Serotonergic Suppression of Interhemispheric Synaptic Potentials in the Rat Prefrontal Cortex

Heather Lynn Read
Loyola University Chicago

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VITA

Heather Read obtained a bachelor of science degree in biochemistry from Texas A & M University in 1982. In 1985, Ms. Read received a masters of science degree in biology under the supervision of Dr. Deborah Armstrong at the University of Texas San Antonio. During the course of her masters work, the author became interested in the functional plasticity of neural networks and pursued that interest by attending a summer course in Neural Systems and Behavior at the Marine Biological Laboratories. The following spring, Ms. Read joined Dr. John Disterhoft's laboratory to investigate hippocampal involvement in Pavlovian or classical conditioning in rabbits.

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<tr>
<td>ACSF</td>
<td>artificial cerebral spinal fluid</td>
</tr>
<tr>
<td>AHP</td>
<td>afterhyperpolarization</td>
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<tr>
<td>APV</td>
<td>D-2-amino-5-phosphono-valerate</td>
</tr>
<tr>
<td>5-CT</td>
<td>5-carboxamidotryptamine</td>
</tr>
<tr>
<td>CNQX</td>
<td>6-cyano-7-nitro-quinoxaline-2-3-dione</td>
</tr>
<tr>
<td>CSC</td>
<td>commissural-schaffer collateral</td>
</tr>
<tr>
<td>DAP</td>
<td>depolarizing afterpotential</td>
</tr>
<tr>
<td>EAA</td>
<td>excitatory amino acid</td>
</tr>
<tr>
<td>DPSP</td>
<td>depolarizing postsynaptic potential</td>
</tr>
<tr>
<td>DPAT</td>
<td>8-hydroxy-2-(di-n-propylamino)-tetraline</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-amino-butyric acid</td>
</tr>
<tr>
<td>5HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>IPSP</td>
<td>inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>NE</td>
<td>norepinephrine</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartic acid</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PSP</td>
<td>postsynaptic potential</td>
</tr>
<tr>
<td>sd</td>
<td>standard deviation of the mean</td>
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<tr>
<td>sem</td>
<td>standard error of the mean</td>
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<tr>
<td>TFMPP</td>
<td>trifluromethylphenylpiperazine</td>
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<td>WM</td>
<td>white matter</td>
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INTRODUCTION

The mammalian prefrontal cortex (PFC) appears to be a neural substrate of attention (Crowne and Pathria, 1982; Fuster, 1980, 1984). Ascribing such a broad cognitive function to a single region of the brain is, of course, misleading if not impossible (Goldman-Rakic, 1984, 1988; Lashley, 1920; Lynch and McLaren, 1989). Nevertheless, the cognitive impairments that develop subsequent to damage to the PFC are remarkably similar in rodents, monkeys and humans. Specifically, animals with unilateral lesions of the PFC develop a hemispatial or contralateral neglect syndrome (Crowne and Pathria, 1982; Fuster, 1980; Heilman and Watson, 1977; Lynch and McLaren, 1989). Frontal lesioned animals can no longer voluntarily attend to or orient towards auditory or visual stimuli in the environment. However reflexive movement of the head and eyes, that is, the vestibulo-ocular reflex, remains intact (Heilman and Watson, 1977; Lynch and McLaren, 1989). A likely neural pathway mediating attentive behavior is the projection from pyramidal neurons in layer V of the PFC to brainstem motor nuclei governing orientation of the head and eyes (Bruce and Goldberg, 1984; 1985; Leichnetz et al., 1987; Neafsey et al., 1986; Sawaguchi et al., 1989; Sikes et al., 1988).

The complement of synaptic inputs to PFC differs from the rest of the cortical mantle, as this region receives dense projections from every known monoaminergic system (Emson and Lindvall, 1979). The density of monoamine containing fibers
from the raphe and midbrain tegmentum is greater in the PFC than in any other region of the cortex, and yet the contribution of serotonergic and dopaminergic inputs to frontocortical processing is unknown. The ascending monoamine and cholinergic projections have been collectively described as a non-specific activating system. This concept was largely derived from the observation that monoaminergic and cholinergic projections to the cortex are diffuse in comparison to the majority of thalamocortical projections which are distributed in restricted cortical domains (Saper, 1987; Diamond, 1979; DeFelipe and Jones, 1988; Fallon and Loughlin, 1982; Lorente de No, 1949). Though diffuse monoamine projections exist, they appear to occur in tandem with projections to restricted topographical and laminar domains across cortices (Morrison et al., 1982; O’Hearn and Molliver, 1984; Rakic et al., 1988; Waterhouse et al., 1983a, 1983b). As monoamine neurons projecting to a region generally do not collateralize to innervate other cortical regions, they could govern the activity of local regions of cortex (Beckstead, 1976; Berger et al., 1976, 1991; Lindvall et al., 1974; Loughlin and Fallon, 1984; O’Hearn and Molliver, 1984; Sobel and Corbett, 1984). In primates, chemical lesions of monoamine input to the PFC disrupt the cognitive processes subserved by this region of cortex without affecting sensory or motor cortical function (Arnsten and Goldman-Rakic, 1985, 1986; Brozoski et al., 1979). Monoamine lesions have been less successful in studying rodent cognitive processes. This may be due to species variation, but experimental conditions and behavioral tasks could also account for these differences (Lorens, 1978). In rodents, characterizing the effects of exogenously applied monoamines on
sensory cue evoked patterns of cortical activity has been a more fruitful approach to investigating monoamine influences on cortical processing.

Monoamines appear to differentially gate sensory afferent activation of cortical neurons. Locus ceruleus activation or exogenously applied norepinephrine (NE) sharpens the receptive field properties of sensory cortical and thalamic neurons without disrupting the basic pattern of activity evoked by sensory stimuli (Rogawski and Aghajanian, 1980; Waterhouse et al., 1986, 1990). In contrast, stimulation of raphe input or application of 5HT disrupts the excitation of cortical and thalamic neurons by sensory afferent activation (Eaton and Salt, 1989; Marks et al., 1987; Waterhouse et al., 1986, 1990; Yoshida et al., 1984). Hence, 5HT and NE can differentially modulate individual cortical neurons. 5HT and NE may exert these effects via actions on spatially remote cortical neurons because there is little overlap in distribution of 5HT and NE fibers in sensory cortices (Morrison et al., 1982). Since activation of raphe and locus ceruleus neurons is linked to the presentation of sensory stimuli, excitation of cortical neurons must rely on the timing and balance of monoamine and sensory afferent input (Heym et al., 1982; Shima et al., 1986).

Serotonergic disruption of cortical activity may be attributed to an attenuation of sensory afferent, thalamocortical, or corticocortical transmission. Attenuation of sensory afferent excitation of cortical and thalamic neurons could be due to a suppression of sensory afferent transmission or to a non-selective suppression of
neuronal excitability. A selective suppression of sensory afferent or thalamocortical transmission within the cortex is likely because 5HT fibers overlap with thalamic and sensory afferent terminal fields (Morrison et al., 1982; Rakic et al., 1988). Unlike the sensory cortices, PFC lacks a layer IV and the highly organized thalamic and sensory afferent projections associated with that layer. Nevertheless, this region does receive thalamocortical and corticocortical afferent input. Furthermore, serotonergic fibers are concentrated in layer V and the lower portion of layer II-III (III) where thalamic and transhemispheric cortical fibers converge on apical dendrites of layer V neurons (Krettec and Price, 1977; Vogt et al., 1981). As corticocortical, thalamocortical and sensory afferent pathways all appear to be excitatory amino acid releasing (EAA), 5HT may selectively modulate EAA transmission in the cortex. There is precedence for such a thesis, as 5HT attenuates monosynaptic EAA transmission between dorsal horn sensory afferents and spinal motor neurons (Wang and Dun, 1990).

A broad objective of this thesis was to understand how monoamines influence cortical processes. The hypothesis posed was that 5HT suppresses interhemispheric EAA transmission in rat PFC. To this end, it was necessary to establish that the EPSP under study was in fact an EAA EPSP. Part I of this thesis was concerned with the isolation and characterization of transhemispheric corticocortical EPSPs recorded in layer V cells of the rat PFC. Part II was concerned with characterizing the site and mechanism of 5HT induced suppression of corticocortical transmission.
BACKGROUND I: Cortical EAA synaptic potentials

Layer V neurons of the rat PFC assimilate excitatory inputs from a variety of sources including the mediodorsal thalamus, the primary sensory and contralateral cortices and from neighboring cells in layers III and V. Cortical and thalamic fibers converge on dendrites of pyramidal and non-pyramidal neurons in layer V. Fibers from contralateral pyramidal neurons course through the dorsal corpus callosum to layer V where they synapse on the soma and proximal dendrites of pyramidal, aspiny stellate, and spiny stellate (multipolar) neurons (DeFelipe and Jones, 1988; Ferino et al., 1987; Gerfen, 1989; Lorente de No, 1949; Sesack et al., 1989; Vogt and Gorman, 1982; Vogt et al., 1981). Thalamic fibers ascend through the striatum (Scheibe! and Scheibe!, 1967) to the PFC where they synapse superficially on dendrites of pyramidal and non-pyramidal neurons in layers III and V (Krettek and Price, 1977; Vogt et al., 1981; Valverde, 1986).

Both thalamocortical and corticocortical fibers apparently release one of the excitatory amino acids (EAAs), glutamate or aspartate (Conti et al., 1989; Mayer and Westbrook, 1987; Ottersen et al., 1983). Thalamic and cortical fibers are glutamate immunoreactive, release glutamate, and have a high affinity EAA uptake system (Baughman and Gilbert, 1981; Beart et al., 1990; Conti et al, 1989; Cotman et al., 1987; Fonnum et al., 1981; Fosse and Fonnum, 1987; Hassler et al., 1982; Monaghan et al., 1989; Peinado and Mora, 1986). Pyramidal and multipolar neurons are
immunopositive for glutamate and the synthetic enzyme glutaminase (Ottersen and Storm-Mathisen, 1984; Kaneko and Mizuno, 1988). Furthermore, the postsynaptic targets of thalamic and cortical terminals express EAA receptors and are depolarized by exogenously applied EAA agonists (De Curtis et al., 1989; Eaton and Salt, 1989; Herling, 1985; Thomson, 1986b).

Multiple EAA receptor subtypes exists and can be pharmacologically distinguished by their relative affinities for aspartate and glutamate (Collingridge and Lester, 1989; Mayer and Westbrook, 1987; Monaghan et al., 1989). The aspartate preferring receptors are activated by the amino acid analogue N-methyl-D-aspartic acid (NMDA) and selectively antagonized by D-2-amino-5-phosphono-valerate (APV). Receptors that have a low affinity for NMDA and are not antagonized by APV are collectively classified as non-NMDA receptors. Non-NMDA receptors are selectively antagonized by quinoxaline derivatives such as 6-cyano-7-nitro-quinoxaline-2-3-dione (CNQX). NMDA and non-NMDA receptors are associated with distinct cation permeable channels.

