Fig. 2.5). A very narrow band within layer V was labeled at intermediate levels (Fig. 2.1 adult). The level of binding in the subplate and subcortical white matter was low at all ages (Fig. 2.1; Fig 2.6A).

5-HT\textsubscript{1C}

\textsuperscript{3}HMesulergine binding exhibited the greatest regional and laminar specificity of the subtypes analyzed in visual cortex (Fig. 2.1, 2.11A). From Figures 2.1, 2.5B, and 2.6B, it is
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apparent that 5-HT1C binding sites exhibited their lowest levels of expression at birth within areas 17 (\( \bar{x} = 9.26 \pm 1.45 \)), 18 (\( \bar{x} = 13.22 \pm 1.45 \)), 19 (\( \bar{x} = 14.51 \pm 1.45 \)) and LS (\( \bar{x} = 12.76 \pm 1.45 \)). Throughout development, the highest density of binding was limited exclusively to the middle cortical layers (Figs. 2.1, 2.6B). A denser band of receptors in the lowest portions of CP, among cells destined for the base of layer IV (Shatz and Luskin, 1986), and upper layer V was distinguished from the equally dense binding in superficial and deep layers at birth (Fig. 2.1; Fig. 2.7B). The number of receptors increased slowly with age in areas 18 (\( \bar{x} = 15.98 \pm 1.45 \)), 19 (\( \bar{x} = 17.24 \pm 1.45 \)) and LS (\( \bar{x} = 17.01 \pm 1.45 \)) but doubled in area 17 (\( \bar{x} = 18.55 \pm 1.45 \)) by PD30 (Fig. 2.5B). A significant increase in specific binding of \(^3\)Hmesulergine was observed at PD40 which was caused, almost entirely, by an increase in area 17 (\( \bar{x} = 31.03 \pm 3.00 \); Fig. 2.5B). Between PD30 and PD75, the pattern of \(^3\)Hmesulergine binding was characterized by a dense band located in deep layer IV / superficial layer V, from which emerged columns of receptors extending vertically into parts of layer III (Fig. 2.1, Fig. 2.6B). These high regional and laminar levels in the expression of 5-HT1C receptors were developmentally transient, peaking at PD40 and becoming reduced through PD75 (\( \bar{x} = 13.02 \pm 0.97 \)) to near adult levels by PD90 (\( \bar{x} = 15.95 \pm 1.46 \)). The reduction of columnar binding appeared gradual, remnants of 5-HT1C receptor columns were present only at the layer III / IV border at PD90 (Fig. 2.1G'). At ages beyond PD120, the distribution of 5HT1C receptors in visual cortex was adult-like, essentially homogenous across all layers (Fig. 2.1, Fig. 2.6B).

5-HT2

The patterns of \(^{125}\)IDOI binding displayed the greatest complexity in regional (Fig. 2.1, 2.5C, 2.11C) and laminar (Fig. 2.6C) distributions, of the four types of 5-HT binding sites examined. The highest levels of 5-HT2 receptors, visualized with \(^{125}\)IDOI, distinguished LS from adjacent cortical areas between PD10 (\( \bar{x} = 16.15 \pm 2.06 \)) and PD75 (\( \bar{x} = 17.11 \pm 2.14 \)). Except for a transient decrease at PD40 (\( \bar{x} = 9.64 \pm 1.05 \)), their level of expression remained high through PD120 (\( \bar{x} = 16.98 \pm 2.17 \)) after which they declined to adult levels (\( \bar{x} = 8.38 \pm 1.42 \)).
The level of 5-HT2-specific binding in areas 17, 18 and 19 was lowest at birth (\( \bar{x} = 1.22 \pm 0.23; 1.91 \pm 0.25; 2.14 \pm 0.14 \)) then increased to intermediate levels by PD40 (\( \bar{x} = 6.73 \pm 0.72; 6.28 \pm 0.67; 7.07 \pm 0.64 \)). Although at very low levels, the expression of 5-HT2 receptors in lower cortical laminae appeared inhomogenous and patchy (Fig. 2.7C). Through early postnatal development (PD0-PD40), the highest concentrations of 5-HT2 receptors in areas 17, 18 and 19 were localized to the deeper cortical layers (IV, V, VI; Fig. 2.1, Fig. 2.6C). The highest laminar-specific density of receptors in area 17 / 18 was found in a band restricted to layer V/VI at birth (Fig. 2.7C) but highest in layer IV from PD10 - PD40 (Fig. 2.6C). A striking augmentation in the numbers of 5-HT2 receptors in all visual cortical regions occurred between PD40 and PD75. They increased to peak levels, to values which were the highest of all cortical regions, in areas 17 / 18 by PD75 (\( \bar{x} = 18.46 \pm 1.79; 14.52 \pm 1.95 \)) and continuing through PD120 (\( \bar{x} = 23.25 \pm 2.23; 18.22 \pm 2.24; \)), before declining to adult levels (\( \bar{x} = 11.19 \pm 1.52 / 7.47 \pm 0.94 \)). The laminar pattern of expression changed significantly over this period, becoming predominant in superficial layers. In area 17 and 18, the highest density of binding was always localized to a denser band within superficial levels of layer IV (Fig. 2.6C). Among the different 5-HT subtypes, the 5-HT2 receptors were found unusual in demonstrating high levels of expression in white matter (Fig. 2.1, Fig. 2.6C). The binding in subcortical white matter was transient, reaching its highest levels at PD30 within a narrow band directly subadjacent to layer VI (Fig. 2.1). This localization of 5-HT2 receptors was observable through PD90 but was never seen in the adult.

5-HT\textsubscript{Up}

The expression of 5-HT\textsubscript{Up} sites closely matched the regional and temporal trends described for the 5-HT2 receptors (Fig. 2.5D); however, unlike 5-HT receptors subtypes, the distributions of 5-HT\textsubscript{Up} did not exhibit a great degree of laminar disparity (Fig. 2.3, Fig. 2.6D). In the later stages of postnatal development there were discernably higher levels of expression in supragranular layers, particularly in area 18 (Fig. 2.3, Fig. 2.6D). It is apparent from Figure 2.5D that, at early postnatal ages, the highest density of 5-HT\textsubscript{Up} was localized to LS (\( \bar{x} = 21.58 \pm 2.24 \)) with the
lowest in area 19 (\( \bar{x} = 9.30 \pm 1.14 \)) followed by area 17 (\( \bar{x} = 9.98 \pm 0.94 \)) and area 18 (\( \bar{x} = 14.39 \pm 1.11 \)). The levels of expression increased progressively in all areas and by PD40 uptake sites were found at high levels, across all cortical areas (17, 44.61 \( \pm \) 2.46; 18, 38.40 \( \pm \) 2.82; 19, 40.47 \( \pm \) 2.12; LS, 39.37 \( \pm \) 2.47). These levels of expression were essentially maintained throughout the remainder of postnatal visual cortical development (Fig. 2.5D). The general trend for all regions to exhibit decreases in receptor expression between PD120 and adult was maintained for the 5-HT\(_{(1p)}\) site, but to a far lesser degree, in areas 17, 19 and LS (Fig. 2.5D). In fact, as is apparent in Figures 2.5D and 2.6D, the density of 5-HT\(_{(1p)}\) sites increased slightly in area 18 from PD120 (\( \bar{x} = 43.48 \pm 3.94 \)) to adult levels (\( \bar{x} = 46.66 \pm 3.94 \)).

**Complementary Patterns of Expression**

A recurring theme in the developmental distribution of the multiple 5-HT receptor subtypes was complementarity of laminar expression. This feature was found to be most conspicuous in area 17. In Figure 2.8, the profile plots illustrate densitometric changes in binding of \([^{3}H]8\text{-OH-DPAT}, [^{3}H]\text{mesulergine, and [}^{125}I\text{]DOI across cortical laminae within area 17 expressed as a proportion of peak binding for eight representative ages between PD0 and adulthood. The distributions of 5-HT\(_{(1p)}\) sites did not exhibit significant laminar variation in area 17 and were not included to simplify visual comparisons. At PD0, only the 5-HT\(_{1C}\) receptors exhibited a specific laminar distribution (layer IV) which would be maintained through subsequent stages in development. While 5-HT\(_{1C}\) receptors were predominant deep within the cortical plate, among prospective layer IV cells (Fig. 2.7B, Fig. 2.8A), the 5-HT\(_{1A}\) (Fig. 2.7A) and 5-HT\(_2\) (Fig. 2.7C) receptors were concentrated within the same, deeper, infragranular laminae (Fig. 2.8A). Beginning at PD10 (Fig. 2.8B) and continuing throughout the remainder of postnatal development, the highest levels of 5-HT\(_{1A}\) receptors were concentrated in supragranular laminae (I-III). On the other hand, the highest levels of 5-HT\(_{1C}\) and 5-HT\(_2\) receptors were consolidated in layer IV. Between PD10 (Fig. 2.8B) and PD20 (Fig. 2.8C) they were localized to the same lamina, but by PD40 (Fig. 2.8D) their relative distributions were changed. 5-HT\(_{1C}\) receptors were maintained in deeper strata
of layer IV, but the 5-HT2 receptors were now concentrated in upper layer IV. This relationship was maintained beyond PD75 (Fig. 2.8E), as long as both receptor subtypes were present. By PD120 the distribution of 5-HT1C receptors was essentially homogeneous across different cortical laminae but the highest density of 5-HT2 receptors was evenly distributed through layers I-IV (Fig. 2.8F). The strong overall tendency in all the panels of Fig. 2.8, is for the concentration of each binding site to peak in different layers and at different cortical depths. The complementarity in the laminar binding profile is most pronounced during intermediate ages examined (Fig. 2.8B - E).
Figure 2.7. Photomicrographs showing the laminar distribution of 5-HT$_{1A}$ (A), 5-HT$_{1C}$ (B) and 5-HT$_{2}$ (C) in near-adjacent sections from kitten visual cortex (Area 17 and 18) at birth (PD0). Autoradiographic images are inset within a photograph of the same section stained with cresyl violet in order to establish laminar boundaries. Peak expression of 5-HT$_{1A}$ (A) and 5-HT$_{2}$ (C) receptors was limited to a single band within infragranular cortical layers (V - VI). 5-HT$_{1C}$ receptors were also limited to a single band (B) but this band was positioned more superficially, extending from upper layer V to deeper levels of the cortical plate (Cp). Scale bar = 500 μm.
**Figure 2.8.** These densitometric profile plots illustrate the changing levels of 5-HT\textsubscript{1A}, 5-HT\textsubscript{1C} and 5-HT\textsubscript{2} receptors as a function of postnatal development. For each age, specific binding density is displayed as a proportion of peak binding. The positions of cortical laminae are indicated by Roman numerals (I-VI); \(cp\) = cortical plate.
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Figure 2.8
Figure 2.9. Higher magnification photomicrographs demonstrating laminar and intralaminar relationships between the distributions of 5-HT$_{1A}$ (A), 5-HT$_{1C}$ (B) and 5-HT$_2$ (C) receptors and 5-HT$_{Up}$ (E) sites in near-adjacent sections from PD50 kitten visual cortex (Area 17). 5-HT$_{1A}$ receptors were densely distributed throughout supragranular (I-III) and to a lesser degree in infragranular cortical laminae (V-VI). The highest densities of 5-HT$_{1C}$ and 5-HT$_2$ receptors were found in middle layers. In addition to displaying a laminar complementarity with 5-HT$_{1A}$ receptors, 5-HT$_{1C}$ and 5-HT$_2$ receptors demonstrated an intralaminar complementarity with the highest density of 5-HT$_{1C}$ receptors located within deeper levels of layer IV and 5-HT$_2$ receptors predominant in superficial layer IV. Moreover, both receptor subtypes exhibited a patchy appearance within layer IV. These patches were ~400 µm in diameter and spaced ~900 µm apart and were localized to the same vertical column (B and C, arrows). The columnar expression of 5-HT$_{1C}$ and 5-HT$_2$ receptors also extended into the deeper portions of layer III. In addition to peak levels in superficial strata of layer IV, 5-HT$_2$ receptors exhibited intermediate levels of expression in layers I-III, lower layer IV and subcortical white matter (wm). Panel E shows that the distribution of 5-HT$_{Up}$ sites was essentially uniform across all cortical laminae. The borders of cortical laminae (I-VI) and subcortical white matter (wm) are indicated at the edge of each panel with reference to the Nissl-stained section (D). Figures B & C are from serially adjacent sections. The distance of each section from that in A is indicated (in µm) in the lower left of each figure. D and E are from the same section, which was Nissl-stained following autoradiographic processing. Scale bar = 1.0 mm.
Figure 2.9
Columnar Distribution of 5-HT Receptors

Between PD30 and PD90, 5-HT\textsubscript{1C} and 5-HT\textsubscript{2} receptors were augmented in a periodic manner within layer IV of area 17. Although the patterned expression of these receptors was first apparent at PD30, it was weak and not always readily demonstrable until after PD40, at ages when both receptor subtypes were found in particularly high abundance in area 17. Because of this developmental profile, we chose to concentrate on PD50 kittens to study the columnar distributions of 5-HT\textsubscript{1C} and 5-HT\textsubscript{2} receptor subtypes.

Columns of 5-HT\textsubscript{1C} receptors emerged from a dense band of 5-HT\textsubscript{1C} receptors at the layer IV/V interface and extended radially, through the entire extent of layer IV, and up into the deepest levels of layer III (Fig. 2.10B, Fig. 2.11A, B). This distinct distribution of 5-HT\textsubscript{1C} receptors was strictly limited to area 17 (Fig. 2.11B). The patterned distribution of 5-HT\textsubscript{2} receptors resembled beady excrescences radiating from a dense band, rather than columns (Fig. 2.10C, Fig. 2.11C). This band was consolidated within upper strata of layer IV (Fig. 2.10C) in area 17 and area 18 (Fig. 2.11A, C). However, the bead-like pattern of expression was not apparent in area 18.

The spatial relationship between the patchy 5-HT\textsubscript{1C} and 5-HT\textsubscript{2} receptor distributions is apparent in Figure 2.10 (C, D) and at higher magnification, in the serially adjacent sections in Figure 2.9 (B, C). The patches of increased 5-HT\textsubscript{1C/2} receptor density were found to be precisely in register, within the same vertical column (Fig. 2.9B, 2.9C; arrows). Although the highest density of each of these 5-HT receptor subtypes was centred within different substrata of layer IV, their patchy pattern of expression was precisely aligned, where they overlapped in upper layer IV (Fig. 2.9B, 2.9C; Fig. 2.10C, 2.10D arrow heads).

The overall columnar distributions of 5-HT receptors in area 17 are best appreciated in parasagittal (Fig. 2.11) and tangential (Fig. 2.12) sections through layer IV of a PD50 kitten. The patchy distribution of 5-HT\textsubscript{1C} receptors at this age was strictly limited, in the neocortex, to visual cortical area 17 (Fig. 2.11A, 2.12A). In sections tangential to the unfolded cortical surface (Fig. 2.12), patches of 5-HT\textsubscript{1C} receptors, with an average diameter of approximately 400 μm (\(\bar{x} = 418\))
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± 18), were found distributed throughout area 17 with an average centre-to-centre spacing of approximately 900 μm (\( \bar{x} = 909 \pm 211 \)). In addition to distinctive distributions in visual cortices (Fig. 2.11D), high levels of 5-HT\(_2\) receptors were observed across a number of different cortical (e.g. FCtx, Cing, Hpc) and subcortical regions in the kitten brain (Fig. 2.11C). Measurements of area 17 from the two hemispheres which were opened and flattened indicate that the surface area of area 17 in PD50 kittens is approximately 312 mm\(^2\) (306, 318) which is similar to values previously published in the adult (Olavarria and Van Sluyters, 1985; Tusa et al., 1978; Van Essen and Maunsell, 1980). The total number of 5-HT\(_{1C}\)-rich patches within area 17 of each hemisphere was 296 and 315, respectively, which suggests a packing density of approximately 1 patch / mm\(^2\). These patches did not appear to be aligned along any particular axis nor was their periodicity significantly different across area 17.
Figure 2.10. Autoradiographic images showing the regional and laminar complementarity of 5-HT$_{1A}$ (B), 5-HT$_{1C}$ (C) and 5-HT$_2$ (D) receptors in near-adjacent sections from PD50 visual cortex. The profile plot in A, generated from a densitometric slice through cortical areas 17 and 18 at the level of the black arrows in B, compares laminar changes in binding density among the three receptor subtypes. 5-HT$_{1A}$ receptors were most highly concentrated in supra-granular layers and of intermediate density within infragranular layers. A high density of 5-HT$_2$ receptors was demonstrated in supragranular and granular layers of both area 17 and 18, with a band of peak binding limited to superficial levels of layer IV. Peak expression of 5-HT$_{1C}$ receptors was localized to the deeper half of layer IV, and was limited to area 17. Both 5-HT$_{1C}$ and 5-HT$_2$ receptors subtypes exhibited a patchy distribution, but within different strata of layer IV, in the same vertical column (filled arrow heads indicate coincident columnar localizations). The borders of areas 17 and 18 are indicated by the open arrows. Scale bar = 2.0 mm.
Figure 2.10
Figure 2.11. The distribution of 5-HT$_{1C}$ receptors, labeled with $[^3]$Hmesulergine (A), and 5-HT$_2$ receptors, labeled with $[^{125}]$DOI (B) in adjacent, parasagittal sections through PD50 kitten brain. Each of these subtypes of the 5-HT2 receptor family exhibited distinctly different regional and laminar localizations. In area 17, high levels of both 5-HT$_{1C}$ and 5-HT$_2$ receptors exhibited a periodic distribution. However, while the highest density of 5-HT$_{1C}$ receptors was limited to visual cortical area 17 at this age, 5-HT$_2$ receptors were more broadly distributed within other cortical (e.g. FCtx, Cing, Hpc) and subcortical regions. The black arrow indicates the anterior extent of visual cortical area 17. Scale bars = 3 mm. (choroid plexus, CP; corpus callosum, cc; suprasplenial sulcus, sspl).
Figure 2.12. The tangential distribution of 5-HT$_{1C}$ receptors in visual cortex at PD50, in sections taken at 400, 640, and 1000 μm (top, middle, bottom panels, respectively) from the surface of a flattened right hemisphere. The overall patchy distribution of 5-HT$_{1C}$ receptors in the neocortex was limited to visual cortical area 17. Patches of 5-HT$_{1C}$ receptors averaged 400 μm in diameter and were separated by an average centre-to-centre spacing of 900 μm. It is apparent from these sections, that columns of 5-HT$_{1C}$ receptors extended from deeper levels of layer III (top) through the entire extent of layer IV and disappeared in a dense band of binding at the layer IV / V border (bottom). The dotted line indicates the approximate position of the area 17 / 18 border. Scale bars = 10 mm and 2 mm (M, ventro-medial; L, dorso-lateral; A, anterior; P, posterior).
The results of this study reveal that 4 serotonin receptor subtypes are expressed in unique and complementary temporal, regional, laminar, and intra-laminar patterns in developing cat visual cortex. 5-HT_{1A} receptors reached peak levels between PD10 and PD30 and were concentrated in superficial (I-III) and in deep (V, VI) laminae of all visual cortical areas. The 5-HT_{1C} and 5-HT_{2} receptor subtypes displayed their highest levels between PD40-PD75 and PD75-PD120, respectively, within different strata of layer IV. While 5-HT_{1C} receptors were restricted to area 17, 5-HT_{2} receptors were highly expressed in area 17, 18 and lateral suprasylvian cortex. Although 5-HT_{Up} sites exhibited significant increases in level of expression throughout postnatal development, they did not demonstrate notable regional or laminar disparity until adulthood.

A striking result of these studies was the finding that peak densities of 5-HT_{1C} and 5-HT_{2} receptors demonstrated transient columnar distributions within layer IV of area 17 in developing visual cortex. Additionally, their differential intra-laminar and regional distributions offered novel evidence regarding a potential relationship of their columnar organization in visual cortex to functional afferent pathways. These results are discussed, in the following sections, with regard to comparative 5-HT receptor-specific binding studies and the functional anatomy of the developing visual cortex.

Columnar Segregation

Perhaps the most significant implication which emerges from the results of this study relates to the transient columnar expression of 5-HT_{1C} and 5-HT_{2} receptors in area 17 and, consequently, the potential role they may play in the formation of functional cortical columns, such as those related to ocular dominance, orientation, or some other columnar property of visual cortex during development.
Other columnar markers. Despite abundant physiological evidence for the existence of functionally similar columnar compartments among primates and carnivores, endogenous anatomical markers of any columnar organization in non-primate striate cortex had not been described (see LeVay and Nelson, 1991 for review). The discovery that cytochrome oxidase-blobs are present in the visual cortex of adult cats (Murphy et al., 1990) and ferrets (Cresho et al., 1992), combined with evidence of a columnar distribution of 5'-nucleotidase (Schöen et al., 1990), cytochrome oxidase (Dyck and Cynader, 1992; Dyck and Cynader, submitted), acetylcholinesterase (Dyck and Cynader, 1992; Dyck and Cynader, submitted), serotonin receptors (Dyck and Cynader, 1990a; Dyck and Cynader, 1990b; Dyck and Cynader, submitted) and synaptic zinc (Dyck et al., 1993; Dyck and Cynader, 1992; Dyck and Cynader, 1989) in young kittens, indicates that this is no longer the case.

The distribution of synaptic zinc (Zn), a vesicular component of a subset of glutamatergic terminals in cat visual cortex (Beaulieu et al., 1991; Beaulieu et al., 1992), is enriched in columns within layer IV of area 17 (Dyck et al., 1993; see Chapter 3). When compared in serially adjacent sections, Zn-rich columns are precisely coaligned with the 5-HT₁C receptor columns described here (Dyck and Cynader, 1992; Dyck and Cynader, submitted; see Chapter 5). Moreover, we have recently demonstrated that CO blobs in kitten visual cortex, are coaligned with patches of increased staining for acetylcholinesterase (AChE), and that the distribution of these CO / AChE blobs is precisely complementary to 5-HT receptor / Zn columns (Dyck and Cynader, 1992; Dyck and Cynader, submitted; see Chapter 5).

