QUANTITATIVE ANALYSIS OF THE GABAERGIC SYSTEM IN CAT PRIMARY SOMATOSENSORY CORTEX AND ITS RELATION TO RECEPTIVE FIELD PROPERTIES

DISSERTATION

Presented to the Graduate Council of the University of North Texas in Partial Fulfillment of the Requirements For the Degree of

DOCTOR OF PHILOSOPHY

By

Jianying Li, M.S.
Denton, Texas
May, 1995
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Sensory neocortex contains a significant number of inhibitory neurons that use gamma-aminobutyric acid (GABA) as their neurotransmitter. Functional roles for these neurons have been identified in physiological studies. For example, in primary somatosensory cortex (SI), blockade of GABA$_A$ receptors with bicuculline leads to expansion of receptive fields (RFs). The magnitude of RF enlargement varies between SI populations of GABAergic neurons were identified by labeling specific calcium binding proteins.

The laminar distributions of GABA-immunoreactive neurons and GABA$_A$ receptors were highly correlated. The densities of GABA-immunoreactive neurons and GABA$_A$ receptors were highest in layers I and II, and decreased to layer VI. This laminar distribution pattern did not resemble the reported pattern of bicuculline effects. The proportions of cortical neurons that were GABA-immunoreactive were similar in areas 3b (29.8%) and 2 (22.8%). In posterior area 3b, layer IV contained significantly more GABA$_A$ receptors than in anterior area 3b. This difference might be related to the slowly- and rapidly-adapting zones of area 3b.

The distribution of cortical neurons characterized by their content of calcium
binding proteins and their relation to GABA neurons were examined. In SI, all of the parvalbumin neurons and 90% of the calbindin neurons contained GABA. Calbindin and calretinin neurons were located in the upper cortical layers, whereas parvalbumin neurons were distributed across all cortical layers except layer I. Layer II contained approximately equal numbers of neurons containing each of the calcium binding proteins.

The extent of horizontal connections varied across cortical layers. Injections of biotin or horseradish peroxidase into layers II, III and V resulted in more widespread labeling than injections into layer IV. The laminar differences in the extent of horizontal connections corresponded well to differences in the magnitude of RF enlargement produced by GABA<sub>A</sub> receptor blockade.
ACKNOWLEDGMENTS

I am grateful to my graduate advisor, Dr. Harris D. Schwark for his excellent guidance, encouragement, and friendship during this work. I wish to thank Dr. Jannon L. Fuchs for her encouragement, advice, and friendship. I would like to thank the other members of my committee Drs. Michael H. Droge, Jacek Kowalski, David C. Tam and Jeffery M. Clarke for the advice and suggestions that they have given me.

Chapters 2 and 3 have been previously published (Journal of Comparative Neurology, 1994, 343:353-361 and 362-369) and are used with permission of John Wiley and Sons.

To my husband, Hong Huang and daughter, Chenchen Huang, and my parents-in-law, Juan Huang, and Weicheng Ding, goes my sincere gratitude for the support, patience, love, and encouragement shown for the successful completion of my graduate education.
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CHAPTER 1

1. INTRODUCTION

Receptive fields of sensory cortical neurons have been used as indicators of neuronal function in perception. Receptive field characteristics such as size, response to stimulus orientation or direction, and inhibitory-excitatory interactions contribute to the performance of the sensory system and probably underlie perceptual characteristics of peripheral stimuli. In the somatosensory system, receptive field size appears to be actively regulated by local inhibitory processes (e.g., Dykes et al., 1984). Perhaps because changes in receptive field size might directly affect the acuity of the sensory system, inhibitory systems have received a great deal of attention in studies of the receptive field properties of sensory neurons. The classical receptive field has been considered to contain an excitatory center and an inhibitory surround (Mountcastle, 1957; Mountcastle and Powell, 1959), although it is now apparent that the inhibitory field spans the entire receptive field, and is coexistent with the excitatory field. More recently it has been shown that somatosensory receptive fields are dynamic, and are largely determined by a balance between excitation and inhibition (Dykes, 1978). Interactions between excitation and inhibition arise through intrinsic and extrinsic cortical connections. Many intrinsic connections arise from cortical interneurons (non-pyramidal neurons), nearly all of which contain gamma-aminobutyric acid (GABA) (Jones, 1984). In the cat and monkey, GABA-immunoreactive (GABA+) neurons comprise 20-25% of cortical neurons (Gabbott
The role of GABAergic inhibitory processes in controlling receptive field properties of neurons in visual and somatosensory cortex is revealed when GABA$_A$ receptor antagonists are applied by iontophoresis (Sillito 1975a; 1977; 1979; 1984; Tsumoto et al., 1979; Batuev et al., 1981; Dykes et al., 1984). In cat visual cortex neurons, bicuculline blockade of GABA$_A$ receptors causes the loss of selectivity for stimulus direction and orientation (Sillito et al., 1975a; 1975b; Tsumato et al., 1979), and antagonistic ON and OFF regions within the receptive field (Sillito, 1975a). In cat SI, bicuculline iontophoresis results in the enlargement of receptive fields of many neurons (Alloway et al., 1989; Dykes et al., 1984). During the GABA$_A$ blockade, the latency of activation from the enlarged portion of the receptive field is longer than that from the original receptive field (Alloway et al., 1989). This observation led the authors to suggest that the receptive field enlargement may be mediated by horizontal connections intrinsic to the cortex which are under inhibitory control.

The magnitude of the effects of bicuculline on receptive fields varies across SI. Zones which have slowly-adapting (SA) and rapidly-adapting (RA) multi-unit responses in the middle cortical layers have been identified in area 3b by Srevetan and Dykes (1983). Neurons which lie in RA zones exhibit greater receptive field expansions during bicuculline iontophoresis than neurons in SA zones. There are also laminar differences in bicuculline’s effects: neurons in the middle cortical layers show the smallest percentage increases in receptive field size (Dykes et al., 1984). Taken together, these observations suggest that there might be regional or laminar differences in the cortical GABAergic
The cortical GABAergic system can be described by the numbers, morphologies and connections of cortical GABAergic neurons, since no extrinsic inhibitory projections to SI have been described. Therefore the present experiments were designed to (1) determine if differences in the numbers and distribution of GABA+ neurons or GABA receptors might correspond to the regional or laminar differences which have been reported for the magnitude of receptive field enlargements produced by bicuculline; (2) compare the extent of intrinsic horizontal connections in different laminae of SI to the laminar differences in the effects of bicuculline; and (3) determine the distributions and morphologies of GABA+ neurons distinguished by calcium binding proteins (CBPs): parvalbumin, calbindin D-28K and calretinin.

1.1 Cortical GABAergic neurons

The role of intracortical, GABA-mediated inhibition in forming receptive fields in cerebral cortex has been studied pharmacologically and physiologically (e.g., Dykes et al., 1984; Kang et al., 1994; Hirsch et al., 1991). Physiological studies of the GABAergic system have been done in cat SI (Alloway et al., 1989; Dykes et al., 1984), but the anatomical circuitry underlying inhibition in this cortex has not been described.

1.1.1 Morphology of cortical GABAergic neurons

Cortical GABAergic neurons are nonpyramidal neurons and make up a variety of morphological classes that include neurogliaform (or spider web) neurons (Kisvárday et
at., 1986; 1990), chandelier neurons (Peters, 1984), double bouquet neurons (DeFelipe et al., 1989; 1990), and basket neurons (Jones, 1975a). Each of these classes has a specific laminar distribution, and forms specific synaptic connections with other cortical neurons. For example, double bouquet neurons are located in layers II and III and have long, thick ascending and descending axons with obvious boutons which probably end on the dendritic spines of pyramidal neurons. Chandelier neurons are small- to medium-sized aspiny neurons lying in layers II and III, with strings of axon terminals ending in symmetric synapses on the axon initial segments of pyramidal neurons. Basket neurons are large multipolar neurons located in all layers but especially in layers III-IV: they have long, aspiny dendrites and ascending axons with long, myelinated, horizontal branches ending in "baskets" around the somata of pyramidal neurons up to 2 mm from their own somata, suggesting that they could mediate inhibition over long distances. Neurogliaform neurons are small aspiny neurons, and are located mainly in layer IV. Small to medium-sized aspiny neurons are found in all cortical layers, especially II-IV (Jones, 1984).

1.1.2 Distribution of cortical GABAergic neurons

In the monkey (Hendry et al., 1987) and cat (Gabbott and Somogyi, 1986; Matsubara et al., 1987) visual cortex GABA + neurons are found in differing proportions throughout the full thickness of the cortex, and are heavily concentrated in layers II, III and IV. GABA+ neurons comprise 95-100% of the neurons in layer I, 15-40% of the neurons in layers II, III and IV, and approximately 10% of the neurons in the remaining layers. Specifically in cat area 17 20.6% of cortical neurons are GABA+ (Gabbott and
Somogyi, 1986). In the areas of monkey cortex which have been examined quantitatively, GABA+ neurons make up 23.4% of the neurons in area 3b and 1-2 and 19.4% of the neurons in areas 17 (Hendry et al., 1987).

1.1.3 Regional differences in inhibitory processes in SI cortex

In both the cat (Rasmusson, 1979; Sretavan and Dykes, 1983) and monkey (Sur, 1981; 1984) SI cortex, regions of area 3b have been classified according to multiunit responses recorded from the middle cortical layers. In the cat these RA and SA regions are organized as strips running in a mediolateral orientation across area 3b (Sretavan and Dykes, 1983). When GABA_A receptors are blocked by iontophoresis of bicuculline, receptive fields of neurons in RA zones increase on average 6.4 times the area of control receptive fields, whereas neurons in SA zones are unaffected by bicuculline (Dykes et al., 1984). Further studies revealed that 85% of RA neurons (not necessarily neurons in RA zones) show large receptive field expansions following bicuculline iontophoresis, while only 21% of SA neurons (not necessarily neurons in SA zones) show receptive field expansions, and these are typically much smaller (Alloway et al., 1989).

Responses of SI neurons to bicuculline also vary across cortical layers. In response to bicuculline iontophoresis, the receptive fields of neurons in the middle cortical layers (those which lie 600-1,100um below the cortical surface) increase, on the average, by two to four times their original size. The receptive fields of neurons which lie above or below this depth increase by six to eleven times (Dykes et al., 1984). These data suggest that there might be differences in the cortical GABAergic system which correspond to
differences in the effects of bicuculline applied to both the RA and SA zones and across cortical layers. The experiment described in Chapter 2 was carried out to determine the numbers and distribution patterns of GABA+ neurons in cat SI. The distribution patterns were analyzed to look for correspondence with the reported effects of bicuculline.

1.2 Cortical GABAₐ receptors

1.2.1 Structural and functional properties of GABA receptors

GABA produces its inhibitory actions by binding to at least two types of receptors: GABAₐ and GABAₐ receptors. Recently, the existence of GABAₐ receptors has been reported in horizontal neurons of the perch retina (Qian and Dowling, 1994). The GABAₐ receptor is a ligand-gated anion channel: it is a hetero-oligomer of 220-400 kDa, composed of two to four different polypeptides (α, β, γ and δ, each about 55 kDa), and a total of four to five subunits. The receptor has at least five different binding sites which bind a variety of drugs, including benzodiazepines and barbiturates, in addition to the binding site for GABA (Stephenson, 1988; Olsen and Tobin, 1990). It is likely that all of the subunits bind GABA and benzodiazepines, perhaps with differing affinities (Olsen and Tobin, 1990). At the GABAₐ receptor the actions of GABA are mimicked by muscimol and blocked by bicuculline, while GABAₐ receptors are insensitive to both bicuculline (Hill and Bowery, 1981) and muscimol (Sivilotti and Nistri, 1990). GABAₐ receptor activation elicits a G-protein mediated response (Bowery, 1983; Bowery et al., 1984; Borman, 1988), and these receptors are sensitive to baclofen. Multiple types of GABAₐ receptors can be defined according to their sensitivity to antagonists. These include 3-
APPA-sensitive/baclofen-sensitive receptors and 3-APPA-sensitive/baclofen-insensitive receptors (Bonanno and Raiteri, 1993). The GABA<sub>c</sub> receptor is insensitive to both bicuculline and baclofen (Polenzani et al., 1991) and has only a single type of subunit (Cutting et al., 1991). This receptor might be a subtype of the GABA<sub>b</sub> receptor (Bonanno and Raiteri, 1993).

1.2.2 Distribution of GABA receptors in the cortex

In most brain regions, GABA<sub>a</sub> binding accounts for 70-80% of total [³H]GABA binding, while GABA<sub>b</sub> binding represents 20-30% (Chu et al., 1990). Although few studies have localized the distribution of GABA<sub>b</sub> receptors, the existing data suggest that it differs from that of GABA<sub>a</sub> receptors. In neocortex, GABA<sub>b</sub> receptors are located primarily in layers I-III (Chu et al., 1990). GABA<sub>a</sub> receptors localized by receptor binding or immunocytochemistry tend to be differentially distributed across cortical layers, but there are differences in these distributions across species and between the methods. In cat visual cortex, immunoreactivity for GABA<sub>a</sub> receptors is rather evenly distributed in all layers except layer I, which has a much lower density (Gu et al., 1993). However, in cat visual cortex receptor binding techniques (using [³H] muscimol) reveal a pattern in which GABA<sub>a</sub> receptors are densest in layers I-IV, least dense in layer V, and intermediate in layer VI (Mower et al., 1986; Needler et al., 1984). In monkey visual cortex, immunoreactivity for GABA<sub>a</sub> receptors is dense in layers II-III, IV<sub>A</sub>, IV<sub>CB</sub>, and VI (Hendry et al., 1990). In monkey SI, muscimol binding is evenly and densely distributed in layers I-III and less dense in layers IV-VI (Lidow et al., 1989). The distribution of
GABA<sub>A</sub> receptor subunits and subtypes also varies with brain regions, and may result from
differences in pharmacological specificity, ligand binding affinity, and physicochemical
properties. These data suggest that differences in the effects of bicuculline on cortical
neurons might arise from differences in the numbers and/or affinities of the receptors
blocked by bicuculline, rather than from differences in the numbers of GABAergic
neurons. The experiment described in Chapter 3 was designed to examine the numbers
and distribution of GABA<sub>A</sub> receptors in SI.

1.3 Intrinsic horizontal connections in the cortex

1.3.1 Dynamic properties of receptive fields

The receptive fields of cortical neurons are largely determined by thalamic inputs
to the cortex (Malpeli et al., 1986; Chapman et al., 1991). However, receptive fields in SI
are smaller than would be predicted from the size of thalamocortical terminal arborization
(Landry and Deschenes, 1981; Landry et al., 1982; Snow et al., 1988). This mismatch
apparently arises from the effects of inhibitory mechanisms intrinsic to the cortex, which
modify receptive field size. Iontophoresis of bicuculline results in expansion of the
receptive fields of SI cortical neurons (Dykes et al., 1984; Alloway et al, 1989).

1.3.2 The role of horizontal connections in forming receptive fields

Studies of cat visual cortex (Gilbert and Wiesel, 1989), monkey SI cortex
(DeFelice et al., 1986) and cat SI cortex (Schwark et al., 1989; 1992) have shown
extensive horizontal connections among cortical neurons, and that these connections can
extend beyond the cortical territories of their receptive fields (Gilbert and Wiesel, 1985).

In cat visual cortex, horizontal connections of pyramidal neurons extend up to 8 mm (Gilbert et al., 1979). Horizontal connections might allow a single cortical neuron to integrate information from an area which is larger than that covered by its receptive field (Gilbert and Wiesel, 1989).