NMDA and non-NMDA receptor mediated changes in membrane potential are physiologically distinct in several respects. Most notably, NMDA induced depolarizations do not increase in amplitude as the membrane is hyperpolarized from resting potentials (Mayer and Westbrook, 1987). This property is an anomaly, as hyperpolarization typically increases cationic potentials presumably by increasing the
electrochemical gradient governing net cation flux across the membrane. As a consequence of the anomalous or unconventional voltage dependence, NMDA potentials contribute relatively little to small amplitude mixed EAA EPSPs when the membrane is hyperpolarized (Collingridge et al., 1986). In contrast, non-NMDA EPSPs are large when the membrane is hyperpolarized. Thus, mixed EAA EPSPs are predominantly non-NMDA potentials under resting conditions. NMDA EPSPs are effectively unmasked by tetanitic activation and membrane depolarizations (Muller and Lynch, 1990). The unconventional voltage dependence of NMDA synaptic potentials has been attributed to blockade of the NMDA ion channel by divalent cations, in particular magnesium. Consequently, when the tissue magnesium ($\text{Mg}^{2+}$) concentration is lowered NMDA potentials display a conventional voltage dependence. NMDA potentials appear to be preferentially enhanced by coincident exposure to a variety of neurotransmitters including glycine, acetylcholine and 5HT (Minota et al., 1990; Markram and Segal, 1990; Nedergaard et al., 1986; Reynolds et al., 1988; Thomson et al., 1989). NMDA potentials are also uniquely associated with the activation of oscillating membrane depolarizations or bursts (Dingledine, 1983; Thomson, 1986b). Comparable depolarizations induced by non-NMDA receptor activation are generally not associated with bursts. Little is known about these bursts, though they may be attributed to a metabotropic effect of the NMDA receptor activation. Furthermore, the facilitation or inhibition of NMDA potentials by some transmitters could be due to modulation of these currents.
EAA pathways in the cortex are physiologically, pharmacologically and anatomically heterogeneous. Of all the cortices, characterization of hippocampal EAA pathways is the most complete. For example, two distinct EPSPs are generated at intrahippocampal EAA synapses. First, the EAA EPSP generated by mossy fiber input to CA3 pyramidal neurons is predominantly mediated by non-NMDA receptors. In contrast, the EAA EPSP generated by the mixed activation of commissural and Schaffer collateral (CSC) inputs to CA1 pyramidal neurons consists of NMDA and non-NMDA components. Accordingly, the CSC-CA1 EPSP is preferentially augmented by conditions that enhance NMDA potentials including membrane depolarization, tetanic stimulation, low extracellular Mg\(^2+\) concentration and saturating extracellular concentrations of glycine (Harris and Cotman, 1986; Harris et al., 1984; Mayer and Westbrook, 1987; Muller and Lynch, 1990; Minota et al., 1989). Furthermore, NMDA receptors are concentrated in the region of CSC termination but sparse in the region of mossy fiber termination (Cotman et al., 1987). Mossy fiber and CSC pathways are anatomically distinct, as only the former is associated with large diameter zinc containing bouton terminals which synapse *en passant* on CA3 pyramidal neurons (Hamlyn, 1962; Haug, 1967).

Few distinctions have been made between EAA pathways in the neocortex. In somatosensory cortex, an EPSP generated by the simultaneous stimulation of thalamic and cortical fibers has both NMDA and non-NMDA components but the source of each component is unresolved (Thomson et al., 1989). Similarly, an
interlaminar corticocortical EPSP observed in the PFC has both EAA components (Hirsch and Crepel, 1990; Jones and Baughman, 1988). In contrast, a putative thalamocortical, possibly intracortical, EPSP in the same region lacks an APV sensitive component (Sutor and Hablitz, 1989b). Thalamocortical and sensoricortical EPSPs generated in the motor cortex appear to be distinct, as only the former is subject to long term potentiation with tetanic activation of both pathways (Iriki et al., 1989). An anatomical dichotomy between EAA pathways also may exist, as some thalamocortical fibers form large bouton synapses akin to those of the mossy fiber projection (Hamlyn, 1962; Vogt et al., 1981). Furthermore, one of the EAA pathways appears to be associated with intensely staining zinc fibers and high densities of kainic acid receptors (Zilles et al., 1990).

The characterization of monosynaptic neocortical EPSPs has been hindered somewhat because polysynaptic intracortical IPSPs often mask the NMDA component of the EPSP. In the visual cortex, stimulation of the optic radiation (sensory afferents) evokes a monosynaptic EAA EPSP which is partially masked by a polysynaptic IPSP (Artola and Singer, 1987). If the IPSP is blocked with bicuculline, a late APV sensitive component is revealed or enhanced. The intralaminar EPSP generated in layer V cells by stimulation of layer III also has a late NMDA component which can be masked by an overlapping IPSP (Hirsch and Crepel, 1990; Jones and Baughman, 1988). Apparently, this bicuculline sensitive IPSP arises from small gamma-amino-butyric acid (GABA) releasing neurons located
in layers III, IV and V that synapse with the somata and dendrites of pyramidal and non-pyramidal neurons (Meinecke and Peters, 1987; McCormick and Prince, 1986). Polysynaptic IPSPs are recruited with stimulation of thalamocortical, corticocortical and sensory afferents (Artola and Singer, 1987; Purpura et al., 1964). The IPSP can be experimentally attenuated by using lower stimulus intensities because it generally has a higher threshold than the monosynaptic EPSPs (Connors et al., 1982; Jones and Baughman, 1988).

Polysynaptic intracortical EPSPs can be evoked with mixed or isolated stimulation of thalamocortical and corticocortical pathways. A polysynaptic EPSP evoked with stimulation of the thalamus has attracted much attention, as it is associated with the synchronous activation of deep cortical neurons (Purpura et al., 1964). This potential was originally believed to be thalamic in origin because there is a direct thalamocortical innervation of layers III, IV and V. However, it is probably generated by cortical neurons, as it persist in thalamic lesioned animals and can be generated by antidromically activating projection neurons in layers V and VI (Ferster and Lindstrom, 1986a, 1986b; Takahashi et al., 1966). Intracortical polysynaptic EPSPs and IPSPs most likely arise from EAA pathways since they are highly sensitive to EAA antagonists (Hirsch and Crepel, 1990; Thomson, 1986; Sutor and Hablitz, 1989b, 1990).

**Conclusion** In spite of the probable heterogeneity of EAA projections to
neocortex, few investigations of isolated cortical or thalamic EPSPs have been made. Neocortical EPSPs are generally elicited by stimulating the subcortical WM where sensory and association cortical fibers merge with interhemispheric cortical and thalamic fibers prior to their ascent. Interhemispheric EPSPs can be evoked in isolation from thalamocortical and sensoricortical EPSPs if stimuli are delivered to the dorsal corpus callosum (Vogt and Gorman, 1982). Interhemispheric connections are particularly prominent in motor and association cortices such as the PFC; however, the EAA receptors mediating interhemispheric transmission have not been characterized.

**Cortical cell types and synaptic inputs** The repertoire of synaptic potentials should vary between cortical cell types according to differences in the complement of afferents innervating distinct populations of cells. For example, cortical association fibers primarily terminate on superficial layer III neurons in motor and sensory cortices. Accordingly, monosynaptic association fiber EPSPs are more frequently observed in superficial layer III neurons (Kosar et al., 1985). Similarly, interlaminar differences in 5HT receptor and fiber distribution exist (Bradshaw et al., 1983; Pazos et al., 1985; Pazos and Palcios, 1985; Steinbusch, 1984; Zilles et al., 1990). Intralaminar differences also exist, as the density of GABAergic synaptic input to layer V callosal projecting pyramidal neurons is greater than that of layer V-VI thalamic projecting pyramidal neurons (Farinas and DeFelipe, 1991). Though distinctions between callosal and thalamic projecting neurons have not been made,
layer V pyramidal cells have been associated with a more prominent IPSP than multipolar cells from the same region (Chagnac-Amitai et al., 1990).

Cortical neurons are typically grouped according to their intracortical and extracortical projections (DeFelipe and Jones, 1988; Chagnac-Amitai et al., 1990; McCormick et al., 1985; Gerfen, 1989; Gilbert, 1983; Hubener et al., 1990; Lorente de No, 1949; Neafsey et al., 1986; Thierry et al., 1983). Pyramidal neurons in layers III, V and VI project through the dorsal callosum to the contralateral cortical and subcortical targets or through the striatum to their respective subcortical target sites (PFC, Vogt and Gorman, 1982; Gerfen, 1989). Pyramidal and multipolar interneurons in layer V project locally to neurons in layers V and VI but do not send collaterals to the callosal or subcortical WM. Hence, antidromic activation from the WM can distinguish between interneurons and projecting neurons but not between neurons in layers III, V and VI. However, different lamina of coronal slices can be visually identified with a field microscope. Layer V is relatively broad in the rat dorsomedial PFC and extends from about 450 to 850 µM below the pial surface (Krettek and Price, 1977).

Cortical neurons can be physiologically categorized according to their pattern of activity observed intracellularly as burst, regular or fast spiking (McCormick et al., 1985). The characteristically large post-spike afterhyperpolarization (AHP) of regular spiking neurons tends to lengthen the interval between action potentials
generated by prolonged depolarizations to threshold. In contrast, the AHP of fast spiking neurons is minimal in comparison to regular spiking neurons, and hence multiple action potentials are generated in rapid succession in response to prolonged depolarizations. Fast spiking neurons are technically difficult to impale due to the small diameter of their soma. The aspiny stellate morphology of fast spiking cells resembles that of gamma-aminobutyric acid decarboxylase or GAD positive inhibitory neurons found in layers II through VI in the rodent neocortex (McCormick et al., 1985). A burst or cluster of action potentials riding on a slow depolarizing envelope is characteristic of burst spiking neurons (McCormick et al., 1985; Montoro et al., 1988). Lucifer yellow filled regular and burst spiking neurons are pyramidal or multipolar in shape (Chagnac-Amitai et al., 1989; Connors et al., 1982; Tseng and Haberly, 1989a). Furthermore, burst spiking neurons are typically found in the deep layers of neo-and-palleocortex and in the CA3 region of the hippocampus. The expression of burst firing is a plastic property, as regular spiking cells display burst firing properties when exposed to bicuculline, kainic acid or high potassium (Artola and Singer, 1987; Westbrook and Lothman, 1983).

As there may be differences in the repertoire of callosal evoked potentials in different cell types, one objective of this thesis was to physiologically characterize superficial layer V cells. Recordings described in Parts I and II were restricted to superficial layer V because these neurons receive prominent projections from the contralateral cortex and the dorsal raphe. Neurons were characterized as callosal
projecting if they were antidromically activated from the dorsal corpus callosum. Furthermore, neurons were physiologically characterized according to membrane firing properties, resting potential and input resistance. Distinctions between cell types are only discussed in Part I since there were no obvious differences in type of synaptic potentials observed between cell type. The lack of differences may have been due to recording from a relatively homogeneous cell population.

The primary aim of the experiments described Part I was to characterize one of the EAA inputs to layer V of the rat PFC, namely the transcallosal cortical input. The PSPs generated at the transcallosal layer V-layer V synapses were recorded in isolation from thalamocortical and sensoricortical EPSPs (Vogt and Gorman, 1982). Short and long latency callosal evoked potentials were physiologically and pharmacologically characterized.
Methods I

Cortical slices were prepared as described previously (Read et al., 1990; Vogt and Gorman, 1982). Adult male Sprague-Dawley rats (175-200 g) were anesthetized with halothane and decapitated. The brain was removed and a frontal block of tissue containing both hemispheres (Figure 1) was prepared and glued to a petri dish containing cold (4-10°C), oxygenated artificial cerebral spinal fluid (ACSF). 500 µm supragenual, coronal slices were cut with a vibratome tissue slicer (Figure 1, A and B). The entire procedure was completed within 10 minutes to insure viability of the tissue. Following a 1 hour incubation at room temperature, the slice was transferred to a submersion recording chamber where it was secured between two silk nets and superfused with warm (32 ± 1°C), oxygenated ACSF of the following composition (in mM): NaCl, 118; KCl, 3; NaH₂PO₄, 1.2; MgCl₂, 1.3; CaCl₂, 2; NaHCO₃, 25 and glucose, 11. ACSF flow rates were maintained between 2 and 4 ml/min.
Glass microelectrodes filled with 4 M potassium acetate (40 to 100 MΩ impedance) were used for intracellular recordings. Current clamp recordings were made with an Axoclamp-2A preamplifier and transformed from analog to digital waveforms for online monitoring and stored for future analysis. Recording electrodes were placed in superficial layer V of the dorsal anterior cingulate and precentral motor areas (Figure 2, Record). Bipolar platinum stimulating electrodes were inserted into the dorsal third of the corpus callosum (Figure 2, CC) for orthodromic and antidromic activation of layer V neurons. Callosal evoked potentials were no longer observed when the callosal white matter between the recording and stimulating electrodes was severed. Hence, these potentials originated from callosal projecting fibers. Higher stimulus intensities were required to evoke responses when the slices were submerged in fluid as opposed to being maintained in a gas-fluid interface. However, the submersion chamber was preferred for our studies, as drug exchange was much more reliable.
EAA antagonists were bath applied in cumulative concentrations. Antagonists caused little or no change in resting membrane potential, input resistance and antidromic potentials. The effects of APV were reversible within 15 minutes of terminating application. In contrast, an hour or more was required to reverse the effects of CNQX (N=2). Hence, in most cases complete reversal was not obtained when cells were exposed to CNQX.
Characterization of Neurons

Neurons impaled in the upper half of layer V were characterized according to their firing pattern evoked by prolonged (≥ 150 ms) depolarization (Figure 3; McCormick et al., 1985). The most common (41/68) cell type was the regular spiking neuron which had a pronounced biphasic post-spike afterhyperpolarization and readily accommodated during prolonged depolarizations. About 40% of the cells displayed a frank burst (14/68) or a post-spike depolarizing afterpotential (DAP; 12/68) upon depolarization from rest (Montoro et al., 1988; Changnac-Amitai et al., 1990). With greater depolarization, the envelope of the DAP was large enough to evoke two or three spikes. When filled with lucifer yellow DAP and burst firing cells have been found to correspond to large pyramidal and multipolar neurons found in layer V (McCormick et al, 1985; Changnac-Amitai et al., 1990). It is likely a similar population was sampled in the present study since large pyramidal and multipolar type neurons were labelled with lucifer yellow in the recording region.