The distribution of numerous columnar markers, and their relationships to CO-blob or interblob zones, and to the functional organization of visual cortex, have been extensively studied in primate striate cortex (see LeVay and Nelson, 1991 for review), but functional correlates of cortical columns in the cat have not yet been described. We have recently demonstrated that Zn is also distributed inhomogenously in primate striate cortex in a manner which is, both tangentially and laminarly, precisely complementary to CO (Dyck et al., 1993; Dyck and Cynader, submitted). In combination with the demonstration that the serotonergic innervation of striate cortex is more
abundant outside of CO-blobs (Hendrickson, 1985), these studies indicate the possibility that a phylogenetic conservation of columnar markers in striate cortex may also reflect parallel functional compartmentalizations. A key question concerns the nature and properties of the columnar system(s) demarcated by the 5-HT receptors. In primates the CO blobs have been associated with several distinct functional and anatomical properties, including colour specificity, ocular dominance, zones of low orientation selectivity, and differential inputs and output connectivity (Livingstone and Hubel, 1984a; Livingstone and Hubel, 1984b). Since the 5-HT receptor columns reported here are precisely complementary to the CO blobs of the cat cortex, some of the same functional properties may be defined by the 5-HT system in development. Of the various features in primate, colour specificity is the least likely candidate to be defined by 5-HT receptors in cats. This is because cats have rather poor colour vision (Daw, 1973), and also because of evidence from nocturnal primates which indicates the presence of CO blobs, even in the absence of colour selectivity (Condo and Casagrande, 1990).

Ocular dominance. In old world monkeys, CO blobs are in the centers of ocular dominance columns and the same relationship may apply in cats. The temporal characteristics of the patchy distribution of 5-HT$_{1C}$ and 5-HT$_2$ receptors parallel the time course for the developmental plasticity of ocular dominance columns. The first signs of 5-HT$_{1C}$ columns are evident around PD30, after the eye-specific geniculate afferents begin to segregate in layer IV (LeVay et al., 1978), and their columnar expression is highest through the developmental period during which ocular dominance columns are most sensitive to manipulations of visual input (Cynader et al., 1980; Hubel and Wiesel, 1962; Jones et al., 1984; Olson and Freeman, 1980). However, the spatial distributions of 5-HT receptor columns are not consistent with a direct relationship to ocular dominance. Anatomical and physiological studies indicate that ocular dominance columns are spaced about 0.5 mm centre-to-centre, only one-half that of 5-HT receptor columns (Anderson et al., 1988; LeVay et al., 1978; Löwel and Singer, 1987; Shatz et al., 1977; Shatz and Stryker, 1978); a result which is substantiated by quantitative data showing that each
cortical hemisphere contains approximately 600 ocular dominance columns (Anderson et al., 1988). Instead we estimate that each visual cortex contains around 300 5-HT receptor columns. Moreover, 5-HT1C receptors are only found in area 17, while ocular dominance columns are represented in areas 17 and 18. Finally, we have shown by direct comparison, in the same cortical section, that the distributions of synaptic zinc-rich-columns, which are coaligned with 5-HT receptor columns in area 17, are not explicitly related to ocular dominance columns labeled transneuronally by intraocular injections of [3H]proline (Dyck and Cynader, 1992; Dyck and Cynader, submitted).

**Orientation.** In terms of their average centre-to-centre spacing, 5-HT receptor columns appear to be more closely related to the orientation column system in cats (Hubel and Wiesel, 1962; Löwel et al., 1988). Metabolic mapping studies of the orientation column system in cat visual cortex using [14C]deoxyglucose, describe a regular system of parallel bands whose trajectory is orthogonal to the area 17 / 18 border (Löwel et al., 1987; Swindale et al., 1987). We do not find that 5-HT receptor columns are found in bands, nor do they appear oriented along any preferred axis (see Fig. 12). However, previous studies have indicated singularities in the cat and monkey cortical orientation maps, zones where different orientation bands coalesce and which contain broadly tuned neurons (Blasdel and Salama, 1986; Bonhoeffer and Grinvald, 1991; Swindale et al., 1987). These singularities are thought to be associated with CO-blobs in monkeys (Blasdel, 1992) and may well have the same association in cat cortex. Since the 5-HT receptor columns are complementary to the CO blobs, these results imply that 5-HT receptor columns may distinguish neurons in the visual cortex which may show a relatively high degree of orientation tuning.

**Parallel processing streams.** In the primate, the segregation of cortical compartments into CO blobs and interblobs, has been functionally related to the hierarchical processing of colour and form perception which are, furthermore, related to similarly segregated geniculocortical pathways (Hubel and Livingstone, 1987; Livingstone and Hubel, 1987a; Livingstone and Hubel,
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1987b; but see Condo and Casagrande, 1990; Lachica and Casagrande, 1992). The lateral geniculate innervation of cat visual cortex arises from three major classes of neurons (X-, Y-, and W-cells), which also form functionally, and anatomically separable processing streams (for review see Sherman, 1985). The differential distributions of 5-HT$_{1C}$ receptors appear to mirror the X-cell innervation of visual cortex, while the 5-HT$_{2}$ distribution appears to reflect non-X-cell geniculocortical projections. Like the peak distributions of 5-HT$_{1C}$ receptors, terminals of all X-cell axons are restricted to area 17, predominantly within deeper levels of layer IV, with lesser projections to lower layer III and layer VI (Ferster and LeVay, 1978; Humphrey et al., 1985). Areas 18, 19 and LS, which receive no X-cell input, also do not contain high levels of $[^{3}H]$mesulergine binding. Similar to the distribution of 5-HT$_{2}$ receptors, Y- / W-cell projections arborize within upper levels of layer IV and lower layer III of areas 17 and 18 (Friedlander and Martin, 1989; Humphrey et al., 1985). Finally, high levels of 5-HT$_{2}$, but not 5-HT$_{1C}$ receptors, transiently demarcate the lateral suprasylvian cortex, which receives the majority of its geniculate input from several classes of Y-cells in the C-laminae (Berson, 1985), and only a transient and sparse innervation from cells in the A laminae (Tong et al., 1991). Furthermore, the development of visual response properties of the suprasylvian cortex occurs between PD9 and PD15 (McCall et al., 1988; Price et al., 1988) at an age when 5-HT$_{2}$ receptors exhibit a striking increase in their levels of expression.

Corticocortical projections. Corticocortical connections among pyramidal neurons in layers II/ III and V in the visual cortex of primates and non-primates are periodically distributed at 1 mm intervals in the tangential plane (see Katz and Callaway, 1992 for review). When related to CO blobs in primate visual cortex, intrinsic cortical connections appear to be made reciprocally among CO-rich patches or CO-poor regions, but not between them (Livingstone and Hubel, 1984b). Preliminary studies indicate that this relationship among CO blobs holds true for corticocortical connections in cat visual cortex as well (Boyd and Matsubara, 1992). This patchy pattern of intrinsic connections emerges early in development from an immature, homogenous
distribution (Callaway and Katz, 1990; Luhmann et al., 1986; Price, 1986), by a process of axon
elimination, which is activity-dependent (Callaway and Katz, 1991; Löwel and Singer, 1992).
Based on the interrelationship between the patchy distribution of 5-HT receptors and CO blobs in
cat cortex, it is possible that the transient columnar distribution of 5-HT receptors is related to the
process of refining cortical interconnections during development. This is substantiated by the
observation that the critical period for the refinement of corticocortical projections ends by 14
weeks (Dalva et al., 1992), at the same time that 5-HT receptor patches disappear.

**Comparative Studies**

**5-HT1 Receptors.** Among the numerous 5-HT receptor subtypes, the 5-HT1A subtype
has been the most intensively studied, primarily as a result of the development of the highly
selective radioligand [3H]8-OH-DPAT (Gozlan et al., 1983). Its specificity has been demonstrated
to be identical in the neocortex of species as diverse as rat, pig and human (Hoyer et al., 1985;
Hoyer et al., 1986a). Using [3H]8-OH-DPAT we have shown that 5-HT1A receptor sites are
highly regulated in the cat visual cortex during development. Consistent with our results, levels of
5-HT1A receptors in the cortex of developing rats (Daval et al., 1987) and humans (Bar-Peled et
al., 1991) exhibit transient, 3- to 4-fold higher densities than in the adult. In the rat, levels
increased markedly during the first 3 weeks after birth. This may be compared to the cat visual
cortex, where the highest levels of 5-HT1A receptors were demonstrated between 2 - 5 weeks
postnatally. In human brain, the highest densities of 5-HT1A receptors were found in the fetal
neocortex, between the 16th and 22nd weeks of gestation (Bar-Peled et al., 1991). Unlike the rat,
where levels remain essentially stable through the remainder of the animals' lifespan (Daval et al.,
1987; Gozlan et al., 1990), our observations of a progressive reduction of [3H]8-OH-DPAT
binding density are consistent with results obtained in the aging human cortex (Cross et al., 1988;
Dillon et al., 1991; Middlemiss et al., 1986).
The ontogenetic expression of 5-HT receptors in cat visual cortex, analysed with [3H]5-HT, is virtually identical to that of 5-HT\textsubscript{1A} receptors, suggesting that the 5-HT\textsubscript{1A} subtype may be the predominant subtype of the 5-HT\textsubscript{1} family found in cat visual cortex. During postnatal development of the kitten visual cortex, levels of [3H]5-HT binding in tissue homogenates increased from low levels at birth, to a peak at 4 weeks of age, and then subsequently declined to adult levels (Jonsson and Kasamatsu, 1983). In rat visual cortex, by contrast, the transient increase of [3H]5-HT binding was apparent during the first postnatal week followed by a progressive decline with increasing age (Uzbekov et al., 1979; Zifa et al., 1988; but see Uphouse and Bondy, 1981; Zilles et al., 1985). Although providing circumstantial support, the usefulness of [3H]5-HT in studies of 5-HT receptor subtypes is obviously constrained by its demonstrated lack of specificity.

The temporal and laminar patterns of the distribution of 5-HT\textsubscript{1A} receptors did not vary significantly between the different visual cortical regions examined. Except for the early postnatal period, 5-HT\textsubscript{1A} receptors were highest in supragranular cortical layers, lowest in middle layers, and attained intermediate levels in infragranular layers. Comparable laminar and ontogenetic analyses of the distribution of 5-HT\textsubscript{1A} receptors in visual cortex of other species have not yet been reported. Unlike the cat, the highest levels of [3H]8-OH-DPAT binding in the rat are localized to deeper cortical layers (Marcinkiewicz et al., 1984; Pazos and Palacios, 1985). On the other hand, the laminar pattern of 5-HT\textsubscript{1A} receptors among non-striate neocortical regions appears similar between adult cats and humans, but a detailed description of human visual cortex findings was not presented (Dillon et al., 1991; Pazos et al., 1991; Pazos et al., 1987a). The laminar pattern of 5-HT\textsubscript{1A} receptors, which differentiates striate from the immediately adjacent, extrastriate visual cortical areas in the adult macaque, appears identical to the results presented here (Parkinson et al., 1989). In the cat, this distinct pattern develops between PD120 and adulthood as a result of a laminar- and region-specific reduction in [3H]8-OH-DPAT binding. Developmental studies in the striate cortex of primates are required to evaluate possible ontogenetic similarities between these species.
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The resolution of in vitro autoradiography is not sufficient to visualize the cell bodies of neurons or to determine the cellular localization of receptors. However, indirect evidence provided by neuron-specific lesions indicates that the 5-HT$_{1A}$ receptors are localized entirely on neurons intrinsic to the kitten visual cortex (unpublished results). The regional colocalizations of 5-HT$_{1A}$ mRNA, assessed with in situ hybridization, combined with in vitro autoradiography, in the adult rat brain, support the idea that 5-HT$_{1A}$ receptors have a predominantly somatodendritic location (Chalmers and Watson, 1991; Pompeiano et al., 1992).

5-HT$_2$ Receptors. The mature distributions of 5-HT$_{1C}$ and 5-HT$_2$ receptor subtypes have been extensively studied in the rodent, porcine, and primate brain (Blue et al., 1988; Gross-Isseroff et al., 1990; Hoyer et al., 1986b; Lidow et al., 1989; McKenna et al., 1989; Pazos et al., 1985; Pazos et al., 1991; Pazos et al., 1984b; Pazos and Palacios, 1985; Pazos et al., 1987a; Pazos et al., 1987b; Rakic et al., 1988). Consistent with the results presented here, 5-HT$_{1C}$ binding sites were reported to be present at low levels, with a slight preference in the middle layers reported in the adult human and rat occipital cortex (Hoyer et al., 1986b; Pazos et al., 1991; Pazos and Palacios, 1985; Pazos et al., 1987a). The localization of mRNA encoding the 5-HT$_{1C}$ receptor in adult rodents, has confirmed and extended these results (Hoffman and Mezey, 1989; Mengod et al., 1990a; Molineaux et al., 1989).

Possibly due to the greater availability of 5-HT$_2$-specific ligands, the distribution of 5-HT$_2$ receptors has been more extensively studied, particularly in striate cortex. In cats, we found that the highest densities of 5-HT$_2$ sites are equally distributed across I-IV in striate cortex, and predominantly in superficial layers in extrastriate regions. In rats, the highest concentration of 5-HT$_2$ receptors was demonstrated in the middle cortical layers (III, IV and V), with highest concentrations at the layer IV/V border (Blue et al., 1988; Mengod et al., 1990b; Pazos et al., 1985). In human and primate material, there are concentrations in layer III, IVa and IVc (Gross-Isseroff et al., 1990; Lidow et al., 1989; Parkinson et al., 1989; Pazos et al., 1991; Pazos et al., 1987b; Rakic et al., 1988). It is possible that these inconsistencies can be attributed to species-
specific localization patterns. Alternatively, the majority of these studies used 5-HT2 antagonists (e.g. \([^{3}H]\)ketanserin, \([^{125}I]\)LSD), which have been shown to label more than one population of 5-HT2 receptors (McKenna and Peroutka, 1989; Pierce and Peroutka, 1989) and/or differential affinity states of multiple receptor subtypes (Leonhardt and Titeler, 1989; Lyon et al., 1987).

Very little is known of the ontogenesis of the 5-HT2 receptor family in other species or cortical areas. In this report we have demonstrated that the distributions, and levels, of 5-HT1C and 5-HT2 receptors are robustly regulated in the kitten visual cortex during postnatal development. The ontogenetic regulation of 5-HT1C and 5-HT2 receptors has also been studied in rat and human brain, but have not been determined with regional specificity. In the whole rat brain, Roth et al. have reported that transient increases in 5-HT1C and 5-HT2 sites during early development (Roth et al., 1991b), were accompanied by parallel increases in the corresponding mRNA (Roth et al., 1991a), which became markedly reduced with age. A marked reduction of 5-HT2 receptors in aged rats has also been reported by others (Battaglia et al., 1988; Gozlan et al., 1990). In addition, although studies of early development have not been reported, age-dependent reductions of 5-HT2 receptors have been reported to occur in the human brain, beyond adolescence, during normal aging and in age-related disease (Biegon, 1991; Cross et al., 1988; Gross-Isseroff et al., 1990; Marcusson et al., 1984; Reynolds et al., 1984; Wong et al., 1984).

The cellular localization of 5-HT1C and 5-HT2 receptors in the adult rodent brain has been inferred, using in situ hybridization and lesion studies, to reside predominantly on non-serotonergic neurons which are intrinsic to the neocortex (Fischette et al., 1987; Hoffman and Mezey, 1989; Mengod et al., 1990a; Mengod et al., 1990b; Molineaux et al., 1989). However, based on lesion studies, and the effects of input-manipulations reported previously (Dyck et al., 1991), it would appear that, at least during a distinct phase of postnatal development, 5-HT1C receptors are associated with axonal terminals of lateral geniculate neurons or possibly with glial cells. In addition, the transient, high levels of 5-HT2 receptors distributed within the subcortical white matter suggests that a significant proportion of these binding sites are localized to glial cells. The resolution of the autoradiographic technique is not sufficiently fine to determine this with
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certainty. A detailed analysis of these receptors using techniques providing cellular resolution are necessary.

As in other species (Hoyer et al., 1986b; Pazos et al., 1984a; Yagaloff and Hartig, 1985; Zilles et al., 1986), we also found very high concentrations of [3H]mesulergine binding sites in the choroid plexus at all stages of development. Our results also indicate high levels of [125I]DOI binding in the choroid plexus of the cat brain (see Fig. 2.11), which are consistent with previous findings (McKenna et al., 1989).

5-HT 3 Receptors. As mentioned in the results, the presence of 5-HT3 receptors in the developing kitten visual cortex was not detected using currently available tritiated ligands. Consistent with binding studies in other species (Barnes et al., 1992; Kilpatrick et al., 1988; Kilpatrick et al., 1989; Waeber et al., 1989), we did find dense labeling in limbic structures, indicating that the lack of binding in visual cortical areas was not due to lack of sensitivity, or other methodological issues. The development of radioligands with higher specific activity may help to allow autoradiographic localization of the low levels of 5-HT3 sites that the cortex may yet possess.

5-HT Uptake Site. A robust developmental regulation of 5-HTU p sites has been previously described for the rat visual and somatosensory cortex (D'Amato et al., 1987). We also found that 5-HTU p sites were developmentally regulated in the postnatal kitten visual cortex. Unlike the kitten, where we observe a gradual increase in expression until adulthood, which is essentially homogenous across cortical laminae, 5-HTU p sites in the developing rat are transiently expressed, during the early postnatal period, at very high levels within layer IV in primary sensory areas (D'Amato et al., 1987). These 5-HTU p sites are presumably localized on axon terminals of serotonergic neurons which arise from the raphe nuclei (Bennett-Clarke et al., 1991; D'Amato et al., 1987). Apart from the apparent lack of a transient increase in expression during early development, our descriptions of the regional and laminar distributions of 5-HTU p sites in the adult
cat are consistent with those found in human (Duncan et al., 1992), primate (Lidow et al., 1989) and rat (Duncan et al., 1992; Kovachich et al., 1988) visual cortices. In each of these studies, the distribution of high affinity $5\text{-HT}_{1P}$ sites was correlated with the density of serotonergic innervation. The relationship of 5-HT receptor subtypes to serotonergic innervation of visual cortex is discussed in greater detail below.

**Relationship to Serotonergic Innervation**

Although the ontogeny of the serotonergic innervation of visual cortex has been investigated in rats (Bennett-Clarke et al., 1991; D'Amato et al., 1987; Descarries et al., 1975; Lidov et al., 1980; Nakazawa et al., 1992; Papadopoulos et al., 1987), ferrets (Voigt and De Lima, 1991a; Voigt and De Lima, 1991b), cats (Gu et al., 1990; Mulligan and Törk, 1988), and primates (de Lima et al., 1988; Foote and Morrison, 1984; Kosofsky et al., 1984; Morrison et al., 1984; Morrison et al., 1982), corresponding data regarding 5-HT receptors during early postnatal development are only available for the rat.

In the rat, a transient hyperinnervation of primary sensory areas by raphe neurons (Bennett-Clarke et al., 1991; D'Amato et al., 1987; Nakazawa et al., 1992), is accompanied by corresponding high levels of $5\text{-HT}_{1P}$ sites (D'Amato et al., 1987) and $5\text{-HT}_{1B}$ receptors (Leslie et al., 1992). Somewhat similar to the rat, the expression of $5\text{-HT}_{1P}$ sites in kitten visual cortex appears to be loosely correlated with its serotonergic innervation. In the second postnatal week (the earliest age studied), serotonin-immunoreactive fibers were equally and sparsely distributed across cortical laminae I - V (Gu et al., 1990), but by postnatal week 6 appeared adult-like, with fiber densities greatest in superficial laminae, less dense in lamina V, and sparse in laminae IV and VI (Gu et al., 1990; Mulligan and Törk, 1988). Among the different 5-HT receptor subtypes investigated, the laminar distribution of $5\text{-HT}_{1A}$ receptors in the cat visual cortex most closely resembled the pattern of its serotonergic innervation. Although their spatial distributions through postnatal development were similar, the marked increases of $5\text{-HT}_{1A}$ receptors between PD20 and
PD40, followed by a reduction to adult levels, does not appear to be reflected by a corresponding change in fiber density (Gu et al., 1990). Biochemical analyses indicate a marked increase in the endogenous concentrations of 5-HT in the developing kitten visual cortex between the third and fifth postnatal weeks (Jonsson and Kasamatsu, 1983), with a parallel transient increase throughout the brain (Daszuta et al., 1979), which is identical to the autoradiographic data, but in conflict with the available immunocytochemical results (Gu et al., 1990). Furthermore, the ontogenesis of serotonergic fibers in the ferret, a related carnivore whose cortical histogenesis is nearly identical to that of the cat, demonstrates an increased density of serotonergic fibers during the equivalent postnatal period (Voigt and De Lima, 1991b). These results suggest that this period of postnatal development is potentially critical for the phenotypic maturation of serotonergic neurons.

The relationship between 5-HT receptors, and particularly the 5-HT1A subtype, with the serotonergic innervation of visual cortex, appears more clearly defined in the adult cat. Immunocytochemical studies in the adult cat, indicate that the arbors of serotonergic axon terminals are mostly restricted to the superficial laminae (I-III) and layer V (Gu et al., 1990; Mulligan and Dirk, 1988). The predominantly superficial distribution of fibers is consistent with the highest levels of the 5-HT1A, 5-HT2 and 5-HTU, but only the 5-HT1A receptor exhibits a higher level of expression specific to layer V. Although not reported in these studies, it would be interesting to see whether the decreased density of 5-HT1A receptors in area 17 and 18, relative to the directly adjacent cortical regions, is reflected by a similarly sharp transition in the density of innervation by serotonergic fibers.

The serotonergic innervation of the mammalian visual cortex arrives via two parallel ascending projections, which are morphologically distinct, and originate from neurons in either the dorsal or median raphe nuclei (Molliver, 1987; Mulligan and Törk, 1988). Their differential distributions and their relative contribution to the innervation of the visual cortex of cats during development are not known, which precludes an analysis of their relative distribution to that of 5-HT receptor subtypes. However, the laminar pattern of 5-HT2 receptors in the neocortex of rats has been described to be associated with the fine axon projections which arise from the dorsal
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raphe nucleus (Blue et al., 1988). Similarly, serotonin axons (Kosofsky and Molliver, 1987; Morrison et al., 1982) and synapses (de Lima et al., 1988) in primate striate cortex, are present in all cortical layers, but form especially prominent, dense bands of arborizing fibers from midlayer III through IVCα (Kosofsky and Molliver, 1987; Morrison and Foote, 1986; Morrison et al., 1982), which is reflected by the distribution of 5-HT2 receptors (Lidow et al., 1989; Parkinson et al., 1989; Rakic et al., 1988). However, without the development of a method with improved cellular resolution, which is compatible with double label studies (e.g. 5-HT receptor-specific antibodies), this relationship cannot be ascertained, nor extended to studies in the cat.