Cortical horizontal connections synapse upon excitatory as well as inhibitory neurons. In visual and auditory cortex the targets of horizontal connections are mostly excitatory neurons; only 5-20% are inhibitory (Kisvárday and Eysel, 1992; McGuire et al., 1991; Elhanay and White 1990). Horizontal connections can modulate the responsiveness of their target neurons. The dynamic properties of cortical neuron responses are at least partly due to modulation through weak synaptic connections (Smits et al., 1991; Zarzecki et al., 1993) formed through long range horizontal connections. Conditioning stimuli presented outside the excitatory receptive fields of SI neurons inhibit the neurons' responsiveness to test stimuli presented inside the receptive field (Laskin and Spencer, 1979). Following bicuculline iontophoresis, the latency of activation is longer from the enlarged portion of the receptive field than from the original receptive field (Alloway et al., 1989). These results suggest that receptive fields can be modulated by horizontal connections, perhaps acting through inhibitory neurons. Further evidence for a role of horizontal connections in forming receptive fields comes from the observation that neurons in layers II, III, V and VI have larger receptive fields than neurons in layer IV (Chapin, 1986; Gilbert, 1977; Sur, 1985). These layers also have more widespread horizontal connections (Rockland and Lund, 1983; Levay, 1988; Albus et al., 1991;
Wallace et al., 1991; Clarke et al., 1993; Lund et al., 1993). Thus, the range of horizontal connections and their interactions with inhibitory neurons might be organized differently across cortical layers, and these differences might correspond to differences in the response sensitivity to bicuculline. The experiment described in Chapter 4 was designed to determine the range of horizontal connections in different layers of SI.

1.4 Classes of GABAergic neurons based on calcium binding proteins

1.4.1 Classes of GABAergic neurons revealed by calcium binding proteins (CPBs)

The population of cortical GABAergic neurons are morphologically (Houser and Hendry et al., 1983) and chemically (Hendry et al., 1984; Jones et al., 1986) heterogeneous. Recent studies have shown that in many of these neurons GABA is co-localized with one of three types of CBPs which have been identified as: parvalbumin, calbindin-D28K or calretinin (Hendry et al., 1989; Hendry and Jones, 1991; Demeulemeester et al., 1991; Miettinen et al., 1992). In cat visual cortex, about 37% of the GABAergic neurons contain parvalbumin, and a smaller fraction (about 18%) contain calbindin-D28K. In monkey neocortex, nearly all GABAergic neurons are also immunoreactive for either calbindin or parvalbumin, except for a small number of GABAergic neurons in layer IV (Hendry et al., 1989). In cat auditory cortex, one of these CPBs is present in 70-75% of the GABAergic neurons (Hendry et al., 1989). In area 17 of humans 7% of the neurons contain parvalbumin, compared to 11.5% in the macaque monkey (Blümcke et al., 1990). Most parvalbumin-immunoreactive (parvalbumin+) neurons also contain GABA, and approximately 80% of calbindin-immunoreactive
(calbindin+) neurons contain GABA. Parvalbumin and calbindin are localized in separate neuronal populations in cat visual cortex (Demeulemeester et al., 1991), rat cerebral cortex (Celio, 1986), and monkey neocortex (Van Brederode, 1990). A third CBP, calretinin, has been identified in rat hippocampus, where 83% of the calretinin+ neurons also contain GABA. Calretinin and parvalbumin are localized in separate groups of neurons, but 5.1% of the calretinin-immunoreactive (calretinin+) neurons and 6.2% of the calbindin+ neurons are immunoreactive for both of these CBPs (Miettine et al., 1992). In monkey prefrontal cortex, calbindin and calretinin are co-localized in the Cajal-Retzius neurons of layer I (Conde et al., 1994).

1.4.2 Distribution of neurons containing calcium binding proteins

In area 3b, as well as in other areas of monkey neocortex, calbindin+ neurons are concentrated in the superficial and deep layers (mostly in layer II, the upper part of layer III, and layer V), whereas parvalbumin+ neurons are concentrated in the middle layers (mostly in deep layer III, layer IV, and upper V) (Hendry et al., 1989; 1991; Van Brederode et al., 1991). Parvalbumin is prominent in the layers which receive thalamic afferents (Blumcke et al., 1990), and the axon terminal staining pattern revealed by parvalbumin antibodies is reminiscent of the distribution of cytochrome C-oxidase reactivity (Carroll and Wong-Riley, 1984) and GAD immunoreactivity (Hendrickson et al., 1981; Fitzpatrick et al., 1987). Most (81.7%) parvalbumin+ terminals form symmetric synapses. The remaining synapses form asymmetric contacts and are located mostly in thalamic recipient layers IVc and IVa (where they comprise 9.9% of all synapses).
Calretinin+ neurons in monkey prefrontal cortex are mainly located in deep layer I and layer II (Conde et al., 1994).

1.4.3 Physiological roles of calcium binding proteins

The physiological roles of CBPs in neuronal function are unknown, but they may be related to neuronal firing patterns. Celio (1990) has suggested that parvalbumin and calbindin-D28K are associated with separate functional systems in the rat brain. The effects of the CBPs may be to influence neuronal excitability (Baimbridge and Miller, 1984) and patterns of neuronal discharge (Kawaguchi et al., 1987). Recently, it was found that parvalbumin+ neurons are fast spiking (Kawaguchi and Kubota, 1993), and metabolically active (McCasland et al., 1993), while calbindin+ neurons are low threshold spiking neurons (Kawagushi and Kubota, 1993). In the ventroposterolateral nucleus of the monkey thalamus, parvalbumin and calbindin are found in separate groups of projection neurons: parvalbumin+ neurons are concentrated in cytochrome-oxidase rich patches, whereas calbindin+ neurons are located in cytochrome-oxidase poor spaces (Rausell et al., 1991). The use of parvalbumin+ and calbindin+ as markers of neuronal plasticity has also been investigated. Transection of the sciatic nerve leads to a 25% reduction in parvalbumin+ neurons in layers II/III, and calbindin+ neurons in layers IV/V (Webster et al., 1993). The different CBPs appear to label classes of neurons with distinct morphologies. Chandelier neurons and basket neurons are immunoreactive for parvalbumin (DeFelipe et al., 1989b), while double bouquet neurons are immunoreactive for both calbindin (DeFelipe et al., 1989a) and calretinin (Conde et al., 1994).
CBP immunostaining to label classes of GABAergic neurons which are distinct in their morphologies, physiological properties and laminar distributions might yield clues to the physiological effects of the GABAergic neurons are related to these subtypes. The experiment described in Chapter 5 was designed to determine the distribution of SI neurons which contain these three CBPs.

The overall goal of this thesis is to understand the inhibitory system of cat SI, and to relate its organization to neuronal receptive fields. Understanding the number, morphology and connections of inhibitory cortical neurons might reveal some of the mechanisms involved in forming receptive fields. Therefore, the specific objectives of the experiments are:

Chapter 2. To determine if the number and distribution of GABA+ neurons in SI correspond to differences in the effects of bicuculline on receptive field size. These differences are seen between RA and SA zones and across cortical layers.

Chapter 3. To describe the distribution of GABA_A receptors in SI, and to relate differences in the receptor distribution to the effects of bicuculline on receptive field size.

Chapter 4. To determine the organization of horizontal cortical connections within SI, and to relate of the extent of these connections to the magnitude of receptive field expansion across cortical layers. Layers in which neurons undergo large receptive field expansions may have more widespread horizontal cortical connections.

Chapter 5. To determine the distribution of classes of cortical GABAergic neurons distinguished by their content of CBPs (parvalbumin, calbindin D-28K or calretinin). GABAergic neurons constitute a heterogenous population with respect to
their anatomical, chemical and physiological properties, suggesting that different classes of GABAergic neurons might have different physiological functions.
CHAPTER 2

2. DISTRIBUTION AND PROPORTIONS OF GABA-IMMUNOREACTIVE NEURONS IN CAT PRIMARY SOMATOSENSORY CORTEX.

2.1 Summary

Certain receptive field properties of cortical neurons depend upon inhibitory, GABAergic inputs. In the somatosensory cortex, iontophoresis of bicuculline, a GABA$_A$ receptor blocker, results in enlargement of receptive fields. However, bicuculline's effectiveness in changing receptive field size varies with the neuron's adaptation characteristics, location within a particular submodality region, and laminar location. To test whether regional differences in the effectiveness of bicuculline are correlated with the distribution of cortical GABAergic neurons, we determined the numbers and proportions of GABA-immunoreactive (GABA$^+$) neurons within cat primary somatosensory cortex. The laminar distribution of GABA$^+$ neurons was similar across all four cytoarchitectonic areas of primary somatosensory cortex, with layer II containing the highest areal density of GABA$^+$ neurons. Numerical proportions of GABA$^+$ neurons in the total neuron population were similar in areas 3b and 2 (29.8% and 22.6%, respectively). Laminar distributions of the proportions of GABA$^+$ neurons were also similar in these two areas; in both areas layer I contained the highest proportion of GABA$^+$ neurons. The laminar distributions of GABA$^+$ neuron densities as well as GABA$^+$ neuron proportions differed from the reported laminar distribution of bicuculline effects on receptive field size.
Moreover, within area 3b, these measures showed no evident patterns that might correspond to rapidly adapting and slowly adapting submodality regions.

2.2 Introduction

The predominant inhibitory neurotransmitter in the cerebral cortex is gamma-aminobutyric acid (GABA). Intracellular recordings reveal inhibitory postsynaptic potentials in most cortical neurons (reviewed by Steriade, '84), and functional blockade of GABA receptors alters the receptive field properties of many cortical neurons (Sillito, '84; Hicks et al., '85). In the cat primary somatosensory cortex (SI), GABA blockade by iontophoretic application of bicuculline increases neuronal responsiveness and results in enlargement of peripheral receptive fields (Dykes et al., '84; Alloway et al., '89). In the cat visual cortex, GABA blockade reduces the selectivity of neurons for stimulus orientation, length and movement direction (Sillito, '75; Sillito et al., '85; Tsumoto et al., '79).

The functional effects of GABA blockade in cat SI cortex appear to vary with the location and type of neuron. Receptive fields of rapidly adapting (RA) neurons show greater expansion following bicuculline iontophoresis than those of slowly adapting (SA) neurons (Alloway et al., '89). Sretavan and Dykes ('83) have described separate RA and SA regions within area 3b, based on multiunit recordings in the middle cortical layers. These RA and SA regions are organized as strips running in a mediolateral orientation across area 3b. Neurons in RA regions are more likely than neurons in SA regions to show receptive field enlargement following GABA blockade (Dykes et al., '84). These results suggest that the GABAergic inhibitory system might differ between these two
regions. Possible laminar differences in the GABAergic system have also been suggested. Compared with neurons in the middle cortical layers (600 - 1100 μm deep), neurons above and below this depth show larger increases in receptive field size in response to GABA blockade (Dykes et al., '84).

We have examined whether the distribution patterns of GABA-immunoreactive (GABA+) neurons in cat SI correspond to submodality regions in area 3b. We plotted the distribution of GABA+ neurons in series of closely spaced sections, and were unable to find patterns in GABA+ neuron density which could be related to submodality region. We also determined the laminar distribution of the proportions of GABA+ neurons in area 3b and in a second forepaw representation in area 2. In both areas, layer I contained the highest proportion of GABA+ neurons, while layer II contained the highest density of GABA+ neurons. Some of these results have appeared in abstract form (Li and Schwark, '91). In a companion paper (Schwark et al., '93), we described the distribution of [3H]muscimol binding in cat SI and motor cortex in relation to the reported distribution of bicuculline effects.

2.3 Methods

2.3.1 Tissue preparation

The material used in this study was derived from three normal adult cats. Each animal was injected with ketamine (30 mg/kg, i.m.), followed by an overdose of pentobarbital (60 mg/kg, i.p.), then perfused through the heart with saline followed by a fixative solution of 2% paraformaldehyde and 0.15% glutaraldehyde in 0.1 M phosphate
buffer. The brain was removed and postfixed for 4-6 hr at 4°C. The brain was then blocked and the blocks were placed in 4°C phosphate buffer containing 30% sucrose until they sank, after which they were rapidly frozen in -35°C isopentane.

Blocks containing SI were sectioned on a sliding microtome in the sagittal plane. Sections were taken through the forearm representation of SI, just medial to the end of the coronal sulcus. Two sets of alternate 16-μm sections were collected in cold phosphate buffered-saline (PBS), one for GABA immunocytochemical staining and one for thionin staining. These sections were used to determine the density distribution of GABA+ neurons throughout SI. Additional sections (300 μm and 50 μm) were cut for postembedding immunocytochemistry to determine the proportions of GABA+ neurons in areas 3b and 2.

To estimate the shrinkage due to immunocytochemical processing of the 16-μm sections, the outlines of 17, wet-mounted sections (sampled from each of the animals) were drawn immediately after sectioning. Twelve of these sections were dehydrated, stained with thionin, and coverslipped, and the remaining 5 sections were processed for GABA immunocytochemistry as described below. The thionin-stained sections showed negligible shrinkage. The sections processed for GABA immunocytochemistry decreased in area by a mean of 18%. To determine if shrinkage of the cortical gray matter was equivalent to shrinkage of the entire section, linear shrinkage in the gray matter was measured in six additional sections by comparing the distances between blood vessels in GABA-stained and adjacent thionin-stained sections. The average areal shrinkage using these measurements was also 18%.
2.3.2 Immunocytochemical staining

2.3.2.1 Free-floating sections

The 16-μm sections were pre-incubated for 2 hr at 4°C in PBS containing 8% normal goat serum (NGS) and 0.3% Triton X-100. The sections were then transferred to the same solution containing rabbit anti-GABA antiserum (Sigma) diluted 1:15,000, and incubated at 4°C for 16-24 hr. After extensive washing in PBS (1 hr at 4°C, then brought to room temperature), the sections were incubated in a 1:100 solution of biotinylated goat anti-rabbit immunoglobulins (Vector Laboratories) for 1.5 hr. The sections were washed for 30 min in several changes of PBS and incubated in avidin-biotin peroxidase complex (Vector Laboratories) for 1.5 hr. After another series of washes, the sections were reacted in PBS containing 0.05% DAB with 0.026% H$_2$O$_2$ and 0.02% nickel ammonium sulfate. The sections were then washed several times in PBS, mounted on gelatin-subbed slides, dried, dehydrated in ethanol, and coverslipped.

2.3.2.2 Post-embedding staining

The 50-μm sections were stained with thionin to aid in localization of cortical laminae and cytoarchitectonic areas. Adjacent 300-μm sections were dehydrated in a progressive ethanol series and then infiltrated for 2 hr in methacrylate resin (Immunobed, Polysciences). The sections were then placed in a mixture of resin and catalyst and flattened between silicone-coated slides and coverslips. After the embedded sections were cured overnight at room temperature, they were removed from the slides, and areas 3b and 2 were dissected out on the basis of cytoarchitectonic criteria (see below), with the aid
of the adjacent thionin-stained sections as guides. The trimmed sections were mounted on a resin block with cyanoacrylate glue and 1-μm sections were cut with an ultramicrotome. The sections were mounted onto slides, dried, then softened in 100% ethanol and rehydrated. For immunocytochemistry, sections were preincubated for 30 min at room temperature in PBS containing 3% NGS and 0.3% Triton X-100, then incubated for 2 hr in the same solution containing rabbit anti-GABA antibody (Sigma) diluted 1:2000. Following a PBS rinse they were transferred to a solution containing 1:50 biotinylated goat anti-rabbit IgG (Vector Laboratories), 3% NGS and 0.3% Triton X-100 for 1 hr. Sections were washed repeatedly in PBS, then incubated for 1 hr in 1:200 avidin biotin-peroxidase complex (Vector Laboratories). After washing in PBS, the sections were incubated in PBS containing 0.05% DAB, 0.026% H₂O₂ and 0.02% nickel ammonium sulfate, washed again in PBS, and placed in 0.1% osmium tetroxide for 30 min. The sections were then dehydrated in ethanol and coverslipped.