As there were no apparent differences with respect to cell type, values for the following membrane properties were pooled. Resting membrane and spike or burst threshold potentials were 76 ± 0.6 and 50 ± 0.1 mV respectively; and action potential overshoot and amplitude were 24 ± 1 and 74 ± 1 mV respectively (mean
Current-voltage (I-V) relations were obtained by measuring the peak steady-state (≥ 150 msec) voltage deflection generated by transient depolarizing or hyperpolarizing current steps. The current was stepped from rest (0 nA) to various levels between -600 and 400 pA. Over this range the I-V relations were linear (R ≥ 0.99) for the majority of cells (Figure 13B). In less than 10% of the cells, the slope of the depolarizing region of the I-V relation was steeper than that of the hyperpolarized region (Thomson, 1985). This non-linearity was not peculiar to any one cell type. The mean input resistance determined from the slope of the I-V relation generated with hyperpolarizing current steps was 55.5 ± 3 MΩ (N=68).

Only 8% (7/68) of the cells studied were antidromically activated by callosal stimulation. Antidromic spikes were identified as all or none potentials displaying a constant latency at various stimulus frequencies. The latency to onset for the antidromic potential was ≤ 1 msec. As the recording electrode was located a distance of 2 mm from the stimulating electrode, the axonal conduction velocity was ≤ 2 m/sec. At resting membrane potential the antidromic spike often failed to invade the soma; but upon depolarization an antidromic spike was observed (Figure 3; Thomson, 1985).

**Intensity Dependence of the Callosal Evoked Potentials**

Four distinct synaptic potentials were elicited by callosal stimulation: 1) a short latency, early peaking, depolarizing postsynaptic potential (e-DPSP); 2) a late
peaking, frequency sensitive, depolarizing postsynaptic potential (l-DPSP); 3) an early peaking hyperpolarizing or inhibitory postsynaptic potential (e-IPSP) and 4) a late peaking IPSP (l-IPSP). As illustrated in Figure 4, the e-DPSP and the l-DPSP were generally the lowest threshold. The e-DPSP amplitude increased with increasing stimulus intensity reaching a maximum of 5 to 10 mV (Figure 3). Recruitment of the l-DPSP was notable due to the large amplitude (10-20 mV) and long duration of this potential (Figure 4; see arrows). At higher stimulus intensities the e-and-l-IPSPs followed the e-DPSP (Figures 3, 6 and 7).

There was often a plateau in the e-DPSP stimulus-response relation prior to detection of an IPSP (Figures 5, 6 and 11; Jones and Baughman, 1988). As the stimulus intensity was increased above the plateau level, the amplitude of the e-DPSP recorded at resting membrane potential continued to rise (Figure 6). However, when the membrane potential was depolarized from rest, the late component (40 msec) of the e-DPSP decreased with increasing stimulus intensities until a net negative e-IPSP was detected (Figure 6). The amplitudes of the e-DPSP at 40 msec and the e-IPSP peak at 25 msec are plotted against stimulus intensity to illustrate the loss of the late component with recruitment of inhibition at higher stimulus intensities (Figure 6). Similarly, the l-DPSP diminished with recruitment of inhibition as shown in Figure 4 (1.2 versus 1.4 mA). Generally, a remnant of the e-DPSP remained at higher stimulus intensities giving rise to an EPSP-IPSP sequence (Figures 4, 6 and 7).
**Late Polysynaptic Potentials**

**EPSP-IPSP Sequence**  If stimulus intensities were adjusted to obtain an EPSP-IPSP sequence, three peaks were evident: the e-DPSP; the e-IPSP and the l-IPSP (Figure 7A). In these experiments, the peak amplitude of each potential was measured at a fixed latency over a range of holding membrane potentials. Though IPSPs were observed in 12 cells with higher stimulus intensities, clear biphasic IPSPs were only observed in 6 of these cells. When the membrane potential was held between -50 and -65 mV, the peak latencies for the e-and-l-IPSPs were $33 \pm 1$ and $167 \pm 6$ msec respectively (mean ± sem; N=6). The e-IPSP was positively directed when the membrane potential was held between -70 and -94 mV and reversed polarity when the membrane potential was depolarized beyond -68 mV (N=6). This reversal potential is near that of the chloride dependent e-IPSP observed throughout the cortex (Connors et al., 1982, 1988; Hirsch and Crepel, 1990; Jones and Baughman, 1988; Scholfield, 1978). The l-IPSP apparently reversed at a membrane potential more negative than -94 mV (N=6).

The amplitude of the depolarizing peak of the EPSP-IPSP sequence was larger at hyperpolarized potentials (Figure 7, filled squares). Therefore, the slope of the voltage relation characterizing the high intensity e-DPSP was conventional. However, the e-IPSP clearly overlapped with and shunted the late component of the e-DPSP (Figure 6). Thus, at higher intensities the e-DPSP shape and voltage dependence
was a product of the e-IPSP as well. If the e-IPSP was somatic in origin it would have greatly influenced the shape and voltage dependence of the e-DPSP (Rall, 1967). Accordingly, when the e-IPSP was eliminated by adjusting the stimulus below threshold for the e-IPSP, the steep negative slope voltage dependence of the e-DPSP was no longer observed.

Initially we had intended to block the e-IPSP with the GABA_A antagonist, bicuculline (0.1 µM). However, in the presence of bicuculline a 1-DPSP and eventually a giant bursting depolarization were evoked by callosal stimulation (N=2; see also, McCormick et al, 1986). We avoided using bicuculline in subsequent experiments because it was virtually impossible to distinguish between synaptic potentials under these conditions. As an alternative, the stimulus intensity was adjusted below threshold for the e-IPSP to study the S-and-1-DPSPs in isolation.

**Physiology and Pharmacology of the 1-DPSP**

A 1-DPSP was evoked with stimulus intensities below threshold for the IPSPs in about 30% of the cells. Callosal evoked 1-DPSPs were not detected in all cells although they were observed in all cell types. In 16% of the cells (11/68) a high threshold 1-DPSP was detected, whereas an isolated low threshold 1-DPSP was evoked in 13% of the cells (9/68). The low threshold 1-DPSP was only observed with anodal stimuli, whereas the high threshold 1-DPSP was observed with either polarity.
Isolating the e-DPSP from the l-DPSP was difficult in some cases because there was some overlap between the two. At resting membrane potential, the average peak latencies for the low and high threshold l-DPSPs were 34 and 30 msec respectively and the average onset latency of the latter was 16.5 msec. Thus, the range of peak latencies for the l-DPSP overlapped with that of the e-DPSP.

Several measures were taken to identify and eliminate the l-DPSP in order to isolate the e-DPSP. In contrast to the e-DPSP, l-DPSPs were extremely labile. The onset and peak latencies fluctuated as much as 30 msec with increases in stimulus intensity and frequency, as is typical of polysynaptic PSPs (not shown). Furthermore, when the stimulus frequency was greater than 0.03 Hz the high threshold l-DPSP amplitude varied considerably with repetitive activation (Figure 4A versus 4B, arrows). l-DPSPs were also particularly sensitive to EAA antagonists. In the example illustrated in Figure 8 the l-DPSP was the lowest threshold potential evoked by anodal stimuli. Conversely, a two component e-DPSP-l-DPSP sequence was evoked with the reverse polarity (same intensity) in the same cell. The e-DPSP decay was shallow and monophasic, whereas, the e-DPSP-l-DPSP sequence was marked by two decay phases. 1 μM CNQX abolished and 10 μM APV reduced or abolished the low threshold l-DPSP. In another cell, the e-DPSP was the lowest threshold response with either stimulus polarity (Figure 9). A high threshold l-DPSP was evoked with anodal stimuli. As in the previous example, the l-DPSP was preferentially abolished by a low concentration of CNQX (1 μM). Thus, the e-DPSP
was isolated from the l-DPSP by testing for frequency sensitivity, lowering the stimulus intensity and by bathing the tissue in 1 \( \mu \text{M} \) CNQX.

**Physiology and Pharmacology of the e-DPSP**

Callosal evoked e-DPSPs were observed in cells displaying regular (N = 42), burst (N = 14) and DAP (N = 12) firing patterns. This finding was anticipated, as contralateral pyramidal neurons project via the callosum and terminate on both multipolar and pyramidal neurons in layer V (Vogt and Gorman, 1982). The range of latencies of the e-DPSP was the similar for all cell types, 2.8 msec ± 0.1 (mean ± sem; N = 68). In antidromically activated cells the e-DPSP immediately followed the antidromic spike.

A stimulus-response curve was generated for each cell prior to determining the voltage dependence and antagonist sensitivity of the e-DPSP (Figure 3). Those cells displaying a steep stimulus response curve were not used, as the threshold for the l-DPSP was near that of the e-DPSP. Stimulus intensities were adjusted to plateau levels in order to make comparisons between cells and to eliminate or reduce the magnitude of overlapping polysynaptic PSPs. Plateau level e-DPSPs were generally observed with stimulus intensities of 2 to 3 mA.

*Voltage Dependence of the Callosal e-DPSP.* In contrast with the e-DPSP evoked
with supramaximal stimuli, the plateau level e-DPSP amplitude increased with depolarization from rest (compare Figs. 5B and 11A, filled squares). Hyperpolarizing and depolarizing current steps (≥ 200 msec; < 1 nA) were administered prior to stimulating the callosum to determine whether the e-DPSP amplitude varied with membrane potential. The e-DPSP was evoked during the steady-state phase of the voltage deflection, as the membrane potential I-V relation was linear in this range (Figure 13B, open squares). The slope of the e-DPSP voltage relation was positive and therefore unconventional. However, the slope of the voltage relation generally approached zero at membrane potentials negative to rest. Furthermore, the I-V relation associated with the peak of the e-DPSP was not significantly different than that of the membrane potential (Figure 13B). Hence, the current flow associated with the e-DPSP had little effect on the somatic membrane input resistance.