The data presented here indicate that the regulation of serotonergic innervation and 5-HT receptors are independently controlled during cortical development. Furthermore, the existence of two pharmacologically and anatomically separate ascending serotonergic systems, combined with a multiplicity of receptors to activate, provides a diverse range of responses which may be necessary to guide the multiple processes involved in visual cortical development.

Conclusions

In the present study we have described the comparative distributions of the 5-HT 1A, 1C, 2 and 3 receptor subtypes as well as the 5-HT uptake site, in the kitten visual cortex during postnatal development. The temporal and spatial complementarity of their respective distributions indicate that 5-HT might play multiple roles in different visual cortical regions and layers at different times during development. Furthermore, this first demonstration that specific 5-HT receptors are transiently organized in columns of kitten visual cortex, argues for a specific role for 5-HT to participate in processes which determine the synaptic development and functional organization of columnar systems in visual cortex. Investigations of the physiological consequences of activating specific 5-HT receptor subtypes in the visual cortex during postnatal development, together with anatomical studies to determine their precise cytological localization, are underway to address fully this role for 5-HT.
ONTOGENIC DISTRIBUTION OF SYNAPTIC ZINC IN THE CAT VISUAL CORTEX

It is apparent that the chelatable pool of zinc plays a complex role in the modulation of neurotransmission in the adult brain (see Chapter 1). Its distribution in the adult neocortex (Haug, 1967; Zilles et al., 1990) and hippocampus (Haug, 1967) is specific to neuronal terminals in a laminar-specific manner which might reflect an underlying functional, rather than just a chemical, specificity. Moreover, ontogenic studies of the hippocampal and striatal distribution reveal developmental gradients which appear to reflect underlying synaptogenic gradients (Crawford and Connor, 1972; Zimmer and Haug, 1978; Vincent and Semba, 1989). However, the ontogenic distribution of zinc in the neocortex of mammals has not been examined.

It has long been established that neurotransmission via the geniculocortical projection is conveyed by an excitatory amino acid (Hagihara et al., 1988; Tsumoto et al., 1986), and that it is likely to be aspartate or glutamate (Erdo and Wolff, 1990; Fosse et al., 1989; Miller et al., 1989; Tamura et al., 1990). However, the precise neurochemical identity has not been established by anatomical methods. We have determined, by ultrastructural colocalization, that glutamate-containing axonal terminals in the cat visual cortex are highly enriched with vesicle-bound zinc (Beaulieu et al., 1992). The histochemical localization of synaptic zinc may provide a procedure for examining the development of, at least in part, the glutamatergic innervation of visual cortex. An anatomical understanding of the ontogenic distribution of zinc-containing, glutamatergic terminals would provide a starting point from which to determine their contribution to activity-dependent
mechanisms of plasticity during development. Using a modification of the selenium-histochemical technique developed by Danscher (1982), the regional, laminar and ultrastructural distribution of the vesicle-bound, synaptic pool of zinc in the postnatal cat visual cortex are described.

Materials and Methods

Light Microscopy

Tissue preparation. The histochemical localization of zinc in the visual cortex of 28 cats ranging in age from birth (postnatal day 0; PD0) to adulthood (> PD360) was assessed by a modification of the selenium method developed by Danscher (1982). We used three cats at PD0 and PD10; two each at PD2, PD20, PD30 and PD40; six at PD50; one each at PD5 and PD75; and six as adults. Under general anaesthesia (PD0 - PD50, halothane to effect; > PD50, sodium pentothal, 28 mg / kg i.v.) an intravenous injection of 20 mg / kg sodium selenite (20 mg / ml) was administered through the cephalic or saphenous vein at a rate of 1 ml / min. After a 15 - 20 min survival period the animals were perfused through the ascending aorta with 50 - 200 ml 0.1M Sorenson’s buffer (SB; pH 7.6). The brains were quickly removed, immediately frozen in isopentane (-70°C) and stored at -20°C. Coronal, cryostat sections (16 μm) were cut through the visual cortex, thaw-mounted onto gelatin-coated glass slides and stored dessicated at -20°C in preparation for histochemical staining. The brains of two PD50 and two adult animals were hemisected, the brainstem removed and the cortex unfolded and flattened according to a method similar to that described by Olavarria and Van Sluyters (1985) in order to assess the tangential pattern of zinc staining within individual visual cortical laminae. Fiduciary landmarks were created, by piercing the block with a 31 gauge needle, in order to facilitate alignment and reconstruction of serial sections. Cryostat sections were cut at 20 - 30 μm, thaw-mounted on glass slides and processed in the same manner as the coronal sections.
In order to assess and control for the degree to which non-specific staining might contribute to optical density measurements, the brains of three additional animals (PD10, PD50 and adult), which had not received sodium selenite infusions, were processed identically to those of the experimental groups.

**Histochemistry.** All glassware was acid cleaned and solutions were prepared with deionized water to prevent contamination by exogenous zinc. In preparation for staining, the sections were thawed and allowed to dry at room temperature, fixed in a descending series of ethanol (95%, 15 min; 70%, 2 min; 50%, 2 min), hydrated, and then dipped in 0.5% gelatin to prevent autocatalytic staining.

Zinc-selenide precipitate was visualized on slides by physical development in 200 ml of freshly prepared developer containing 50% Gum arabic (120 ml), 2.0 M sodium citrate buffer (20 ml), 0.5 M hydroquinone (30 ml) and 37 mM silver lactate (30 ml). In complete darkness, sections were incubated in the developing solution at 26°C for 50 - 150 min, depending on the age of the animal and desired staining intensity. The histochemical detection of zinc in the PD0 brain required a development time of 150 min while animals of ages greater than PD50 required only 60 minutes in the developer to achieve optimum staining intensity. These differences in developing time precluded a quantitative analysis of zinc distribution across different age groups and allowed us to only make within group comparisons.

After staining, slides were washed for 20 min in running tap water at 40°C to remove the gelatin coat, rinsed in distilled water (2 X 2 min), and stabilized in 5% thiosulphate for 12 min. Slides were postfixed in 70% ethanol (EtOH) for at least 30 min, dehydrated in 100% EtOH, cleared in xylene and coverslipped with Permount.

**Regional and Laminar Boundaries.** Several approaches were utilized to relate the pattern of zinc staining to laminar and regional borders. Selected sections were counterstained with cresyl violet which were then used to establish laminar borders in the various visual cortical areas based on cytoarchitectonic criteria established by Otsuka and Hassler (1962) in adult cats and Shatz and Luskin (1986) for early postnatal development. The borders of visual cortical areas were also
determined according to the electrophysiologically defined maps of Tusa et al. (1978, 1979, 1980), which describe the relationships of regional borders and gyral patterns. Additional information was derived from a number previous studies in which we utilized autoradiographic markers with laminar-specificity or were found on lateral geniculate nucleus terminals in developing cat visual cortex (Prusky et al., 1987; Shaw et al., 1984; Shaw et al., 1986).

**Densitometric analysis and photography.** Video images were captured and optical density profiles across cortical layers of stained sections were generated on a Macintosh IIfx-based image analysis system (Panasonic BD400 CCD camera; Data Translation DT-2255 quick capture board) running Image software (NIH, v 1.42). Unless otherwise indicated, density profiles were obtained across cortical laminae from area 17 at the level of the suprasplenial sulcus. Because the variability in the developing time for the zinc staining process made it impossible to make quantitative comparisons across ages, the densitometric data were used only to establish within-animal differences in staining intensity between cortical areas and across cortical laminae.

The reverse contrast photographic images of silver-stained brain sections were produced by projecting the slide-mounted histological image from a photographic enlarger directly onto photographic paper.

**Electron Microscopy**

**Tissue Preparation.** The electron microscopic localization of zinc in developing cat visual cortex was undertaken in 12 cats (PD0-1, n = 3; PD10-15, n = 2; PD30, n = 1; PD50-60, n = 2; adult, n = 4). The cats were prepared as in the light microscopic studies except that the SB wash was followed by perfusion with 200 - 500 ml SB containing 1.0 - 2.5% glutaraldehyde. The brain was removed and the visual cortex (area 17 / 18) was blocked into 4 mm slabs which were postfixxed for 1 hour in the fixative. Tissue blocks were then washed for 15 min in several changes of 0.1M phosphate buffer (PB, pH 7.4). Vibratome sections were cut at 50 and 100 μm and then stored, floating, in PB in preparation for the histochemical visualization of zinc.
**Histochemistry.** Two different methods were employed to visualize the zinc-selenide precipitate at the ultrastructural level in developing cat visual cortex. In the first, a series of sections was incubated, free-floating and in the dark, in the identical developer solution with developing times similar to those found optimal for light microscopic visualization. Developed sections were washed twice, 5 min each, in PB prior to embedding.

The zinc-selenide reaction product was also visualized in alternate series by silver enhancement using the IntenSE M kit (Jannsen). Physical development by this method provided several advantages over traditional methods for zinc visualization; namely the tissue is processed for significantly shorter times in a solution that is light insensitive and of neutral pH (7.3). The tissue was processed as directed in the accompanying brochure except that the sections were washed in either distilled H$_2$O, PB, or 0.1M citrate buffer (CB, pH 7.4) prior to development. Sections were incubated for times ranging from 6 - 30 minutes to obtain optimal staining. After development, the sections were washed for 1 min in each of 3 changes of the appropriate pre-incubation solution (dH$_2$O, PB, or CB). Several sections were mounted on gelatin-coated slides, dehydrated in ethanols and xylene, and coverslipped with Permount for light microscopic evaluation. The remaining stained sections developed by either technique were prepared for embedding by washing in distilled H$_2$O, PB, or CB and then postfixing for 30 min in 1.0% OsO$_4$ dissolved in either distilled H$_2$O, PB, or CB. Following osmium fixation, the sections were again washed in distilled H$_2$O, PB, or CB and then dehydrated in an ascending series of ethanol; 50% and 70% (5 min each), 1.0% uranyl acetate in 70% (20 min), 90% and 95% (5 min each), 100% (2 x 10 min). The dehydrated sections were immersed in two changes of propylene oxide (10 min each) and embedded between glass slides in Durcupan ACM resin (Fluka) which was allowed to polymerize at 56°C for 2 days. Individual blocks representing supragranular, granular and infragranular cortical laminae were then microdissected and re-embedded.

Ultrathin sections of silver interference colour were cut on an ultramicrotome Ultracut E, Reichert) and mounted on Pioloform coated, single slot copper grids. Some sections were contrast enhanced with a dilute solution of lead citrate. Grids were viewed under a Phillips model 410
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electron microscope and photographs of zinc-containing profiles were taken at magnifications ranging from 7,700X to 16,500X.

Results

Technical Considerations and Controls. Our modification to the route of administration of sodium selenite from that initially described by Danscher (1982) was not found to reduce the sensitivity of the selenite histochemical technique for visualizing zinc. Intravenous administration of sodium selenite following induction of general anaesthesia required shorter survival periods than were necessary with i.p. or i.m. administration while maintenance of anaesthesia for the duration of the survival period eliminated the behavioural stress of selenium toxicity we had previously noted in the awake animal. These modifications were also found to lead to greater consistency of the histochemical results both among and within the various age groups studied.

The ultrastructural distribution of zinc in the developing cat visual cortex was assessed and compared by physical development and IntenSE M silver intensification (Jannsen). At the light microscopic level, no obvious qualitative or quantitative differences were observed between the two methods. However, the Jannsen method resulted in much better preservation of the tissue when viewed at the ultrastructural level. This was particularly evident when citrate- or phosphate-buffered washes were applied rather than distilled H2O. Physical development according to the original method did, however, result in silver particles within positive structures whose sizes tended to be more uniform. Similar observations regarding these two methods have been recently made by Stierhof et al (1991).

Tissue sections from the visual cortex of control animals which were not injected with sodium selenite, but were otherwise identically processed, were found to be devoid of silver reaction product regardless of the age of the animal or the development procedure used. In addition, densitometric measurements from these sections did not reveal any variation across cortical laminae. Neither intrinsic lamina-specific differences in optical density or non-specific background
staining contributed to the densitometric measurements reported in this study. The laminar staining observed in the developing visual cortex thus reflects the intrinsic zinc distribution and is not a result of artifactual, nonspecific staining.

**Ultrastructural Distribution of Zinc**

At the ultrastructural level, almost all of the histochemical reaction product was concentrated over synaptic vesicles regardless of the animal's age (Fig. 3.1). In the younger animals (<PD30) silver particles were, infrequently, observed outside the axon terminal. In these cases, they were usually associated with either multivesicular bodies (Fig. 3.1B, open arrow) or with microtubules (not illustrated).

When the synaptic contact of the positive terminal was cut at the appropriate angle, it always had an asymmetric appearance (Fig. 3.1, large arrows). This distinct distribution confirms previously published results (Faber et al., 1989; Frederickson and Danscher, 1988; Haug, 1967; Perez-Clausell and Danscher, 1985a), and is consistent with the observation that zinc-containing vesicles are colocalized with glutamate in presynaptic terminals (Beaulieu et al., 1992). In Figure 3.1D, the zinc-positive asymmetric synapse faces a symmetric, non-reactive contact (small arrow) on the same post-synaptic dendritic spine. At all ages, the majority of positive synapses are apposed to dendritic spines (Fig. 3.1A, B, D) and to a lesser extent onto small, distal dendritic shafts (Fig. 3.1C). Dendritic shafts were identified by the presence of mitochondria and microtubules. In contrast, dendritic spines were distinguished by their small diameter, absence of mitochondria and microtubules, and the occasional appearance of a spine apparatus. No zinc-positive contacts were found on proximal dendrites or cell somata.

The differential laminar distribution of zinc, which we describe at the light microscopic level, is reflected, at the ultrastructural level, by an apparent increase both in the total number of positive terminals as well as in the density or number of zinc-positive particles within each terminal. However, a complete analysis is underway to test this observation critically.
Figure 3.1. These electron microscographs demonstrate the ultrastructural localization of zinc in visual cortex of kittens at postnatal day 1 (A), 10 (B), 30 (C) and in adult cat (D). At all ages studied, histochemically visualized zinc was found over synaptic vesicles. These zinc-containing terminals always made an asymmetric synaptic contact on postsynaptic elements (large, solid arrows) which were most often found to be spines, based on their size and lack of microtubules. The zinc-containing vesicles were never found in terminals making symmetric synapses (D, small arrow). In younger animals (Fig. 1A) the contact was often opaque and less well defined. Only occasionally, and more often in young animals, when the zinc was visualized outside the axon terminal, it was either associated with microtubules or multivesicular bodies (B, open arrow). Scale bar in A, B, C = 0.125 μm and in D = 0.25 μm.
Figure 3.1
Adult visual cortex. The distribution of histochemically reactive zinc in a representative coronal section through one hemisphere of the adult cat brain is pictured in reverse contrast in Figure 3.2. Intense, zinc-positive staining (white) was essentially limited to hippocampus (Hpc) and cerebral cortex. Representative diencephalic (lateral geniculate nucleus, Lgn; medial geniculate nucleus, Mgn) and brainstem nuclei (superior colliculus, Sc) found in this plane of section are relatively unstained, as are fiber tracts (corpus callosum, cc).

In the adult cat cerebral cortex, the typical laminar pattern of synaptic zinc was characterized by intense staining in layers I, II, III and V while layer VI was moderately stained and layer IV was only very lightly stained. Several borders between adjacent cortical areas were revealed by zinc staining although a general pattern of zinc staining throughout the cortex is evident (e.g. lateral suprasylvian cortex, PMLS, PLLS). Areas 17 and 18 of visual cortex (boundaries indicated by arrows in Figure 3.2) were distinctly differentiated from adjacent cortical area 19 laterally and cingulate cortex ventro-medially by a conspicuous lack of staining in layer IV. Unlike areas 17 & 18, the lateral boundary of visual cortical area 19 was not distinguished from adjacent cortical regions by zinc histochemistry.

Figure 3.3 is a higher power view which displays the laminar pattern of synaptic zinc differentiating adult visual cortical areas 19 (Fig. 3.3A, left), 18 (Fig. 3.3A, right), and 17 (Fig. 3.3B). The distinct 19 / 18 border is indicated with an arrow in Figure 3.3A. The tissue-section in Figure 3.3A was counterstained with cresyl violet to demonstrate that, when compared to Figure 3.3B, zinc-barren profiles in the zinc-stained tissue represent unstained neuronal somata. Optical density profiles plotted in Figure 3.3C delineate the relative changes in staining intensity across layers and between the three visual cortical areas. In each cortical area, layers I, II, III and V clearly contain the highest concentrations of histochemically-reactive zinc while in layer VI, the staining intensity is reduced by about 20%. The major difference in staining intensity between adjacent visual cortical areas is that layer IV in area 19 stains as darkly as layer VI while in area 17 /
18, layer IV contains only slightly more zinc than the subcortical white matter, only 30% of that in layers I-III & V. No staining was evident in the visual cortex of animals processed without sodium selenite treatment. The laminar staining difference thus reflects the intrinsic zinc distribution and is not a result of artifactual, nonspecific staining.

**Developing visual cortex.** At birth (postnatal day 0, PD0; Fig. 3.4), visual cortical areas 17 and 18 were distinguished from adjacent areas 19 and cingulate cortex (Cg) by the near-absence of histochemically-reactive zinc. The cingulate cortex and area 19 contained a single lamina of zinc-positive staining deep within the cortical plate (CP) and layer V. The most intensely staining area in the brain at birth was the region of mossy fiber terminals which originate from the granule cells of the hippocampal dentate gyrus (Hpc). At this age, and at all other ages studied, the histochemical reaction product was not seen in cell bodies but was limited to the neuropil.

Qualitative developmental changes in the distribution of zinc in the visual cortex of postnatal cats ranging in ages from PD2 through PD75 are illustrated in Figure 3.5. Although these are representative figures, the laminar patterns and intensity of staining in visual cortex did not differ substantially between individuals within each age group. The first indication of zinc staining within visual cortical areas 17 / 18 appeared between PD2 (Fig. 3.5A) and PD5 (not shown) and was manifested as a single faint lamina. Analysis of area 17 in the PD2 kitten at higher magnification (Fig. 3.6A, 3.6B) revealed that the single lamina containing the densest concentration of synaptic zinc represents the lower CP and upper layer V. The optical density plot (Fig. 3.5A) confirms a peak distribution in this lamina and shows that layers I, VI and upper CP were evenly and equally stained. The bottom of layer I was characteristically punctuated by an unstained compact zone of cells at the top of CP.

The laminar differentiation, which distinguished areas 17 and 18 from adjacent cortex, became more apparent at PD10 (Fig. 3.5B, arrows). While most other neocortical areas contained a single lamina of zinc-positive staining, visual cortex began to manifest a trilaminar pattern. The higher magnification photomicrographs of area 17 at PD10 (Fig. 3.6C, D) demonstrate that the highest
concentrations of histochemically-reactive zinc were found in layers III, V and the top of layer I. The corresponding optical density profile (Fig. 3.5B) confirmed a lesser relative distribution of zinc in layer IV compared to that stained in layers III and V. This trilaminar pattern was essentially maintained at PD20 with the exception that layers II and III became equally stained and a narrow gap of reduced staining distinguished layer I from layer II (Fig. 3.5C).

A considerable increase in the amount of zinc in the visual cortex was apparent between PD20 and PD30 (Fig. 3.5D). Only a qualitative statement, however, based on staining intensity and differences in developing time, can be made in this regard. The distribution of zinc in the cat visual cortex at this age appeared to have attained its adult pattern of distribution with layers I-III and V staining intensely, and layers IV and VI less stained. However, as revealed in the optical density plot, the relative intensity of zinc staining in layer IV was still at a moderate level, only slightly less than that of layer VI. In addition, a thin zone of reduced staining at the top of layer II could still be detected at this age, particularly along the medial bank of area 17. This feature disappeared in the older kittens.