2.3.2.3 Immunocytochemical controls

Method and antibody specificity were tested for both procedures by: 1) running a dilution series of the primary antibody; 2) eliminating the primary antibody and incubating the tissue in the antibody diluent alone; 3) using a high concentration of normal goat serum; and 4) preadsorbing the antibody with glutaraldehyde-fixed GABA before incubation. The results of each of these procedures indicated that the primary antibody as used in our procedures was specific for GABA.
2.3.3 Data collection

2.3.3.1 Distribution of GABA+ neurons

The locations of all GABA+ neurons, together with the laminar and cytoarchitectonic boundaries in SI (from adjacent thionin sections), were plotted from five series of six alternate 16-μm sections with the aid of a camera lucida at 125X magnification. Each series of sections was taken from a different hemisphere. Criteria for identifying the boundaries of the cytoarchitectonic areas were based on the description of Hassler and Mühs-Clement ('64) as described previously (Schwark et al., '92). The following were the primary criteria used to distinguish the areas in the SI forepaw representation: Area 3b had a thick, dense layer IV, and layer V was mostly clear, with a few large pyramidal neurons. In area 1, layer IV was thinner and higher than in area 3b, and the large pyramidal neurons in layer V were found in nests. The superficial layers of area 2 were thicker than those in area 1, so that at the boundary with area 1 there was a step-like shift in the boundary between layers III and IV. Layer IV was invaded by larger cells from layers III and V. Layer III of area 5a had no sublayers, and layer IV was thin but denser than in area 2. According to these criteria, area 3b was slightly narrower and area 1 was slightly wider in the anterior-posterior dimension than in the figures of Hassler and Mühs-Clement ('64). However, our boundaries were in good agreement with the locations of labeled corticocortical cells described previously (Schwark et al., '92).

The location of each GABA+ neuron was entered into a computer with a digitizing tablet. To determine the density of GABA+ neurons in SI, the area of each layer in each cytoarchitectonic region of SI was measured (minus the area of major blood vessels) and
the number of GABA+ neurons per square millimeter was calculated. These values were corrected for the 18% shrinkage resulting from the immunocytochemical procedures. To derive overall distribution patterns, the number of GABA+ neurons lying in each bin of a two-dimensional grid overlying SI was calculated and plotted as a two-dimensional contour plot. From plots of various bin sizes (25, 50, 75, 100, and 125 μm on a side), an optimal resolution was found to be 100 x 100 μm.

To test for periodicities in the distribution of GABA+ neurons in area 3b, Fourier analyses of binned data were performed using procedures similar to those of Schwartz et al. ('88). Because of the curvature of the cortex through area 3b, these analyses were performed on only three hemispheres in which the degree of curvature was small. In addition, the analyses were restricted to layers III and IV in order to minimize distortions due to curvature. The number of GABA+ neurons in 50 or 100 μm-wide bins running vertically through layers III and IV were counted, and the distributions were compared across adjacent sections. These comparisons were used to look for any consistent patterns in the distribution of GABA+ neurons in these layers. To reveal any periodicities in the distributions, a Fast Fourier Transform (Wilkinson, 1990) was performed on the binned data. Periodograms, or plots of average squared amplitude of sinusoidal components as a function of frequency, were constructed for each section. Periodograms from adjacent sections were then compared to look for peaks which were consistent across sections.

2.3.3.2 Proportion of GABA+ neurons

The proportions of GABA+ neurons in areas 3b and 2 were determined by the
method described by Montero and Zempel ('86). Profiles of GABA+ somata were drawn from 1-μm plastic sections at 1250X magnification. The coverslips were then removed and the sections were counterstained with toluidine blue, after which the profiles of GABA-negative (GABA-) somata were plotted on the same drawings. Eighteen columns, each 100 μm wide and spanning the thickness of the cortex, were sampled from each area in four hemispheres taken from three different animals. For analysis, each column was subdivided into 100 μm x 50 μm sample regions. The outline of each neuronal profile was entered into a computer using a digitizing tablet (resolution 0.06 μm for the present drawings), and the area (A) and perimeter (p) were calculated. Reliability of data collection was assessed by selecting 10 neurons and digitizing their outlines 10 times. The average coefficient of variation of these data was 2.9 (range 1.7 - 6.3).

The mean diameter (d) of each profile was calculated from the profile area (A) according to the formula $d = \sqrt{4A/\pi}$. The mean tangent diameter of the somata (D) was calculated from the formula $D = 4/\pi \times d$. Because GABA+ and GABA- cortical neurons differ in size (Gabbott and Somogyi, '86), the formula of DeHoff and Rhines (1961) was used to calculate the number of somata per unit volume (volume fraction, $N_v$) from the number per unit area ($N_a$) according to the formula $N_v = N_a/D$. Finally, the proportion of GABA+ neurons was calculated by dividing $N_v$ of GABA+ neurons by $N_v$ of all neurons within the sample area. The coefficient of circularity of each profile was calculated according to the formula $\text{circ} = 4\pi A/p^2$, where A is the area and p is the perimeter of the profile.

In six additional columns, the method described above was compared with the
physical dissector method (Sterio, '84). To calculate the proportion of GABA+ neurons using the physical dissector method, adjacent sections were stained for GABA and counterstained with thionin. GABA+ nuclei which were present in one section (the reference section) but not in the adjacent (look-up) section were counted and entered into the following formula: \[ N_v = \frac{(\Sigma Q_\text{eq})}{(a \times h)} \], where \( Q_\text{eq} \) is the number of nuclei in the reference section but not in the look-up section. The volume sampled was calculated from the area sampled \( (a) \) multiplied by the distance between the sections \( (h) \). The same procedure was then used to determine \( N_v \) of GABA- neurons, and the proportion of GABA+ neurons calculated. Because the accuracy of the dissector method depends upon accurate measure of section thickness (to derive \( h \)), the ultramicrotome was calibrated by measuring a blank block with a microscope graticule, cutting a series of 100 sections, and remeasuring the block. The section thickness was then adjusted and the procedure repeated, until 1-\( \mu \)m sections were produced reliably.

2.4 Results

2.4.1 Distribution of GABA-immunoreactive neurons in SI cortex

GABA+ neurons in 16-\( \mu \)m sections were easily distinguished from background (Fig. 1), although the density of staining varied somewhat among cells. The proximal dendritic processes of GABA+ neurons were frequently stained, and GABA+ axonal terminal branches could often be identified. GABA+ neurons with large cell bodies were located in lower layer III and occasionally in layers V and VI. There was little apparent clustering of GABA+ neurons, although there were many regions of 100-150 \( \mu \)m diameter
which were devoid of GABA+ cells. Such regions were apparent in every cortical layer.

A total of 57,889 cells were plotted from 30 parasagittal sections which included areas 3a, 3b, 1 and 2 of SI cortex. The staining quality was quite uniform among the series (coefficients of variation of areal densities ranged from 3 to 11.7). The pattern of GABA+ neuron densities across cortical layers was similar in the four cytoarchitectonic areas (Fig. 2). The highest areal density of GABA+ neurons was in layer II, followed by layer III. The lowest density was in layer VI, followed by layer V (and in some areas, layer I). Because these data were obtained from cell counts in 16-μm sections, regional variation in the sizes of GABA+ neurons could affect estimates of areal densities. However, an analysis of variance performed on GABA+ neuron diameters in 1-μm sections through areas 3b and 2 revealed no significant differences in cell size between these areas or among layers. This result is also reflected by the similarity between the laminar distribution of the volume fraction (Nv) of GABA+ neurons derived from 1-μm plastic sections (Fig. 3) and the distribution of areal densities (Fig. 2).

Analysis of variance of the areal densities revealed significant main effects of cytoarchitectonic area (F_{3,48}=5.18, p<0.005), layer (F_{5,48}=47.74, p<0.001), and animal (F_{2,48}=11.91, p<0.001). There were no significant interactions among these factors. Post-hoc Tukey tests revealed a significantly lower density of GABA+ neurons in one of the three animals. The series of sections from this animal were uniformly stained, and density distributions across laminae and areas were similar to those of the other animals, suggesting that this was a real difference rather than the result of incomplete staining. Post-embedding staining of thin sections from this animal similarly revealed low overall
numbers of neurons, but the proportions of GABA+ neurons were similar to the other animals (see below). Post-hoc Tukey tests also revealed a significantly lower areal density of GABA+ neurons in area 2 compared to areas 1 or 3b (p<0.05). No other differences between areas were significant. Differences in the density of GABA+ neurons between adjacent layers of the cortex were all significant except for layers IV and V (Tukey test, p<0.05).

The correspondence between the density of GABA+ neurons and the pattern of submodality regions of area 3b (as described by Sretavan and Dykes, '83) was examined in series of sections from five hemispheres of three animals. We did not attempt to correlate GABA+ neuron distribution with physiological recordings because electrode penetrations compromise the quality of immunocytochemical staining. According to the maps of Sretavan and Dykes (1983), the submodality regions of area 3b lie in an approximately mediolateral orientation and range in width from 200 μm to more than 1 mm. If there are consistent differences in the density of GABA neurons between submodality regions, they should be apparent as repeated patterns within series of closely-spaced sections. Although the presence of such patterns would not permit direct correlation with the physiologically defined regions, their absence would suggest a lack of relationship between GABA neuron density and the submodality regions.

The distribution of GABA+ neurons in a single section is shown in Figure 4. This section is shown again in Figure 5, at the top of the middle column, to permit comparison between the distribution of the neurons and the contour plot. Series of alternate sections through the right hemispheres of three cats are shown in Figure 5. Each contour plot was
derived from sections separated by 16 μm, so that the entire series spans a total of 176 μm. These plots illustrate the higher density of GABA+ neurons in superficial layers (refer to Fig. 2). The plots also show that the pattern of GABA+ neuron density varies from section to section, in an apparently nonsystematic manner. Comparisons of sections within each series revealed no evident pattern in the density of GABA+ neurons which might be related to submodality regions in area 3b.

The distributions of GABA+ neurons in area 3b were also assessed by constructing histograms of the number of GABA+ neurons which fell into 50 or 100 μm vertical bins through layers III and IV (Fig. 6). No obvious patterns in GABA+ neuron densities were evident in comparisons of the distributions across series of sections. Fast Fourier Transforms were performed to look for periodicities in GABA+ neuron distributions. Although small peaks were apparent in some of the resulting periodograms, their positions (corresponding to the frequency of the underlying sinusoid) varied from section to section. The most consistent peak, seen in 9 of 18 sections, appeared at a period of approximately 200 μm. However, the peak was not very prominent, and because there were low numbers of GABA+ neurons (2-12) per bin, it is not certain whether this is significant.

2.4.2 Proportions of GABA-immunoreactive neurons in areas 3b and 2

GABA+ and GABA- neurons in a 1-μm plastic section which has been counterstained with toluidine blue are shown in Figure 7. The proportions of GABA+ neurons in areas 3b and 2 were determined for 18 columns (for each area) from each of four hemispheres (Table 1 describes the data set). For each area, the proportion of
GABA+ neurons was calculated in two different ways. In the first method, an overall proportion was calculated from the entire sample area. In the second method, proportions were first calculated for each cortical layer, and an overall proportion was derived from the average of these proportions. These two methods yielded different results due to differences in the thickness of cortical laminae between the two areas. Calculated for the entire sample area, the overall proportion of GABA+ neurons in area 3b (29.8% ± 1.5 (SEM)) was significantly higher than in area 2 (22.6% ± 2.6) (paired t-test, tₚ=2.44, p<0.05). The variability in these measures was greater for area 2 than for area 3b (coefficient of variation: area 2 = 21, area 3b = 11.5). This variability may have arisen from sample heterogeneities introduced by sulcal variations between hemispheres or by differences in the relative thickness of cortical layers.

Table 1. Parameters of GABA+ and GABA- neurons in areas 3b and 2.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Sample area (mm²)</th>
<th>d (µm)</th>
<th>D (µm)</th>
<th>%xV¹</th>
<th>Circ.² Coeff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 3b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GABA+</td>
<td>1106</td>
<td>11.75</td>
<td>12.4</td>
<td>15.8</td>
<td>29.1</td>
<td>0.77</td>
</tr>
<tr>
<td>GABA-</td>
<td>3302</td>
<td>11.75</td>
<td>15.2</td>
<td>19.4</td>
<td>70.9</td>
<td>0.77</td>
</tr>
<tr>
<td>Area 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GABA+</td>
<td>826</td>
<td>13.36</td>
<td>12.9</td>
<td>16.4</td>
<td>23.7</td>
<td>0.83</td>
</tr>
<tr>
<td>GABA-</td>
<td>3274</td>
<td>13.36</td>
<td>15.9</td>
<td>20.2</td>
<td>76.3</td>
<td>0.84</td>
</tr>
</tbody>
</table>

¹ Volume fraction was calculated from the total sample area. See text for details.
² Coefficient of circularity.

The proportions of GABA+ neurons in each cortical layer in areas 3b and 2 are
shown in Figure 8. Analysis of variance revealed a significant main effect of layer
($F_{5,33}=120.95, p<0.001$), but not area. The lack of a significant effect of area in this
analysis, compared to the results of the t-test described above, appears to be due to
differences in the relative thicknesses of cortical layers in these areas. In both areas the
majority of the neurons in layer I (88-91%) were GABA+. The proportions of GABA+
neurons in layers II and III were lower in area 2 than in area 3b. However, there was no
significant interaction between area and layer, indicating that in the present sample the
laminar patterns in these two areas were not significantly different.

In six columns through area 3b, the present quantification procedures were
compared with the physical dissector method. The present quantification methods and the
dissector method yielded similar results ($15.0\% \pm 1.9$ (SEM) and $16.3\% \pm 1.5$,
respectively) (paired t-test, $t_5=0.82, p<0.45$). These proportions were somewhat lower
than the values presented above because layer I was excluded from the analyses.

2.5 Discussion

The results of the present study revealed no correlation between the density of
GABA-immunoreactive neurons and the submodality regions in area 3b of cat SI cortex.
Areas 3b and 2 contained similar proportions of GABA+ neurons, and in these areas the
proportions of GABA+ neurons were uniformly distributed across all cortical layers
except layer I.

2.5.1 GABA+ neuron density in relation to bicuculline effects in SI
Rapidly adapting and slowly adapting responses to peripheral stimulation characterize single neurons at all levels of the somatosensory system. Mapping studies suggest that neurons of these response classes are spatially segregated within each level: dorsal column nuclei, thalamus, and cortex (Dykes, '83). In cat and monkey SI cortex, descriptions of this segregation have been based on multiple unit recordings from the middle layers (probably layer IV) (Sretavan and Dykes, '83; Sur et al., '84). However, even in the SA regions most of the neurons have RA responses (Dykes and Gabor, '81; Dykes et al. '84). Thus, two different classification schemes have been used to describe RA and SA properties in SI cortex: one is based on responses of individual cells, and the other is based on regions mapped by multiple unit recordings from thalamic-recipient layers.