**Sensitivity to APV and CNQX.** The effects of CNQX and APV on the callosal e-DPSP were qualitatively different. The NMDA antagonist, APV (20 µM), reduced the mean e-DPSP (8.7 ± 1.4 mV) by 20 % (i.e., 1.8 mV) and the total area by 41% (N=5; Table I; Figure 10). The onset latency and rising slope of the e-DPSP were not reduced by APV, whereas, the slope of decay and total duration were. Furthermore, the APV sensitive late component of the e-DPSP diminished with hyperpolarization, as expected for an NMDA receptor mediated potential (Figure 10). In contrast, the non-NMDA antagonist, CNQX, invariably reduced both the peak amplitude and the rising slope of the e-DPSP (N=7; Table I; Figure 11).
Furthermore, the effects of CNQX were concentration dependent. As illustrated in Figure 11, submaximal concentrations of CNQX (0.3 and 1 µM) decreased the rising slope and plateau amplitude of the e-DPSP. The stimulus-response relation was shifted to the right but the threshold was unaffected (Figure 11A). The mean plateau level e-DPSP amplitude, rising slope and total area were reduced by 52%, 37% and 61% in 1 µM CNQX (N=7) and by 79%, 67% and 78% in 3 µM CNQX (N=4; Table I). Up to 5 µM CNQX caused no further reduction in the e-DPSP.

In the presence of CNQX, APV reduced the rising slope and peak amplitude of the remaining e-DPSP (Figure 12; Table I; N=5). Together 3 µM CNQX and 10 µM APV, reduced the mean plateau level e-DPSP recorded at resting potentials by 93% (N=2). In the presence of 1 µM CNQX and 10 µM APV the mean reduction was only 70% (N=3; Table I). As 10 µM is a relatively high concentration of APV, it was assumed that the e-DPSP remaining in 1 µM CNQX and 10 µM APV was in fact APV insensitive. Thus, under the three conditions tested, about 20% of the e-DPSP peak amplitude was APV sensitive. In some cells, APV reduced a greater proportion of the e-DPSP recorded at depolarized potentials (Figure 13). The two components were most likely generated by the same set of fibers since the threshold and onset latency of the APV and CNQX sensitive components were the same.
Discussion I

The majority of neurons in the present study were probably local or ipsilateral projecting neurons. More than 90% of the superficial layer V neurons were not antidromically activated from the callosum. Thus, these neurons did not project contralaterally or their contralateral projections were not within the plane of the slice. Ferster and Lindstrom (1985b) found that superficial layer V neurons were not antidromically activated from the superior colliculus or the thalamus, thus a subpopulation of intracortical projecting neurons may exist in superficial layer V. The morphologic and physiologic properties of neurons characterized in this study resembled those of neurons that give rise to local or horizontal projections within layers V and VI (McCormick et al., 1985; Changnac-amitai et al, 1990; Vogt and Gorman, 1982).

Polysynaptic Potentials

*EPSP-IPSP sequence* As our aim was to characterize the direct excitatory connections between cortical neurons, it was necessary to differentiate these from the polysynaptic potentials evoked with callosal stimulation. The e-DPSP, generally the lowest threshold potential, increased in amplitude with increasing stimulus intensity to a plateau or maximal level above which polysynaptic inhibitory and excitatory potentials were evoked.
The callosal EPSP-IPSP sequence, evoked with higher stimulus intensities, was the same as previously described grey or white matter evoked potentials recorded throughout the rodent neocortex. The early inhibitory component (e-IPSP) reversed between -68 and -78 mV, whereas, the late inhibitory component (l-IPSP) apparently reversed at a potential negative to -90 mV. The mean peak latencies of early and late IPSPs were 33 and 167 msec respectively. The time course and reversal potential of the e-IPSP resembled those of the chloride mediated IPSPs generated by GABA$_A$ receptor activation (Connors et al., 1988; Scholfield, 1978). The l-IPSP resembled the slow, potassium mediated IPSP that has been characterized as GABA$_B$ receptor mediated (Connors et al., 1988; Howe et al., 1987; Karlson et al., 1988; Newberry and Nicoll, 1984). Early and late IPSPs presumably arose from polysynaptic EAA pathways since callosal projections arise almost exclusively from pyramidal neurons. Accordingly, blockade of EAA transmission with CNQX blocked IPSPs evoked with stimulation of the grey or white matter (Hirsch and Crepel, 1990; Hablitz and Sutor, 1990). Because of the overlap in time course, the e-IPSP dramatically influenced the shape and voltage dependence of the e-DPSP (Figures 7 and 13). The depolarizing peak of the EPSP-IPSP sequence increased with hyperpolarization apparently due to an increase in the reversed (depolarized) e-IPSP which shunts the late APV sensitive component of the e-DPSP (Figure 6; Hirsch and Crepel, 1990; Jones and Baughman, 1988).

I-DPSPs Polysynaptic EPSPs have been observed under a variety of conditions and
may even be characteristic of foci generating synchronous cortical activity. In the present study, we restricted our recording to the superficial band of layer V which receives direct excitatory inputs from contralateral layer V and superficial layer III. Layer III in turn receives a monosynaptic input from neurons in ipsilateral layer VI. The polysynaptic potentials recorded in the present study could have been generated by the layer VI-layer III-layer V pathway, as some cells in layer VI project via the callosum and receive projections from contralateral layer VI. In addition, 1-DPSPs could have arisen from reverberating excitations between hemispheres as the callosal WM between the stimulating electrode and the opposite hemisphere was not routinely severed. A 1-DPSP resembling the high threshold 1-DPSP has been evoked in layer III cells with stimulation of the underlying grey matter (Sutor and Hablitz, 1989a, 1989b). As in the present case it was difficult to determine the source of the layer III 1-DPSP, however, it did not arise from recurrent excitations between hemispheres because monohemispheric slices were used in that study.

Paradoxically, in some cells the threshold for the 1-DPSP was below that of the e-DPSP whereas, in other cells it was above. Low threshold callosal evoked 1-DPSPs have been observed in layers III and VI of the somatosensory cortex with latencies ranging from 7 to 20 msec (Thomson, 1986). Low threshold 1-DPSPs observed in the present study may have been generated by the same pathway as 1-DPSPs observed by Thompson, as the range of onset latencies were similar. Furthermore, both potentials were sensitive to APV. One pathway was eliminated as a source for the
low threshold l-DPSP, that is, it was not a serial product of the e-DPSP.

Our major concern in the present study was to eliminate the l-DPSPs in order to record the e-DPSP in isolation. This was achieved by using low stimulus intensities and by eliminating those cells with low threshold l-DPSPs from the study. However, in some cells the peak of the l-DPSP was very near that of the e-DPSP making it difficult to distinguish between the two. Varying the stimulus frequency was one means of differentiating between the two, as the peak amplitude of high and low threshold l-DPSPs varied considerably with repetitive stimulation at frequencies above 0.03 Hz. Furthermore, l-DPSPs were highly sensitive to EAA antagonist, as apparently all polysynaptic intracortical PSPs are (Hablitz and Sutor, 1990; Hirsch and Crepel, 1990; Thomson, 1986). 1 µM CNQX abolished and APV reduced or abolished the high and low threshold l-DPSPs. Thus, polysynaptic pathways generating l-DPSPs utilized both NMDA and non-NMDA transmission, predominantly the latter. Though 1 µM CNQX partially blocked the e-DPSP, it preferentially blocked low and high threshold l-DPSPs. Hence, CNQX was an effective tool for isolating the e-DPSP from polysynaptic potentials.

Callosal evoked e-DPSP

Source The callosal e-DPSP was most likely generated by monosynaptic somatodendritic input from contralateral layer V pyramidal neurons (Vogt and Gorman,
The e-DPSP was assumed to be monosynaptic because the onset latency did not vary with stimulus parameters (i.e., intensity, frequency or polarity). Recordings were restricted to layer V to eliminate parallel, albeit less prominent, monosynaptic transcallosal connections between contralateral neurons in layers III and VI (Gerfen, 1989; Vogt et al., 1981). Stimuli were delivered to the dorsal corpus callosum, not the white or grey matter beneath the recording site, to exclude thalamic fibers as a source for the e-DPSP. It is possible that the e-DPSP was generated in part by the antidromic activation of neighboring callosal projecting pyramidal neurons since a similar phenomenon has been observed in visual and somatic sensory cortices of the cat (Ferster and Lindstrom, 1985a, 1985b; Takahashi et al., 1966). However, antidromically activated inputs from neighboring layer V pyramidal neurons should have been physiologically identical to orthodromically activated input from contralateral layer V neurons because the same type of neurons give rise to both inputs and the synapses are presumably distributed over the same somato-dendritic region. Antidromic activation of layer III callosal fibers would have generated a monosynaptic e-DPSP, if the same fibers collateralized to innervate layer V cells. Though collaterals of callosal projections exist, collateralization of interhemispheric fibers is generally not observed in adult rats (Catsman-Berrevoets et al., 1980, 1981; Ivy and Killackey, 1982; O'leary et al., 1981; Sarter and Markowitsch, 1985; Ferino et al., 1987).

Voltage sensitivity  When the membrane potential was progressively depolarized
from rest, the amplitude of the isolated e-DPSP increased. Though the slope of the voltage relation at depolarized membrane potentials was positive (i.e., unconventional), it was shallow and approached zero at membrane potentials negative to rest. A similar voltage dependency has been described for NMDA receptor mediated synaptic potentials (Mayer and Westbrook, 1987). Alternatively, an unconventional voltage dependence has been ascribed to the influence of voltage dependent currents which also generate a non-linearity in the somatic membrane I-V relation (Sutor and Hablitz, 1989b). In the present study the somatic membrane I-V relations were generally linear over the range of voltage tested. Furthermore, the slope of the I-V relation associated with the peak of the e-DPSP was not significantly different than that of the membrane potential. Hence, it is likely that the somatic membrane potential was not greatly influenced by the dendritic synaptic currents and visa versa.

**EAA Antagonist Sensitivity** Callosal e-DPSPs were predominantly non-NMDA receptor mediated. The non-NMDA antagonist, CNQX (0.3 to 3 µM), reduced the rising slope and peak amplitude of the plateau level e-DPSP in a concentration dependent manner. CNQX (3 µM) reduced the mean e-DPSP peak amplitude, rising slope and total area by 79%, 67 % and 78% respectively. Thus, it would appear that non-NMDA receptors were responsible for most of the callosal e-DPSP. Kainate receptors may have mediated the CNQX sensitive component, as CNQX (1 to 5 µM) preferentially antagonizes the effects of kainic acid in cortical slices.
(Andreasen et al., 1989). Accordingly, a high density of kainic acid receptors has been observed in layer V where contralateral layer V afferents terminate (Monaghan and Cotman, 1982; Patel et al., 1986; Zilles et al., 1990). In contrast, quisqualate and NMDA receptors apparently predominated in layers III and IV where thalamic fibers terminate (Monaghan and Cotman, 1985; Monaghan et al., 1984; Maragos et al., 1988).

An APV sensitive component of the e-DPSP was observed in the absence and presence of CNQX. APV alone reversibly reduced the peak amplitude and late component of the e-DPSP by about 20%, however the rising slope was unaffected. Similar findings have been reported for monosynaptic e-DPSPs recorded in cultured spinal and hippocampal neurons (Forsythe and Westbrook, 1988). However, the APV sensitive late component could have been an APV sensitive 1-DPSP in the present study. As an alternative, the APV sensitivity was tested in the presence of CNQX (Figures 12 and 13; Table I 1) which eliminated the 1-DPSP, as well as the e-IPSP (Hirsch and Crepel, 1990; Hablitz and Sutor, 1990). CNQX concentrations of up to 5 µM failed to completely block the e-DPSP. Residual e-DPSPs recorded in the presence of 1 to 3 µM CNQX were reversibly attenuated by 10 µM APV. It was assumed that the APV and CNQX sensitive components were generated by the same set of callosal fibers, as the threshold and latencies of the two were the same. NMDA receptors greatly influenced the shape of the residual e-DPSP, as APV reduced the rising slope of e-DPSPs when 1 to 3 µM CNQX was present. If the
CNQX and APV sensitive components summed linearly, then the APV sensitive component was responsible for as much as 20% of the peak e-DPSP amplitude under all conditions tested. NMDA receptors may contribute relatively more to the total e-DPSP under physiologic conditions, as no measures were taken to optimize NMDA potentials in the present study.