After PD30, changes in the relative intensity and pattern of zinc staining appeared to be confined to layer IV of areas 17 and 18. The density of zinc staining in layer IV declined at PD50 to approximately 75% of that in the most intensely staining layers I-III and V (Fig. 3.5E). The relative reduction from layer IV at this age, however, was not uniform and appeared patchy (Fig. 3.5E; Fig. 3.7, arrows). This patchy appearance in layer IV was not readily apparent in coronal sections at the other ages studied, including PD30 (Fig. 3.5D) and PD75 (Fig. 3.5F). The staining of synaptic zinc within layer IV became reduced, at PD75, to almost 50% of that found in layers I-III and V (Fig. 3.5F). The relative reduction of synaptic zinc from layer IV compared to that of layers I-III and V in adult cortex (~30%, Fig. 3.3C), however, was still not attained at this, the latest pre-adult age studied.
**Figure 3.2.** Distribution of synaptic zinc in a frontal section through the cortex of the adult cat. Histochemically reactive zinc, demonstrated here in reverse phase (white), is predominant in telencephalic areas such as cerebral cortex and hippocampus (Hpc) but avoids diencephalic and mesencephalic structures and fiber tracts such as the corpus callosum (cc). In the cerebral cortex, zinc staining distinctly differentiates visual cortical areas 17 and 18 from the subadjacent cingulate cortex (CG) and lateral area 19 (boundaries indicated by solid arrows), by avoiding layer IV (bounded by open arrows). (Lgn, Lateral geniculate nucleus; Mgn, Medial geniculate nucleus; Sc, Superior colliculus; cc, corpus callosum; PMLS, postero-medial lateral suprasylvian cortex; PLLS, postero-lateral lateral suprasylvian cortex; Scale bar = 2.0 mm).
Figure 3.2
**Figure 3.3.** Higher power view of areal and laminar distribution of zinc in adult cat visual cortex. (A) The boundary between area 19 on the left and area 18 on the right is indicated by the arrow. (B) The distribution of zinc across cortical layers (I-VI) in area 17 is similar to that in area 18 but is distinguished from that in area 19 by a relative lack of zinc in layer IV. The section in A was counterstained with cresyl violet to demonstrate that the zinc-barren "holes" in B represent unstained cell somata for figs. 3A & B. (C) Optical density profiles taken across visual cortical layers indicate little difference in staining intensity between layers I-III, V or VI of the different visual cortical areas; but the intensity of staining in layer IV of areas 17 and 18 is 50% less than that in area 19, at levels near that found in the white matter. (Scale bar = 250 μm).
Area 19
Area 18
Area 17

Figure 3.3
Figure 3.4. Staining of synaptic zinc, which appears in this reverse phase photograph as white, avoids visual cortical areas 17 & 18 (boundaries indicated by arrows) of kittens at birth, but is demonstrable in area 19, cingulate cortex (Cg) and hippocampus (Hpc). (Scale bar = 1.0 mm)
**Figure 3.5.** Postnatal changes in the distribution of synaptic zinc in kitten visual cortex are pictured in reverse-phase. Profile plots (inset) represent optical density measurements across cortical laminae (I-VI) in area 17 at the level of the suprasplenial sulcus, and are included to facilitate the interpretation of laminar descriptions here and in the text. The boundaries between areas 17 / 18 and adjacent cortex are indicated by arrows. Synaptic zinc in areas 17 / 18 appeared soon after birth, just below CP, as a single, dense layer of staining (A). At PD10 (B), the highest concentrations of synaptic zinc are found within laminae V, III and the top of layer I. By beginning to avoid layer IV, the distribution of synaptic zinc differentiates areas 17 / 18 from adjacent cortex. This trilaminar appearance is still evident at PD20 (C); however, layers II / III have become more intensely stained. At PD30 (D) layers I, II, III and V were most densely stained while layers IV and VI contained relatively less, and equal, densities of synaptic zinc. Changes in zinc staining between PD30 and adult were limited to layer IV of area 17 / 18. This was seen as a relative reduction of staining to about 75% of that seen in supra- and infragranular layers at PD50 (E) which decreases further to 60% at PD75 (F). (Scale bar = 2.0 mm).
**Figure 3.6.** Zinc-stained (A, C) and near adjacent, Nissl-stained sections (B, D) through visual cortical laminae (I-VI) of area 17 at PD2 (A, B) and PD10 (C, D). Between PD2 and PD5, synaptic zinc first appears, and is most dense in the neuropil just below the cortical plate (CP). By PD10 synaptic zinc is localized primarily to layers III, V and the top of layer I. The staining of zinc in layer IV is slightly less intense than that of layers III and V, giving the PD10 visual cortex a trilaminar appearance. (Scale bar = 500 μm).
**Patchy Distribution in Layer IV.** In cutting sections parallel to the surface of unfolded and flattened cortex, the patchy distribution of zinc staining in layer IV of the PD50 visual cortex became much more apparent (Fig. 3.8A, C, E). Although an irregular pattern of staining in layer IV of adult visual cortex was not distinguishable in coronal sections (Fig. 3.1 & 3.2), sections cut tangential to the surface revealed a patchy pattern, similar to that of the younger animals, but of much lower relative intensity and contrast (Fig. 3.8B, D, F). The periodic pattern of zinc-staining in tangential sections, robust in 20 μm sections from PD50 cortex (Fig. 3.8C), was only clearly apparent, in sections from adult cortex which were at least 30 μm thick (Fig. 3.8D).

In addition to clearly revealing the inherent patchy distribution of zinc in layer IV, sections cut in the tangential plane provided information regarding the periodicity and areal distribution of the zinc-rich patches in layer IV. From the light-field micrographs in Figure 3.8, at levels near the top (A, B), middle (C, D) and bottom (E, F) of layer IV, it was apparent that patches of darker staining in area 17 extended through layer IV and appeared to form spotted irregular bands whose direction of elongation varied with location in the cortex. The pattern of zinc-rich zones in area 17 appeared periodic, with patches being, on average, around 400 μm in diameter and spaced 900 μm apart. Moreover, the patchy staining was restricted to area 17, while in area 18, the staining appeared homogeneous.
Figure 3.7. In this coronal reverse-phase photograph of PD50 visual cortex, staining for synaptic zinc appears patchy in layer IV of area 17. Zones of decreased staining in layer IV are indicated by arrows. (Scale bar = 1.0 mm).
Figure 3.8. Zinc stained sections at three levels through layer IV from opened and flattened cortex of a PD50 kitten (A, C, E) and an adult cat (B, D, F). At both ages, the columnar staining in layer IV was manifested as patches of darker staining at approximately 0.9 mm intervals, organized in spotty bands oriented mediolaterally or obliquely. The appearance of zinc patches was coincident with that of layer IV and limited to visual cortical area 17; in contrast, zinc staining of layer IV in area 18 appeared homogeneous. The boundaries of areas 17 & 18 are indicated by the dotted lines in D and E. The patchy pattern of staining in layer IV of the adult visual cortex was only visible in 30 μm thick sections and was of much lower contrast and intensity than that of the younger animal. The position of fiduciary landmarks, used to facilitate section alignment, are indicated by arrows. (A, anterior; L, lateral; M, medial; P, posterior; III, IV, V indicate cortical laminae; Scale bar = 4.0 mm)
Discussion

In the adult cat, visual cortical areas 17 and 18 were conspicuously distinguished by the reduced staining density of zinc in layer IV. Layers I, II, III and V exhibited intense staining while layer VI was moderately stained. The pattern of staining in the laterally-adjacent visual cortical area 19 more closely resembled that of most other neocortical areas; layers I, II, III and V were most intensely staining, while layers IV and VI appeared equally and moderately stained.

At birth (PD0) visual cortical areas 17 and 18 were distinguished from adjacent cortex by a complete absence of staining for synaptic zinc. Soon after birth synaptic zinc in the visual cortex was localized to a single lamina within and just below the cortical plate. By PD10, zinc-positive staining was visible at the top of layer I and throughout layers III and V, while it was reduced in layer IV. The first indication of a differentiation of area 17 and 18 from adjacent cortical areas by a relative reduction of zinc in layer IV was apparent at this age. At PD30 the distribution of synaptic zinc appeared to attain its adult distribution, except that the relative density of zinc in layer IV was almost as high as in layer VI. The relative reduction of synaptic zinc from layer IV represented the only observable, qualitative change in distribution from this age through adulthood. The reduction from layer IV was uneven and demonstrated a banded, patchy pattern in area 17.

The laminar localization of zinc predominantly to layers I, II, III and V in the neocortex of adult mammals has been described for several other species (see Frederickson, 1989 for survey). Developmental changes in the distribution of histochemically reactive zinc have been demonstrated in the developing rat olfactory cortex (Friedman and Price, 1984), striatum (Vincent and Semba, 1989) and amygdala (Mizukawa et al., 1989); however, the distribution and ultrastructural localization of zinc in the adult and developing cerebral neocortices have not been reported previously. Several groups have demonstrated general increases in the total amount and concentration of zinc per gram of wet weight brain tissue during the life span of humans (Völk et al., 1974) and rats (Crawford and Connor, 1972), but these do not differentiate synaptic from nonsynaptic sources. The hippocampal mossy fibers, which contain the highest density of synaptic
Ontogeny of Synaptic Zinc

Zinc in the brain, are the best characterized of the zinc-containing neuronal systems. In the rat (Zimmer and Haug, 1978) and cat (Frederickson et al., 1981), developmental gradients in the distribution of histochemically localizable zinc in the hippocampal mossy-fiber region have been reported to reflect developmental synaptogenetic gradients and may be related to synaptic maturity.

The vesicular localization of zinc within terminals of zinc-containing neurons in the adult rat brain has been well established (Haug, 1967; Holm et al., 1988; Perez-Clausell and Danscher, 1985a). We have confirmed this in the cat visual cortex, and, in addition, have demonstrated that histochemically-reactive zinc is present over synaptic vesicles in a subset of axon terminals of zinc-containing neurons making asymmetric contacts with postsynaptic targets, throughout postnatal development. Although a quantitative stereological analysis was not performed in these studies, age-related increases of synaptic zinc in the developing rat amygdala have been described as being due primarily to an increase in the number of zinc-positive particles within each terminal (Mizukawa et al., 1989). It is likely that developmental variations in zinc staining observed in the cat visual cortex at the light microscopic level can be accounted for similarly.

The vast majority of cortical zinc-containing terminals in the adult rat appears to be from local cortical interneurons rather than from long projection neurons (Hill and Frederickson, 1988). This appears true of cat visual cortex as well, since lesions which interrupt ascending brainstem, callosal, or geniculate afferents to visual cortex in the adult cat are unsuccessful in altering the overall laminar pattern of zinc staining (Dyck and Cynader, unpublished data). However, in light of the results presented here, an assessment of the effect of manipulations of input and/or visual experience on the expression of zinc-rich patches in layer IV should prove interesting (Chapter 4).

The postnatal changes in zinc staining in kitten visual cortex appear to reflect known anatomical and functional developmental landmarks. In supragranular layers, the cells which will inhabit layers II and III are still migrating at birth, and do not assume their final positions until about 3 weeks postnatal (Shatz and Luskin, 1986). Functional corticocortical synapses are established in supragranular layers around PD30 (Toyama and Komatsu, 1987), at the age when the mature, patchy distribution of local cortical connections becomes defined (Callaway and Katz, 1990), and
Ontogeny of Synaptic Zinc

the essentially mature pattern and density of zinc staining in the superficial layers are established. In layer IV, the first signs of the geniculocortical projection can be detected shortly after birth (Shatz and Luskin, 1986). Around 3 weeks postnatal, these inputs begin to segregate into ocular dominance columns within layer IV (LeVay et al., 1978). This segregation, which is input- and activity-dependent, is complete by eight or nine weeks of age. The transient, patchy staining of zinc in layer IV suggests that synaptic zinc may contribute in the mechanisms of column formation, such as ocular dominance or orientation, in the visual cortex during the critical period. Only two other endogenous molecules have, to date, been shown to be expressed transiently in patches during the critical period for cat visual cortical development (Dyck and Cynader, 1990a; Schöen et al., 1990; but see Chapter 5). When compared in adjacent sections, the transient columnar distribution of serotonin receptors localized with autoradiographic techniques, is coextensive with zinc positive patches (Dyck and Cynader, 1992; Chapter 5). In addition, the CNS glycoprotein 5'-nucleotidase, which is a major component of myelin (Cammer et al., 1980), and regulated by zinc (Mallol and Bozal, 1983), is transiently localized to ocular dominance columns with the same periodicity and laminar distribution as that described for zinc patches (Schöen et al., 1990). Finally, histochemical zinc has been demonstrated to transiently demarcate barrels in somatosensory cortex of the developing rat (Akhtar and Land, 1990). Thus, the transient, patchy staining of zinc in area 17 further indicates a potential contribution of zinc-related neurotransmission to cortical column formation.

Functional Considerations. The precise physiological role of a releasable pool of zinc in the adult central nervous system has not yet been established. However, zinc has been implicated in regulating excitatory and inhibitory neurotransmission via interaction with opiateergic (Stengaard-Pedersen, 1982; Stengaard-Pedersen et al., 1981a; Stengaard-Pedersen et al., 1981b; Stengaard-Pedersen et al., 1983), GABAergic (Legendre and Westbrook, 1991; Smart and Constanti, 1990; Smart et al., 1991; Westbrook and Mayer, 1987) glutamatergic (Christine and Choi, 1990; Legendre and Westbrook, 1990; Peters et al., 1987; Westbrook and Mayer, 1987), serotonergic
Ontogeny of Synaptic Zinc

(Peters et al., 1988) and glycinergic (Yeh et al., 1990) receptors. The Zn-sensitivity of opiate receptors has prompted the suggestion that Zn may act as the endogenous ligand at \( \sigma_2 \) sites in the rat hippocampus. Recent evidence suggests that, in the developing brain, endogenous zinc modulates GABAergic neurotransmission by acting on both GABA_A (Smart and Constanti, 1990; Smart et al., 1991) and GABA_B (Xie and Smart, 1991) receptor subtypes. In these and other studies (Draguhn et al., 1990), the sensitivity of GABA receptors to zinc is described as decreasing with age and to be receptor subunit specific, suggesting a specific role for synaptically released zinc in modulating inhibitory processes in development.

An important characteristic of the cat visual cortex which has been extensively studied is the marked modifiability of neuronal responsiveness by visual experience early in development. The phenomenon of long-term potentiation (LTP), a possible mechanism responsible for synaptic malleability and best characterized in the hippocampus (Bliss and Lynch, 1988), has also been described in both adult (Artola and Singer, 1987) and developing visual cortex (Connors and Bear, 1988; Komatsu et al., 1981). In the adult visual cortex, Toyama and Komatsu found that synaptic potentiation was greater in the superficial layers, which receive corticocortical synapses, than in layer IV, which is the recipient of geniculocortical synapses (Toyama and Komatsu, 1987). Ocular dominance plasticity is predominantly localized to layer IV in the young kitten but in the zinc-rich layers II, III, V and VI in older kittens (Fox et al., 1989); these layers also contain the highest abundance of NMDA receptors (Fox et al., 1989). Although excitatory neurotransmission from the lateral geniculate to layer IV of cat visual cortex is mediated by both NMDA and non-NMDA receptors (Tsumoto et al., 1986), the induction of LTP requires the activation of postsynaptic NMDA receptors, presumably by glutamate. Some additional factor, perhaps zinc, co-released with glutamate may be essential (Kauer et al., 1988; Weiss et al., 1989), or zinc could simply potentiate LTP induction by the blockade of GABAergic inhibition (Westbrook and Mayer, 1987). In the adult visual cortex, the induction of LTP requires activation of NMDA receptors with the concomitant reduction of GABAergic inhibition (Artola and Singer, 1987), a phenomenonon
which has been well characterized in the hippocampus (Douglas et al., 1982; Wigström and Gustafsson, 1983).

The transduction of extracellular signals to functional messages within the cell is thought to be linked to controlled fluctuations in the levels of the intracellular messenger calcium. As a divalent cation, zinc can replace or modify the effects of calcium at specific intracellular binding sites and thereby further influence signal transduction pathways (Csermely et al., 1989; Csermely et al., 1988). Several calcium binding proteins whose functions have been reported as significant regulators of intracellular calcium, such as calcyclin (Filipek et al., 1990), calmodulin (Baudier et al., 1983) and S100 (Baudier, 1988; Baudier and Gerard, 1986; Baudier and Gérard, 1983; Baudier et al., 1986; Baudier et al., 1984; Baudier et al., 1983; Baudier et al., 1985), actually bind zinc with higher affinity than calcium, or alter their affinity to calcium in the presence of zinc. The concentrations of these and several metallothionin-like zinc binding proteins appear to be developmentally regulated, implicating important zinc-protein and zinc-calcium interactions in the development and the maturation of the brain (Ebadi, 1986; Ebadi and Hama, 1986). Calcium ions also mediate the activation of one branch of the phospholipase C - protein kinase C (PKC) signal transduction cascade (Nishizuka, 1986). Zinc has been shown to be able to enter postsynaptic terminals via voltage-gated Ca\(^{2+}\) channels (Wang and Quastel, 1990). Once inside, zinc has the ability to activate PKC, either directly (Murakami et al., 1987), or by regulating the interaction of phorbol dibutyrate with PKC (Forbes et al., 1990) to induce PKC translocation to membranes (Csermely et al., 1988). In the developing cat visual cortex, PKC is transiently expressed in presynaptic terminals of layer IV at a time when the concentration of zinc is highest in layer IV and, furthermore, is found predominantly in superficial and deep layers in the adult cortex (Jia et al., 1990). Zinc has also been demonstrated to produce a strong blockade of Ca\(^{2+}\) current through dihydropyridine-sensitive Ca\(^{2+}\) channels (Winegar and Lansman, 1990). This effect appears to be channel-selective (Büsselberg et al., 1992). In the cat visual cortex, the expression of these sites, visualized by in vitro autoradiography using PN200, is limited to layer IV at birth but is high in
superficial and deep layers after P20 (Cynader et al., 1990), following a similar distribution and
time course as the distribution of synaptic zinc.

The establishment during development, and maintenance in maturity, of the synaptic form of
the CNS is to some degree dependent on a group of molecules which mediate trophic interactions
between individual cellular components. Zinc plays an integral role in the function of one of the
best characterized of such molecules, namely the nerve growth factor (NGF). Zinc ions are
involved in the activation process of the gamma-subunit esteropeptidase, and that activation
involves removal of zinc ion from the native 7S NGF (Pattison and Dunn, 1976a; Pattison and
Dunn, 1976b). The NGF proprotein contains and is stabilized by zinc ions; removal of zinc from
the 7S component of NGF alone, without dissociation, is sufficient to allow expression of its
esteropeptidase activity (Greene and Shooter, 1980; Pattison and Dunn, 1976a; Pattison and Dunn,
1976b). In this sense, zinc serves to act as a control ion which keeps the NGF protein in an
inactive form (the zymogen) until it recognizes its naturally occurring substrate (Young and
Koroly, 1980). A role for zinc as a critical factor in the regulation of NGF-mediated trophic
phenomena in the hippocampal formation has been reported, but such a role has not yet been
recognized in the cerebral cortex. Whether zinc plays an analogous role for other members of the
NGF family, such as BDNF and NT-3, remains unknown.

**Conclusions.** Patterns in the distribution of synaptic zinc appear to be developmentally
regulated and reflect the process of synaptic maturation of a subset of glutamate-containing neurons
which project to the developing visual cortex in a laminar- and region-specific manner.
Furthermore, the patchy distribution of synaptic zinc in layer IV of area 17 suggests that zinc may
contribute to the mechanisms of column formation during the critical period in development within
which the visual cortex exhibits use-dependent plasticity. This anatomical description of the
ontogenetic localization of zinc and the redistributions involved in attaining the adult pattern place
important constraints on the precise role of zinc-containing neurons in the processes of visual
cortical development and plasticity. These possibilities are further addressed in Chapter 4.
The anatomical description, in Chapters 2 and 3, of distributions of serotonin (5-HT) receptors and zinc (Zn) which are transiently expressed in columns at high levels, are suggestive of their participation in processes whereby particular columnar domains are defined. Moreover, the columnar expression of these molecules was found to be highest during the period within which the visual cortex exhibits an enhanced sensitivity to activity-dependent modifications of its organization. The decline in sensitivity to visual deprivation closely parallels the reduction in levels of the columnar expression of these molecules. As outlined in Chapter 1, the segregation of lateral geniculate axons into ocular dominance columns, is susceptible to modifications of visual input-and experience. If 5-HT receptors and Zn participate in activity-dependent mechanisms of column formation, such as those for ocular dominance, then their expression in visual cortex should, like these columnar systems, be constrained by early visual experience. This hypothesis is addressed by the studies described in this chapter.

The deprivation procedures were divided into two general categories; first, those which modified visual experience, but left visual pathways intact; and second, those which interrupted visual input, by inactivating successive levels of the visual processing pathway. Figure 4.1 outlines, in schematic form, the different procedures utilized to determine the effects of manipulating visual experience or input, on the expression of 5-HT receptors and Zn. The dependence of 5-HT receptor and Zn expression, on normal binocular visual experience was
assessed by subjecting kittens to: 1) a short period of monocular deprivation (MD) by eyelid suture (Fig. 4.1B), 2) long-term MD either by eyelid suture (Fig. 4.1B) or by unilateral enucleation (Fig. 4.1C), or 3) binocular deprivation, by rearing kittens from birth in complete darkness (Fig. 4.1D).

The role of binocular visual input in the normal development of the neurochemically defined columns was determined by selectively eliminating the participation of different visual processing pathways by: 1) unilateral enucleation (Fig. 4.1C), 2) unilateral optic tract section (Fig. 4.1E), 3) LGN aspiration or inactivation of intrinsic neurons with tetrodotoxin (Fig. 4.1F). The effects of restricting cortical visual experience on the columnar expression of 5-HT<sub>1C/2</sub> receptors and Zn was assessed in these kittens at the age during which they display the highest levels of their column-specific expression, around PD50. If related to ocular dominance, for example, then modifications in the columnar appearance of these molecules might be expected to vary from normal (Fig. 4.1A), in the same manner ocular dominance columns are affected (cortical schematics, Fig. 4.1B-F).

If any of these molecules are on cells which receive direct input from lateral geniculate neurons or alternatively, on geniculate terminals, then their participation in processes involved in columnar segregation might be substantiated. Although the columnar expression of 5-HT<sub>1C/2</sub> receptor subtypes in geniculate recipient laminae support this possibility, direct evidence is not available due to the poor resolution of the autoradiographic method. In a preliminary assessment, the cytological localization of 5-HT receptor subtypes to cells intrinsic to visual cortex or on afferent axon terminals, was inferred from the effects of neuron-specific lesions produced by an infusion of quinolinic acid. The utility of this toxin, which is reported to be selective to neurons, while leaving glial cells and the fibers and axon terminals of neurons outside the lesion zone intact (Schwarcz et al., 1983), has been determined previously for other neurotransmitter systems (Prusky et al., 1988; Shaw et al., 1989).
Figure 4.1. Schematic diagram illustrating the manipulations of visual input and experience imposed on kittens. The outcome of the columnar expression of 5-HT receptors and Zn, following each of these manipulations, is schematically predicted for visual cortical area 17, by the effects of monocular deprivation (B, C), binocular deprivation (D), optic tract section (E) and LGN lesions (F); based on the assumption that their distributions are affected the same as those of eye-specific inputs into ocular dominance columns. A. The normal columnar projection of red and green eye-specific afferents through the lateral geniculate nucleus to their respective cortical compartments in the visual cortex is shown. Note that there is normally a contralateral bias (exaggerated here) of the representation of each eye in the visual cortex. B. The elimination of patterned visual input from one eye during early development, induced by eyelid suture, results in a reduction of cortical space innervated by and influenced by the deprived eye. C. Cortical representation is exclusively maintained by the remaining eye following early enucleation. D. The deprivation of any photic stimulation during the animals' lifetime, results in a reduction of the normal segregation of ocular dominance columns (exaggerated here, for effect). E. Limiting visual cortical input to that generated spontaneously by neurons in the lateral geniculate nucleus, was assessed by completely depriving geniculate neurons of visual input, by early optic tract transection. F. The contribution of the lateral geniculate nucleus, to the columnar compartmentalization of these markers in visual cortex, was assessed by eliminating the influence of even spontaneous activity from neurons in the lateral geniculate.
Figure 4.1

A

1-3
4a
4c
5/6

Lateral Geniculate Nucleus

Optic Tract

Dark Rear

Monocular deprivation

Figure 4.1

B

1-3
4a
4c
5/6

Monocular deprivation

Enucleation

Figure 4.1

C

1-3
4a
4c
5/6

Lateral Geniculate Nucleus

Figure 4.1

D

1-3
4a
4c
5/6

Activity-Dependent Expression

Figure 4.1

E

1-3
4a
4c
5/6

Activity-Dependent Expression

Figure 4.1

F

1-3
4a
4c
5/6

Activity-Dependent Expression

Figure 4.1

LGN Lesion

Figure 4.1
Materials and Methods

Animals and Deprivation Procedures

Twenty-two kittens served as subjects in the various experiments described here. A summary of the kittens and the procedures of visual input-/experience-deprivation are summarized in Table 2 and Figure 4.1, respectively. The procedures used for perfusion, tissue preparation and for the autoradiographic and histochemical staining techniques were described previously, in detail in Chapters 2 & 3.