Blockade of GABA<sub>A</sub> receptors by iontophoretic application of bicuculline results in receptive field enlargement and enhanced responsiveness in some SI neurons, and these effects appear to be related to adaptation rates, although the exact nature of this relation is not clear. Dykes et al. ('84) reported a relationship between the effectiveness of bicuculline and the neuron's location within cortical submodality regions: bicuculline iontophoresis results in receptive field enlargement in a higher proportion of neurons in RA regions than in SA regions, regardless of the adaptation characteristics of the individual neurons. Alloway et al. ('89) reported a relationship between the effectiveness of bicuculline and the adaptation characteristics of individual neurons: bicuculline produces receptive field enlargement in both RA and SA neurons, but the degree of enlargement is much greater in RA neurons. Similar results have been seen in monkey SI
cortex, where bicuculline produces receptive field enlargements in both RA neurons and the dynamic response components of SA neurons, but has little effect on the static response components of SA neurons (Alloway and Burton, '91). In these studies, neurons were not assigned to specific submodality regions. It may be the case, therefore, that GABA plays different roles in different cortical regions as well as in different cell types.

We hypothesized that differences in the effects of GABA receptor blockade on receptive field size could be due to differences in the cortical inhibitory circuits in RA and SA regions, perhaps reflected in the density of GABAergic neurons. Indeed, periodicities in GABA+ neuron densities have been described in monkey cortex in area 18 (Hendry et al., '87) and prefrontal cortex (Schwartz et al., '88), although the functional correlates of these patterns are not known. The results of the present study suggest, however, that differences in the density of GABA+ neurons are not correlated with submodality regions in area 3b, and therefore probably do not underlie the differential effects of bicuculline iontophoresis on neurons in these regions. A potential limitation in the present study is that, to preserve staining quality, we did not physiologically define the submodality regions prior to taking sections. Nevertheless, in two preliminary mapping experiments on different animals we confirmed the presence of these regions, and our immunocytochemical samples were taken from the same region of area 3b that was mapped by Sretavan and Dykes ('83). Moreover, by sampling from five hemispheres, it is likely that we sampled from both RA and SA regions in at least some of the series of sections. None of the series contained a consistent pattern of GABA+ neuron density across sections.
The basis for differential bicuculline effects in RA and SA regions is still unknown. Alloway and Burton ('91) have suggested that differences in bicuculline effects might result from differences in the extent of divergence of thalamocortical afferent axon terminals. SA afferents, for example, might form smaller or narrower terminal arborizations, such that inhibitory circuits play a lesser role in determining receptive field size in this system. There is little evidence, however, for morphological differences between RA and SA thalamic afferents to SI. In the cat, the terminal fields of RA and SA afferents are similar in size (Landry and Deschenes, '81). Only a single SA afferent to area 3b has been described in the monkey, and it formed a small terminal arborization (Garraghty and Sur, '90). Another factor which might account for the differences in the effects of bicuculline is the distribution of GABA$\alpha$ receptors. It may be that neurons in SA regions contain fewer GABA$\alpha$ receptors than neurons in RA regions. To examine this possibility we have examined in a separate study the distribution of $[^3]$Hmuscimol binding in SI cortex (Schwark et al., '93).

2.5.2 Proportions of GABA$+$ neurons in areas 3b and 2

Somatosensory cortex appears to contain a higher proportion of GABA$+$ neurons than visual cortex. We found that 26.4% of the neurons in cat SI cortex (areas 3b and 2 combined) were GABA$+$, compared to 20.6% reported in cat area 17 (Gabbott and Somogyi, '86). Because differences in the relative thickness of laminae between cortical areas can contribute to differences in overall proportions, comparisons between different cortical areas must be made with caution. Nevertheless, plots of the proportions of
GABA+ neurons as a function of cortical depth also suggest that SI cortex contains a higher proportion of GABA neurons than visual cortex. This difference parallels that seen in the monkey, where GABA+ neurons make up 23.4% of the neurons in areas 3b and 1-2 and 19.4% of the neurons in area 17 (Hendry et al., '87). It may be possible to determine the origin of these differences in the cat by using the data on total neuron numbers in these areas reported by Beaulieu and Colonnier ('89). Area 17 contains more neurons under 1 mm² of surface area than area 3b (78,400 and 58,900, respectively) (Beaulieu and Colonnier, '89). Multiplying these values by 20.6% (for area 17, Gabbott and Somogyi, '86) and 29.1% (for area 3b, present study) yields 16,150 GABA+ neurons in area 17 and 17,140 GABA+ neurons in area 3b. The difference in proportions of GABA+ neurons therefore appears to arise primarily from differences in the numbers of GABA- neurons.

The pattern of the proportion of GABA+ neurons as a function of cortical depth in the cat was different from that in the monkey, where layer IV stands out as having higher proportions of GABA+ neurons than layers III or V in both areas 17 and 3b (Hendry et al., '87). Species differences were also apparent in the areal densities of GABA+ neurons: the density distributions of GABA+ neurons have peaks in layer IV in both cortical areas in the monkey (Hendry et al., '87), whereas such peaks are not evident in area 17 (Gabbott and Somogyi, '86) or area 3b in the cat. However, qualitative observations of cat auditory cortex suggest that, in this area, layer IV contains a higher density of GABA+ neurons than adjacent layers (Hendry and Jones, '91).

We compared the proportions of GABA+ neurons in areas 3b and 2 because area 2 contains a separate, cutaneous representation of the forepaw (Iwamura and Tanaka, '78;
McKenna et al., '81) which, based on anatomical connections and functional properties, appears to lie between area 3b and motor cortex (Iwamura and Tanaka, '78; Waters et al., '82; Schwark et al., '92). Area 2 contained a lower areal density of GABA+ neurons than area 3b, and the proportion of GABA+ neurons in area 2 was also lower than in area 3b, but this difference was not present when the proportions were calculated from laminar averages. We did not attempt to determine the proportion of GABA+ neurons in area 1 because of the curvature of the cortex in this area, but the areal density of GABA+ neurons in area 1 was similar to that in area 3b. It therefore appears that in the cat, areas 3b, 1, and 2 contain similar proportions of GABA+ neurons.
Fig. 1. Photomicrograph of GABA+ neurons (A) in a 16-μm parasagittal section through the forearm representation of area 3b in SI cortex. An adjacent, thionin stained section is shown in (B). Scale bar = 250 μm.
Fig. 2. Mean areal density of GABA+ neurons by cortical layer in each of the four cytoarchitectonic areas of SI cortex. Error bars represent standard errors of the means derived from five hemispheres.
Fig. 3. Volume fraction of GABA+ neurons by cortical layer in area 3b. These data were derived from counts of GABA+ neurons in 1-μm plastic sections. Error bars represent standard errors of the mean derived from six hemispheres.
Fig. 4. Plot of the positions of all GABA+ neurons in a 16-μm parasagittal section through the forearm representation in SI cortex. A 2-dimensional contour plot of this section is shown at the top of the middle column in Figure 4.
Fig. 5. Two-dimensional contour plots of the numbers of GABA+ neurons in series of alternate, 16-μm parasagittal sections through SI cortex of the right hemispheres of three cats. Sections were taken through the forearm representation of SI, just medial to the end of the coronal sulcus. Cytoarchitectonic areas are delineated in the top section of each series. The plots are based on the number of GABA+ neurons which fall in 100 x 100 μm bins, and contour lines surround bins which differ by one or more neurons. Solid lines of increasing thickness correspond to increasing numbers of neurons in the bins. This organization gives the appearance of darker areas in regions of higher GABA+ neuron densities. Scale bar = 1 mm.
Fig. 6. Horizontal distribution of GABA+ neurons in layers III and IV in a parasagittal section through area 3b. The histogram depicts the number of GABA+ cells in adjacent 50 μm bins spanning layers III and IV. The starting point for the histogram is the boundary between areas 3b and 1, and the stopping point is the boundary between areas 3a and 3b.
Fig. 7. GABA+ neurons in a counterstained 1-μm plastic section. Examples of GABA+ neurons are indicated by closed arrows, GABA- neurons are indicated by open arrows. Scale bar = 10 μm.
Fig. 8. Proportion of GABA+ neurons as a function of cortical layer in areas 3b and 2.

Error bars represent standard errors of the means derived from six hemispheres.
CHAPTER 3

3. REGIONAL DISTRIBUTION OF GABA$_A$ RECEPTOR BINDING SITES
IN CAT SOMATOSENSORY AND MOTOR CORTEX.

3.1 Summary

Inhibition in primary sensory cortex plays a role in neuronal responses to peripheral stimuli. For many neurons in cat primary somatosensory cortex, blockade of GABA$_A$ receptors by bicuculline results in receptive field enlargement. The magnitude of this effect varies with the neuron's adaptation characteristics and its location in particular laminae and submodality regions. To test whether these variations are correlated with the distribution of GABA$_A$ receptors, we analyzed $[^3]$Hmuscimol binding in cat primary somatosensory and motor cortical areas. The highest levels of binding were in layers I-III, and the lowest levels were in layers V-VI. In somatosensory cortical areas, layer IV was distinguished by higher levels of binding than in adjacent layers. Within layer IV, levels of binding were significantly higher in posterior area 3b than in anterior area 3b. These differences may correspond to the rapidly adapting and slowly adapting submodality regions which have been described in this area. The laminar distribution of $[^3]$Hmuscimol binding differed from that of $[^3]$Hflunitrazepam, and neither resembled the distribution of the magnitude of bicuculline's effects on receptive field size. The laminar distribution of $[^3]$Hmuscimol binding was highly correlated with the areal density of GABA-immunoreactive neurons described in a companion study.
3.2 Introduction

The predominant inhibitory neurotransmitter of the cerebral cortex, gamma-aminobutyric acid (GABA), binds to at least two subtypes of the GABA receptor, GABA\textsubscript{A} and GABA\textsubscript{B} (Sivilotti and Nistri, '90). At the GABA\textsubscript{A} receptor, the actions of GABA are mimicked by muscimol and blocked by bicuculline, while GABA\textsubscript{B} receptors are insensitive to both bicuculline (Hill and Bowery, '81) and muscimol (Sivilotti and Nistri, '90).

Studies with bicuculline indicate that inhibition mediated by GABA\textsubscript{A} receptors plays a role in shaping the receptive fields of cortical neurons. In many neurons in cat primary somatosensory cortex (SI), bicuculline blockade of GABA\textsubscript{A} receptors results in expansion of the receptive field and increased responsiveness to somatosensory stimuli (Dykes et al., '84; Alloway et al., '89). The degree to which bicuculline affects receptive field size appears to be related to the neuron's location, adaptation characteristics, or both. Alloway et al. ('89) reported that the receptive fields of rapidly adapting neurons in cat SI show greater expansion following bicuculline application than those of slowly adapting neurons. Dykes et al. ('84) reported that bicuculline results in enlargement of receptive fields of neurons in rapidly adapting, but not slowly adapting regions of area 3b defined by multiple unit recordings in the middle cortical layers. Their study also revealed laminar differences in responses to bicuculline: neurons in the middle cortical layers show smaller increases in receptive field size than neurons outside these layers (Dykes et al., '84). The results of these studies suggest that there may be functional differences in the GABA circuitry between cortical layers or submodality regions. Associated with these differences
may be regional variation in GABA<sub>\alpha</sub> receptors.

Motor cortex also contains a large population of GABA<sub>\alpha</sub>ergic neurons (Hendry et al., '87). A role for GABA<sub>\alpha</sub> receptors in motor cortex is suggested by the observation that iontophoresis of bicuculline into one part of the motor map can enhance the ability of electrical stimulation to produce movements from neighboring representations (Jacobs and Donoghue, '91). However, the role of GABA<sub>\alpha</sub> receptor-mediated inhibition in shaping the responses of motor cortical neurons is not known, and there is currently no information on the distribution of GABA<sub>\alpha</sub> receptors in cat motor cortex.

In the present study we analyzed the distribution of [\textsuperscript{3}H]muscimol binding in film autoradiographs of parasagittal sections through cat SI. Comparisons were made between laminar density profiles from each cytoarchitectonic area of SI and from motor cortical areas 4 and 6. Further analysis of layer IV in area 3b revealed that posterior regions contained significantly higher levels of [\textsuperscript{3}H]muscimol binding than anterior regions. The distribution of [\textsuperscript{3}H]muscimol binding was also compared to the benzodiazepine [\textsuperscript{3}H]flunitrazepam, which binds to a subunit of some GABA<sub>\alpha</sub> receptors, where it acts as a GABA<sub>\alpha</sub>ergic agonist by promoting GABA binding to GABA<sub>\alpha</sub> receptors (Barnard et al., '87). Portions of these results have appeared in abstract form (Li and Schwark, '92).

3.3 Methods

Four normal adult cats were used in these experiments. Each animal was injected with ketamine (30 mg/kg, i.m.), followed by an overdose of pentobarbital (60 mg/kg, i.p.). The brain was then rapidly removed, blocked, and frozen in -30°C isopentane.
Parasagittal sections through somatosensory and motor cortex were cut at 16 μm on a cryostat and thaw-mounted onto gelatinized slides. Additional sections from one animal were cut in the frontal plane through primary visual and auditory cortex. The sections were stored desiccated at -80°C until they were used in the binding procedures.

3.3.1 Ligand binding

3.3.1.1 [3H]Muscimol binding

The procedure for [3H]muscimol binding was similar to that of Mower et al. (1986). Sections were brought to room temperature and preincubated in 0.31 M Tris-citrate buffer (pH 7.1) at 4°C for 30 min, followed by incubation for 40 min at 4°C in the same buffer containing 10 nM [3H]muscimol (20 nCi/mm, New England Nuclear). The sections were then rinsed twice (15 sec each) in 4°C buffer alone, briefly dipped in 4°C dH₂O, and dried in a stream of air. Nonspecific binding was assessed in the presence of 1 mM GABA. Following incubation, the sections were post-fixed for 15 min in formaldehyde vapors.

Scatchard analysis was carried out on a series of 16 μm sections. Sections were treated as described above, using seven concentrations of [3H]muscimol ranging from 1.6 nM to 90 nM. At each concentration, nonspecific binding was determined in the presence of 1 mM GABA.

3.3.1.2 [3H]Flunitrazepam binding

The binding procedure for [3H]flunitrazepam was similar to that of Unnerstall et al.
Sections were incubated for 40 min at 4°C in 1 nM [3H]flunitrazepam (87 Ci/mmol, New England Nuclear) in 0.17 M Tris-HCl, pH 7.4. Sections were then rinsed twice (1 min each) in 4°C buffer, dipped briefly into 4°C dH2O, and dried in a stream of air. Nonspecific binding was assessed in the presence of 1 μM clonazepam.

3.3.2 Autoradiography and data analysis

Brain sections and tritium Microscale standards (Amersham) were apposed to tritium-sensitive Hyperfilm (Amersham). Exposure times were 60 days for [3H]muscimol and 25 days for [3H]flunitrazepam. The films were developed in Kodak D19 and processed further according to the manufacturer's instructions. Following preparation of the autoradiographs, the sections were stained with thionin.

Cortical areas 2, 1, 3b, 3a, 4 and 6 were identified on thionin-stained sections according to the criteria of Hassler and Milhs-Clement ('64) as described previously (Schwark et al., '92; Li and Schwark, '93). Density profiles were constructed using a video-based image analysis system (MCID, Imaging Research, St. Catherines, Ontario, Canada). The profiles were obtained by superimposing on the autoradiographic image a rectangle approximately 500 μm wide, spanning the thickness of the cortex. Optical density values, calibrated in nCi/mg wet weight, were then averaged over the width of the rectangle to plot binding density by cortical depth. Laminar boundaries were then drawn on the plots, and the average binding densities were calculated for each layer. On average, six density profiles were constructed from four sections for each cortical area in each hemisphere of three animals. Comparisons of binding densities between cortical areas
were based on laminar averages. Additional analysis of layer IV in area 3b was performed by constructing density histograms along the horizontal dimension on layer IV and averaging density values across the thickness of the layer. Sections from the fourth animal were used to compare patterns of $[^3H]$muscimol and $[^3H]$flunitrazepam binding in primary sensory cortical areas.