**EAA pathway heterogeneity** The contribution of NMDA receptors to the callosal e-DPSP characterized in the present study is similar to that of EPSPs evoked with stimulation of the subcortical WM (Jones and Baughman, 1988; Thompson, 1989). This could be due to the activation of a common set of fibers, as callosal fibers ascend via the subcortical WM *en route* to layer V (Scheibel and Scheibel, 1967). EPSPs presumably arising from neurons in layers III, V and VI are all mixed EAA EPSPs (DeCurtis et al., 1989; Hirsch and Crepel, 1990; Jones and Baughman, 1988). In contrast, a putative thalamocortical EAA EPSP resembles the callosal e-DPSP in voltage dependence but not EAA antagonist sensitivity (Hablitz and Sutor, 1990; Sutor and Hablitz, 1989a, 1989b). That is, the thalamocortical EPSP does not have a significant APV sensitive component.

The dichotomy in EAA pathways may be functionally relevant for the dissemination of converging EAA input, as NMDA and non-NMDA potentials are differentially affected by converging transmitter input, pharmacologic insult and tetanic stimulation (Mayer and Westbrook, 1987; Muller and Lynch, 1990; Reynolds
et al., 1988; Markram and Segal, 1990). According to the classical columnar view of cortical processing, excitatory input can activate superficial neurons which in turn activate deep neurons which in turn activate subcortical targets. In theory, excitatory input can bypass superficial layers directly activating output from the deep layers or bypass deep layers altogether, as superficial neurons also project subcortically. Thus, the circuitry of the cortex allows for serial or parallel processing via superficial and deep cortical layers. If serial or parallel cortical circuits are comprised of distinct EAA pathways, then convergent non-EAA transmitter influence may gate the flow of information through one or the other cortical circuits. In addition, physiologic conditions would influence simple modes of cortical activity such as synchronous rhythmic bursting which apparently rely on the integrity of EAA input and at some synapses NMDA receptor activation. Support of such a thesis awaits further characterization of EAA cortical pathways, as well as their modulation by non-EAA transmission.
Figure 3. Three firing patterns observed in superficial layer V cortical neurons with prolonged current injections. Prolonged (≥150 msec) hyperpolarizing and depolarizing current steps (between -300 and 200 pA; 100 pA increments) were made from resting potential (-80 mV). Top left: Regular spiking cells displayed pronounced biphasic post-spike hyperpolarizations and readily accommodated to prolonged depolarization. Right: DAP cells were characterized by pronounced post-spike depolarizing afterpotentials (DAPs). With larger depolarizations from rest, the envelope of the DAP increased to evoke a small cluster (2-3) of spikes (not shown). Bottom left: Burst spiking cells displayed all or none bursts in response to depolarization from rest. Right: A small number (7/67) of the cells in superficial layer V were antidromically activated from the callosum. The antidromic spike generally failed to invade the soma unless the membrane was depolarized from rest. The response of a regular spiking cell to callosal stimuli is shown; three successive responses to callosal stimulation at -78 mV (rest) and at -60 mV are shown.
Figure 4. Callosal evoked synaptic responses in two layer V neurons. Three successive synaptic responses recorded at rest are superimposed for each stimulus intensity shown. (A) A short latency depolarizing potential (e-DPSP) was the lowest threshold response in this regular spiking neuron. The amplitude of the e-DPSP grew with increasing stimulus intensity. At high intensities (3 mA), a late depolarizing peak (l-DPSP) was observed (arrow). When the stimuli were delivered once every 30 seconds (0.03 Hz), there was little variation in the l-DPSP amplitude. (B) In another regular spiking neuron, the threshold for the l-DPSP (1 mA) was near that of the e-DPSP. When the stimulus intensity was increased beyond threshold for the l-DPSP (1.2 mA), the amplitude of the l-DPSP was attenuated and ultimately replaced by a hyperpolarizing potential (3 mA). Note in B, the amplitude and peak latency of the l-DPSP (arrows) varied as the stimulus frequency was > 0.03 Hz. Resting membrane potentials for cells in A and B were -80 and -74 mV respectively.
Figure 5. The callosal evoked e-DPSP amplitude increased with increasing stimulus intensity in 5 different layer V neurons. e-DPSP amplitudes were measured at rest in each cell. The stimulus-response relation generally reached a plateau with stimuli between 2 and 3 mA. The e-DPSP on the far left (squares) apparently summated with the 1-DPSP generating a steep stimulus-response curve.
Figure 6. IPSPs recruited with high stimulus intensities reduce the late component of the DPSP recorded at depolarized potentials. Top: Callosal evoked responses recorded in a DAP cell. Upper traces: responses evoked by 3, 4, and 8 mA at a holding potential of -57 mV. Note the reduction in the late component observed at intensities above 3 mA. Lower traces: responses evoked by 1, 2, 3 and 4 mA at rest (-78 mV). Each trace is an average of three synaptic responses. Bottom: Graphic summary of the same data. The stimulus-response relation for the peak of the DPSP (10 msec) recorded at rest (-78 mV) reached a plateau with stimuli between 2 and 3 mA (filled circles). Beyond the plateau, the amplitude of the depolarizing potential recorded at rest continued to rise. The stimulus response relation for the late component (40 msec) of the e-DPSP recorded at -57 mV also peaked between 2 and 3 mA (open circles). However, at higher stimulus intensities the amplitude of the late component decreased and was ultimately replaced with a net hyperpolarizing potential (filled squares).
Figure 7. The voltage dependence of EPSP-IPSP sequence evoked with high intensity stimulation of the callosum. When the stimulus intensity was supramaximal for the e-DPSP, a biphasic IPSP followed the e-DPSP. (A) Both peaks of the IPSP increased with depolarization from rest (-73 mV). The early (25 msec) IPSP (e-IPSP; arrow head) was hyperpolarizing when the membrane potential was held between -50 mV and -68 mV. At membrane potentials negative to -68 mV, the e-IPSP was depolarizing. In contrast, the late (120 msec) IPSP (l-IPSP; arrows) was hyperpolarizing at all membrane potentials shown. (B) Voltage relations for the synaptic responses illustrated in A are shown graphically in B. The e-IPSP reversed between -65 and -70 mV, whereas, the l-IPSP was nearly reversed at -94 mV. Note that the supramaximal e-DPSP displayed a conventional voltage relation (squares).
Figure 8. Effects of EAA antagonists on e-DPSP-l-DPSP sequence and low threshold l-DPSP.
A e-DPSP-l-DPSP sequence and a l-DPSP were recorded at various holding membrane potentials (-87 to -65 mV) in a regular spiking neuron. **Left**: e-DPSP-l-DPSP sequences evoked with cathodal stimuli. **Right**: Low threshold l-DPSP evoked with anodal stimuli. **Left top**: At -65 mV the l-DPSP (solid triangles) evoked several regenerative sodium spikes (spikes are truncated). **middle**: The l-DPSP of the e-DPSP-l-DPSP sequence was attenuated but not abolished following exposure to APV (20 µM). **bottom**: The l-DPSP was abolished by 1 µM CNQX. **Right top**: An isolated l-DPSP was evoked with anodal stimuli (same intensity). **middle & bottom**: Low threshold l-DPSPs were abolished by APV (20 µM) or CNQX (1 µM). Resting membrane potential of this cell was -72 mV. Three successive responses were averaged for each trace, with the exception of traces right middle set of single trial traces. Stimulus frequency was 0.033 Hz.
Figure 9. Effects of CNQX on an isolated e-DPSP and a e-DPSP-l-DPSP sequence evoked in a DAP neuron. Top left: Isolated e-DPSPs evoked with cathodal stimuli and recorded at various membrane potentials. Right: e-DPSP-l-DPSP sequence evoked with anodal stimuli and recorded at the same membrane potentials. Bottom: Isolated e-DPSPs (left) and e-DPSP-l-DPSP sequences (right) recorded at resting membrane potential (-82 mV) in the presence or absence of CNQX (1 µM) are superimposed and subtracted (bottom traces) to illustrate the degree of antagonism. Note that the l-DPSP was abolished by 1 µM CNQX but e-DPSPs evoked with anodal or cathodal stimuli were not.
Figure 10. APV reversibly attenuated the e-DPSP late component. Plateau level e-DPSPs recorded at various membrane potentials in the presence or absence of APV (20 µM) are superimposed. Top Trace: At a membrane potential of -58 mV, the late component of the e-DPSP reached action potential threshold (spike is truncated). In the presence of APV the e-DPSP no longer evoked a spike. Bottom Traces: The APV sensitive late component was larger at membrane potentials depolarized to rest (-70 mV) and essentially non-existent at more negative membrane potentials (-80 mV).
Figure 11. CNQX antagonism of the plateau level e-DPSP was concentration dependent. (A) CNQX (1 µM) reduced the plateau level e-DPSPs (arrow) from 5 mV to 2 mV and the suprplateau level e-DPSP from 7.8 to 2.5 mV. Note that the threshold did not change. (B) CNQX (0.3 and 1 µM) reduced the e-DPSP rising slope, peak amplitude and total area in a concentration dependent manner. Plateau level e-DPSPs recorded at rest in the presence and absence of CNQX are superimposed. Note that the e-DPSP onset latency is not affected by CNQX. Cells in A and B were both regular spiking with resting potentials of -80 and -73 mV respectively.
Figure 12. An APV sensitive component persisted in the presence of 3 µM CNQX. All recordings were obtained from the same regular spiking neuron. Each trace is an average of three consecutive e-DPSPs. (A) The amplitude and total area of the e-DPSP increased with depolarization from rest (-72 mV). CNQX (1 and 3 µM) antagonism of the e-DPSP was concentration dependent. e-DPSPs evoked at depolarized potentials were particularly sensitive to EAA antagonists. (B) A residual e-DPSP persisted in the presence of 3 µM CNQX. The residual e-DPSP was attenuated by 10 µM APV. (C) APV reduced the rising slope, peak amplitude and total area of the e-DPSP recorded in the presence of CNQX (3 µM). e-DPSPs recorded in the presence of CNQX (a) and CNQX plus APV (b) are superimposed. e-DPSPs recorded at a holding potential of -50 mV (top traces) were more sensitive to the antagonist action of APV than those recorded at rest (bottom traces). Note that the e-DPSP onset latency is the same under all conditions.
Figure 13. EAA antagonists reduced the e-DPSP amplitude in a voltage and concentration dependent manner. (A) The control (solid squares) e-DPSP voltage relation was unconventional, as the amplitude (mV) increased with depolarization but not hyperpolarization of the membrane potential (mV). CNQX, 1 and 3 µM (solid triangles and circles), decreased the e-DPSP recorded at any membrane potential to approximately 4 and 2.5 mV respectively. Hence, the CNQX antagonism was greater for e-DPSPs evoked at potentials depolarized from rest (-72 mV). APV reduced the residual e-DPSP persisting in the presence of 3 µM CNQX. (B) Current voltage (I-V) relations were linear over the range of voltages examined. Steady state voltage deflections (mV) were elicited by transient (200 msec) depolarizing or hyperpolarizing current steps (nA). The slope of the I-V associated with the e-DPSP peak depolarization (solid squares) was the same as the membrane potential I-V (open squares). Hence, the e-DPSP was not associated with a detectable increase in input resistance. Data was obtained from the regular spiking neuron described in Figure 12.
Table I: Effects of EAA Antagonists on the e-DPSP

<table>
<thead>
<tr>
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<th>Amplitude (mV)</th>
<th>Slope (V/S)</th>
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<td>37%</td>
<td>61%</td>
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<tr>
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<td>1.9 (0.2)</td>
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</tr>
<tr>
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<td>90%</td>
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Background II: Serotonergic modulation of cortical neuron function

Serotonergic input to the neocortex arises predominantly from the dorsal raphe (Lorens, 1974; Steinbusch, 1984). Dorsal raphe fibers ascend with the medial forebrain bundle to innervate discrete regions of cortex, whereas median raphe fibers in the cortex or more diffuse (O’Hearn and Molliver, 1984). Serotonergic fibers, 5HT and 5HT metabolites are particularly concentrated in the supragenual dorsomedial PFC (Emson and Lindvall, 1979; Reader and Grondin, 1987). Within this region the serotonergic fibers are somewhat concentrated in layers III and V where they terminate almost exclusively on dendrites of cortical neurons (Steinbusch, 1984; Zilles et al., 1990). Serotonergic fibers in the superficial layers (III-IV) are anatomically distinct from those in the deep layers. Morphologic differences of serotonergic fibers in layers III and V are mirrored by differences in 5HT receptor subtypes concentrated in each layer. 5-HT\(_2\) receptors are highly concentrated in the superficial layers, whereas 5-HT\(_1\) receptors are particularly concentrated in layer V and VI (Pazos et al., 1985; Pazos and Palacios, 1985; Zilles et al., 1990).