**Monocular Deprivation.** Monocular visual deprivation was induced either by eyelid suture (MD) or by enucleation (ME). Eyelid suture was performed under halothane anaesthesia (to effect) either near the age of eye-opening (n=4) or 1 week prior to perfusion (n=2). All animals were checked daily to assure the integrity of sutures. For ME, kittens (n=6) were anaesthetized with halothane, the extraocular muscles and optic nerve were transected, and the entire globe was excised. The eyelids were sewn together and the animals were reared normally.

**Binocular Deprivation.** Two kittens were raised in complete darkness from birth until PD51 (raised by Dr. D. Mitchell, Dalhousie University). On the day of perfusion, one kitten was brought into the light and allowed 2 hours of unrestricted vision prior to perfusion. The other was anaesthetized while still in the dark and its eyes were kept covered until the brain was removed.

**Optic Tract.** Kittens (n=2) were anaesthetized with halothane, the optic tract was accessed using the trans-buccal approach (Lepore et al., 1983), and then transected unilaterally under direct visual guidance (performed by Dr. F. Leporé, Université de Montréal).
Table 2. Summary of Animals and Manipulation of Visual Experience

<table>
<thead>
<tr>
<th>Cat</th>
<th>Procedure</th>
<th>Age at Procedure</th>
<th>Age at Perfusion</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK209</td>
<td>Right MD</td>
<td>PD49</td>
<td>PD55</td>
<td>1A, 1C, 2, Zn</td>
</tr>
<tr>
<td>K91-12</td>
<td>Right MD</td>
<td>PD40</td>
<td>PD52</td>
<td>1A, 1C, 2, Zn</td>
</tr>
<tr>
<td>MDZn</td>
<td>Right MD</td>
<td>PD8</td>
<td>PD50</td>
<td>1A, 1C, Zn</td>
</tr>
<tr>
<td>K91-21</td>
<td>Right MD</td>
<td>PD8</td>
<td>PD50</td>
<td>1A, 1C, 2, Zn</td>
</tr>
<tr>
<td>K92-6</td>
<td>Right MD</td>
<td>PD10</td>
<td>PD50</td>
<td>1A, 1C, 2, Zn</td>
</tr>
<tr>
<td>K91-29</td>
<td>Right MD</td>
<td>PD5</td>
<td>PD50</td>
<td>1A, 1C, 2, Zn</td>
</tr>
<tr>
<td>K91-0</td>
<td>Right ME</td>
<td>PD5</td>
<td>PD50</td>
<td>1A, 1C, Zn</td>
</tr>
<tr>
<td>K91-17</td>
<td>Right ME</td>
<td>PD3</td>
<td>PD52</td>
<td>1A, 1C, Zn</td>
</tr>
<tr>
<td>K91-27</td>
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<td>PD8</td>
<td>PD50</td>
<td>1C, Zn</td>
</tr>
<tr>
<td>BK369</td>
<td>Left ME*</td>
<td>PD2</td>
<td>PD56</td>
<td>1C, Zn</td>
</tr>
<tr>
<td>BK370</td>
<td>Left ME*</td>
<td>PD2</td>
<td>PD56</td>
<td>1C, Zn</td>
</tr>
<tr>
<td>BK431</td>
<td>Left ME*</td>
<td>PD5; PD53**</td>
<td>PD66</td>
<td>Zn, Proline</td>
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<tr>
<td>C502</td>
<td>Dark Rear</td>
<td>PD0</td>
<td>PD51</td>
<td>1A, 1C, 2</td>
</tr>
<tr>
<td>C503</td>
<td>Dark Rear</td>
<td>PD0</td>
<td>PD51 + 2 hr***</td>
<td>1A, 1C, 2</td>
</tr>
<tr>
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<td>Left Optic Tract</td>
<td>PD10</td>
<td>PD50</td>
<td>1A, 1C, Zn</td>
</tr>
<tr>
<td>BK240</td>
<td>Left Optic Tract</td>
<td>PD20</td>
<td>PD51</td>
<td>1A, 1C, Zn</td>
</tr>
<tr>
<td>RC77</td>
<td>TTX Right LGN</td>
<td>PD23</td>
<td>PD37</td>
<td>1C, 2</td>
</tr>
<tr>
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<td>TTX Left LGN</td>
<td>PD23</td>
<td>PD37</td>
<td>1C, 2</td>
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<tr>
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<td>TTX Right LGN</td>
<td>PD23</td>
<td>PD37</td>
<td>1C, 2</td>
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<td>Asp Right LGN</td>
<td>PD40</td>
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<td>QA</td>
<td>PD58</td>
<td>PD65</td>
<td>1A, 1C, 2, Up</td>
</tr>
<tr>
<td>UC567</td>
<td>QA</td>
<td>PD57</td>
<td>PD64</td>
<td>1A, 1C, 2, Up</td>
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* Tangential sections ** Intracellular [3H]proline injection *** 2 hr visual exposure
1A, 1C, 2, Up: Autoradiography for 5-HT 1A, 1C, 2 receptor subtypes, and 5-HT uptake site respectively.
Zn, zinc histochemistry; PD, postnatal day; QA, quinolinic acid; TTX, tetrodotoxin; Enuc, enucleation;
MD, monocular deprivation by eyelid suture; Asp, lesion by aspiration.
Lateral Geniculate Nucleus. One kitten was anaesthetized with halothane and the right LGN was accessed and aspirated via a lateral approach using visual and stereotactic guidance (performed by Dr. M. Cynader). The other three kittens had a cannula implanted in the LGN (A, 3.5 mm; L, 8.0 mm, H, 11.0 mm) which was attached to an osmotic minipump (Alzet 2002), and tetrodotoxin (TTX, 100 µM in ACSF) infused at a rate of 0.5 µl / hr for 2 weeks (performed by Dr. Qiang Gu, University of British Columbia).

Quinolinic Acid. Two kittens were anaesthetized with halothane to effect and small holes were drilled through the skull at three antero-posterior levels overlying area 17 in one hemisphere. One microliter of quinolinic acid dissolved in saline (pH 7.4; 0.3 µM / µl) was infused, over 5 min, through a 30 gauge cannula lowered 1.2 mm below the cortical surface at each site. The animals survived 7 days prior to perfusion.

Results

The effects of visual deprivation were most clearly reflected by changes in the distribution of the 5-HT1C receptor subtype. The normal, characteristic pattern of [3H]mesulergine binding at PD50 consisted of a continuous band of dense binding at the base of layer IV. From this band, periodic, radially oriented columns of receptors emerged and extended through layer IV into the lower part of layer III. This pattern became progressively more distinct with age, and peaked at PD50. At this age, the greatest density of 5-HT1C receptors in neocortex was strictly limited to layers III/IV of visual cortical area 17 (see Figs. 2.1, 2.9, and 2.10, Chapter 2). When compared to the patterns observed following the various manipulations in Figure 4.2, it is apparent that the incidence and contrast of the columns of [3H]mesulergine binding in visual cortex was unaltered by a short period of MD (Fig. 4.2A). An extended period of MD resulted in a reduction of binding in the columns perforating layer IV of both hemispheres (Fig. 4.2B). The normal organization of 5-HT1C-rich columns in layer IV of area 17 exhibited an even greater reduction, bilaterally, with long-term ME (Fig. 4.2C). The complete restriction of visual information imposed by dark-rearing
kittens from birth to PD51, prevented the segregation of 5-HT\textsubscript{1C} receptors into columns in layer IV of visual cortex (Fig. 4.2D). Instead, a thicker than normal, dense band of binding, extended throughout layer IV of area 17 in these kittens. This pattern of binding was unchanged even when 2 hours of visual exposure was given prior to perfusion. These results indicate the importance of visual exposure in general and of binocular vision in particular, in the formation of 5-HT\textsubscript{1C} receptor columns during development.

In none of the manipulations of visual experience, did the pattern or levels of 5-HT\textsubscript{1A} receptors appear different than normal. The effects of restricting both visual experience and input, were non-existent or negligible (compare Figs. 4.3A, 4.6B; with Figs. 2.1, 2.10 in Chapter 2). By contrast, the effects of visual deprivation on the columnar patterns of 5-HT\textsubscript{2} receptors and Zn, although less well-defined than for 5-HT\textsubscript{1C} receptors, appeared to be similarly affected. The effects of the various manipulations on the distribution of Zn, and 5-HT\textsubscript{1A} and 5-HT\textsubscript{2} receptor subtypes are described in the following sections, and are compared and contrasted to the effects observed on 5-HT\textsubscript{1C} receptor expression.

**Unilateral Enucleation.** Long-term ME resulted in effects on Zn and 5-HT\textsubscript{1C/2} receptors which were apparent even in sections cut in the frontal plane (Fig. 4.3). The binding pattern of $[^{125}\text{I}]$DOI to 5-HT\textsubscript{2} receptors (Fig. 4.3C), was distinctly different than that of kittens raised normally to PD50 (Figs. 2.1, 2.10; Chapter 2). The densest binding was expressed at the layer III / IV border, as was normally observed; however, the periodic pattern of binding was not clearly apparent (Fig. 4.3C). In addition, a reduced density of binding within this lamina in area 18, combined with a relative increase in the levels of binding within lower strata of layer IV clearly demarcated area 17 from area 18 (compare to adjacent section labeled with $[^{3}\text{H}]$mesulergine, Fig. 4.3D). Similarly, the distribution of Zn in layer IV of kittens raised to PD50 with only one eye (Fig. 4.3B), appeared more homogenous than that observed normally (see Fig. 3.8, Chapter 3). Although apparent in the frontal plane, the effects were more clearly seen in sections through layer IV which were cut tangential to the cortical surface (Fig. 4.4, Fig. 4.5). These figures demonstrate
the distribution of 5-HT$_{1C}$ receptors and Zn in near adjacent sections, from two kittens (who were also littermates) raised from PD2 to PD56 with only the left eye removed. In both kittens there was a marked decrease in the level and contrast of 5-HT$_{1C}$ receptors and Zn expressed in patches in area 17 bilaterally. This reduction appeared to be represented by a decrease in the level of contrast between patch and interpatch regions, and also by a reduction in the size of the patches (compare to Fig. 2.12 for 5-HT$_{1C}$ and Fig. 3.8 for Zn). However, the total number of 5-HT$_{1C}$-rich columns did not appear to be different from normal (enucleates (n=4), $\bar{x} = 292$; control (n=2), $\bar{x} = 305.5$).

The effect of early enucleation was not identical in each animal examined. The results which are depicted for three different animals in Figures 4.3-4.5 (K91-0, Fig. 4.3D; BK369, Fig. 4.4; BK370, Fig. 4.5), are representative of the range of effects observed, among all the kittens studied thus far. A bilateral asymmetry in the residual levels of the columnar expression of both Zn and 5-HT$_{1C}$ receptors was observed in only 2 of the 6 animals (K91-0, Fig. 4.3D; BK369, Fig. 4.4). This disparity could not be attributed to whether the deprived eye was ipsilateral or contralateral. Furthermore, although the column-specific labeling for both Zn and 5-HT$_{1C}$ receptors was attenuated in all animals, the effects of enucleation appeared to exert a greater effect on the expression of Zn patches (Fig. 4.5).

**Dark-Rearing.** As described above, and depicted in Figure 4.6, the effect of binocular deprivation by dark-rearing, on the distribution of 5-HT$_{1C}$ receptors was marked (Fig. 4.6C, C'). This was also the only manipulation which produced an effect on the normal pattern of 5-HT$_{1A}$ receptors (Fig. 4.6B). During early postnatal development (PD10 - PD40, but not beyond PD40; Fig. 2.1B-F & 2.10B, Chapter 2), 5-HT$_{1A}$ receptors demarcate visual cortical areas 17, 18 and 19 from adjacent cortical regions by relatively increased levels of expression. In Fig. 4.6B, the distribution of 5-HT$_{1A}$ receptors clearly demarcate area 17 from adjacent regions. However, dark-rearing did not change the complementary laminar-specificity exhibited by the relative distributions of each 5-HT receptor subtype described in Chapter 2 (compare Fig. 4.6A with Fig. 2.10A). Similar to the changes observed in the 5-HT$_{1C}$ receptors, the dense band of binding at the layer III
Activity-Dependent Expression

/ IV border, characteristic of 5-HT2 receptors at this age, appeared more homogenous. The effect of binocular deprivation, by dark rearing, on Zn-staining in visual cortex was not examined because neither of the two animals studied thus far was treated with sodium selenite prior to perfusion.

Optic Tract Lesion. The sections in Figure 4.7, through the visual cortex of two different kittens (kitten OPZn, A-D; kitten BK240, E, F), are representative of the effects of unilateral optic tract transection on the distribution of 5-HT1C receptors (Fig. 4.7A, D, E) and Zn (Fig. 4.7B, D, F). The overall effect of transecting the optic tract, in both animals, was to reduce the columnar expression of 5-HT1C receptors in the ipsilateral hemisphere (hemisphere on right side of panel). Columns of 5-HT1C receptors in layer IV, emerging from the dense band at the layer IV / V border, were clearly present in both hemispheres of kitten OPZn (Fig. 4.7 A, C), but they were greatly reduced in the ipsilateral hemisphere, particularly in the anterior-most sections of area 17 (Fig. 4.7C, right hemisphere). In kitten BK240 (Fig. 4.7E, F), the normal columnar appearance of 5-HT1C receptors in the left hemisphere of panel E, was contrasted by the complete lack of columnar segregation in layer IV on the lesion side (right hemisphere). In addition to differing in their age at time of surgery (see Table 2), the two kittens differed in the extent of their lesions; the transection was incomplete in kitten OPZn (Fig. 4.7A-D), but complete in BK240 (Fig. 4.7E, F). In the near-adjacent sections stained for Zn, changes in the frequency of Zn-positive columns appeared to mirror those of 5-HT1C columns, but not nearly as clearly (Fig. 4.7 B, D, F). The effect of unilateral optic tract transection on the expression of 5-HT2 receptors has not yet been examined.
Figure 4.2. The effect of manipulating visual experience on the subsequent columnar development of 5-HT₁C binding sites in visual cortex was assessed in kittens who were monocularly deprived for one week by eyelid suture (A); from the time of eye-opening by eyelid suture (B) or unilateral enucleation (C); or were dark-reared from birth (D). All animals survived until PD50. [³H]Mesulergine-labeled columns were still present, with normal contrast, in area 17 of kittens monocularly-deprived by eyelid suture for a short duration (A) but were somewhat reduced following long-term deprivation (B). The expression of 5-HT₁C columns in layers III / IV of area 17 was significantly reduced in both hemispheres of the unilaterally enucleated kittens, but the lamina-specific binding at the bottom of layer IV appeared unaffected (C). The segregation of 5-HT₁C receptors into columns was prevented in kittens who were raised in the dark from birth (D). Scale bar = 2 mm.
Figure 4.3. The effect of long-term monocular deprivation by enucleation, on the distribution of 5-HT$_{1A}$ (A), Zn (B), 5-HT$_2$ (C), and 5-HT$_{1C}$ (D) in near-adjacent sections from the visual cortex of kitten K91-0 (sections A, C, D are serially adjacent). A. The laminar- and regional-specificity of 5-HT$_{1A}$ receptors was left unchanged following unilateral enucleation from PD5 (see Fig. 10). B. Although not as clear as in tangential sections (see Fig. 24; Fig. 25), the distribution of Zn in layer IV of area 17 appeared more homogenous than in the normal PD50 visual cortex (see Chapter 2). C. A reduction in the binding of [¹²⁵I]DOI to 5-HT$_2$ receptors, along the dense band at the layer III / IV border in area 18 relative to area 17, combined with increased levels of expression in lower strata of layer IV, distinguished area 17 from area 18 in the unilaterally enucleated kitten. This pattern was not present in normal animals of the same age. D. The expression of 5-HT$_{1C}$ columns in layers III / IV of area 17 was significantly reduced in both hemispheres of the unilaterally enucleated kittens, but the lamina-specific binding at the bottom of layer IV appeared unaffected. Scale bar = 2 mm.
Figure 4.3
**Figure 4.4.** The effect of enucleation of the left eye at PD2 on the tangential distribution of 5-HT₁C receptors (top) and synaptic zinc (bottom) in the right (R) and left (L) hemispheres of kitten BK369 at PD 56. The columnar expression of both 5-HT₁C receptors and Zn was significantly reduced relative to normal PD50 visual cortex (Chapters 2, 3). This reduction, was reflected in a reduced diameter, and contrast, of 5-HT₁C columns, because the absolute number of columns did not vary significantly from normal. In this animal, the attenuation of column-specific labeling for both Zn and 5-HT₁C receptors was bilaterally asymmetric, with losses greater in the hemisphere ipsilateral to the enucleated eye. Scale bars = 10 X 2 mm.
Figure 4.5. The effect of enucleation of the left eye at PD2 on the tangential distribution of 5-HT$_{1C}$ receptors (top) and synaptic zinc (bottom) in the right (R) and left (L) hemispheres of kitten BK370 at PD 56. The columnar expression of both 5-HT$_{1C}$ receptors and Zn was significantly reduced relative to normal PD50 visual cortex (Chapters 2, 3). This reduction, was reflected in the diameter, and contrast of 5-HT$_{1C}$ columns, because the absolute number of columns did not vary significantly from normal. In this animal, a greater effect was observed on the column-specific expression of Zn, where patches of increased Zn-staining in area 17 were rarely observed. Scale bars = 10 X 2 mm.
Figure 4.5
Figure 4.6. The effect of dark-rearing on the expression of 5-HT$_{1A}$ (B), 5-HT$_{1C}$ (C, C') and 5-HT$_2$ (D, D') receptors, in serially adjacent sections (B, C', D'; C, D), at two caudo-rostral levels (C, D). The relative laminar distribution of 5-HT receptors was not affected by dark-rearing. The characteristic complementarity of peak expression levels in area 17 were not different from normal (see Fig. 2.10A). However, compared to normal, the columnar segregation of either 5-HT$_{1C}$ or 2 receptors was not apparent. Instead, each exhibited virtually homogenous levels throughout their respective laminar strata. Moreover, the expression of 5-HT$_{1A}$ receptors was abnormally high in area 17, relative to adjacent visual cortex, compared to that seen normally at this age (compare with Fig. 2.1E, F & 2.10B) Scale bar = 2 mm.
Figure 4.6
Figure 4.7. The effect of a unilateral optic tract section on the expression of 5-HT$_{1C}$ receptors (A, C, E) and synaptic zinc (B, D, F) in two animals (OPZn, A-D; BK240, E, F). Although the optic tract section in the kitten OPZn (A-D) was incomplete, a marked reduction in the columnar expression of both Zn and 5-HT$_{1C}$ receptors was apparent, particularly at rostral-most portions of area 17 (C-D). With a complete transection (E, F) the columnar segregation of 5-HT$_{1C}$ receptors (E) and Zn (F) was completely repressed. Scale bar = 2 mm.
Lateral Geniculate Nucleus. Intrageniculate infusions of TTX compromised both the levels and patterns of 5-HT$_{1C}$ and 5-HT$_{2}$ receptor binding in kitten visual cortex (Fig. 4.8). In all cases, the effect on binding appeared to be limited to the visual cortex ipsilateral to the infused LGN. A reduction in the laminar-specific binding of [$^{125}$I]DOI to 5-HT$_{2}$ (A), and of [$^{3}$H]mesulergine to 5-HT$_{1C}$ receptors (B), in kitten RC78 was virtually complete, except for a small region of 5-HT$_{1C}$ receptors in the ventral-most portion of area 17 (B, arrow). Similarly, in a different animal (RC77), a distinct border in the reduction of 5-HT$_{2}$ receptors, suggests that alterations in binding levels are directly related to the extent of LGN inactivated. These results were substantiated by a complete reduction of the laminar-specific binding pattern of 5-HT$_{1C}$ receptors observed following removal of the LGN by aspiration (Fig. 4.8D).