Differences in average binding levels across cortical areas and layers were assessed by analyses of variance and appropriate post-hoc tests. Scatchard analyses were performed using a non-linear regression program (LIGAND, Munson, '83), and differences between binding values were tested by analyses of variance.

3.4 Results

The laminar patterns of $[^3H]$muscimol binding in somatosensory and motor cortical areas were quite similar (Fig. 9). Labeling was densest in the upper layers (I and II) and decreased gradually with increasing depth below these layers. In the somatosensory areas, layer IV was distinguished by higher levels of binding than in the adjacent layers.

Scatchard analyses of $[^3H]$muscimol binding revealed that the differences in binding densities between upper (II-III) and lower (V-VI) cortical layers were primarily due to differences in the total number of binding sites ($B_{\text{max}}$) (Fig. 10). $B_{\text{max}}$ in the upper layers (242 fmol/mg) was significantly greater than in the lower layers (105 fmol/mg) ($F_{1,9}=13.40, p<0.015$), while there was no significant difference in dissociation constant ($K_d = 13.3$ nM in upper layers vs 8.0 nM in lower layers; $F_{1,9}=5.81, p<0.06$).
3.4.1 Distribution of $[^{3}H]$muscimol binding sites

Representative density profiles for each of the six cortical areas are shown in Figure 11. In every area, binding density was highest in layers I and II, decreased through layer III, and was lowest in layers V and VI. The average levels of $[^{3}H]$muscimol binding by layer and cortical area are shown in Figure 12. Because motor cortex lacks layer IV, separate analyses were done on somatosensory and motor cortical areas. In somatosensory cortical areas, there was a significant main effect of area ($F_{3,79}=6.84$, $p<0.01$), which reflected significantly lower binding in area 2 than in areas 3b or 3a, and in area 1 compared to area 3a (Bonferroni test (Wilkinson, '90) $p<0.05$). There was also a significant animal-by-layer interaction ($F_{10,79}=2.12$, $p<0.05$), apparently due to 12.6% higher density of binding in layers I and II in one animal than in the others, and which precluded further comparisons by analysis of variance. Motor cortex showed a significant main effect of layer ($F_{4,325}=483.69$, $p<0.01$). Post hoc analysis of this effect revealed significant differences for all possible pairwise comparisons between layers (Bonferroni test, $p<0.05$). Analysis of motor cortex also showed a significant animal-by-area interaction ($F_{2,325}=18.36$, $p<0.01$), due to different relative levels of binding in the two motor areas in different animals. Although somatosensory and motor cortex were analyzed separately, examination of the distributions in Figure 12 suggest that layer I in motor cortical areas contained somewhat higher binding than layer I in somatosensory cortex. Binding levels in layer III in the motor cortical areas fell between the levels in layers III and IV in the somatosensory cortical areas.

In each of the somatosensory cortical areas, there was a small peak in
\[^{3}\text{H}\]muscimol binding in layer IV. In area 3b, this peak was especially pronounced in some profiles, but absent in others. To determine the origin of this variation, we collected a complete sample of density profiles through area 3b from series of six sections through each of two hemispheres. In each section four profiles, which together covered the entire extent of area 3b, were examined for the presence or absence of a peak in layer IV. Of the 48 profiles, 31 (65%) showed a peak in \[^{3}\text{H}\]muscimol binding in layer IV. Additional density histograms were obtained from layer IV alone in order to evaluate \[^{3}\text{H}\]muscimol binding in this layer in more detail. In many of these profiles through layer IV, there was an apparent difference in the level of \[^{3}\text{H}\]muscimol binding between anterior and posterior regions. Therefore we defined two regions in area 3b by examination of averaged density profiles (example shown in Fig. 13), and determined the average binding levels for each region. The average density of \[^{3}\text{H}\]muscimol binding was lower in the anterior part of area 3b than in the posterior part in five of six hemispheres (Fig. 14). On average, anterior area 3b contained 6.3% lower \[^{3}\text{H}\]muscimol binding levels than posterior area 3b, a difference that was statistically significant \((F_{1,60}=4.20, \ p<0.05)\). Analyses of the locations of layer IV density peaks in individual density profiles (compared across series of six sections) revealed no consistent pattern other than these anterior-posterior differences.

3.4.2 Distribution of \[^{3}\text{H}\]flunitrazepam binding

The laminar distributions of \[^{3}\text{H}\]flunitrazepam and \[^{3}\text{H}\]muscimol binding were compared in area 3b of one animal (Fig. 15). Compared to levels of \[^{3}\text{H}\]muscimol binding, the absolute levels of \[^{3}\text{H}\]flunitrazepam binding was lower in layers I and II,
similar in layer III, and higher in layers IV-VI (Fig. 16).

3.4.3 $[^3]$H]Muscimol binding in visual and auditory cortex

To determine whether the laminar pattern of $[^3]$H]muscimol binding in primary somatosensory cortex was representative of other primary sensory cortical areas, we examined the distribution of $[^3]$H]muscimol binding in primary visual cortex and primary auditory cortex (Fig. 17). Although the distributions were similar, primary auditory cortex had somewhat lower levels of binding in the upper layers than visual or somatosensory cortex, and primary visual cortex had somewhat higher levels of binding in the lower layers than auditory or somatosensory cortex (Fig. 18).

3.5 Discussion

Analysis of $[^3]$H]muscimol binding in somatosensory and motor cortical areas revealed significant differences in binding densities between cortical areas, between cortical laminae, and within layer IV of area 3b. In all of the somatosensory and motor cortical areas examined in the present study, $[^3]$H]muscimol binding levels were highest in layers I and II and lowest in layers V and VI. Except for layer I, which contains few neurons, this same pattern was seen in the density of GABA-immunoreactive neurons in SI (Li and Schwark, '93). There was a strong positive correlation between the density of $[^3]$H]muscimol binding and the density of GABA-immunoreactive neurons (Li and Schwark, '93) across laminae in SI (Pearson's $r=0.88$, $p<0.05$) (Fig 19). The correlation coefficient becomes 0.97 if layer I is excluded from the analysis. This correlation between
numbers of GABAergic neurons and GABA$_A$ receptors probably reflects the limited axonal arborizations of most cortical GABAergic neurons (Houser et al., '84).

3.5.1 Distribution of [$^3$H]muscimol binding across cortical areas

Comparisons of [$^3$H]muscimol binding across cortical areas were based on laminar averages to control for variations in laminar thickness among areas. Among the cytoarchitectonic areas of SI, area 2 had significantly lower overall levels of [$^3$H]muscimol binding than areas 3b and 3a, and area 1 had lower levels than area 3a. The density of [$^3$H]muscimol binding in area 2 was 5.8% lower than in area 3b. Similarly, we found in a companion study that the proportion of GABA-immunoreactive neurons in area 2 was 18.6% lower than the proportion in area 3b, although this difference was not statistically significant (Li and Schwark, '93). The relationship between these cytoarchitectonic areas and the body map in cat SI is not well established, but our sections through area 2 probably included a region that contains a separate cutaneous representation of the forepaw and forearm (Iwamura and Tanaka, '78; McKenna et al., '81; Waters et al., '82; Garraghty et al., '87). Based on the present data, it appears that the system of cortical inhibition may be organized differently in the forearm representations in areas 3b and 2. In the monkey, where each cytoarchitectonic area of SI appears to contain a separate representation of the body (Kaas et al., '79), such differences have not been reported; although not compared directly, areas 3 and 1-2 appear to have similar levels of [$^3$H]muscimol binding (Lidow et al., '89).
motor and somatosensory cortical areas (214.8 ± 1.9 (SEM) and 208.0 ± 5.8 fmol/mg, respectively (p<.78)). This also appears to be true for the monkey (Lidow et al., '89). In the present experiments, the levels of $[^3]$Hmuscimol binding in layer III of motor cortex fell between the levels in layers III and IV of somatosensory cortex. The levels might reflect the dual roles of this layer in motor cortex: whereas in sensory cortex layer III gives rise to corticocortical connections and layer IV receives most of the thalamocortical input (e.g., Kosar and Hand, '81; Schwark et al., '92), in motor cortex layer III gives rise to corticocortical connections (Yumiya and Ghez, '84) and also receives most of the thalamocortical input (Strick and Sterling, '74).

In both somatosensory and motor cortex, analyses of variance revealed significant interactions involving animal as a factor. It seems unlikely that such variability arose from methodological factors. No perfusion was performed, all sections were incubated in the same ligand solution, and the order of incubation was randomized. Moreover, differences in such factors would most likely produce overall differences between animals, rather than significant interactions between cortical area and animal. It may be that such interactions can arise from differences in rearing conditions. For example, it has been shown that GABA$_A$ receptors can be affected by sensory deprivation (Hendry et al., '90). The life histories of the animals used in the present study are unknown. Few studies using receptor autoradiography have examined inter-animal variability, and additional studies will be necessary to explore this issue.

Primary auditory and visual cortex had patterns of $[^3]$Hmuscimol binding somewhat similar to that of somatosensory cortex, except that binding densities in layers
IV-VI of visual cortex were approximately twice that of the other cortical areas. The 
$[^{3}H]$muscimol binding pattern in visual cortex was similar to that described previously (Mower et al. '86). Although in the somatosensory cortex levels of $[^{3}H]$muscimol binding were highly correlated with the density of GABA immunoreactive neurons, this correlation may not hold for visual cortex. Compared to somatosensory cortex, visual cortical layers V and VI contain lower densities of GABA-immunoreactive neurons (Gabbott and Somogyi, '86; Li and Schwark, '93) but higher levels of $[^{3}H]$muscimol binding. Such differences might arise if GABAergic neurons in the deep layers of visual cortex form more extensive axonal arborizations than those in somatosensory cortex.

3.5.2 Distribution of $[^{3}H]$flunitrazepam binding

The distribution of $[^{3}H]$flunitrazepam binding in area 3b differed from that of $[^{3}H]$muscimol binding. Compared to $[^{3}H]$muscimol, absolute levels of $[^{3}H]$flunitrazepam binding were lower in layers I and II, similar in layer III, and higher in layers IV-VI. This distribution is different from that in monkey area 3, where the number of $[^{3}H]$flunitrazepam binding sites are somewhat higher than the number of $[^{3}H]$muscimol binding sites, particularly in upper layer III and layer IV (Lidow et al., '89). Differences between the distributions of $[^{3}H]$muscimol and $[^{3}H]$flunitrazepam binding have been reported previously (Unnerstall et al., '81) and are not unexpected, given that not all GABA_A receptors bind benzodiazepines with equal affinity (Seeburg et al., '90). Since benzodiazepines can enhance the action of GABA at GABA_A receptors (Study and Barker, '81), it could be that GABA_A receptors with benzodiazepine binding sites mediate
the effects of bicuculline on receptive field size. However, iontophoresis of flunitrazepam decreases receptive field size in neurons throughout SI (though more effectively in rapidly adapting subregions) (Oka and Hicks, '90), and our data suggest that the laminar distribution of \[^{3}H\]flunitrazepam binding does not correspond to the effects of bicuculline.

3.5.3 \[^{3}H\]Muscimol binding in relation to bicuculline effects

In somatosensory cortex, bicuculline's effects on receptive field size are specific to rapidly adapting neurons (Alloway et al., '89) and vary in magnitude across layers (Dykes et al., '84). Layer IV of area 3b contained 6.3% lower \[^{3}H\]muscimol binding in the anterior region than in the posterior region. The region of area 3b sampled here probably contains the wrist and forearm representation (Felleman et al., '83). On the basis of detailed maps, this region has been divided into rapidly adapting and slowly adapting zones (Dykes et al., '80; Sretavan and Dykes, '83) organized in a wavy, interdigitating pattern. In the forearm representation, the slowly adapting region tends to lie in anterior 3b, next to the border with area 3a (Dykes et al., '80; Sretavan and Dykes, '83). Thus, the region of layer IV that contains lower \[^{3}H\]muscimol binding levels might correspond to the slowly adapting region in the forearm representation of area 3b. This conclusion must be tentative because, to preserve the integrity of receptor binding in the present experiments, we did not map these areas.

The laminar distribution of \[^{3}H\]muscimol binding in area 3b did not correlate with the degree of receptive field expansion resulting from bicuculline iontophoresis (Dykes et al., '84). This was especially obvious in layers IV-VI, where \[^{3}H\]muscimol binding was
higher in layer IV than in V or VI, and the reverse relationship holds for bicuculline iontophoresis (greater percent increase in receptive field size of neurons in layers V and VI than in layer IV; Dykes et al., '84).

The degree of receptive field enlargement following inhibitory blockade might reflect the spread of thalamocortical afferents (Alloway and Burton, '91). Indirect support of this in monkey comes from an intracellularly labeled, slowly adapting thalamocortical afferent which had a more restricted distribution than rapidly adapting thalamocortical afferents (Garraghty and Sur, '90). In the cat, however, there appear to be no evident differences in the size of slowly and rapidly adapting thalamocortical afferents (Landry and Deschênes, '81). Moreover, since bicuculline's effects on receptive field size are specific for rapidly adapting neurons regardless of their laminar position (Alloway et al., '89), differences due to divergence that arise in layer IV must be maintained throughout the cortical circuitry. Evidence for specificity of inhibitory circuitry at the level of individual neurons was seen in cat visual cortex, where neurons projecting to different targets receive different numbers of inhibitory synapses on their somata (Fariñas and DeFelipe, '91).

Alternatively, laminar differences in the magnitude of bicuculline's effects might arise from differences in horizontal, intracortical connections. The horizontal extent of these connections is greatest in the deep layers (Gilbert & Wiesel, '79; Schwark and Jones, '89; Ojima et al., '92), where the greatest receptive field enlargements are seen (Dykes et al., '84), and least in layer IV, where the smallest enlargements are seen.
Fig. 9. Distribution of $[^3H] \text{muscimol}$ binding in a 16 $\mu$m parasagittal section through somatosensory and motor cortical areas of the cat. The section was taken just medial to the end of the coronal sulcus, in the forearm representation of SI. Scale bar = 2 mm.
Fig. 10. Scatchard analysis of $[^3]H$-muscimol binding in upper layers (II-III) and lower layers (V-VI) of somatosensory cortex. Binding values are expressed per mg wet weight. There was a significant difference between these sets of layers in $B_{\text{max}}$, but not $K_d$ ($B_{\text{max}}$: $F_{1,5}=13.40$, $p<0.015$, $K_d$: $F_{1,5}=5.81$, $p<0.06$).
Fig. 11. Representative density profiles of $[^3]$Hmuscimol binding in six areas of cat somatosensory (areas 2, 1, 3b and 3a) and motor (areas 4 and 6) cortex. In each graph, the total length of the vertical axis represents 2500 μm. The horizontal axis shows binding levels in fmol/mg wet weight.
Fig. 12. Average levels of $[^3H]$muscimol binding by cortical layer in six somatosensory and motor cortical areas. Within each group of bars, layers I through VI are represented from top to bottom. Bars represent averages of 32 - 42 density profiles. Standard errors shown are based on means values for each hemisphere.
Fig. 13. Density profile of $[^3]H$-muscimol binding in layer IV of area 3b in animal C012L (see Fig. 6). The profile represents an average derived from six sections, and was smoothed using a 5-bin moving boxcar average. The arrow shows the division between anterior and posterior regions.
Fig. 14. Average levels of $[^3H]$muscimol binding in layer IV in anterior and posterior regions of area 3b. Labels under the horizontal axis denote the animal number and hemisphere. Each average is based on data from six sections. Overall, the density of binding in layer IV was significantly lower in anterior than in posterior regions ($F_{1,60} = 4.20$, $p < 0.05$).
Fig. 15. Distribution of $[^3\text{H}]$flunitrazepam binding in a parasagittal section through somatosensory and motor cortex of the cat. The section was taken just medial to the postcruciate dimple. The long, thin line marks the boundary between areas 3a and 4 to distinguish SI (to the left) from motor cortex (to the right). Scale bar = 1 mm.
Fig. 16. Average binding densities of $[^3\text{H}]$muscimol and $[^3\text{H}]$flunitrazepam in area 3b as a function of cortical layer. Values for $[^3\text{H}]$muscimol were taken from the data for Figure 4. Values for $[^3\text{H}]$flunitrazepam were derived from a representative density profile, 1 mm wide, through area 3b.
Fig. 17. Distribution of $[^3]$Hmuscimol binding in primary somatosensory (A, sagittal plane), auditory (B, frontal plane), and visual cortex (C, frontal plane) of the cat.