5HT receptors can be categorized according to their relative affinities for tritiated 5HT where 5HT\(_2\) and 5HT\(_1\) receptors have a low and high affinity for 5HT respectively (Nelson, 1990, review). Potent selective 5-HT\(_2\) or 5-HT\(_1\) receptor antagonists are presently not available (Bradley et al., 1986; Pazos et al., 1985; Pazos and Palacios, 1985; Peroutka, 1986). 5-HT\(_2\) receptors can be distinguished from 5-HT\(_1\)
receptors, as the former are antagonized by ketanserin (Bradley et al., 1986). Spiperone antagonizes 5-HT$_2$ receptors, but it also antagonizes 5-HT$_1$ receptors (Bradley et al., 1986; Peroutka, 1986). Both spiperone and ketanserin have little affinity for 5-HT$_3$ receptors. Thus, if a serotonin receptor-mediated response is antagonized by both spiperone and ketanserin, then it is most likely mediated by 5-HT$_2$ receptors. Alternatively, if the response is antagonized by spiperone but not ketanserin, then it is probably mediated by 5-HT$_1$ receptors. Finally, 5-HT responses that are resistant to ketanserin and spiperone may be mediated by 5-HT$_3$ receptors. 5-HT$_3$ receptor-mediated responses are selectively antagonized by ICS 205-930 and MDL 7222 and are mimicked by 2-methyserotonin in the rat PFC (Ashby et al., 1989; Bradley et al., 1986). 5-HT$_{1A}$ receptors are selectively activated by low concentrations of the 5-HT$_{1A}$ agonist, 8-hydroxy-2-(di-n-propylamino)-tetraline (DPAT), whereas 5HT$_{1A}$ and 5HT$_{1B}$ receptors are non-selectively activated by trifluromethylphenylpiperazine (TFMPP) and 5-carboxyamidotryptamine or 5-CT (Beck, 1989; Colino and Halliwell, 1986; Hamon et al., 1986; Nishimura et al., 1988; Sills et al., 1984). Thus, if the physiologic response to 5HT is antagonized by spiperone but not by ketanserin and is mimicked by low concentrations of TFMPP, 5-CT or DPAT, then the receptors mediating that response can be classified as 5-HT$_{1A}$. In theory, 5HT$_{1B}$ receptors are activated by 5HT, TFMPP, or 5-CT and insensitive to ketanserin or spiperone (Sills et al., 1984). There is no selective 5HT$_{1B}$ antagonist to date but 5HT$_{1B}$ receptors are antagonized by $B$-adrenergic antagonist such as pindolol.
Much of our understanding of how monoamines act on neural processes is derived from the discovery that neurotransmitters can modify voltage dependent ion conductances (Adams and Galvan, 1986; Kupfermann, 1979). By altering the voltage dependent membrane properties of neurons, neurotransmitters can dramatically change a neuron's responsiveness to converging synaptic inputs. For example, the passive conduction of an EPSP from the dendritic tree to the soma can be facilitated by enhancing voltage dependent inward currents or by suppressing voltage dependent outward currents that are activated by the EPSP induced membrane depolarization (Adams and Galvan, 1986). Thus, the EPSP that reaches the hilar region would be larger and closer to firing (action potential) threshold. Dopamine, 5HT, and acetylcholine may increase the amplitude and duration of an EAA EPSP via such a mechanism (Davies et al., 1987; Legendre et al., 1989; Ma and Dun, 1986; McCormick and Williamson, 1989; Penit-Soria et al., 1987; Rasmussen and Aghajanian, 1990; Sheldon and Aghajanian, 1990; Spain et al., 1990). The reduction in net outward current by these transmitters is generally associated with an apparent increased input resistance and in some cases a membrane depolarization. A variety of transmitters alter voltage dependent potassium conductances that shape the action potential and prevent repetitive discharge with prolonged depolarization. 5HT, norepinephrine, histamine, dopamine, and acetylcholine agonists all decrease the post-spike train afterhyperpolarizing potential (AHP) in cortical tissue (Colino and Halliwell, 1986; Foehring et al., 1989; Lancaster and Adams, 1986; Slater and Larson-Prior, 1987; Madison and Nicoll, 1986; McCormick and Williamson, 1989; Storm,
1989). A reduction in AHP amplitude can dramatically alter the input/output ratio of a cell since the affected neuron fires repetitively in response to prolonged depolarization to firing threshold. Transmitter induced reduction of AHPs or net outward conductances is not 'excitatory' in the classical sense, as it is not necessarily associated with a membrane depolarization and generally lasts for several minutes following a brief exposure to the transmitter.

In addition to increasing the membrane excitability, 5HT can hyperpolarize the membrane via an increase in outward potassium conductance (Andrade and Nicoll, 1988; Baskys et al., 1989; Colino and Halliwell, 1987; Jahnsen, 1980; Joël et al., 1987; Segal, 1981; Wang and Dun, 1990). In this respect the action of 5HT resembles that of the inhibitory neurotransmitter, GABA. In fact, the two transmitters act via a common mechanism (McCormick and Williamson, 1989).

The 5HT induced membrane hyperpolarization and reduction in AHP are mediated via different 5HT receptor subtypes. The membrane hyperpolarization is mediated by 5HT\textsubscript{1A} receptors, whereas the excitatory effects of the amine are not (Andrade and Nicoll, 1988; Beck et al., 1985; Colino and Halliwell, 1987; Davies et al., 1987; Joël et al., 1987; Wang and Dun, 1990). The postsynaptic response to activation of putative raphe fibers is associated with a membrane hyperpolarization which is 5HT\textsubscript{1A} receptor mediated and a depolarization of unknown receptor origin (Grantyn and Grantyn, 1972, 1973; Pan et al., 1989; Park et al., 1982; Wang and Dun,
1990). The receptor subtype mediating the reduction in AHP and net outward conductances has been described as 5HT$_2$, 5HT$_{1c}$ and 5HT$_1$-like (Davies et al., 1987; Legendre et al., 1989; Ma and Dun, 1986; McCormick and Williamson, 1989; Pan et al., 1989; Penit-Soria et al., 1987; Rasmussen and Aghajanian, 1990). Hence, as is the case for other amines and acetylcholine, the facilitatory and inhibitory effects of 5HT are mediated via distinct receptor subtypes.

Monoamine induced transient hyperpolarizations and prolonged increases in excitability often occur in tandem and may serve to increase the signal to noise ratio for processing convergent synaptic potentials. It has been suggested that the brief hyperpolarization serves to attenuate all isolated small amplitude EPSPs, whereas the AHP reduction would promote the summation of a barrage of EPSPs and the repetitive action potential discharge upon reaching threshold. Thus, the output (action potentials) generated by tonic isolated input would be attenuated, while the output generated by intense phasic input would be augmented. Accordingly, several groups have attributed the 5HT induced attenuation of synaptic potentials and spontaneous cortical activity to postsynaptic effects of the monoamine (Jahnsen, 1980; Segal, 1990). However, an alternative mechanism may be proposed if this data is interpreted in a different light.

A dissociation between the effects of 5HT on the membrane potential or input resistance and synaptically evoked potentials has been observed in sympathetic and
parasympathetic ganglia, spinal cord, locus ceruleus and somatosensory cortex. In the sympathetic and parasympathetic ganglia, 5HT blocks cholinergic transmission without affecting the postsynaptic response to exogenously applied acetylcholine (Dun and Karczmar, 1980; Nishimura et al., 1988). Furthermore, this effect is partially reversed by increasing the extracellular calcium concentration (Dun and Karczmar, 1980). In addition, 5HT reduces the somatic calcium spike and the associated underlying current (Holz et al., 1986; Kelly and Penington, 1989; Penington and Kelly, 1990). A reduced calcium influx would account for the 5HT induced reduction transmitter release from sympathetic and cerebellar nerve endings (Maura et al., 1986; Feniuk et al., 1979; Watts et al., 1981). Thus, the 5HT induced attenuation of synaptic potentials may be attributed to a presynaptic site of action.

A presynaptic site of action also has been proposed for the effects of 5HT on EAA EPSPs in the rat spinal cord and locus ceruleus (Bobker and Williams, 1989; Wu et al., 1991). In both tissues bath applied 5HT does not appreciably change the resting membrane potential nor does it alter the postsynaptic response to pressure applied glutamate. 5HT induced attenuation of both EAA EPSPs is antagonized by spiperone but not ketanserin; and low concentrations of DPAT (≤ 1 µM) mimic the inhibitory effect. Thus, the pharmacologic profile of the receptors mediating suppression of EAA EPSPs resembles that of 5HT1A receptors. Maximal suppressive effects of 5CT and TFMPP were similar to those of 5HT; however, the actions of 5CT and TFMPP were insensitive to spiperone antagonism (Bobker and Williams,
Hence, Bobker and Williams suggested that 5HT suppresses EAA EPSPs via actions on both 5HT$_{1A}$ and 5HT$_{1B}$ receptors. It is difficult to determine whether 5HT$_{1A}$ and 5HT$_{1B}$ receptors or distributed on a common set of fibers (or neurons), since additivity of 5HT$_{1A}$ and 5HT$_{1B}$ receptor activation has not been investigated.

5HT suppresses the spontaneous activity of layer V neurons in the PFC (Bradshaw et al., 1983; Lakoski and Aghajanian, 1985); however, the site and mechanism of action is unclear. In the PFC, there are dense, overlapping projections from contralateral PFC and dorsal raphe in layer V where 5HT$_1$ receptors are concentrated. Thus, input from the raphe may modulate interhemispheric EAA transmission. The primary aim of Part II of this dissertation was to characterize the effects of 5HT on interhemispheric transmission. Based on previous investigations, it is hypothesized that 5HT attenuates cortical activity via a suppression of EAA transmission. Specifically, it is hypothesized that 5HT suppresses interhemispheric EAA transmission in layer V cells of the rat PFC.
Methods II

Cortical slices were prepared and maintained as described in the Methods I. Callosal potentials were evoked and characterized as described in Methods I. In order to test the postsynaptic effects of 5HT on glutamate potentials, glutamate was pressure applied to the proximal dendrites and soma of the impaled neuron. 5HT creatine sulfate was bath applied to the tissue in single or cumulative concentrations up to 300 \( \mu \text{M} \). Cumulative concentration-effect curves were generated in the presence or absence of APV (10 \( \mu \text{M} \)), spiperone (1 \( \mu \text{M} \)), DPAT (10 \( \mu \text{M} \)) or ketanserin (1 \( \mu \text{M} \)). Cumulative exposures to 5HT were limited to 12 minutes to reduce or eliminate the excitatory effects of the monoamine. Concentration-effect curves for 5HT induced decreases in e-DPSP and glutamate potential amplitudes were not normalized, as PSP amplitudes were similar between cells. The EC\(_{50r}\), E\(_{\text{max}}\) and slope (N) of concentration-effect curves were derived from the following hyperbolic function which was used to fit the data: \( E = E_{\text{max}} / [1 + (\text{EC}_{50}/ [5HT])^N] \).