Quinolinic Acid. The effect of neuron-specific lesions in visual cortical area 17, on the distribution of 5-HT receptor subtypes at PD50, is shown in Figure 4.9. The differential effect of quinolinic acid lesions on the levels of expression suggests that these receptors might be localized to different cytological compartments within area 17. The virtual elimination of all 5-HT$_{1A}$ receptors within the lesion zone suggests that these receptors are localized on neurons intrinsic to area 17 (Fig. 4.9A). However, the 40% reductions in the binding of [$^{3}$H]CN-IMI and [$^{125}$I]DOI to 5-HT$_{Up}$ (Fig. 4.9C) and 5-HT$_{2}$ receptors (Fig. 4.9D), suggest that they are, in part, localized to extrinsic elements or glial cells. Although the expression of 5-HT$_{2}$ receptors was reduced to background levels in superficial and deep cortical laminae, 60% remained within the dense band at the layer III / IV border, perhaps an indication of expression on two different cytological elements. On the other hand, the expression of 5-HT$_{1C}$ receptors, although slightly reduced (< 10%), is still expressed in columns, in laminar-specific manner, potentially indicating a localization on afferent fibers (Fig. 4.9B).
Figure 4.8. The effect of unilateral LGN aspiration (D) or inactivation by TTX (A-C), on the expression of 5-HT_{1C} (B, D) and 5-HT_{2} (A, C) receptors. A reduction in the laminar-specific binding of 5-HT_{1C} and 5-HT_{2} receptors was observed in all animals, in the hemisphere ipsilateral to the TTX infusions and lesion. The binding indicated by the arrows in B and C, observed in two different animals (RC78, A, B; RC77, C), probably reflects residual activity of 5-HT_{2} (C) and 5-HT_{1C} (B) receptors in the LGN resulting from incomplete TTX infusion. Residual binding of 5-HT receptors was not observed in any portion of the visual cortex of kitten RC68, where complete LGN aspiration was confirmed (D). Scale bar = 2 mm.
**Figure 4.9.** Effect of quinolinic acid lesion on binding of $[^3\text{H}]8$-OH-DPAT (A), $[^3\text{H}]$mesulergine (B), $[^{125}\text{I}]$DOI (C) and $[^3\text{H}]$CN-IMI (D) in near adjacent sections through area 17 of PD50 visual cortex. The effect of the lesion on levels of 5-HT receptors, compared with the sham injected hemisphere, is reflected in each panel by the density plots. The outline of each densitometric slice used to generate these profiles is indicated, in each panel, by the rectangle. The limited effect of a quinolinic acid lesion on the binding of $[^3\text{H}]$mesulergine in area 17 suggests that the 5-HT$_{1C}$ receptors are located on terminals of neurons projecting to area 17, or on glial cells. On the other hand, the complete reduction in binding of 5-HT$_{1A}$ receptors, implies that these receptors are strictly localized to cortical cells intrinsic to area 17. The significant reduction in the binding of $[^{125}\text{I}]$DOI and $[^3\text{H}]$CN-IMI within the lesion site suggest that 5-HT$_{2}$ receptors and 5-HT$_{1p}$ sites are also, at least partially, localized to elements intrinsic to cortex. Arrows indicate borders of the primary necrotic zone. Scale bar = 2.0 mm.
Figure 4.9
Discussion

The results of the various experiments described in this chapter indicate that the normal levels of expression, and the distributions, of several neuroactive molecules which demarcate a particular columnar organization of visual cortex, are constrained by early visual experience. The high levels of expression of Zn, 5-HT1C and 5-HT2 receptors in columns in geniculate recipient laminae of the developing visual cortex, described in Chapters 2 & 3, were suggestive of the possibility that these molecules could be regulated by visual experience. The effects of altering visual experience during early development on an animals' visual abilities in later life are profound. Anatomical changes to the visual pathway which underlie the obvious behavioural deficits have been extensively examined (for reviews see Movshon and Van Sluyters, 1981; Sherman and Spear, 1982). It is highly likely that they also represent the basis for the changes in 5-HT receptor expression and Zn distribution that have been described here.

As indicated in Chapter 1, abnormalities produced by monocular deprivation (MD) have been proposed to result from a competitive disadvantage placed on the deprived eye, by the non-deprived eye, in acquiring cortical territory (Wiesel and Hubel, 1965). After several weeks of monocular deprivation, the majority of cells in the visual cortex are responsive to visual stimulation only through the eye which had been left open (Wiesel and Hubel, 1963b). The locus of effect is not in the eye, or in the lateral geniculate nucleus, but in the visual cortex, at the site where binocular inputs converge (reviewed in Movshon and Van Sluyters, 1981; but see Sherman and Spear, 1982, and discussion below). The predominant anatomical effect seen in the cortex is that the normal geniculocortical projection is physically rearranged as a consequence of MD, with a corresponding shift in ocular dominance represented by a reduction of afferents representing the deprived eye and a corresponding increase in cortical space occupied by the nondeprived eye (Shatz and Stryker, 1978). Although responses of visual cortical neurons to visual stimulation are driven almost exclusively by the non-deprived eye, a significant visual cortical projection of geniculate axons representing the deprived eye is still present (Garey and Blakemore, 1977; Lin
and Sherman, 1978; Shatz and Stryker, 1978; Spear and Ganz, 1975). Similar to that seen for
ockular dominance, the columnar distributions of 5-HT receptors and Zn also appear to reflect the
level of competitive activity arising binocularly. The levels of Zn and 5-HT$_{1C/2}$ receptors in
columnar compartments were unaffected by short periods of monocular deprivation and were only
slightly reduced following eyelid suture before eye-opening. By contrast, a much greater, bilateral
reduction in the degree of columnar expression of both 5-HT$_{1C}$ receptors and zinc was apparent as
a result of unilateral enucleation prior to eye-opening. In one kitten showing no evidence of
residual Zn columns, the organization of non-deprived eye inputs were homogenously distributed
throughout the visual cortex bilaterally (Fig. 1.1). Thus, a reflection of the severity with which
competitive interactions are reduced appears to be mirrored in the residual columnar expression of
these molecules. Recently Bliss-Tieman has shown that deprived geniculocortical cells make fewer
and abnormal synapses in layer IV of visual cortex, but similar to the expression of 5-HT$_{1C}$ and
Zn described here, they are restricted to ocular dominance columns which are faint and usually fail
to extend into extragranular layers (Bliss-Tieman, 1991). These results substantiate transneuronal
data which indicate that the distribution of deprived eye terminals appears more widespread in
lower layer IV (Shatz and Stryker, 1978). These data, combined with the results of monocular
deprivation presented here, indicate that diffuse visual information, provided through the sutured
eyelid (Spear et al., 1978), appears sufficient to drive the columnar segregation of 5-HT receptors
and Zn. Moreover, the observation that columnar segregation continues, albeit abnormally, with
retinal input from only one eye, indicates that spontaneous activity in the deprived laminae of the
lateral geniculate itself might be sufficient to organize cortical comparments based on binocular
competition.

Many lines of evidence indicate that the effects of visual deprivation on the LGN are
minimal; however, a selective vulnerability of geniculocortical Y-cell pathways to both monocular
and binocular deprivation has been reported (Kratz et al., 1979; Sherman et al., 1972; reviewed in
Sherman and Spear, 1982). Based on pharmacological studies, this physiological phenomenon has
been attributed to the formation of abnormal excitatory synaptic connections. When inhibitory
neurotransmission is antagonised in visual cortex by infusion of bicuculline, the ability of the deprived eye to drive cortical cells is reversibly restored (Burchfiel and Duffy, 1981; Duffy et al., 1976; Sillito et al., 1981). These data are supported by the observation that early MD produces a reduction in intracortical inhibition (Tsumoto and Suda, 1981; Wilson and Sherman, 1976), attributed to a loss of Y-cells, which are thought to mediate such inhibition in normal cats (Singer et al., 1976; Tsumoto, 1978; see also discussion of Y-cells below). That many cortical cells would receive functional excitatory synaptic contacts from a greater proportion of X-cells, might provide a functional basis for the homogeneity of Zn-containing fibers in layer IV observed to result following enucleation (Figs. 4.3-4.5). Based on the precise colocalization of Zn columns, with the X-like distribution of 5-HT_{1C} receptor columns (Dyck and Cynader, 1992; Dyck and Cynader, submitted; see Chapter 5), Zn-containing glutamatergic terminals and X-cell terminals may be one and the same. Although the results provided by physiological recording are apparently confounded by problems of sampling error, a large number of anatomical reports are indicative of a selective reduction of Y-like cells in the LGN and, correspondingly, Y-cell terminals in the visual cortex (reviewed in Sherman and Spear, 1982). Much of this support stems from the reduced incidence of Y-cells labeled retrogradely from tracer injections in area 18. The geniculate projection to area 18 arises exclusively from Y-cells in the A lamina, whereas area 17 receives mixed X- and Y- inputs, albeit to different laminar strata (see Discussion, Chapter 2). Furthermore, the expression of CAT301, a Y-cell specific antigen, is significantly reduced with monocular and binocular deprivation (Sur et al., 1988). Some support for this hypothesis is provided in this study by the selective abnormal reduction of 5-HT_{2} receptors in area 18 following early unilateral enucleation (Fig. 4.3B). The data presented in Chapter 2, although inferential, indicate that 5-HT_{1C} and 5-HT_{2} receptors likely differentiate geniculocortical X- and Y-pathways, respectively. Further support for these inferences might be provided by assessing the effect of deprivation on the transient expression of 5-HT_{2} receptors expression in the lateral suprasylvian cortex, where receptive fields of neurons are abnormal (Spear and Tong, 1980), and which receives exclusive input from Y- and possibly W-cells (Berson, 1985).
The normal laminar-specific expression of 5-HT1C/2 receptors prevailed following the elimination of all photic stimulation during development but their segregation into normal columnar compartments was prevented. The segregation of geniculate terminals into ocular dominance columns in the visual cortex does develop, to a limited extent, in dark-reared and binocularly lidsutured cats (Stryker and Harris, 1986; Swindale, 1981). Considering that ocular dominance columns do not segregate when the retinal ganglion cells have been silenced by intraocular infusion of TTX (Stryker and Harris, 1986), it is apparent that spontaneous activity of retinal ganglion cells provides a sufficiently patterned input to permit some aspects of the activity-dependent molding of neural connectivity recognized in visual cortex. However sufficient spontaneous activity is for the segregation of ocular domains, it does not appear to be adequate for the columnar segregation of 5-HT receptors.

The predominant effect of dark-rearing in the kitten visual cortex is the prolongation of the critical period during which functional changes can be induced by monocular deprivation (Cynader and Mitchell, 1980; Mower et al., 1981). This delay in maturation is reflected by numerous physiological (reviewed in Sherman and Spear, 1982) and morphometric parameters (O'Kusky, 1985; Takács et al., 1992). The present results, where we describe the altered distribution of 5-HT receptors after 7 weeks of dark-rearing, confirm and extend those described previously (Mower, 1991). There, the effects of dark-rearing cats to an age of 4 - 5 months were described by an increase in the number of receptors labeled with [3H]5-HT, which was specific to the supragranular and infragranular laminae of visual cortex (visual cortical regions were not differentiated, nor were temporal data presented). In animals raised in parallel, immunocytochemical results demonstrated that this increase in binding was not reflected by an increase in serotonergic fiber density (Mower, 1991). Due to the nonselectivity of [3H]5-HT, Mower was not able to discriminate the effect of dark-rearing on individual receptor subtypes. However, together with the results described thus far in this thesis, it is apparent that the delay in maturation of the visual cortex is reflected by a parallel delay in the temporal patterns of each of the 5-HT receptors examined. The high density of 5-HT1A receptors in area 17 relative to adjacent
visual areas after 7 weeks of dark-rearing, is similar to that seen at earlier stages of development but not normally at PD50 (see Fig. 2.1A-E), and probably represent the majority of receptors described by Mower. Similarly, the homogenous distributions of 5-HT1C and 5-HT2 receptors are similar to those found in younger kittens, where levels of binding were homogenous in layer IV (Fig. 2.1A'-E'; Fig. 2.1A"-E"). On the basis of studies indicating the recovery of orientation selectivity and ocular dominance column formation (Cynader and Mitchell, 1980; Swindale, 1988), these results might predict that the process of 5-HT1C/2 receptor segregation would proceed with subsequent exposure to light.

The effects of dark-rearing were not evaluated with respect to the normal columnar distribution of synaptic zinc, but several indices of glutamatergic neurotransmission have been found to be dramatically altered in kitten visual cortex as a result of dark-rearing. Physiological evidence for a delay in the developmental decrease in visual cortical NMDA-receptor efficacy in layers IV, V, and VI has been reported for kittens raised to PD43-49 in the dark (Fox et al., 1991). The normal distribution of NMDA-receptors labeled with $^{125}$I MK-801 progresses from a homogenous distribution across laminae during the first few postnatal weeks, but by PD50 is predominant in supragranular layers. Ligand binding data from the same kittens as those reported in this chapter, indicate that glutamatergic receptors labeled with $^{125}$I MK-801 are homogenously distributed across all laminae (Cynader et al., 1991). Thus, the similar effect of dark-rearing, to delay the redistribution of 5-HT and glutamatergic receptors, might also be imposed on the columnar segregation of glutamatergic, zinc-containing fibers. This remains to be seen.

The effects of early optic tract transections on the columnar expression of 5-HT receptors have been determined only from two animals. Even though they might be considered preliminary, as such, the effects observed on the expression of 5-HT1C/2 receptors contribute significantly to an understanding of the observed effects of visual deprivation. The consequences of an optic tract section could be considered to be analogous to dark rearing one cortical hemisphere, except with respect to the contribution of spontaneous retinal ganglion cell input. The observation that some columnar segregation of 5-HT1C/2 receptors occurred in the animal with an incomplete lesion of
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the optic tract, compared to no apparent segregation in the animal with a complete optic tract transection, provides evidence that columnar segregation in the cortex requires normal binocular input. Residual columnar expression of 5-HT receptors was higher in posterior portions of the visual cortex (Fig. 4.7A), which might be correlated with the specificity of the optic tract fibers cut; however, the high degree of retinotopic order found in the LGN and visual cortex is not reflected by a similar organization in the pathway of fibers in the optic tract (Horton et al., 1979).

From the results of direct manipulation of geniculate input, it is apparent that the contribution of spontaneous activity by neurons of the deafferented lateral geniculate nucleus was sufficient for the laminar-specific expression of 5-HT1C/2 receptors, but not for their segregation into columnar compartments. The selective reduction of 5-HT1C/2 receptors in layer IV of visual cortex following LGN inactivation, either by lesion or TTX infusion, indicates that their laminar-specific expression at this age is dependent on the integrity of the LGN. Evaluation of the lateral geniculate nucleus, in Nissl-stained sections near the infusion site, indicates that neurons near the infusion site are not pyknotic, suggesting that the effects of TTX were not due to neurotoxicity. The question arises as to whether 5-HT1C and 5-HT2 receptors are localized directly on LGN terminals, or are highly regulated in an activity-dependent manner, on cells directly under the influence of geniculate input. The lack of a significant effect of quinolinic acid lesions on the laminar-specific distribution of 5-HT1C receptors, contrasted by a complete reduction of 5-HT1A receptors, are highly indicative of the cytological distribution of 5-HT1C receptors on afferent fibers, compared to a possible localization of 5-HT1A receptors on intrinsic neurons. Although there is evidence for 5-HT receptors on cortical astrocytes, they are believed to belong to the 5-HT1 family (Whitaker-Azmitia, 1988; Whitaker-Azmitia and Azmitia, 1986). The significant reduction in the levels of high affinity 5-HT uptake sites following quinolinic acid lesions is disconcerting because reuptake mechanisms for 5-HT are thought to be localized predominantly on the same nerve terminals from which it is released. However significant evidence exists for high affinity uptake of 5-HT into cortical astrocytes (Kimelberg, 1988; Kimelberg and Katz, 1985). The reduction of uptake sites by lesions induced by quinolinic acid puts into question the interpretability
of the varied effects on the expression of the other receptor subtypes observed here. However, limited evidence indicates that quinolinic acid, a tryptophan metabolite which is endogenous to the cortex, and whose levels are developmentally regulated (Moroni et al., 1984), has a neurotoxic action on serotonergic terminals in the hippocampus (El Defrawy et al., 1986). If serotonergic terminals in the kitten visual cortex are damaged by quinolinic acid, then the reduction of 5-HT$_2$ receptors and 5-HT$_{Up}$ sites by almost 40%, indicates their representation on serotonergic terminals. The remaining 5-HT$_{Up}$ sites could be on glial cells (Kimelberg, 1988; Kimelberg and Katz, 1985), and the residual 5-HT$_2$ receptors could either be on glial cells or afferent fibers. Further investigation is required to address this question.

The majority of studies assessing the consequences of visual deprivation have emphasized the effects imposed on ocular dominance column formation, although detrimental effects on other columnar features of visual cortex have also been described. The arrangement of orientation columns is similar to ocular dominance in that they are likely to be determined by the arrangement of geniculocortical afferent inputs (Stryker et al., 1990; Chapman et al., 1991). However, their columnar segregation is impaired by binocular (suture or dark-rearing), but not monocular restriction of visual experience (Blakemore and Price, 1987; Cynader et al., 1980 but see Fregnac et al., 1981).

The differential effects of monocular, versus binocular, deprivation are very similar to those observed in the organization of horizontal connections during development. Although disputed, there is some evidence that columnar compartments, defined by patchy horizontal connections in supragranular layers, link zones of like orientation selectivity (Gilbert and Wiesel, 1989; but see Matsubara et al., 1985; Matsubara et al., 1987). The secondary disruption of activity in supragranular layers of the visual cortex, resulting from reduced synaptic input from the poorly driven cortical cells in layer IV (Bliss-Tieman, 1991), would also be expected to affect the organization of intracortical patches. Interestingly, these patches have a periodicity similar to that described by 5-HT receptors and Zn (see Katz and Callaway, 1992 for review). The formation of the patchy pattern of intracortical connections arises from an activity-dependent, selective axon
retraction during early postnatal development (Callaway and Katz, 1990; Lübke and Albus, 1992; Luhmann et al., 1986; Price, 1986). Similar to the effects of dark-rearing described here, where 5-HT$_{1C/2}$ receptors fail to segregate, the effects of binocular lid suture prior to eye-opening, results in the maintenance of an homogenous distribution of intracortical projections ( Löwel and Singer, 1992). In addition, the critical period for the effects of deprivation extends to about 14 weeks of age (Dalva et al., 1992), when the 5-HT receptor columns are no longer present (see Chapter 2).

The results of this study clearly indicate that the columnar expression of 5-HT receptors and Zn are dependent on normal binocular visual experience and activity. However, arguments for an implicit relationship between the columnar system(s) described by these molecules, and those of either ocular dominance columns, orientation columns and/or patchy horizontal connections could be made based on the results of the various deprivation paradigms employed here.

The experiments in Chapter 5 attempt to address the possibility of explicit functional relationships using double label procedures to determine whether the columnar systems described thus far are related to the geniculocortical ocular dominance columns which have been intensively studied.
The columnar arrangement of functionally similar elements has long been considered to be a fundamental principle of neocortical organization (Lorente de Nó, 1949; Mountcastle, 1978). The demonstration that cytochrome oxidase-enriched blobs overlie eye-specific columns in primate visual cortex (Horton and Hedley-White, 1984) has proven to be invaluable in providing an anatomical reference point from which theories regarding the functional compartmentalization of mammalian visual cortex have evolved (reviewed in LeVay and Nelson, 1991). Several other metabolic enzymes (Horton, 1984; Sandell, 1986) as well as a variety of molecules which are associated with the function of GABA-ergic (Hendrickson, 1985; Hendrickson et al., 1981), peptidergic (Kuljis and Rakic, 1990), and cholinergic (Graybiel and Ragsdale, 1982; Horton, 1984) neurotransmission, among others (see LeVay and Nelson, 1991), have been found to be selectively localized within blobs, or to the interblob region, in primate visual cortex. These results suggest a potential relationship between neurotransmitter systems, and functionally specialized compartments in visual cortex. Cytochrome oxidase-blobs were initially reported to be unique to primates (Horton, 1984; Horton and Hubel, 1981), leading to hypotheses which constrained functional relationships of these anatomically-defined compartments to features of visual processing also unique to primates, such as colour (Livingstone and Hubel, 1984a). Despite numerous attempts, anatomical evidence for the periodic distribution of any endogenous molecule in the visual cortex of non-primates had not been provided until recently (Schöen et al., 1990).
Over a decade after having been discovered in primates, preliminary reports indicate that a blob-like pattern of cytochrome oxidase-staining is present in the visual cortex of adult carnivores (Cresho et al., 1992; Murphy et al., 1990). These data, in combination with the data presented thus far in this body of work, indicate that anatomical correlates for the columnar organization of visual cortex might not be species-unique, and, furthermore, provide the first evidence that functional correlates of the columnar distribution of neuroactive molecules might be conserved.

As a first step toward understanding the functional relationships of Zn and 5-HT receptor columns, the first series of experiments described in this chapter establish that cytochrome oxidase (CO) and acetylcholinesterase (AChE; its anatomical relationship with CO in primate visual cortex is known) are present in patches in developing cat visual cortex. Their relationship to the columnar distributions of Zn and 5-HT receptors described in Chapters 2 and 3 are also established. Secondly, the columnar distributions of Zn, 5-HT, CO and AChE are compared to the distribution of ocular dominance columns, visualized by transneuronal autoradiography, in kitten visual cortex. Finally, the possibility that the distribution of these neuroactive molecules describe cortical compartments which are functionally conserved across species is addressed by comparing the distribution of Zn to CO in the striate cortex of monkeys.

Materials and Methods

Animals and Surgical Procedures

Cats. Ten kittens between the ages of 50 and 60 postnatal days were anaesthetized to effect with halothane, the saphenous vein was cannulated and an intravenous injection of sodium selenite (10 mg / kg) was administered at a rate of 1 ml / min. Following a 15 - 20 min survival period the animals were killed with an overdose of sodium pentobarbital and perfused through the ascending aorta with 100 ml of 0.1M Sorenson's buffer. The brain was quickly removed and the visual cortex from each hemisphere was blocked, opened, flattened between glass slides and then quickly
frozen on a bed of dry ice. Prior to cutting sections, fiduciary landmarks were placed using a 30 gauge needle, at approximately 10 mm intervals, to facilitate section alignment. Serial sections were cut at a thickness of 25 μm on a cryostat at -20°C, thaw-mounted on gelatin-coated glass slides and stored at -20°C.

Monkeys. Three adult vervet monkeys (Cercopithicus aethiops) were heavily sedated with ketamine and slowly administered an intravenous injection of sodium selenite (10 mg / ml; 10 mg / kg). After 15 minutes, the monkeys were killed with an overdose of sodium pentobarbital and perfused transcardially with Sorenson's buffer (0.1M, pH 7.4). The brain was quickly removed and the visual operculum from one hemisphere was dissected and flattened between glass slides in order to cut sections tangential to the cortical surface. The other hemisphere was left intact for coronal sections. The tissue blocks were quickly frozen as previously described. Prior to sectioning, pin holes were placed in the flattened hemisphere to provide fiduciary landmarks. Sections were cut on a cryostat (-20°C), at a thickness of 25 μm, and thaw-mounted on gelatin-coated slides.