A. The line marks the boundary between areas 3a and 4 to distinguish SI (to the right) from motor cortex (to the left). B. Primary auditory cortex lies on the ectosylvian gyrus. In the figure dorsal is to the left. Part of the suprasylvian sulcus is visible at lower right. C. The entire cortex in this figure is primary visual cortex (area 17). Dorsal is to the right. Scale bar = 1 mm.
Fig. 18. Representative density profiles of $[^3]$Hmuscimol binding through cat primary sensory cortical areas. Labels on the horizontal axes denote binding levels in nCi/mg wet weight.
Fig. 19. Comparison of the laminar distribution of $[^3H]$muscimol binding (in fmol/mg wet weight) and GABA(+) neuron densities in area 3b. The data for GABA(+) neuron densities were taken from Li and Schwark (‘93).
CHAPTER 4

4. HORIZONTAL CONNECTIONS IN CAT SI
AND THEIR RELATION TO RECEPTIVE FIELD SIZE

4.1 Summary

Horizontal cortical connections were studied following injections of neuronal tracers into different cortical layers of area 3b in primary somatosensory cortex (SI) of the cat. The positions of retrogradely labeled neurons were plotted and related to the representation of the body surface. Most labeled neurons were distributed near the injection site, but some labeled neurons were found far from the injection site. The furthest extent of labeling varied across cortical layers. Injections into layers II, III and V resulted in more widespread labeling than injections in layer IV. This pattern corresponds well with laminar differences in the magnitude of receptive field enlargement produced by blockade of GABA_A receptors.

4.2 Introduction

Receptive fields (RFs) of sensory neurons arise from excitatory input from peripheral receptors. As early as the first synaptic relay in the somatosensory system, inhibitory neurons contribute to the organization of the RFs, so that they contain inhibitory as well as excitatory components (Golovchinsky, 1980). Thus, the receptive fields of central neurons arise from a combination of excitation and inhibition. The balance
between excitation and inhibition is apparently dynamic. Blockade of local inhibition by bicuculline iontophoresis (to block GABA_A receptors) in cat SI results in the expansion of the RFs of many SI neurons (Dykes, 1984; Alloway et al., 1989). The magnitude of this effect varies across cortical layers. Bicuculline iontophoresis results in smaller RF enlargements for neurons in the middle cortical layers than for neurons in upper and lower cortical layers (Dykes, 1984). This pattern of bicuculline effects is not related to the laminar distribution patterns of GABA neurons or GABA_A receptors (Li and Schwark, 1994; Schwark et al, 1994), which are mainly located in layer II, then decrease in number to layer VI.

There is accumulating evidence that horizontal cortical connections may play an important role in forming and modulating RFs. Conditioning stimuli (brief air puffs) presented outside the excitatory RFs of SI neurons inhibit the responsiveness to stimuli inside the RF (Laskin & Spencer, 1979). Following RF enlargement induced by GABA_A blockade, the latency of activation from the enlarged portion of the RF is longer than that from the original RF (Alloway et al., 1989). These results suggest that RFs can be modulated by horizontal connections within SI, perhaps acting through inhibitory neurons. Thus, the magnitude of RF enlargement in SI neurons caused by bicuculline might be determined by the length of the horizontal connections which synapse on the neuron.

Horizontal connections in sensory cortex are abundant and extensive. They are formed primarily by the axon collaterals of pyramidal neurons (Gilbert, 1983; Schwark and Jones, 1989), although some non-pyramidal neurons send axon collaterals up to two millimeters from their somata (Jones, 1984). Pyramidal neuron axon collaterals in SI can
extend many millimeters (Schwark and Jones, 1989). Such an organization suggests that a
target neuron in SI might receive input from neurons whose RFs lie outside the boundaries
of the target neuron's RF. These inputs might be under inhibitory control, so that loss of
inhibition would reveal the full extent of inputs to the neuron. To determine if the extent
of horizontal connections which a neuron receives might be related to the magnitude of
RF enlargement that is produced by bicuculline, the present experiments coupled
physiological mapping of RFs with retrograde tracer labeling to examine the extent of
horizontal connections and their relation to the body representation in SI.

4.3 Methods

4.3.1 Animal preparation

The experiments were conducted on six adult cats. Each cat was injected with
atropine (0.04 mg/kg i.m.) and ketamine (30 mg/kg, i.m.), and cannulae were inserted into
a femoral vein and artery. Surgical levels of anesthesia were induced with sodium
thiopental (20 mg/kg i.v.) and maintained by continuous infusion (3.5 mg/kg, i.v.). A
tracheostomy was done, the cat was mounted in a stereotaxic frame, and craniotomies
were made over the forelimb representations in SI of both hemispheres.

4.3.2 Tracer injections

The RFs at the injection site in the distal forelimb representation were mapped by
first recording multiple unit responses to natural peripheral stimulation. A small deposit of
Neurobiotin (5% Neurobiotin in saline, 1 μA for 30 min, tip diameter 10-20 μm) or
horseradish peroxidase (5% HRP in saline, 1 μA for 1 hr, tip diameter 10-50 μm) was then made by iontophoresis. After the injection, a new electrode filled with 2% NaCl was used to map the body representation around the injection site within a range of 2-3 mm. The cortex was then covered with agarose followed by melted Vaseline. The cats did not recover from anesthesia. After 24 hr the cats received a lethal overdose of anesthetic (rapid i.v. injection of 50 mg/kg sodium thiopental) and were perfused with physiological saline followed by a fixative solution containing 2% paraformaldehyde and 0.15% glutaraldehyde in 0.1 M phosphate buffer. The brain was removed and postfixied for 4-6 hr at 4°C. The brain was then blocked and the blocks were placed in 4°C phosphate buffer containing 30% sucrose until they sank, after which they were rapidly frozen in -35°C isopentane.

4.3.3 Histology

For tissue blocks containing Neurobiotin injections, two sets of 25-μm thick sections were cut on a sliding microtome in the parasagittal or tangential plane. One set of sections was incubated in standard avidin-biotin peroxidase complex for 1 hr. After a series of washes the sections were processed in phosphate-buffered saline containing 0.05% DAB with 0.026% H₂O₂ and 0.02% nickel ammonium sulfate. The second set of sections was stained with thionin to localize laminar and area boundaries. For tissue blocks containing HRP injections, 50-μm thick sections were cut in the parasagittal plane. Sections were reacted by the cobalt- and nickel-enhanced diaminobenzidine (DAB) method of Adams and Warr (1976), and selected sections were counterstained with
4.3.4 Data collection

The size and laminar location of each injection was determined. The locations of all neurons retrogradely labeled with Neurobiotin or HRP, together with the laminar and cytoarchitectonic boundaries in SI, were plotted with the aid of a camera lucida at a final magnification of 125X. The criteria for distinguishing boundaries between areas and laminae were those of Hassler and Muhs-Clement (1964) as described previously (Schwark et al., 1992). The position of every labeled neuron was entered into a computer with a digitizing tablet, and three-dimensional plots of the labeled neurons were then constructed.

4.4 Results

Details of the injections are presented in Table 2. All of the injections were 80-90 μm in diameter except for one, which was 30 μm. This injection was analyzed separately. Two aspects of the pattern of labeling were analyzed: the extent of local labeling (near the injection site) and the extent of distant labeling.

4.4.1 Patterns of local labeling

In all injections, the majority of the labeled neurons were located near the injection sites. Approximately 70-85% of all labeled neurons were located within 500 μm of the injection site. The labeled neurons were not evenly distributed around the injection site.
However, the irregularities in the distribution patterns were not obviously related to the laminar position or RF of the injection site.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Location</th>
<th>Injection size (μM)</th>
<th>Tracer</th>
</tr>
</thead>
<tbody>
<tr>
<td>C063LSI</td>
<td>II</td>
<td>90</td>
<td>Neurobiotin</td>
</tr>
<tr>
<td>C050</td>
<td>III</td>
<td>80</td>
<td>Neurobiotin</td>
</tr>
<tr>
<td>C063RSI</td>
<td>III</td>
<td>30</td>
<td>Neurobiotin</td>
</tr>
<tr>
<td>C059LSI</td>
<td>IV</td>
<td>90</td>
<td>Neurobiotin</td>
</tr>
<tr>
<td>C061LSI</td>
<td>IV</td>
<td>90</td>
<td>Neurobiotin</td>
</tr>
<tr>
<td>C555</td>
<td>V</td>
<td>90</td>
<td>HRP</td>
</tr>
</tbody>
</table>

4.4.2 Patterns of distant labeling

The location and extent of labeled neurons which were located farther away from the injection site varied with injection site. The distributions of these neurons were irregular, rather than evenly distributed around the injection sites. For example, an injection into layer II resulted in more labeled neurons posterior and lateral to the injection than anterior and medial (Fig 20A). An injection into layer III labeled a tight cluster of neurons which were located approximately 650 μm lateral to the injection site (Fig 20B). These neurons were all located in layer III. Anterior and slightly medial to the injection, labeled neurons were scattered more diffusely throughout many cortical layers, and were found up to 1600 μm distant. Irregularities in the pattern of labeled neurons were less evident following an injection into layer IV. 85% of the neurons labeled by this injection were clustered around the injection site. However, a few, more distantly labeled neurons
were found posterior and lateral to the injection site (Fig 20C). Following an injection into layer V (Fig 20D), labeled neurons were less tightly clustered around the injection site, and were located up to 1800 μm from the injection site. Other labeled neurons were located in layer III, anterior and medial to the injection site.

The furthest extent that labeled neurons were found from the injection site was measured for all injections. Injections into layers II, III, and layer V resulted in more widespread labeling than the injection in layer IV (Fig 21).

4.4.3 Retrograde labeling and the body map

Irregularities in the distribution patterns of retrogradely labeled neurons might reflect the organization of the body map in SI. In one animal the forelimb representation in area 3b was mapped after the injection was made into layer IV. Neurons at the injection site responded to stimulation of hair at the lateral edge of the dew claw. Neurons in a range of approximately 500 μm around the injection site responded to stimulation from hair on the lateral forearm, adjacent to the dew claw. The injection into layer IV labeled neurons around the injection site in a range of approximately 500 μm. Other labeled neurons were found posterior to the injection site, RFs at this site were proximal to those at the injection site and responded to stimulation of hair.

4.4.4 Laminar distribution of labeled neurons

All injections, regardless of laminar location, resulted in greater numbers of labeled neurons in layer III than in the other layers. However, the laminar distribution of labeled
neurons varied across injections in different laminae (Fig 22). Injections into layers II or III labeled neurons primarily in layers II and III, with only a few labeled neurons located in the other layers. Most of the neurons labeled by layer IV injections were in layers III and IV, with only approximately 16% of the labeled neurons in layer VI. Following an injection into layer V, labeled neurons were mainly located in layers III, V and VI.

4.5 Discussion

4.5.1 Organization of horizontal cortical connections in SI

The results of the present study revealed the local and distant distributions of neurons which were labeled following injections of retrograde tracers into area 3b. In both the cat (Schwark et al., 1992) and monkey (DeFelipe et al., 1986), long-distance horizontal connections link the four cytoarchitectonic areas of SI. In cat SI, areas 3b and 1 contain a single body representation (McKenna et al., 1981; Felleman et al., 1983). Although the organization of connections between the cytoarchitectonic areas of SI have been described (Schwark et al., 1992), the organization of shorter, horizontal connections which are formed within the cytoarchitectonic areas is less well described.

In the present study of area 3b, injections resulted in higher numbers of local labeled neurons than of distant labeled neurons. Between 70-85% of the labeled neurons were located within 500 µm of the injection site. Neurons in area 3b form extensive local axon collaterals (Schwark and Jones, 1989), and these may underlie short-range interactions between neurons which respond to adjacent body regions. However, these interactions might not be uniformly specified between adjacent body regions, because the
present data suggest that these local labeled neurons were not evenly distributed around the injection site. Irregularity was even more apparent in the distribution patterns of distant labeled neurons. These neurons were located in an anisotropic distribution which may reflect the organization of the body representation in SI.

Unlike other cortical areas, the horizontal connections within area 3b did not appear to arise from a patchy distribution of neurons. Instead, the labeled neurons were evenly distributed, but were positioned in a particular direction from the injection site. In the primary visual and auditory cortex of the cat, and in many cortical areas of the monkey, horizontal connections arise from a patchy network of neurons (Rockland & Lund 1983; Levay 1988; Albus et al., 1991, Wallace et al., 1991; Clarke et al., 1993). In the visual cortex, these horizontal connections appear to link neurons which have similar orientation selectivities (Gilbert & Wiesel, 1989). Individual neurons give rise to clustered axon collaterals as they pass through cortical columns with similar orientation selectivity (Gilbert and Wiesel, 1983). Patchy labeling seen in SI underlies connections between cytoarchitectonic areas, rather than within a single area (Schwark et al., 1992). Although we observed a small cluster of neurons 650 µm from one of the injection sites, in general the labeled neurons were diffusely distributed. Anterograde labeling studies in monkey SI suggest that axon terminals form a patchy network of connections (DeFelipe et al., 1986 and Lund et al., 1993), while the retrograde labeling patterns revealed in the same studies were not patchy (Lund et al., 1993).

4.5.2 Horizontal connections and RFs
The role of horizontal connections in modulating RFs is becoming more apparent. In the visual and auditory cortex, horizontal connections synapse primarily upon excitatory neurons: only 5-20% of the synapses are made upon inhibitory neurons (Kisvárday & Eysel, 1992; McGuire et al., 1991; Elhanay and White, 1990). Modulation of the RF by horizontal connections is apparently under local inhibitory control. Blockade of GABA\(_A\) receptors by bicuculline iontophoresis results in expansion of the RFs of SI cortical neurons (Dykes et al., 1984). These expansions may be due to unmasking of existing, weak synaptic connections (Smits et al., 1991; Zarzecki et al., 1993), at least some of which are formed by long range horizontal connections. Therefore, neurons which form long horizontal connections may modulate distant receptive fields. Under normal situations, the horizontal excitatory input may not be strong enough to elicit action potentials in the target neurons because of tonic, surround inhibition. When the inhibition is blocked, stimulation of an adjacent RF would ultimately reach and activate the neuron through horizontal connections. This would appear as an expansion of the RF. Therefore, neurons which receive more widespread horizontal connections would tend to exhibit larger expansions of their receptive fields during blockade of GABA\(_A\) receptors.