Apparent K\(_d\)'s (or the K\(_B\)'s) for spiperone antagonism were determined according to the following relation: \( [A]_{+\text{spip}}/[A] = 1 + B/K_B \). \( [A]_{+\text{spip}}/[A] \) is the ratio of EC\(_{50r}\)s in the presence and absence of spiperone. B is the concentration of antagonist used and K\(_B\) is the estimated dissociation constant for that antagonist.
Results II

Effects of 5HT on postsynaptic membrane potentials

Brief (≤ 3 minutes) applications of 5HT (≤ 100 µM) did not alter the resting membrane potential or input resistance of superficial layer V neurons. In some cells the membrane was hyperpolarized or depolarized by 1 to 3 mV during 12 minute period following a 3 minute exposure to 5HT or during prolonged (12 minutes) exposures to 5HT. As similar fluctuations in membrane potential were observed in the absence of drug application, it was difficult to attribute these changes in membrane potential to actions of 5HT. 5HT (≤ 100 µM) elicited little or no change in membrane input resistance as determined from the amplitude of voltage deflections generated by long duration (150 msec) hyperpolarizing or depolarizing current steps (Figure 14). In contrast, 5HT greatly suppressed cortical synaptic transmission. In the first example, a brief exposure to 10 µM 5HT reversibly suppressed callosal evoked e-DPSP by 90 % without affecting the input resistance or membrane potential. In the second example, cumulative concentrations (≤ 100 µM) of 5HT were applied over a 12 minute period. During that twelve minute period, there was some attenuation of tonically induced voltage deflections and a small membrane hyperpolarization (≤ 3 mV; the membrane was manually clamped to the baseline potential). Note that suppression of the e-DPSP was greater than that of the tonic depolarizing voltage deflections. Furthermore, maximal attenuation of the voltage deflections preceded that of the e-DPSP.
Effects of 5HT on callosal and subcortical WM evoked synaptic potentials

In this series of experiments, stimulus evoked PSPs were characterized as described in Part I with one exception. About half (4) of the high threshold l-DPSPs were elicited via stimulation of the subcortical WM, as polysynaptic potentials were more reliably evoked from this site. The inhibitory effects of 5HT on the low threshold callosal evoked e-DPSP were characterized in detail, as this was the primary aim of the present series of experiments.

Though 5HT had little effect on the resting membrane potential or input resistance, it consistently attenuated PSPs elicited by callosal or subcortical WM stimulation. Only two PSPs in the entire study were not attenuated by a maximally effective concentration of 5HT, and both of these e-DPSPs were elicited with stimulation of the subcortical WM. The time course for the effects of 5HT on WM evoked PSPs is illustrated in Figure 15. All WM evoked potentials were partially attenuated within seconds of switching to 5HT containing solutions and maximally attenuated within 3 minutes. Full recovery from the inhibitory effects of 5HT required 15 to 20 minutes. Following recovery from the 5HT induced suppression about 10% of the callosal or subcortical WM evoked potentials were increased in amplitude. The facilitation of transmission may have been due to the actions of 5HT, as a 5HT induced facilitation of cortical EPSPs has been observed following prolonged (≥ 20 minutes) exposures to the monoamine (Reynolds et al., 1988). Recovery from the facilitation required about an hour.
**Polysynaptic Potentials.** 5HT reduced or abolished 1-DPSP component of the e-DPSP-1-DPSP sequence leaving much of the e-DPSP intact. In these experiments, the stimulus intensities were adjusted to obtain the e-DPSP-1-DPSP sequence and stimuli were delivered at frequencies of 0.1 to 1 Hz to illustrate the characteristic stimulus induced augmentation of the 1-DPSP (Figure 15A). PSPs were recorded at resting membrane potential, as the S-and-l-DSPPs were sufficiently large at these potentials. The 1-DPSP generally reached action potential threshold, hence eliciting several regenerative sodium spikes. APV was applied to some cells prior to exposure to 5HT to lower the 1-DPSP amplitude below the action potential threshold. As illustrated in Figure 15, 5HT (25 µM) abolished the 1-DPSP without appreciably affecting the rising slope of the e-DPSP (Table II; N=12). Partial recovery was generally obtained within 12 minutes of switching to control solutions and full recovery was obtained within 15 to 20 minutes. The mean peak amplitude, latency and total area of the control 1-DPSP were 14 ± 1 mV, 29 ± 2 msec and 590 msec x mV respectively (mean ± sem; N=7). Maximally effective concentrations of 5HT reduced the mean peak latency, amplitude and area to 6 ± 1, 16 ± 1 and 196 ± 60 respectively (mean ± sem; N=7; Table II). 5HT also reduced the rising slope and amplitude of the e-DPSP, however the e-DPSP was relatively less sensitive to 5HT. As illustrated in Figure 16, lower concentrations of 5HT (1 µM) preferentially suppressed the 1-DPSP component, whereas higher concentrations (≥ 10 µM) attenuated the e-DPSP as well. Thus, the inhibitory effects of 5HT resembled those of CNQX (Part I).
5HT (10 to 50 µM) greatly attenuated both components of the IPSP, hence revealing the underlying e-DPSP late component or the l-DPSP (N=8). In these experiments, stimulus intensities were adjusted to evoke an e-DPSP-IPSP sequence. Stimuli were delivered once every 20 or 30 seconds (0.1 or 0.03 Hz), as the IPSP amplitude was attenuated when the stimuli were delivered more frequently (Figure 17A). The membrane potential was held above the apparent reversal potential for the e-IPSP to maximize the amplitude of the IPSP. Once a baseline IPSP amplitude was obtained, cortical slices were transiently (3 minutes) exposed to a maximally effective concentration of 5HT (10 to 50 µM). As illustrated in Figure 17, 5HT reduced the early and late components of the IPSP. Underlying l-DPSPs or a e-DPSP late components were similarly revealed following 5HT or frequency induced suppression of the IPSP. The reduction in IPSP amplitude was accompanied by a reduction in the e-DPSP component of the e-DPSP-IPSP sequence. Again, the effects of 5HT on polysynaptic potentials resembled those of CNQX described in Part I.

Monosynaptic Potentials. 5HT suppressed NMDA and non-NMDA components of the callosal e-DPSP. In this and subsequent series of experiments, stimulus intensities were adjusted below threshold for the l-DPSP and IPSP to isolate the e-DPSP. The membrane potential was maintained at rest or slightly depolarized from rest (∼-75 mV) to maximize the baseline e-DPSP amplitude. Frontocortical slices were exposed to 5HT (≤30 µM) in the presence or absence of APV in order to
demonstrate the effects of 5HT on the NMDA and non-NMDA components of the e-DPSP. 5HT attenuated the late, decay phase to a greater extent than the early, rising phase of the e-DPSP. The high sensitivity of the late component to 5HT was reflected in the greater loss of total area as compared to other parameters measured (Table II). The e-DPSP evoked in the presence of 5HT was subtracted from the control e-DPSP to illustrate the 5HT sensitive component. The slow time course of the 5HT sensitive component resembled that of the APV sensitive component (Figure 18A). In order to demonstrate the effects of 5HT on the early APV insensitive component, the tissue was bathed in APV (10 or 20 µM) prior to switching to solutions containing both APV and 5HT (3 to 30 µM). As illustrated in Figure 18B, 5HT suppressed the residual APV insensitive component of the e-DPSP (N=6). Hence, both NMDA and non-NMDA components of the e-DPSP were attenuated by 5HT.

Suppression of EAA transmission by 5HT was concentration dependent. In these experiments, cumulative concentrations of 5HT (≤ 100 µM) were applied over a 12 minute period. As illustrated in Figure 19, 5HT reduced the peak amplitude, total area and rising slope of the e-DPSP in a concentration dependent manner. Maximally effective concentrations of 5HT (≥ 10 µM) reduced the rising slope, peak amplitude and total area by 44%, 50% and 68% respectively (Table II; N=11). A similar concentration dependent suppression of the e-DPSP was observed in the presence of APV (Table II; N=4). The reduction in e-DPSP rising slope by maximal
concentrations of 5HT was much greater when low stimulus intensities were employed. That is, the mean e-DPSP rising slope was reduced by 18% when supramaximal stimulus intensities were employed and by 44% when plateau level stimulus intensities were employed (Tabel 1).

Callosal e-DPSPs and depolarizing responses to the EAA, glutamate, were concomitantly attenuated by 5HT in a dose dependent manner (Figure 20). The membrane depolarization induced by local application of glutamate and the e-DPSP were monitored concurrently in eight cells. For two of the eight cells, APV (10 µM) was present to block NMDA potentials (Figure 21). Glutamate (10 mM) was pressure applied to the somata and proximal dendrites of superficial layer V neurons and the amplitude of the glutamate potential was adjusted to match that of the e-DPSP by manipulating the application pressure. 5HT reduced the peak amplitudes of the e-DPSP and the glutamate potential, though suppression of the latter was generally less pronounced (Figure 22). 5HT (≥ 10 µM) facilitated or had no effect on the glutamate depolarizations observed in three of the eight cells, whereas all eight e-DPSPs were suppressed. Data from several cells were averaged to generate a concentration effect curve for inhibitory effects of 5HT on e-DPSPs and glutamate depolarizations (Figure 22). Cells lacking a 5HT induced suppression of the glutamate potentials were eliminated so comparisons could be made between e-DPSP and glutamate potential suppression. Prior to exposure to 5HT, the matched control e-DPSP and glutamate potential amplitudes were 9.97 ± 1 and 12.5 ± 0.89 mV,
respectively (means ± sem; N=6). The maximal reduction ($E_{\text{max}}$) of glutamate depolarization and e-DPSP amplitudes were 4.6 ± 1.6 mV and 1.9 ± 0.23 mV respectively (mean ± sd; N=6). Maximally effective concentrations of 5HT (10 to 100 µM) reduced the mean e-DPSPs and glutamate potentials by approximately 47% and 21% respectively. In the presence of APV, matched e-DPSP and glutamate potential amplitudes were reduced by 44% and 26% respectively (N=2). Hence, 5HT induced suppression of the glutamate depolarization was less than that of the callosal e-DPSP in the presence or absence of APV.

The 5HT$_{1A,2}$ antagonist, spiperone, differentially affected the 5HT induced suppression of the e-DPSP and the glutamate potential. In these experiments, the effects of 5HT were determined in the presence and absence of spiperone. As illustrated in Figure 23, spiperone antagonized the effects of 5HT on the e-DPSP (N=6). In four of these cells the glutamate potential and the e-DPSP were monitored simultaneously during exposures to 5HT in the presence and absence of spiperone. Spiperone shifted both concentration effect curves to the right (Figure 24). Furthermore, the spiperone antagonism was surmounted by higher concentrations of 5HT. Spiperone dramatically affected the glutamate potential concentration effect curve, increasing the estimated EC$_{50}$ from 4.3 ± 1 to 49.5 ± 1. In contrast, the EC$_{50}$ associated with e-DPSP inhibition was increased from 5 ± 1 to 10 ± 5 µM. Assuming that 1 µM spiperone competitively antagonized the effects of 5HT, the K$_b$'s for spiperone were 95 nM and 1000 nM respectively. Hence, it was
assumed that 5HT inhibited the e-DPSP through two mechanisms one of which was sensitive to spiperone.