Transneuronal Autoradiography. Transneuronal labeling of eye-specific inputs to visual cortex was performed in 8 kittens (PD45 - PD53) by intra-ocular injection of [3H]proline (Wiesel et al., 1974). Under halothane anaesthesia 2.0 - 2.5 mCi of [3H]proline (Amersham), in 20 μl of saline, was injected slowly (15 min) into one eye through a 27 gauge cannula whose tip was positioned near the optic disc via a lateral approach. An equivalent volume of saline was injected into the other eye to control for possible asymmetric effects on the ocular dominance distribution. The animals survived 8 - 13 days and were then perfused as above. In two of the animals, the brain was frozen intact for horizontal sections to be cut. In the remaining kittens, the visual cortex from both hemispheres was prepared for tangential sections as described in Chapter 2.
Autoradiography and Staining

For comparisons among the different columnar markers, slide-mounted sections were divided into sets, comprising near-adjacent sections, which were processed for either 5-HT\textsubscript{1C} receptor autoradiography, or zinc, acetylcholinesterase or cytochrome oxidase histochemistry. Sections from proline injected animals were divided into 4 sets, which were either: 1) stained for synaptic zinc, 2) stained for synaptic zinc and then apposed to Hyperfilm (4 weeks), 3) apposed to Hyperfilm (4 weeks) then stained for zinc or, 4) apposed to Hyperfilm (4 weeks), unprocessed. In this way, the distribution of eye-specific label could be directly compared to the distribution of zinc patches in near-adjacent, directly adjacent or the same sections. Naturally, the last provided the best results.

**Serotonin Receptor Autoradiography.** 5-HT\textsubscript{1C} and 5-HT\textsubscript{2} receptors were visualized using methods identical to those described in Chapter 2.

**Zinc Histochemistry.** The distribution of synaptic zinc in sections from kitten and monkey visual cortex was assessed using the methods described in Chapter 3.

**Acetylcholinesterase Histochemistry.** Sections intended for the detection of acetylcholinesterase (AChE) were fixed in 4% paraformaldehyde in PB for 10 min. The staining procedure was as described by Karnovsky and Roots (1964) (Karnovsky and Roots, 1964). The fixed sections were preincubated for 30 min in PB containing 30 μM tetraisopropylpyrophosphoramide (iso-OMPA) to inhibit butyrylcholinesterase activity. The AChE-positive reaction was visualized following incubation for 6 - 8 hours (at room temperature) in 300 ml of a 50 mM Tris-maleic buffer (pH 6.0) containing 150 mg acetylthiocholine iodide, 441 mg sodium citrate, 225 mg cupric sulphate and 49 mg potassium ferricyanide. The slides were rinsed in distilled water, allowed to air dry overnight, cleared in xylene, and coverslipped with Permount.
Cytochrome Oxidase Histochemistry. The slide-mounted cryostat sections were processed within 24 hours of sectioning in order to minimize enzyme degradation. Prior to staining, the sections were fixed for 5 min in 4% paraformaldehyde in 0.1M phosphate buffer (PB, pH 7.4). Two different staining methods were used with equivalent results; however, the cobalt enhanced version proved superior for visualizing cytochrome oxidase enriched patches.

The first method was described by Horton (1984) and is based on a modification of a procedure developed by Wong-Riley (1979). Following fixation, the slides were rinsed twice for 5 minutes in PB and then immediately incubated at 37°C, for 2 - 8 hours, in a solution consisting of 50 mg DAB, 30 mg cytochrome C (Type III, Sigma) and 20 mg catalase (Sigma) in 100 ml PB. After incubation, the slides were rinsed in buffer, dehydrated in an ascending series of alcohol, cleared in xylene and coverslipped with Permount.

The second method utilized the same histochemical principles but enhanced the visibility of the reaction product, and the contrast between differentially stained regions, by heavy metal intensification (Silverman and Tootell, 1987). Following fixation, the sections were transferred through 4 serial changes of PB containing 10% sucrose and then placed for 10 min into a 0.05 M Tris-HCl buffer (pH 7.6) containing 275 mg cobalt chloride / l buffer, 10% sucrose and 0.5% dimethylsulfoxide (DMSO). Following a brief rinse in PB (1 min), the sections were incubated in the reaction medium made up of 50 mg DAB, 15 mg cytochrome C, 5 g sucrose, 10 mg catalase and 0.25 ml DMSO in 100 ml PB (bubbled with oxygen @ 37°C). The reaction time varied from 2 - 3 hours. Following incubation, the slides were rinsed in buffer, dehydrated in an ascending series of alcohol, cleared in xylene and coverslipped with Permount. For either method, control sections incubated in the presence of sodium cyanide (0.01M) showed complete inhibition of reaction product formation.

Image Analysis. Histochemically stained sections and autoradiographic images were digitally captured using a Cohu CCD camera (4915) and a Data Translation (DT-2255) frame grabber card installed in a Macintosh IIfx computer running Image (NIH, v 1.47) software. Serial
sections were aligned using an *Image* module programmed in house, which allowed precise alignment of successive images on line. The relationship of the autoradiographic and histochemical features in sections of kitten visual cortex was determined by arithmetically blending, or transparently superimposing, digital images using Adobe Photoshop (v 2.1, Adobe Systems Inc.). The captured and aligned figures were lettered and labeled using Canvas (v 3.1, Deneba Software) and hard copies were digitally processed by printing onto black and white negative film (Ilford FP4) using a slide processor, and then conventionally printed onto photographic paper (eg. Figs. 5.3 - 5.7).

**Results and Discussion**

The laminar and tangential distributions of 5-HT<sub>1C</sub> receptors (A, B), Zn (C, D), CO (E, F) and AChE (G, H) are compared in Figure 5.1 in frontal (A, C, E, G), and tangential sections (B, D, F, H), through the visual cortex of PD50 kittens. It is clear from this figure that the columnar distribution of each of these molecules was more readily apparent in the tangential plane than in the frontal plane. For CO, and particularly AChE, the patterned appearance is virtually invisible in the frontal plane.

The characteristics of the periodic pattern of 5-HT<sub>1C</sub> receptors in layer IV of area 17, were described in Chapter 2. The distribution of Zn, which also exhibited column-specific labeling in layer IV of area 17, was described in Chapter 3. In comparing their columnar distribution in directly adjacent sections (Fig.5.1A, C; Fig. 5.3A, B), these molecules were found to be precisely overlapping, and localized to the same column (note arrows).

The distribution of these markers was compared with that of CO and AChE in serially adjacent sections (Fig. 5.3). As in the adult cat (Murphy et al., 1990), the patchy distribution of CO in PD50 kittens was not readily apparent in coronal sections (Fig. 5.1E), but was clear in the tangential plane (Fig. 5.1F; Fig. 5.3A'-C'). We also found AChE to exhibit a periodic pattern in tangential sections (Fig. 5.1H, Fig. 5.3A"-C"), which was not apparent in the coronal plane (Fig.
5.1G). AChE-rich patches were limited to a thin band at the layer III/IV border (Fig. 5.1H, Fig. 5.2 A"-C"), which were aligned with CO blobs (Fig. 5.3), but complementary to Zn / 5-HTIC columns (Fig. 5.4). The complementary relationship between CO / AChE-enriched blobs and the Zn / 5-HTIC patches in layer IV can be seen in Figure 5.5 at higher magnification. Here, digitally captured images showing the patchy distribution of Zn (Fig. 5.5A) and CO (Fig. 5.5B) are shown individually, and then superimposed and summed in Figures 5.5C and 5.5D. The homogeneous image resulting from the summation of carefully aligned Zn and CO stained sections (Fig. 5.5C), contrasted with the reappearance of the distinct periodicity when the overlying image was shifted to the right by a distance equal to one-half of the average patch spacing (450 µm, see arrows) and then summed (Fig. 5.5D), indicating that these two systems were precisely complementary in the tangential domain.

The distributions of several enzymes and other molecules associated with the functions of neurotransmitters have been shown to be either aligned with, or complementary to, CO-blobs in primate visual cortex (see LeVay and Nelson, 1991 for review). These data, and those reported here, suggest that column-specific molecules might demarcate common functional domains in the visual cortex of species as phylogenetically diverse as carnivores and primates. Indeed, we found that the laminar-specific distribution of Zn in striate cortex of adult primates (Cercopithecus aethiops) was exactly complementary to that of CO (CO, Fig. 5.6A, C, F; Zn, Fig. 5.6B, D, G), just as it was in the cat. In the tangential plane of section, CO-rich blobs (Fig. 5.6A) were clearly shown to be interdigitated within a Zn-stained matrix (Fig. 5.6B) in layers II and III. This precise complementarity was evident, in the frontal plane, between laminae as well (Fig. 5.6C, 5.6D; Fig. 5.6F, 5.6G). Preliminary results indicate that this complementary relationship between Zn and CO patches in vervet monkeys is present as early as 4 weeks of age (Dyck and Cynader, unpublished results). The distribution of serotonin receptors in the visual cortex of developing primates has not yet been examined, although previous studies have shown the distribution of serotonergic afferents to be more abundant in interblob zones (Hendrickson, 1985).
Figure 5.1. Multiplicity of markers for columnar domains in PD50 kitten visual cortex. The columnar distributions of 5-HT\textsubscript{1C} receptors (A, B) and synaptic zinc (C, D) in layer IV of area 17 were apparent even in the frontal plane (A, C). The registration of several columns is apparent in the frontal plane (arrow heads). The distributions of cytochrome oxidase (E, F) and acetylcholinesterase (G, H), which are also cytochemical markers of the columnar architecture in primate striate cortex, did not appear patchy in frontal sections (E, G), but clearly demarcated columnar domains in the tangential plane (F, H). The most marked columnar distribution of each of the four markers was found in upper layer IV; however, their individual distributions were contained within distinct layer III / IV substrata. Nevertheless, their organization into patches of increased density all exhibited the same periodicity in area 17 (900-1000 μm, centre-to-centre). Scale bars = 3.0 mm.
Figure 5.1
Figure 5.2. Compared in serially adjacent sections, patches of Zn (A), and those of 5-HT$_1$C (B) and 5-HT$_2$ (C) receptors were found localized to the same vertical columns in layer IV of area 17 (note arrows). The patchy distributions of Zn and 5-HT$_2$ receptors were greater within the more superficial strata of layer IV. Levels of 5-HT$_1$C receptors were highest as they emerged from the dense band at the layer IV / V border, and became less dense as they extended through the entire extent of layer IV into layer III. Note that panel A is slightly shifted to the right, relative to B and C. Scale bar = 1 mm.
Figure 5.3. The distribution of Zn (A-C), CO (A'-C'), and AChE (A''-C'') in serial sections from levels near the top (A, A', A''), middle (B, B', B'') and bottom (C, C', C'') of layer IV in PD50 kitten visual cortex. Patches of Zn and CO were predominant in upper strata of layer IV and waned in deeper strata, although Zn was found at deeper levels than CO. AChE-rich patches, on the other hand, were limited to a thin region at the layer III / IV border. Note that none of these molecules exhibited a periodic pattern in area 18. The area 17 & 18 borders are indicated by the white dotted lines in A-C. The positions of selected laminae are indicated by Roman numerals in A'-C'. Fiduciary landmarks are indicated by the black arrows in A''-C''. Scale bar = 3 mm.
Figure 5.4. The relationship between patches of Zn (A) and AChE (B) in adjacent, tangential sections through the visual cortex of PD50 kitten. The negative correlation observed between Zn- and AChE-rich patches are indicated in several places by the black arrows. Selected laminae are indicated by Roman numerals. White arrows indicate fiduciary landmarks. (M, ventro-medial; P, posterior; L, dorso-lateral; A, anterior) Scale bar = 5 mm.
Figure 5.5. High magnification digital images of cytochrome oxidase (A) and zinc (B) staining in serially adjacent sections through area 17 of PD50 cat visual cortex. The periodic, patchy distributions of both cytochrome oxidase and synaptic zinc are evident in layer IV. When the two sections were superimposed and summed, the precise laminar and columnar complementarity of these two markers was manifested by the disappearance of the patchy pattern in layer IV(C). Only the microtome blade scratches showed an increased density following their spatial summation (C, diagonal streaks). When the overlying cytochrome oxidase image was shifted to the right by 450 µm, and then summed (D), the patchy pattern reappeared, indicating that synaptic zinc and cytochrome oxidase are distributed with precise laminar and intra-laminar complementarity. White arrows indicate fiduciary landmarks used for section alignment. The dotted lines and Roman numerals in C outline and indicate laminar boundaries. Scale bar = 1 mm.
Figure 5.5
In primates, the CO-rich zones are associated with: (1) the centers of eye dominance columns, (2) areas of high colour selectivity, (3) zones of broad orientation selectivity, and (4) preferential input from various processing streams originating in the lateral geniculate nucleus (LeVay and Nelson, 1991; Livingstone and Hubel, 1988). To begin to examine the cross-species generality of these functional relationships, we compared the distribution of Zn patches with ocular dominance (OD) columns in kitten cortex. Figures 5.7 and 5.8 illustrate the tangential distribution of Zn patches (Fig. 5.7A', B'; Fig. 5.8C, D), compared with ipsilateral eye-specific patches labeled autoradiographically (Fig. 5.7A, B; Fig. 5.8A, B), in the same sections through layer IV of kitten visual cortex. At higher magnification in Figure 5.8C, the white, dotted lines indicate the outline of the OD pattern superimposed on the Zn-stained section, and the outlines of Zn-rich patches are indicated on the OD map of Figure 5.8B. Although some overlap between the two patchy patterns was evident, they were not explicitly aligned with one another.

These findings demonstrate that the relationship between the CO/AChE and 5-HT receptor/Zn system and eye-specific innervation observed in primates, is not obligatory in cats. Likewise, the association with colour is unlikely to be obligatory, given the cat's poor colour vision capacities (Daw, 1973), and the presence of CO-blobs in primates without colour vision (Condo and Casagrande, 1990). Our findings are consistent with at least two of the remaining functional interpretations. (1) Previous studies have indicated singularities in the cat and monkey cortical orientation maps, zones where different orientation bands coalesce and which contain broadly tuned neurons (Blasdel and Salama, 1986; Bonhoeffer and Grinvald, 1991; Swindale et al., 1987). These singularities are thought to be associated with CO blobs in monkeys (Blasdel, 1992) and may well have the same association in cat cortex. (2) In primates, the CO-blob / interblob system has been reported to contain geniculate inputs representing different processing streams (LeVay and Nelson, 1991). This is consistent with the findings described in Chapter 2 which indicate, on the basis of spatial characteristics of 5-HT receptor expression, that 5-HT1c and 5-HT2 receptors are individually related to different, parallel processing streams. The precise relationship and the extent to which functional homology is maintained among species, await further examination.
Figure 5.6. The distribution of cytochrome oxidase (CO; A, C, F) and synaptic zinc (Zn; B, D, G) in striate cortex of an adult Vervet monkey. The precise complementarity of CO-blobs within a Zn-stained matrix in laminae II / III of V1 was clearly seen in the near-adjacent sections which were cut tangential to the cortical surface (A, B). The complementary relationship was maintained in lamina IVa, but in this layer, Zn-stained blobs were, instead, surrounded by a CO matrix. The periodic patterns of CO and Zn were not robust in coronal sections (C, D, F, G; note arrows), but these panels demonstrate that the laminar-specific distributions of these two molecules also exhibited a high degree of complementarity in V1 and V2 (C, D). In contrast with CO (A, C, F), the highest levels of Zn (B, D, G) were found in layers I, IVb and VI, while layers II / III and V were moderately stained. Nissl-stained sections (E) were used to establish laminar boundaries. The white arrows in A & B indicate fiduciary landmarks, used to facilitate section alignment. Roman numerals in B & E indicate the relative locations of cortical laminae. Scale bars in A - D = 2.0 mm; in E-G = 500 μm.
Figure 5.6
Figure 5.7. The overall distribution of eye-specific "columns" (A, B), labeled using $^{3}$Hproline injected in the ipsilateral eye, are compared with Zn-rich patches (A', B') in adjacent sections (A-A'; B-B') at two depths from the cortical surface (700 & 800 μm) through visual cortical areas 17 and 18. These figures clearly indicate that the spatial patterns, of their respective patchy distributions, is very similar in extent. Note that the ocular dominance bands double their spacing in area 18 and are clearly oriented perpendicular to the 17 / 18 border. There is a weak indication that Zn may similarly change its pattern in area 18. Common fiduciary landmarks are indicated by the arrows. Medial is down. Scale bars = 10 X 2 mm.
Figure 5.8. The relationship between the neurochemically-defined columnar architecture in layer IV of kitten visual cortex, and ocular dominance columns was determined in the same section stained first for synaptic zinc (C, D) and then processed for $[^3\text{H}]$proline autoradiography (A, B). In C, the outline of the pattern of ipsilateral-eye projections is superimposed on the zinc patches in layer IV, and in D the zinc patch outline is superimposed on the ocular dominance distribution in B. There does not appear to be an explicit relationship between these columnar systems. Fiduciary landmarks used for image alignment are indicated by the arrows in A and D. Scale bar = 4 mm X 1 mm.
GENERAL DISCUSSION

Summary

The studies described in the previous four chapters have provided important anatomical and functional information regarding the expression of serotonin receptor subtypes and synaptic zinc in the visual cortex of cats during postnatal development; and have specifically indicated that:

1. The levels of serotonin 1A, 1C and 2 receptor subtypes and the high affinity serotonin transporter were developmentally regulated, with each receptor exhibiting unique temporal and spatial profiles in their levels of expression.

2. The serotonin 1C and 2 receptor subtypes were transiently expressed at high levels in the same cortical columns, but within different geniculate recipient sublaminae.

3. Synaptic terminals containing vesicular zinc were developmentally regulated and highly enriched in the same cortical columns and during the same temporal window described by serotonin receptor columns.

4. The columnar expression of synaptic zinc and serotonin receptors required normal binocular visual input and was activity-dependent.

5. Cytochrome oxidase and acetylcholinesterase were localized to cortical compartments which were precisely complementary to those demarcated by synaptic zinc and serotonin receptors.

6. The precise laminar and columnar complementary relationship of zinc and cytochrome oxidase observed in kitten visual cortex was also found in primate visual cortex.

These points are summarized in schematic form in Figures 6.1 and 6.2.
Figure 6.1 Schematic summary diagram of the relative distribution of synaptic zinc, serotonin receptors, and acetylcholinesterase in the kitten visual cortex during postnatal development. The interdigitated columnar mosaic formed by 5-HT receptors/zinc and CO/AChE is not present at birth but becomes prominent during a critical period of development during which the synaptic organization of the visual cortex is susceptible to activity- and experience-dependent modifications. The columnar expression of 5-HT_{1C/2} receptors and AChE is transient and restricted to this period of development. However, a remnant of the compartmentalized organization of CO and Zn is still present in the adult visual cortex.
General Discussion

Figure 6.1
**Figure 6.2** Schematic diagram summarizing the effects of manipulating visual input and experience on the columnar expression of Zn / 5-HT₁C and 5-HT₂ in the kitten visual cortex. The columnar compartmentalization of zinc and 5-HT₁C&₂ receptors is dependent on the integrity of the lateral geniculate nucleus and, in particular, normal binocular input during development. The effect of dark rearing and compromising the lateral geniculate input on the columnar distribution of zinc was not assessed and is, therefore not indicated.
Figure 6.2
Discussion

The results of the experiments outlined here raise important issues with regard to the mechanisms of activity-dependent plasticity in the nervous system. Many of the implications of these findings, with respect to the anatomical and functional development of kitten visual cortex, are discussed extensively within the individual chapters. As such, they will not be raised again here. However, several methodological issues and specific functional implications which were not dealt with, or only briefly, warrant further review.

Methodological Considerations

Autoradiographic procedures. The ligand concentrations and incubation parameters which were used to label the different 5-HT receptor subtypes were determined from previously published studies, which had characterized these ligands in a number of different species, but predominantly in adult animals. In addition, all of the binding procedures were carried out at concentrations which were below the level of saturation for each receptor. As a consequence, one can not be certain whether the variations in binding density that were observed reflect changes in receptor number (Bmax), or in receptor affinity (Kd), or both. Studies in other species, which have characterized developmental changes in 5-HT receptor expression, have reported ontogenetic changes in binding to be exclusively due to changes in their number, and not affinity (Biegon, 1991; Gross-Isseroff et al., 1990). However, this cannot simply be assumed to be true in cats as well. In fact, changes in the binding affinity (Kd) of a number of neurotransmitter receptors have been described in kitten visual cortex during postnatal development (Shaw et al., 1984; Shaw et al., 1985). Regardless of whether they reflect changes in affinity or number, the densitometric changes in receptor binding that have been described here are likely to reflect specific indices of receptor sensitivity during development. However, a complete pharmacological characterization of these ligands at each age would be required to address these questions definitively.
The pharmacological and molecular characteristics of 5-HT$_1C$ and 5-HT$_2$ receptors are very similar, and several studies have indicated that currently available ligands lack subtype specificity (Closse, 1983; Glennon et al., 1992). However, the reported lack of specificity of $[^3H]$mesulergine for 5-HT$_1C$ receptors in rats (Closse, 1983; Pazos et al., 1988), differs from studies in human and porcine brain, where the binding of $[^3H]$mesulergine was determined to be 5-HT$_1C$-specific (Lyon and Titeler, 1988; Pazos et al., 1988; Pazos et al., 1984b). Several lines of evidence indicate that the specificity of $[^3H]$mesulergine and $[^125$I]DOI binding for the 5-HT$_1C$ and 5-HT$_2$ subtypes, respectively, holds true in cats as well. First, the distinctly different temporal and spatial distributions of $[^3H]$mesulergine and $[^125$I]DOI binding indicate that these ligands label different populations of receptors. The inclusion of spiperone, which has a 1000-fold lower affinity to 5-HT$_1C$ than 5-HT$_2$ receptors, in the incubation medium has been used to confer greater specificity of $[^3H]$mesulergine for 5-HT$_1C$ receptors. In the present studies, no differences were found in the regional and temporal binding patterns exhibited by $[^3H]$mesulergine binding in cat visual cortex with, or without, the inclusion of spiperone. Finally, the temporal and regional patterns in the expression of 5-HT$_2$ sites were identical when either $[^125$I]DOI or $[^3H]$DOB was used. These data do not, however, rule out the possibility that $[^3H]$mesulergine and $[^125$I]DOI differentiate different affinity states of either the 5-HT$_1C$ and/or 5-HT$_2$ receptor, or the existence of additional subtypes within the 5-HT$_2$ family (Hartig et al., 1990; Leonhardt and Titeler, 1989; Lyon and Titeler, 1988; Pierce and Peroutka, 1989; Teitler et al., 1990). Further pharmacological characterization, combined with molecular biological studies, are required to fully address these issues.