The present data suggest that neurons in the upper and lower cortical layers receive more widespread horizontal connections than neurons in layer IV. This pattern corresponds very well with the pattern of bicuculline effects across cortical layers described by Dykes et al. (1984). The RF expansions produced by bicuculline iontophoresis are much smaller for layer IV neurons than for neurons in the other cortical layers.
The functional implications of the irregular labeling patterns are less clear. A small number of SI neurons, primarily in area 2, respond best to stimuli moving across their receptive fields in a particular direction (Hyvarinen and Poranen, 1978; Gardner, 1989). It has been proposed that directional selectivity in SI is determined by input from presynaptic neurons whose receptive fields overlap (Gardner, 1989). In this model, each afferent innervates particular dendritic branches, and these afferents are somatotopically organized. Thus, the somatotopic organization of afferents ending on a neuron's dendritic branches could control the information flow in particular dendritic branches, and determine response selectivity to stimulus. In the case of afferents arising from horizontal connections, these inputs might contribute to interactions across regions of the body surface.
Figure 20. Three-dimensional plots of neurons labeled by injections into various cortical layers. The locations of the injections were layer II (A), III (B), IV (C), and V (D).
Figure 21. The furthest extent of labeled neurons following injections in various cortical layers.
Figure 22. Proportions of labeled neurons across cortical layers.
CHAPTER 5

5. THE DISTRIBUTION OF NEURONS CONTAINING PARVALBUMIN, CALBINDIN D-28K AND CALRETININ IN CAT PRIMARY SOMATOSENSORY CORTEX.

5.1 Summary

In many areas of neocortex, the calcium binding proteins (CBPs) parvalbumin, calbindin, and calretinin, are contained in separate populations of neurons which differ in their laminar distributions. In the present study, laminar distributions of neurons which contained parvalbumin (PV+), calbindin (CALB+) and calretinin (CALR+) were determined for the four cytoarchitectonic areas of cat primary somatosensory cortex (SI).

For each CBP the laminar distributions were similar across the cytoarchitectonic areas of SI. PV+ neurons were found in all layers except layer I, with highest densities in layer IV. CALB+ neurons were found in highest densities in layers II and III. CALR+ neurons were found in all layers, with highest densities in layers I and II.

Within layer III the CBPs tended to vary in density across the cytoarchitectonic areas of SI. PV+ neurons were found in higher densities in areas 3b and 1 than in area 2. CALB+ neurons were found in higher densities in anterior SI than in posterior SI. The pattern for CALR+ neurons was the reverse, with higher densities in posterior regions of SI. These differences in regional distributions of CBPs within the cytoarchitectonic areas of SI may reflect functional differences between these areas.
5.2 Introduction

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the cerebral cortex. Immunocytochemical studies have shown that GABA neurons comprise 20%-30% of the cortical neurons in areas 17 and SI (Gabbot and Somogyi, 1986; Hendry et al., 1987; Li and Schwark, 1994). Cortical GABAergic neurons are a morphologically diverse group, and have distinct synaptic relations with other cortical neurons (Houser et al., 1983; Jones, 1984). Cortical GABAergic neurons are also chemically diverse: many contain other classic neurotransmitters (Conti et al., 1987a; Kosaka et al., 1988; and Trottier et al., 1989) or neuropeptides (Demeulemeester et al., 1991; Hendry et al., 1984; Jones and Hendry, 1986).

GABAergic neurons play important roles in cortical function. Blockade of GABA\(_A\) receptors results in increased receptive field size of neurons in area 3b of SI (Alloway et al., 1989; Dykes, 1983), and these effects vary according to area and the laminar position of the neuron (Dykes et al., 1983). It may be that some of the subpopulations which can be identified by chemical markers correspond to functionally distinct groups of GABAergic neurons.

It has recently been shown that three types of calcium binding proteins (CBPs) are present in separate populations of GABA neurons (Demeulemeester et al., 1991; Miettinen et al., 1992; Gulyás et al., 1992). It has been proposed that the presence of these CBPs (parvalbumin (PV), calbindin D-28 (CALB) and calretinin (CALR)) may be related to the level (Baimbridge & Miller, 1984) or pattern of a neuron's discharge.
(Kawaguchi et al., 1987). PV has been localized to fast spiking (Kawaguchi and Kubota, 1993), metabolically active (McCasland et al., 1993) cortical neurons. CALB has been localized in low threshold spiking cortical neurons (Kawaguchi and Kubota, 1993). If the presence of a particular CBP marks a functionally distinct population of neurons, then differences in the relative numbers and distributions of neurons which contain these three calcium binding proteins might reflect functional differences among cortical areas. The distributions of neurons containing CBPs have not been characterized in cat SI, a sensory cortical region with functionally-distinct cytoarchitectonic areas. Moreover, the distribution of CALR+ neurons has not been quantitatively examined in any cortical area.

In the present study, the laminar distributions of neurons containing PV, CALB, or CALR were determined for each of the four cytoarchitectonic areas of SI. In addition, colocalization experiments were performed to determine if CBPs were found only in GABAergic neurons, and if any CALR+ neurons contained additional types of CBPs. Neurons containing CALB or CALR were mainly located in superficial cortical layers. PV+ neurons were located mainly in layers III and IV. Layer I contained no PV+ or CALB+ neurons. Almost all PV+ neurons and 90% of the CALB+ neurons also contained GABA. CALR+ neurons never contained PV or CALB.

5.3 Materials and methods

5.3.1 Tissue Preparation

The material used in this study was derived from 7 normal cats. Each animal was injected with ketamine (30 mg/kg, i.m.) and given an overdose of pentobarbital (60 mg/kg,
i.p.), then perfused through the heart with saline followed by a fixative solution containing 2% paraformaldehyde and 0.15% glutaraldehyde in 0.1 M phosphate buffer. The brain was removed and postfixed for 4-6 hr at 4°C. The brain was then blocked and the blocks placed in 4°C phosphate buffer containing 30% sucrose until they sank, after which they were rapidly frozen in 35°C isopentane.

Blocks containing SI were sectioned on a sliding microtome in the parasagittal plane. Four sets of 16 μm sections were collected in cold phosphate buffered-saline (PBS): one set for each calcium binding protein antibody and one set for thionin staining to determine laminar and cytoarchitectonic borders.

5.3.2 Immunocytochemical Staining

5.3.2.1 Distribution of CBPs

Three sets of 16 μm sections were washed for 10 min in 4°C PBS containing 0.4% Triton X-100, and incubated for 2 hr at 4°C in PBS containing 3% normal horse serum (NHS) and 0.3% Triton X-100. The sections were then transferred to a PBS solution containing 0.1% NHS, 0.1% Triton X-100 and the appropriate antibody: mouse monoclonal anti-parvalbumin (1:12000, Sigma), mouse monoclonal anti-calbindin D-28K (1:1000, Sigma) or 1:8000 anti-calretinin (1:8000, gift from Dr. M.R. Celio). After 24 hrs the sections were washed in PBS at 4°C for 10 min (several changes), then incubated in a 1:100 solution of biotinylated horse anti-mouse (PV, CALB) or goat anti-rabbit (CALR) immunoglobulins (Vector) at 4°C for 1 hr. The sections were washed for 10 min in room temperature PBS (several changes) and then incubated in avidin-biotin peroxidase
complex (Vector) for 1 hr. After another series of washes the sections were reacted in PBS containing 0.05% DAB with 0.026% $\text{H}_2\text{O}_2$ and 0.02% nickel ammonium sulfate. The sections were then washed several times in PBS, mounted on subbed slides, dried, dehydrated through a graded series of alcohols, cleared, and coverslipped.

5.3.2.2 Double labeling

Four sets of sections were stained using immunofluorescent methods to co-localize CBPs and GABA. Labeling procedures were similar to those described above except for the concentrations of primary and secondary antibodies (Table 3). After incubation in fluorescein secondary antibodies for 2 hr at 4°C, sections were washed for 10 min in PBS at room temperature, mounted on subbed slides, dried, cleared in xylene and coverslipped.

Table 3. Antibodies used for co-localization of CBPs and GABA.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Animal</th>
<th>Dilution</th>
<th>Mono/polyclonal</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GABA</td>
<td>rabbit</td>
<td>1:15000</td>
<td>polyclonal</td>
<td>Sigma</td>
</tr>
<tr>
<td>PV</td>
<td>mouse</td>
<td>1:3000</td>
<td>monoclonal</td>
<td>Sigma</td>
</tr>
<tr>
<td>CALB</td>
<td>mouse</td>
<td>1:500</td>
<td>monoclonal</td>
<td>Sigma</td>
</tr>
<tr>
<td>CALR</td>
<td>mouse</td>
<td>1:1500</td>
<td>polyclonal</td>
<td>Dr. Celio</td>
</tr>
<tr>
<td>Fluorescent secondary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mouse IgG</td>
<td>horse</td>
<td>1:600</td>
<td>fluorescein</td>
<td>Vector</td>
</tr>
<tr>
<td>rabbit IgG</td>
<td>goat</td>
<td>1:600</td>
<td>Texas red</td>
<td>Vector</td>
</tr>
</tbody>
</table>

5.3.3 Data Collection
5.3.3.1 Distribution of labeled neurons

The locations of neurons containing CBPs, together with the laminar and cytoarchitectonic boundaries in SI (from adjacent thionin sections) were plotted from four series of six, alternate sections (80 µm between sections) using a camera lucida at 125X magnification. Each series of sections was taken from a different hemisphere. Criteria for identifying the boundaries of the cytoarchitectonic areas were based on the description of Hassler and Muhs-Clement (1964) as described previously (Schwark et al., 1992; Li and Schwark, 1994).

The location of every labeled neuron in the sample area was entered into a computer with a digitizing tablet. To determine the density of labeled neurons in SI, the area of each layer in each cytoarchitectonic area of SI was measured (minus the area of major blood vessels) and the number of labeled neurons per square millimeter was calculated. Differences in the densities of labeled neurons were assessed using Friedman’s analysis of variance based on ranks and post hoc tests using Bonferroni’s correction for repeated tests.

5.3.3.2 Double labeling.

The proportions of neurons which contained combinations of CBPs and GABA were determined using a fluorescent microscope and switching filters to identify double-labeled neurons. Neurons were sampled across all six cortical layers in the four cytoarchitectonic areas of SI.
5.4 Results

5.4.1 Morphological characteristics of neurons containing calcium binding proteins

Neurons which contained PV were of various sizes, ranging from 2.7 to 23.2 \( \mu m \) in diameter (Fig 23). The average diameter of PV+ neurons was 12.56 ± 0.29 \( \mu m \). The larger neurons (Fig 24A) had features typical of classical basket neurons (Jones, 1975; DeFelipe et al., 1986) with numerous dendritic processes radiating from their somata. These neurons were located mainly in deep layer III, layer IV and layer VI. Neurons which contained CALB or CALR were small to medium in size, with average diameters of 8.35 ± 0.39 and 9.01 ± 0.41 \( \mu m \) respectively (Fig 23). The diameters of CALB+ neurons ranged from 3.94 to 14.27 \( \mu m \), and those of CALR+ neurons ranged from 5.11 to 15.96 25 \( \mu m \). Some CALR+ neurons, and most CALB+ neurons, were characteristic of classic double bouquet neurons (Jones, 1975; Somogi and Cowey, 1984) (Fig 24B). The dendrites of these neurons were bitufted in appearance and extended vertically through the cortical depth. The ascending and descending dendrites of these neurons extended into the adjacent layers. Some of the CALR+ neurons had characteristics of small basket neurons (Jones, 1984) or Cajal-Retzius neurons (Marin-Padilla, 1986). The Cajal-Retzius type neurons had stout processes at the bases of their somata and were located in layer I. These neurons sent projections horizontally into deep layer I and superficial layer II (Fig 24C).

5.4.2 Distributions of neurons containing calcium binding proteins

Neurons which were immunoreactive for CBPs could be easily distinguished from
background. In each of the cytoarchitectonic areas of SI neurons immunoreactive for PV, CALB or CALR were differentially distributed across layers (Fig 25). For each CBP, the laminar distribution was similar across the cytoarchitectonic areas.

The laminar distribution of PV+ neurons is illustrated in Figure 26. PV+ neurons were located in all cortical layers except layer I. Layer IV contained the highest density of PV+ neurons. Within layer III, the density of PV+ neurons varied across areas of SI. Areas 3b and 1 contained more PV+ neurons than area 2 ($F_r=9.9$, $p<0.02$).

The laminar distribution of CALB+ neurons was very different from that of PV+ neurons (Fig 27). The highest density of CALB+ neurons was in layer II, followed by layer III. Layers I, IV, V and VI contained few CALB+ neurons. Differences in labeled neuron densities between cytoarchitectonic areas were seen in layers II and III. In layer II, area 3a contained higher densities of CALB+ neurons than areas 1 or 2 ($F_r=9.9$, $p<0.02$). In layer III, area 3a contained higher densities of CALB+ neurons than area 1 ($F_r=9.9$, $p<0.02$).

The laminar distribution of CALR+ neurons more closely resembled that of CALB+ neurons than PV+ neurons (Fig 28). However, unlike CALB+ neurons, the highest density of CALR+ neurons was in layer I, followed by layer II. The densities of CALR+ neurons decreased from layer III to layer VI. As with the other CBPs, differences in the densities of CALR+ neurons across cytoarchitectonic areas were seen in layer III. Areas 1 and 2 contained higher densities of CALR+ neurons than area 3a ($F_r=9.9$, $p<0.02$).

Another comparison of the distributions of neurons containing the three CBPs is
presented in Figure 29. Comparing the distributions of the three CBPs within each layer, it can be seen that only CALR+ neurons are located in layer I. The three CBPs are represented in similar numbers in layer II. Layer III contains all three CBPs, but PV+ neurons are found in greater numbers. PV+ neurons dominate the distributions of CBPs in the remaining layers.

5.4.3 Co-localization of CALR with PV or CALB

Co-localization of CALR and PV was determined by two methods: counting the proportions of CALR+ neurons which were also PV+, and the number of PV+ neurons which were also CALR+. Out of 247 CALR+ neurons studied, none were PV+. Only 1 out of 400 PV+ neurons were CALR+. Out of 228 CALR+ neurons studied, none were CALB+. However, 2 out of 151 CALB+ neurons were CALR+ (Fig 30).

5.4.4 Co-localization of GABA with PV or CALB

Almost all (99.4%) PV+ neurons in layers II-VI were GABA+, and 90% of CALB+ neurons were GABA+ (Fig 30).

5.5 Discussion

5.5.1 Distribution of cortical neurons containing calcium binding proteins

PV, CALB, and CALR labeled three distinct populations of GABAergic neurons in SI. For each CBP, the laminar distribution was similar across the four cytoarchitectonic
areas of SI. However, these patterns varied among the CBPs. CALB+ and CALR+ neurons were located primarily in superficial cortical layers (CALR in layers I and II, CALB in layers II and III), whereas PV+ neurons were located throughout layers II-VI (most densely in layers III and IV). Within the superficial layers, especially layer III, there was a tendency for the density of each CBP to vary across the cytoarchitectonic areas of SI. In general, anterior SI contained more CALB+ neurons and fewer CALR+ neurons than posterior SI. PV+ neurons were densest in areas 3b and 1.

The laminar distribution patterns of PV+ neurons were consistent with studies of other cortical areas. In monkey visual cortex (Blumcke et al., 1990; Hendry et al., 1991) and rat sensorimotor cortex (Van Brederode et al., 1991), PV+ neurons are concentrated in the middle layers (deep layer III, layer IV and upper layer V). These layers receive direct projections from the thalamus.

The laminar distribution patterns of CALB+ neurons was similar to those described in previous reports except for layer V. Studies in area 3b and other areas of the monkey neocortex show that CALB+ neurons are concentrated in superficial (layer II and upper layer III) and deep (layer V) layers (Hendry et al., 1989; 1991; Van Brederode et al., 1991). In the present study, 92% of the CALB+ neurons were located in layers II and III. It is possible that these differences are due to species differences. Pyramidal CALB+ neurons have been reported in monkey SI (DeFelipe & Jones, 1992; van Bederode et al., 1991), but no apparent pyramidal-shaped CALB+ neurons were identified in the present study.

There are no data on the distribution of CALR+ neurons in sensory cortex. In
monkey prefrontal cortex, CALR+ neurons are mainly located in deep layer I and layer II (Conde et al., 1994). CALR+ neurons in layer I have characteristics of Cajal-Retzius cells, and CALR has been used as a marker to study the development of Cajal-Retzius cells in the cortex (Weisenhorn et al., 1994; del Rio et al., 1995).

5.5.2 Colocalization of calcium binding proteins and GABA

Almost all PV+ neurons, and 90% of the CALB+ neurons, in cat SI also contained GABA. These results are in agreement with previous studies in cat visual cortex (Demeulemeester et al., 1991), rat cerebral cortex (Celio, 1986), and monkey striate cortex (van Brederode, 1990).

PV, CALB and CALR were localized in separate neuronal populations, but in rat hippocampus 5.1% of CALR+ and 6.2% of CALB+ neurons are immunoreactive for both CALR and CALB (Mittine et al., 1992). In monkey prefrontal cortex CALB and CALR are co-localized in Cajal-Retzius neurons of layer I (Conde et al., 1994). These differences in co-localization may arise from differences among species, or between non-sensory cortex and sensory cortex.

5.5.3 The correlation of the distribution patterns with the possible function of CBPs

Neurons which contain PV have the characteristics of basket neurons and chandelier neurons (DeFelipe et al., 1989b) while those which contain CALB or CALR resemble double bouquet neurons (DeFelipe et al., 1989a). Each of these morphological classes of neurons has specific synaptic interactions with other cortical neurons. For
example, chandelier neurons form synapses with axon initial segments of pyramidal neurons, whereas basket neurons form synapses around somata. The correspondence of CBP content with morphology suggest that neurons which contain different CBPs may also have different functions.

The physiological roles of these CBPs in neuronal function are not known, but it has been suggested that they may influence neuronal excitability (Baimbridge & Miller, 1984) and patterns of neuronal discharge (Kawaguchi et al., 1987). Recently it was found that PV neurons are fast spiking (Kawaguchi & Kubota, 1993), and metabolically active (McCasland et al., 1993), whereas CALB neurons are low threshold spiking neurons (Kawaguchi & Kubota, 1993). Given these physiological differences, the presence of different types of CBP-containing neurons in different cortical regions or layers might imply function differences. Indeed, Celio (1990) has suggested that PV and CALB might be associated with separate functional systems in the rat brain. In subcortical brain regions PV+ and CALB+ neurons are not exclusively GABAergic, but may instead identify separate thalamic pathways (lateral geniculate nucleus: Johnson & Casagrande, 1993; ventroposterior nucleus: Rausell et al., 1992).

In the cortex PV and CALB are almost exclusively localized in GABAergic neurons. The cortical GABAergic system plays an important role in information processing by exerting strong inhibition on its target neurons. The strength of inhibition varies across cortical regions, layers and types of neuron. For example, blockade of GABA_A receptors on SI neurons results in expansion of receptive fields, but the sizes of the expansions vary between submodality zones and across cortical layers (Dykes et al.,
Bicuculline also has a differential effect on directional selectivity of simple, complex and hypercomplex neurons in cat visual cortex (Sillito, 1977).

Electrical stimulation experiments in cortical slices suggest that laminar distribution differences between CBP+ neurons might underlie laminar differences in inhibitory processes. Stimulation of layer II results in slow IPSPs in pyramidal neurons of all layers. The amplitudes of these slow IPSPs decrease as deeper layers are stimulated. In contrast, fast IPSPs reach maximum amplitude when the stimulus is delivered to the same layer as the target neuron soma, and decrease in amplitude when more superficial layers are stimulated (Hirsch et al., 1991; Kang et al., 1994). The slow IPSPs may arise from GABA<sub>B</sub> receptors located in distal dendrites, whereas the fast IPSPs may arise from GABA<sub>A</sub> receptors located on proximal dendrites and somata. These data, together with the laminar differences of CBP+ neurons described in the present report, suggest that there may be distinct populations of inhibitory neurons which underlie these different forms of IPSPs. For example, CALR+ and CALB+ neurons, which are mainly located in layer II, might act selectively on GABA<sub>B</sub> receptors. PV+ neurons, which are distributed in all cortical layers except for later I, might specifically act on GABA<sub>A</sub> receptors.
Figure 23. Frequency distributions of soma sizes for three types of calcium binding protein-immunoreactive neurons: parvalbumin (PV), calbindin (CALB), and calretinin (CALR).
Figure 24. Camera lucida drawings of examples of PV- (A), CALR- (B) and CALB- (C) immunoreactive neurons.
Figure 25. Photomicrographs of PV- (A), CALB- (B) and CALR- (C) immunoreactive neurons in 16 μm parasagittal sections through area 3b of SI cortex. An adjacent thionin-stained section is shown in (D). Scale bar = 200 μm
Figure 26. Density of PV neurons, by cortical layer, in each of the four cytoarchitectonic areas of SI cortex. Each symbol represents the value from a single animal.
Figure 27. Density of CALB+ neurons, by cortical layer, in each of the four cytoarchitectonic areas of SI cortex. Each symbols represent the value from a single animal.
Figure 28. Density of CALR+ neurons, by cortical layer, in each of the four cytoarchitectonic areas of SI cortex. Each symbols represent the value from a single animal.
Figure 29. Comparison of distributions of three calcium binding protein-containing neurons by cortical layer and area.
Figure 30. Double-labeled sections to illustrate co-localization. A-B, C-D, E-F, and G-H are single sections viewed with different filters. A-B are PV and GABA, C-D are CALB and GABA, E-F are PV and CALR, and G-H are CALB and CALR.
6. CONCLUSIONS

The experiments described in this thesis provide a quantitative description of the GABAergic system in SI. It has been shown previously that blockade of GABA_A receptors by bicuculline iontophoresis results in expansion of the receptive fields of many neurons in SI (Dykes et al., 1984; Alloway et al., 1989). This effect of bicuculline on receptive field size varies with cortical region and layer (Dykes et al., 1984). Neurons in slowly adapting zones of area 3b, and in the middle layers of cortex exhibit smaller enlargements than neurons that lie outside these regions.

The laminar distribution patterns of GABA+ neurons were similar in the four cytoarchitectonic areas SI. The density of these neurons was highest in layer II, then decreased steadily to layer VI. Overall, 25-30% of the neurons in SI were GABA+. The proportions of GABA+ neurons were uniformly distributed throughout all cortical layers except layer I, in which 90% of cortical neurons were GABA+. The distribution of GABA+ neurons was not related to differences in the effects of bicuculline on receptive field size.

Analysis of [^3^H] muscimol binding revealed similar patterns in somatosensory, auditory, and visual cortex, except that binding densities in layers II-VI of visual cortex were approximately twice that of the other areas. In each of the four cytoarchitectonic areas of SI [^3^H] muscimol binding levels were highest in layers I and II and lowest in
layers V and VI. Except for layer I, which contains few neurons, the binding pattern was highly correlated with the laminar distribution patterns of GABA+ neurons. The positive correlation between numbers of GABA+ neurons and GABA$_A$ receptors probably reflects the limited axonal arborization of most cortical GABAergic neurons (Houser et al., 1984).

In SI, receptive field enlargements produced by bicuculline are larger in neurons in rapidly adapting zones than for neurons in slowly adapting zones (Dykes et al., 1984). The present studies revealed that layer IV in the anterior region of area 3b contained 6.3% fewer muscimol binding sites than the posterior region. The rapidly adapting and slowly adapting zones which have been described in area 3b are organized in mediolateral strips (Dykes, 1980; Sretavan and Dykes, 1983). The slowly adapting zone tends to lie in anterior 3b, next to the border with area 3a (Sretavan and Dykes, 1983). Thus, the region of layer IV that contains lower [$^3$H]muscimol binding levels might correspond to the slowly adapting region of area 3b.

The results of the studies of GABAergic neurons and GABA$_A$ receptors suggest that the distribution of these inhibitory markers are not closely related to the degree of receptive field enlargement produced by GABA$_A$ receptor blockade. Instead, receptive field enlargement may depend on the extent of horizontal cortical connections. Conditioning stimuli presented outside the excitatory receptive fields of SI neurons inhibit the responsiveness to stimuli presented inside the fields (Laskin and Spencer, 1979). During receptive field enlargement induced by GABA$_A$ receptor blockade, the latency of activation from the enlarged portion of the receptive field is longer than that from the original receptive field (Alloway et al., 1989). The authors of this study suggested that
horizontal cortical connections might play an important role in controlling receptive field. These horizontal connections might be modulated by inhibitory neurons distributed within and around the cortical representation of the receptive field (Albus et al., 1991).

Injections of retrograde tracers in different cortical layers of area 3b revealed that 71-85% of the labeled neurons are clustered near the injection sites. In contrast to primary auditory and visual cortex, where labeled neurons located further from the injection site lie in clusters (Rockland and Lund, 1983; Levay 1988; Wallace et al., 1991; and Clarke et al., 1993), in SI these neurons were more uniformly distributed. The horizontal extent over which labeled neurons were located varied across layers. Injections into layers II, III and V resulted in more widespread labelling than injections in layer IV. This pattern of horizontal connections corresponded quite well to the pattern of the magnitude of receptive field enlargement produced by bicuculline.

The physiological functions of horizontal cortical connections could be further revealed in future experiments by measuring the effects of horizontal interactions in extracellular recordings from pairs of SI neurons. The strength of excitatory or inhibitory connections formed between pairs of neurons could be detected by cross-correlation analysis. If the balance between excitation and inhibition could be altered, for example, by bicuculline iontophoresis, the inputs from surrounding neurons might change the strength of correlated firing. In other experiments, the origins of horizontal connections could be correlated with body representation.

Although the population of GABA+ neurons was uniformly distributed across the layers of cortex, different classes of cortical GABAergic neurons may have distinct
functions. Cortical GABAergic neurons constitute a heterogenous population with respect to their morphological, chemical and physiological properties, suggesting that there are different classes of GABAergic neurons. In the present experiments, GABAergic neurons were characterized by their content of one of three CBPs: parvalbumin, calbindin or calretinin. Parvalbumin+ neurons had characteristics of classical basket neurons which form pericellular synapses around pyramidal neurons (Jones, 1984). Calbindin+ and calretinin+ neurons had characteristics of classical double bouquet neurons which have synapses on a number of different sites, including the dendritic shafts and somata of nonpyramidal cells (Somogyi and Cowey, 1981).

Each type of CBP marked classes of neurons which had unique distribution patterns. The present experiments are the first to describe these patterns in SI, and the first to describe the distribution pattern of calretinin neurons in any sensory cortical region. The laminar distribution pattern of each of the calcium binding proteins was similar across the four cytoarchitectonic areas of SI. Parvalbumin+ neurons were located in all cortical layers except layer I, with highest densities in layer IV. Calbindin+ neurons were mainly located in layers II and III, with low densities in layer I and the deep cortical layers. Calretinin+ neurons were mainly located in layers I and II, and in decreasing densities to layer VI. These distribution patterns are compatible with the idea that different classes of GABAergic neurons may be responsible for different types of inhibitory postsynaptic potentials in the cortex. Neurons which contain calretinin and calbindin may act specifically on GABA_\text{\textsubscript{A}} receptors to produce slow IPSPs, whereas neurons which contain parvalbumin may act selectively at GABA_\text{\textsubscript{A}} receptors to produce fast IPSPs. It may be
possible to test this idea using co-localization techniques and electron microscopy.

Experiments of the type presented in this thesis may begin to reveal the organization of cortical circuits which control receptive field size. Control of receptive field size may be an essential function of the cortical circuitry within somatosensory cortex. Modulation of receptive field size will affect the performance of the sensory system, and thus perception.
BIBLIOGRAPHY


Hassler, R. and K. Mühs-Clement (1964) Architektonischer Aufbau des

Hendrickson, A.E., S.P. Hunt and J.-Y. Wu (1981) Immunocytochemical localization of

Hendry, S.H.C., E.G. Jones, J. DeFilipe, D. Schmechel, C. Brandon and P.C. Emson
(1984) Neuropeptide containing neurons of the cerebral cortex are also

Hendry, S.H.C. and E.G. Jones (1986) Reduction in number of immunostained GABA
neurons in deprived-eye dominance columns of monkey area 17. Nature
320:750-753.

of GABA immunoreactive neurons in different areas of monkey cerebral cortex. J.
Neurosci. 7:1503-1519.

(1989) Two classes of cortical GABA neurons defined by differential calcium

of immunocytochemically localized GABA_A receptors in adult monkey visual

Hendry, S.H.C. and E.G. Jones (1991) GABA neuronal subpopulations in cat primary
auditory cortex: Co-localization with calcium binding proteins. Brain Res.
543:45-55.
Hicks, T.P., P. Landry, R. Metherate, and R.W. dykes (1985) Functional properties of
neurods mediated by GABA in cat somatosensory cortex under barbiturate and
urethane anesthesia. In M.J. Rowe and W.D. Willis (eds): Development,
Organization, and Processing in Somatosensory Pathways, New York: Alan r.Liss,
pp.265-276.

Hill, D.R. and N.G. Bowery (1981) 3H-Baclofen and 3H-GABA bind to


diversity of immunocytochemically identified GABA neurons in the monkey

Houser, C.R., Vaughn, J.E., Hendry, S.H.C., Jones, E.G. and Peters, A. GABA neurons
in the cerebral cortex. In: Cerebral Cortex: Functional Properties of Cortical
63-89.

Hyvarinen, J. and A. Poranen (1978) Movement-sensitive and direction and
orientation-selective cutaneous receptive fields in the hand area of the post-central
gyrus in monkeys. J. Physiol. (Lond.) 283:523-537.

Iwamura, Y. and M. Tanaka (1978a) Functional organization of receptive fields in the cat
somatosensory cortex. II: Second representation of the forepaw in the ansate


71:280-293.

spiking cells in rat hippocampus (CA1 region) contain the calcium binding protein

Kawaguchi, Y. and Y. Kubota (1993) Correlation of physiological subgroupings of
nonpyramidal cells with parvalbumin- and calbindin_{D_{28k}}-immunoreactive neurons in

Keinanen, K., W. Wisden, B. Sommer, P. Werner, A. Herb, T. Verdoorn, B. Sakmann and
P. Seeburg (1990) A family of AMPA-selective glutamate receptors. Science
249:556-560.

Somogyi (1986) Synaptic targets of HRP-filled layer III pyramidal cells in the cat
striate cortex. Exp. Brain Res. 64:541-552.


and /or glutamic acid decarboxylase-like immunoreactivities in various brain
regions of the rat. Exp. Brain Res. 70:605-617.


Calretinin is present in non-pyramidal cells of the rat hippocampus—II. 
Co-existence with other calcium binding proteins and GABA. Neurosci. 48:29-43.

nurons in the magnocellular and parvocellular layers of the lateral geniculate 

Mountcastle, V.B., P.W. Davies and A.L. Berman (1957) Response properties of neurons 

Mountcastle, V.B. and T.P.S. Powell (1959) Neural mechanisms subserving cutaneous 
sensibility, with special reference to the role of afferent inhibition in sensory 

 receptors in the visual cortex of normal and monocularly deprived cats. Brain Res. 
380:253-260.

Needler, M.C., C. Shaw and M. Cynader (1984) Characteristics and distribution of 
muscimol binding sites in cat visual cortex. Brain Res. 308:347-353.

 infragranular pyramidal neurons in cat primary auditory cortex. Cereb. Cortex 
2:197-216.

Oka, J.-I. and T.P. Hicks (1990) Benzodiazepines and synaptic processing in the spatial 
68:1025-1040.