The differential absorption of tritium emissions in the brain, based upon tissue variations in myelin content (Geary and Wooten, 1985; Herkenham and Sokoloff, 1984), could provide a source of variation to the laminar binding patterns observed in the autoradiographic studies carried out in this thesis. The process of myelination in visual cortex of the kitten starts around 4 weeks postnatal (Looney and Elberger, 1986; Remahl and Hildebrand, 1990) and proceeds progressively in a deep-to-superficial laminar gradient, reaching adult levels around 12 weeks of age (Daw,
The highest levels of myelin are in layers IV-VI. The issue of quenching is not significant with regard to $[^{125}\text{I}]$-labeled ligands, but the signal provided by the tritiated ligands used to detect $5\text{-HT}_{1A}, 1\text{C}, 3 \& \text{Up}$ binding, might have been attenuated, particularly at ages beyond 4 weeks of age. This might be significant, particularly with regard to the columnar representation of $5\text{-HT}_{1C}$ receptors, whose increasing numbers during this period of time, might have been underestimated in these studies. It should be noted that, in autoradiographic analyses of major neurotransmitter receptors in the primate visual cortex, where laminar variation in myelination is much more extreme than in the cat, the total effect of quenching was no greater than 20% (Lidow et al., 1989; Rakic et al., 1988).

**Histochemical procedures.** It is estimated that 85-90% of the zinc in the brain is bound into the tertiary structure of over 50 different zinc-containing enzymes (Wallwork, 1987). The basis of selectivity for the methods used here to indicate the remaining 10-15% of zinc, which is localized to presynaptic vesicles of zinc-containing neurons, is conferred by the fact that the metabolic pool of zinc is bound within the tertiary structure of proteins and is, therefore, inaccessible to chelation by sulphide or selenite ions (Vallee, 1983; Vallee and Galdes, 1984). Although initial versions of the zinc histochemical method stained heavy metals in addition to zinc (Danscher et al., 1976; Haug, 1973), the selenium-histochemical method utilized in this study has demonstrated specificity for that portion of zinc in the brain which is localized to presynaptic vesicles of zinc-containing neurons (Danscher et al., 1985; Frederickson, 1989; Frederickson and Danscher, 1988). However, if any portion of the staining presented here is due to labeling of other heavy metal ions, then, based on the ultrastructural analyses which find histochemically-labeled silver particles almost exclusively limited to vesicles within synaptic terminals, their role in synaptic modulation should be studied as well.

One of the most significant methodological issues raised in these, and in other studies which have succeeded in demarcating columnar compartments in mammalian neocortex (e.g. primate, Tootell et al., 1988; cat, Murphy et al., 1990; ferret, Cresho et al., 1992; rat, Nakazawa
et al., 1992) is with regard to the plane within which the various markers are visualized, and the sensitivity and contrast of the staining method employed. Thus, by cutting tissue sections in such a way as to maximize the view of the particular region of interest (eg. tangential to the cortical surface for laminar and intralaminar analyses), the levels of acetylcholinesterase and cytochrome oxidase, whose developmental expression has been studied previously in kitten visual cortex without an indication of columnar compartmentalization (CO, Kageyama and Wong-Riley, 1986; Price, 1985; AChE, Bear et al., 1985a; Bear et al., 1985b), were found here to vary in a periodic manner. The most robust markers of the columnar compartmentalization of visual cortex were those demonstrated by zinc histochemistry and 5-HT receptor autoradiography. In initial studies, the periodic distributions of AChE and CO were not always readily seen, due to the limited contrast provided by brown reaction products of unenhanced DAB or thiocholine, unless compared in adjacent sections using 5-HT autoradiography or zinc as a metric. When heavy metal enhancement techniques were used (CO, Silverman and Tootell, 1987; AChE, Schatz et al., 1992; our preliminary results not reported here), the periodic patterns became easily and clearly distinguishable.

To achieve higher cytological resolution with the currently available anatomical markers utilized in these studies was not possible due, in part, to an incompatibility of paraformaldehyde fixation with the procedures used to localize receptors and for zinc histochemistry. The detrimental effects of fixation on receptor integrity are obvious, but those on synaptic zinc are obscure. Because glutaraldehyde fixation has no effect on the integrity of zinc-staining, it is likely that paraformaldehyde adversely affects the bond between zinc and selenite ions (Danscher and Zimmer, 1978), which is a requirement of physical development for visualizing synaptic zinc. Serendipitously, by being unable to use paraformaldehyde in studies comparing the distribution of zinc with the other molecules in adjacent sections, the column-specific cytochrome oxidase staining was found to be much more robust in unfixed tissue, as long as the tissue was flash-frozen, sectioned and stained within 24 hours or, alternatively, stored at -70°C until it was processed.
Functional Considerations

The data presented in this thesis have advanced existing studies which have assessed the anatomical distributions and potential functional contribution of serotonin receptors, zinc, acetylcholinesterase and cytochrome oxidase to various aspects of information processing in the mature and developing mammalian visual cortex. The primary goals of this thesis, as described in Chapter 1, were to assess anatomical distributions during postnatal development, and to determine the functional significance of serotonin receptors and synaptic zinc in the cat visual cortex. As far as these goals have been advanced, they still remain inadequately answered. Determination of the precise cytological distributions of serotonin receptors and synaptic zinc, combined with an understanding of how their actions are transduced to signals which are developmentally and functionally meaningful, is crucial. The remainder of this discussion provides a preliminary discussion of these important issues, as well as directions for future studies.

Where are these molecules? Available evidence regarding the cytological expression of the different 5-HT receptor subtypes on glial cells or neuronal somata, dendrites and axon terminals was provided in Chapters 2 and 4. A significant implication, provided by the results of Chapter 4, was that 5-HT$_1$C/2 receptors might be localized on geniculocortical afferents to visual cortex. Similarly, inferential support for a similar source of zinc-containing fibers in layer IV was provided by the data presented in Chapters 3 and 4. The idea that 5-HT receptors might be localized on zinc-containing geniculocortical fibers, albeit speculative, is attractive.

The obvious directions for the next generation of studies are to distinguish clearly the precise location and source of 5-HT receptors and synaptic zinc with respect to afferent input to the visual cortex at different ages. These studies would require using techniques which permit tract-tracing combined with receptor detection and/or histochemistry, with subcellular resolution.

The distribution of cells intrinsic to the cortex which express 5-HT receptors could be detected by immunocytochemistry or with in situ hybridization studies (ISH). The 5-HT$_1$A (Albert
et al., 1990; Fargin et al., 1988), 5-HT_{1B} (Hamblin et al., 1992; Mochizuki et al., 1992), 5-HT_{1D} (Weinshank et al., 1992), 5-HT_{1E} (McAllister et al., 1992), 5-HT_{1C} (Lübbert et al., 1987; Saltzman et al., 1991), and 5-HT_{2} (Chen et al., 1992; Julius et al., 1990; Pritchett et al., 1988) receptors have all been cloned and their nucleic acid sequences are published. The utility of receptor-specific antibodies to localize 5-HT receptors would be substantial, however, the production of antibodies against members of the G-protein-coupled receptor family has proven extremely difficult because of the tremendous levels of homology between receptors for different neurotransmitters, even at the protein level (D. Julius; F. Lübbert, personal communication). Some, however, are becoming available. Preliminary studies with 5-HT_{1A} receptor-specific antibodies provided by J. Raymond (Duke University) have confirmed the receptor binding results provided here (Dyck, unpublished results). The use of antibodies against the other subtypes will prove useful, as they become available.

One of the primary implications suggested by the unique temporal, regional and laminar distributions of the neuroanatomical markers utilized in the studies presented in this thesis, is the possibility that these molecules differentiate functionally distinct compartments in the developing visual cortex (see Chapters 2 and 5 for discussion). The functional nature of these new columnar systems remains uncertain, but several lines of evidence indicate that they may be related to specific features of visual information processing such as those described by geniculocortical processing streams. These possibilities could be addressed with the application of methods which can identify these functional domains in combination with receptor localization. For example, the temporally and spatially unique distributions of 5-HT_{1C} and 5-HT_{2} receptors appear to differentiate the anatomically and functionally distinct X and Y geniculocortical pathways, respectively (see Chapter 2). An evaluation of the distribution of Cat-301, a neuronal surface-associated proteoglycan which demarcates Y-like pathways in the cat and primate visual system (Hendry et al., 1988; Sur et al., 1988), combined with 5-HT receptor identification would provide support for this hypothesis. Although an anatomical marker of the X-cell pathway has not yet been found, individual X- and Y-cells in the lateral geniculate nucleus can be physiologically identified and labeled (Freund et al.,
1985; Friedlander et al., 1985) in a manner which might be compatible with methods of receptor colocalization.

The synthesis and release of transferrin from choroid epithelial cells (Aldred et al., 1987), which has been shown to have growth factor-like activity on CNS neurons (Beach et al., 1983), is regulated by 5-HT1C receptors (Esterle and Sanders-Bush, 1992). Epithelial cells of the choroid plexus express the highest levels of 5-HT1C/2 receptors in the kitten brain (see Chapter 2). Activation of 5-HT1C receptors leads to an increase in the production of transferrin (Esterle and Sanders-Bush, 1992), and would, thereby, provide a trophic influence on brain development, conveyed humorally by the CSF. The localization of 5-HT and transferrin receptors with cytological resolution might reveal other distinct cell populations which provide a significant contribution to the processes involved in visual cortical development and plasticity.

The discussion thus far, concerning the locus of expression of 5-HT receptors in the cat brain, has been biased by the assumption that most of their function would be conferred by their location on neurons, glial cells or epithelial cells of the choroid plexus. However, the physiological control of cerebral blood flow by serotonergic mechanisms is thought to be mediated by high affinity serotonin receptors (see Edvinsson et al., 1991; Parsons, 1991 for reviews) and the complex action of 5-HT on cerebral blood vessels is possibly attributable to an heterogeneous occupation by multiple subtypes. This possibility is suggested by the observation of different vascular responses to varied pharmacological manipulations, but a thorough understanding is lacking. Additional complexity is brought about by apparent species differences in the particular receptor subtypes found on blood vessels. Pharmacological profiles indicate that cerebrovascular tone is mediated by 5-HT1A-like receptors in guinea pig and dog, 5-HT1B/1D-like receptors in cat, and 5-HT2-like receptors in the rat, dog and cat (for details see Bonvento et al., 1991). The contribution of vascular serotonin receptors to the binding levels of the various ligands examined in the cat visual cortex is unknown, but could be addressed by studies utilizing anatomical methods providing greater cellular resolution.
The precise localization of the cell bodies of zinc-containing terminals in the visual cortex has not yet been determined. Indirect evidence provided by lesion studies have indicated that the major source of zinc-containing terminals arises from local projections neurons intrinsic to the cortex (see Discussion of Chapter 3); however, these studies were performed in adult animals. The high levels of synaptic zinc found in columnar compartments within layer IV of younger kittens indicates the potential for an extrinsic source, perhaps even from neurons originating in the lateral geniculate nucleus. It would be important to tackle this question, possibly with the judicious application of specific lesion and tract-tracing studies combined with zinc histochemistry in young kittens (c. PD50).

What are they doing, wherever they are? The existence of multiple receptors responsible for transducing the signal provided by 5-HT was apparent, in retrospect, from the first studies which assessed the physiological consequences of applying 5-HT onto neurons in the cat visual cortex (Roberts and Straughan, 1967). Thus the effects of 5-HT were reported to both inhibit and excite cortical neurons (Basant et al., 1990; Roberts and Straughan, 1967), or remarkably, to change from one to the other in response to a change in their basal firing rate (Waterhouse et al., 1990). The cellular mechanisms of the inhibitory response are thought to be due to the activation of a hyperpolarizing $K^+$ current, resulting from the activation of $5-HT_{1A}$ receptors. Excitation on the other hand, is thought to be mediated by the activation of $5-HT_2$ receptors which reduce the two distinct $K^+$ currents $I_{AHP}$ and $I_M$ (Andrade and Nicoll, 1987; Colino and Halliwell, 1987; Davies et al., 1987; for reviews see Aghajanian et al., 1990; Andrade and Chaput, 1991; Bobker and Williams, 1990). The activation of these specific membrane currents is mediated by two intracellular signalling cascades: the adenylyl cyclase / cyclic AMP pathway, and the phospholipase C / phosphoinosotide hydrolysis pathway (PI). The specific serotonin receptor subtypes examined in this thesis are cumulatively linked to both of these transduction systems. Studies examining 5-HT stimulated adenylyl cyclase in the mammalian brain indicate that the $5-HT_{1A}$ receptor mediates this response. Occupation of the receptor binding site of
5-HT$_{1C}$ and 5-HT$_2$ receptors results in the activation of phosphoinositide hydrolysis (reviewed in Sanders-Bush, 1988a; Sanders-Bush, 1988b; Zifa and Fillion, 1992).

All of the members of the 5-HT1 family, in addition to 5-HT$_{1A}$ receptor, are coupled to the cAMP / adenylyl cyclase signal transduction pathway. The potential significance of this relationship in the context of cortical development is embodied by their transient levels of expression during cortical development in both rats and cats. Thus, the transient expression of 5-HT$_{1A}$ receptors, which was described in Chapter 2, is coincident with the peak period of synaptogenesis (PD8-37; Cragg, 1975), the establishment of functional corticocortical synapses (PD30; Toyama and Komatsu, 1987), and the age when the mature distribution of local cortical connections in kittens becomes defined (Katz and Callaway, 1992). In the rat, a transient serotonergic hyperinnervation of rodent somatosensory and visual cortex (D'Amato et al., 1987; Nakazawa et al., 1992) is combined with a transient, increased expression of 5-HT$_{1B}$ receptors (Leslie et al., 1992) and is coincident with the period of increased growth and synaptogenesis in rat cortex (Blue and Parnavelas, 1983b). There is, therefore, a significant possibility that 5-HT1-related stimulation of adenylyl cyclase might provide integral mechanisms for the neuronal differentiation, neuropil formation, and synaptogenesis induced in neocortical cells in vitro by the application of 5-HT (Chubakov et al., 1986).

Additional support for a role for cAMP mediated 5-HT mechanisms in synaptic plasticity has been provided by studies in invertebrate models. Facilitatory processes exerted by serotonin, such as those seen in LTP, which are governed by Hebbian rules (eg. concurrent pre-and postsynaptic changes), have been demonstrated using the gill-withdrawal response in *Aplysia*. The application of 5-HT to sensory neurons triggers two intracellular mechanisms which result in increased presynaptic transmitter release. The first facilitates release by prolonging the action potential thereby allowing more Ca$^{2+}$ entry, and a second improves the efficiency of Ca$^{2+}$-dependent release processes, possibly by increasing the number of vesicles available for release or by an increased efficiency of release machinery. These actions are dependent on the stimulation of the adenylyl cyclase / cAMP transduction pathway. Long-term changes require protein synthesis.
which may be maintained by the persistent activation of cAMP-dependent protein kinases (reviewed in Carew, 1989; Goelet et al., 1986; Greenberg et al., 1987; Kandel, 1976). Similar serotonin-mediated facilitatory mechanisms could be acting at synapses in the kitten visual cortex.

Several neural receptors, including both members of the 5-HT2 family, have mitogenic potential and can induce a state of uncontrolled growth when transfected into fibroblasts, a feature which is correlated with the activation of the PI second messenger pathway (Hanley, 1989; Julius et al., 1989). The transient distributions of 5-HT1C and 5-HT2 receptors, may have growth stimulating functions conferred by a linkage to PI; however, this requires further investigation. Nevertheless, it is apparent that 5-HT receptors, which are coupled to different effector systems capable of supporting mechanisms of growth and plasticity, are found at specific loci of enhanced growth and synaptogenesis in the developing kitten visual cortex.

Many different neurotransmitter / neuromodulator receptors have demonstrated laminar-specific distributions in the postnatal kitten visual cortex. It is highly likely that two, or more, different receptors coupled to the same, or different second messenger system(s) could be localized on the same cells. Their synergistic activation of neurons during distinct temporal windows, within different laminae and cortical regions may be necessary to mediate the long-term plastic changes observed in developing visual cortex (see Cynader et al., 1989; Cynader et al., 1990; Cynader et al., 1991). Consistent with a particular role for 5-HT1C/2 receptors and the PI-signalling cascade in contributing to mechanisms of visual cortical plasticity is the observation that, among over 30 receptor subtypes which have been examined in the developing visual cortex, those receptors which are differentially expressed in layer IV during the critical period, are either coupled to PI-turnover, or related to the mobilization of calcium or zinc (Cynader et al., 1994).

The postsynaptic, molecular mechanisms which culminate in the expression of LTP involve an NMDA receptor-activated Ca^{2+}-influx which stimulates the activity of protein kinases and a further triggering of intracellular Ca^{2+} mobilization. The hydrolysis of phosphatidylinositol bisphosphate in the PI-signal transduction pathway, with the production of diacylglycerol (DAG), acts synergistically with Ca^{2+} to activate protein kinase C (PKC). The activation of PKC together
with type II Ca\(^{2+}\) / calmodulin-dependent protein kinase (CaM) has been shown to be necessary for LTP induction in the hippocampus (see Bliss and Lynch, 1988; Kennedy, 1989; Madison et al., 1991 for reviews). In recent studies, 5-HT has been demonstrated to modulate excitatory amino acid responses in cat neocortex (Nedergaard et al., 1987). A facilitatory action of 5-HT was not apparent alone, but only when combined with glutamate-receptor-specific agonists. This observation is also supported by studies indicating that 5-HT is a direct positive modulator of glutamate-binding to NMDA receptors (Mennini and Miari, 1991), suggesting that 5-HT may participate in the modulation of LTP. Furthermore, the possibility that 5-HT\(_{1C/2}\) receptors might mediate PI-related effects in LTP-like processes in visual cortex has been proposed (reviewed in Fields and Nelson, 1991), but remains to be investigated. The columnar colocalization of 5-HT\(_{1C/2}\) receptors and synaptic zinc during the critical period, combined with the demonstrated ability of Zn\(^{2+}\) to replace Ca\(^{2+}\), or to modify the response produced by Ca\(^{2+}\) in many cellular responses traditionally thought to be exclusively mediated by Ca\(^{2+}\) (see Discussion Chapter 3), provide significant potential for 5-HT and zinc to contribute to the mechanisms of activity-dependent synaptic changes in visual cortex. Studies assessing the relative ability to induce LTP within 5-HT / Zn columns, as opposed to CO / AChE blobs, would provide an interesting perspective on this hypothesis.

Many neurotransmitters perform special roles during early development, which are not normally in their repertoire in the mature nervous system (see review by Lipton and Kater, 1989). Among them glutamatergic and serotonergic neurotransmitter systems have both been implicated in providing trophic and growth cues for neurons and glial cells during development (reviewed in Mattson, 1988; Whitaker-Azmitia, 1991). An interesting link between 5-HT and zinc has recently surfaced, based on the colocalization of zinc and 5-HT receptors, and their similar modulation in columnar compartments in visual cortex by visual input and activity during sensitive periods of visual cortical development. Recently, cortical astrocytes have been demonstrated to provide specific trophic support for serotonergic neurons (Whitaker-Azmitia and Azmitia, 1989). The neurotrophic factor has been identified as S100B (Azmitia et al., 1990), whose primary role in the
nervous system was assumed to be conferred by its' Ca^{2+}-binding ability (reviewed in Kligman and Hilt, 1988; Van Eldik and Zimmer, 1988). S100β might be called, more appropriately, a Zn^{2+}-binding protein which binds more molecules of Zn^{2+} with higher affinity than Ca^{2+} (Baudier et al., 1986; Baudier et al., 1983). Moreover, the Ca^{2+} and Zn^{2+} binding sites are distinct, with Zn^{2+} binding increasing the affinity of Ca^{2+} for its binding site (Baudier, 1988; Baudier and Gerard, 1986; Baudier and Gérard, 1983). The expression of S100β in visual cortical astrocytes is developmentally regulated and highest levels are selectively, and transiently expressed in layer IV between 3 and 6 weeks postnatally (Dyck et al., 1993). At ages beyond PD50, the highest levels of the S100β protein in visual cortex are limited to superficial and deep laminae, thereby avoiding layer IV and precisely mirroring the distribution of synaptic zinc described in Chapter 3. An understanding of the specific functional relationships between 5-HT, S100β, and zinc in visual cortex during development would be provided by colocalization studies and might reveal an underlying cooperativity among these molecules which is necessary for maintaining plasticity in the developing visual cortex.

The functional significance of a columnar compartmentalization of neuroactive molecules, such as zinc, 5-HT receptors, and acetylcholinesterase, in the developing visual cortex has been extensively discussed in this manuscript; however, the manner in which these molecules might participate in the processes of visual information processing is unknown. An understanding of the explicit contribution of these molecules to the development of the mammalian visual cortex will not be provided by anatomical or physiological studies alone, but will require an interdisciplinary approach, utilizing a combination of anatomical, molecular biological, pharmacological and physiological approaches as has been eluded to in the foregoing discussion.
Conclusion

The experiments described in this thesis provide novel anatomical and functional indices of the development and plasticity of mammalian visual cortex. The disparate, yet complementary temporal and spatial distributions of serotonin receptor subtypes and synaptic zinc indicate their potential for participating in diverse developmental processes. In particular, this initial description of the columnar compartmentalization in the cat visual cortex of molecules which are integral for intercellular communication, and whose expression is influenced by visual input and experience, affords a new perspective into the subcellular mechanisms which mediate activity- and experience-dependent changes in the synaptic organization of the developing brain. The similarity in the organization of this neurochemically-defined columnar mosaic in visual cortex of both cats and monkeys might underlie phylogenetically homologous mechanisms which participate in establishing particular features of the functional organization of mammalian visual cortex.
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