Spiperone sensitive effects of 5HT were most likely mediated by 5HT₁ type receptors, as the 5HT₂ antagonist, ketanserin failed to block the inhibitory effects of 5HT (N = 4 of 4). In these experiments, the inhibitory effects of 5HT were monitored in the presence and absence of 1 µM ketanserin (Figures 25 and 26). In the first example the 1-DPSP of a e-DPSP-1-DPSP sequence was abolished by 5HT, whereas the e-DPSP was largely unaffected (Figure 25). A similar inhibition was obtained in the presence of ketanserin, though the inhibition appeared to be greater. That is, suppression of the e-DPSP amplitude was greater when ketanserin was present. Similarly, ketanserin enhanced the inhibitory action of 1 µM 5HT on an isolated e-DPSP (Figure 26). 5HT reduced the e-DPSP area by less than 10% in the absence of ketanserin and by more than 40% in the presence of ketanserin. Similar enhancements were only observed in 2 of 4 cells tested.

The 5HT₁A ligand, DPAT (0.3 to 10 µM) caused little suppression of WM evoked cortical potentials, however, high concentrations of DPAT antagonized the 5HT induced suppression of WM evoked cortical potentials (Figure 27). The antagonist action of DPAT was tested in three cells and a full 5HT concentration response relation was generated for one of these cells. The antagonist action of DPAT was overcome by higher concentrations of 5HT (not shown).
Discussion II

At concentrations that maximally suppressed interhemispheric transmission, 5HT had little or no effect on the membrane potential or input resistance in the majority of layer V neurons tested. When observed, 5HT induced hyperpolarizations (or depolarizations) were no greater than 3 mV. Small amplitude, 5HT induced hyperpolarizations associated with decreases in input resistance have been observed in somatosensory neocortical neurons and are presumably due to actions of 5HT through activation of $5\text{HT}_{1A}$ receptors (Davies et al., 1987; McCormick and Williamson, 1989). As in the present study, the 5HT induced hyperpolarizations observed by Davies et al. (1987) only occurred in a small percent of the neurons tested. Reynolds et al (1988) reported a 5HT induced suppression of a e-DPSP which was not associated with an apparent change in input resistance or membrane potential. Similar dissociations between 5HT induced suppression of transmission and postsynaptic membrane polarization have been observed in the spinal cord and locus ceruleus; hence, 5HT may suppress transmission via a mechanism independent of its effects on membrane potential (Wu et al., 1991; Bobker and Williams, 1989).

Several observations indicate that effects of 5HT were receptor mediated. Suppressions of e-DPSPs and the glutamate induced depolarizations by 5HT were concentration dependent, saturable and reversible. The suppressive effect of 5HT was antagonized by spiperone but not ketanserin. Hence, suppression of
interhemispheric transmission was receptor mediated.

Monosynaptic Potentials

Suppression of glutamate and synaptic potentials by 5HT may have been mediated via actions of the monoamine on distinct target sites. The maximal 5HT suppression, $E_{\text{max}}$, of the e-DPSP was greater than that of the glutamate depolarizing potential. The $E_{\text{max}}$ for suppression of e-DPSPs was $\sim 5$ mV, whereas, that for the glutamate potentials was $\sim 2$ mV. If the effects of glutamate were independent of transmitter release, then the effects of 5HT on the glutamate potential reflected postsynaptic effects of the monoamine. In contrast, suppression of the e-DPSP by 5HT probably reflected presynaptic, as well as, postsynaptic actions of the monoamine. Though measures were taken to isolate the e-DPSP from the e-IPSP, the $E_{\text{max}}$ for 5HT induced suppression of the e-DPSP may have reflected effects of the agonist on overlapping excitatory and inhibitory potentials. Thus, suppression of the e-DPSP may have been due to actions on multiple target sites.

Attenuations of synaptic and glutamate potentials by 5HT were apparently mediated via actions of the amine on distinct receptor subtypes. The suppressive effects of 5HT on e-DPSPs and glutamate potentials were differentially sensitive to antagonism by spiperone. The apparent $K_{d}$s for spiperone against 5HT induced suppression of the glutamate potential and the e-DPSP were 95 and 1000 nM
respectively. Thus, 5HT induced suppression of the glutamate potential was ten fold more sensitive to spiperone antagonism. Spiperone sensitive receptors mediating suppression of the DPSP and glutamate potentials were most likely of the 5HT\textsubscript{1} subtype since the suppressive effects of 5HT were insensitive to antagonism by ketanserin. With an apparent \( K_d \) of 95 nM, spiperone was less effective as an antagonist than has been reported for other 5HT\textsubscript{1A} receptor mediated events (Beck et al., 1985; DeVivo and Maayani, 1986; Pan et al., 1989; Shenker et al., 1987). High concentrations of the 5HT\textsubscript{1A} agonist, DPAT, induced little suppression of the e-DPSP (or glutamate potentials) but antagonized further suppression by 5HT. Thus, the spiperone sensitive receptor in this system had an atypical 5HT\textsubscript{1A} pharmacologic profile. The receptors mediating suppression of glutamate potentials by 5HT were not likely to be of the 5HT\textsubscript{1B} type, as spiperone is a \( \geq 100 \) fold less potent at 5HT\textsubscript{1B} than at 5HT\textsubscript{1A} receptors (Nelson, 1990). However, 5HT\textsubscript{1B} receptors could have contributed to the spiperone/ketanserin insensitive suppression of transmission by 5HT. Dual receptor/mechanisms have been proposed for suppressive effects of 5HT on DPSPs in the spinal cord and locus ceruleus (Bobker and Williams, 1989; Wu et al., 1991). In those systems, the maximal suppression of transmission by DPAT was a fraction of that induced by 5HT or 5CT. Furthermore, spiperone more effectively antagonized the actions of DPAT than those of 5HT or 5CT. The receptors and/or effectors mediating suppression of corticocortical transmission may be distinct from those mediating suppression of transmission in the spinal cord and locus ceruleus, since the rank order of agonist potencies were different in the present study.
Polysynaptic potentials

In the present study, the degree of inhibition by 5HT was related to the stimulus intensities employed. Maximally effective concentrations of 5HT suppressed the high threshold IPSP without abolishing the overlapping e-DPSP or the l-DPSP. Similarly, 5HT (≥ 10 µM) abolished the high threshold l-DPSP and the associated stimulus induced augmentation of that potential without appreciably affecting the e-DPSP rising slope. In contrast, 10 to 30 µM maximally attenuated the rising slope, peak amplitude and total area of the e-DPSP when stimulus intensities were adjusted to plateau levels. Thus, the l-DPSP and e-DPSP appeared to be more resistant to the inhibitory effects of 5HT when stimulus intensities were supramaximal for the respective potentials.

It was difficult to ascertain the site or mechanism of action causing the 5HT induced suppression of polysynaptic potentials. Extrapolating from the observed effects of 5HT on the e-DPSP, it may be assumed that 5HT reduced polysynaptic inhibition and excitation via a suppression of EAA transmission. Accordingly, EAA antagonists suppressed polysynaptic IPSPs and l-DPSPs at doses that submaximally suppressed the e-DPSP (Part I; Hirsch and Crepel, 1990). Alternatively, 5HT induced reductions of polysynaptic potentials have been attributed to the hyperpolarization of the neurons generating that input (Segal, 1990) or to a selective action on GABAergic and EAA transmission (Bobker and Williams, 1989).
The high sensitivity of polysynaptic potentials to 5HT is of particular relevance to the integrative function of layer V, as neurons in this layer appear to rely on polysynaptic excitatory coupling for synchronous activation and output. Afferent activation of large regions of cortex has been associated with the frequency and intensity sensitive recruitment of large polysynaptic potentials (Ferster and Linstrom, 1986a, 1986b; Klee and Offenloch, 1964; Morison and Dempsey, 1942; Purpura et al., 1964; Schlag and Villablanca, 1967). These polysynaptic potentials are apparently generated via the extensive lateral innervation of deep cortical neurons by deep cortical neurons, that is, the horizontal plexuses described by Cajal (DeFelipe and Jones, 1988). Accordingly, layer V has been described as the focus or generator of synchronous cortical activity in the adult neocortex (Perkins and Teyler, 1988; Silva et al., 1991). Similar foci exist in the deep layers of entorhinal and olfactory cortex and in the CA3 region of the hippocampus (Alonso and Garcia-Austt, 1987; Prince and Connors, 1986; Schwartzkroin, 1986; Miles et al., 1984). These foci share several common properties including extensive intralaminar EAA synaptic coupling and a high incidence of burst or DAP firing neurons. Polysynaptic l-DPSPs resembling those described here, are associated with the synchronous ‘bursting’ of cells in layers III and V of sensory cortices and the CA3 region of the hippocampus (Luhmann and Prince, 1990; Miles et al., 1984). As 5HT preferentially attenuates polysynaptic potentials, it may shift layer V neurons from a synchronous mode of activity to an asynchronous one. Accordingly, there have been numerous reports suggesting that raphe serotonergic input raises the threshold for synchronous cortical activity (Kovacs
and Zoll, 1974; Lerner-Natoli et al., 1986; Munkenbeck and Schwark, 1982; Simon et al., 1973; Vanderwolf, 1988, 1989). Of course, this is a simplistic scheme as it does not account for the excitatory effects of the monoamine observed following prolonged exposures nor does it account for the possibility that 5HT may preferentially attenuate IPSPs under some conditions.

**Conclusions** This study provides evidence that 5HT modulates interhemispheric transmission. Isolated interhemispheric corticocortical DPSPs were found to consist of NMDA and non-NMDA components. Serotonin effectively suppressed both components of the monosynaptic potentials as well as higher threshold polysynaptic potentials. The effects of 5HT on monosynaptic potentials are mediated through activation of 5HT$_1$-like receptors which are apparently located pre-and postsynaptically on layer V neurons of the PFC.
Figure 14. Effects of 5HT on membrane potential, input resistance and callosal e-DPSP. (A) Brief bath applications of 5HT (10 µM; 3 minutes) reduced the e-DPSP amplitude but not the input resistance monitored from membrane potential deflections (mV) elicited by 150 msec depolarizing current steps (0.1 nA). Note that the membrane potential was not hyperpolarized during the 3 minute exposure to 5HT. (B) The membrane potential of another cell was hyperpolarized (2 mV) during a 12 minute exposure to cumulative concentrations of 5HT (3 and 10 µM; 3 minutes each). The membrane potential was manually clamped to resting levels prior to testing the input resistance (0.2 nA current steps) and the DPSP amplitude (*). In the presence of 10 µM 5HT, there was a 20% reduction in input resistance and a 90% reduction in e-DPSP amplitude. The e-DPSP recovered (R) from the suppressive effects of 5HT in both neurons. Neurons in A and B were both regular spiking, with resting membrane potentials of -73 mV.
Figure 15. 5HT abolished the frequency sensitive 1-DPSP. A e-DPSP-l-DPSP sequence was evoked with high intensity stimulation of the subcortical WM. All synaptic potentials were recorded at rest from the same DAP neuron. **Top left:** A control e-DPSP-l-DPSP sequence elicited with low frequency (0.03 Hz) stimulation. **right:** A control e-DPSP-l-DPSP sequence elicited with a stimulus frequency of 1 Hz. Note the stimulus induced augmentation of the l-DPSP. **Middle left:** 1 Hz stimulus induced augmentation prior to exposing the tissue to 5HT. **right:** The effects of 25 µM 5HT on the e-DPSP-l-DPSP sequence and the associated frequency induced augmentation were obtained 3 minutes after switching to a 5HT solution. **Bottom left:** Partial recovery was obtained within 12 minutes of switching to control solutions. **right:** The l-DPSP was fully recovered following an 18 minute wash.
Figure 16. 5HT reduced the l-DPSP of high threshold e-DPSP-l-DPSP sequences. Stimulus threshold for the e-DPSP-l-DPSP sequence was greater than that required to evoke a maximal amplitude e-DPSP. Exposure to 5HT was non-cumulative. Control and 5HT (1 to 30 µM) treated potentials are superimposed. **Top traces:** Low concentrations of 5HT (1 µM) reduced the l-DPSP peak amplitude by 50% without affecting the e-DPSP rising slope or peak amplitude (*). **Middle traces:** 5 µM 5HT reduced the l-DPSP by 70% and attenuated the e-DPSP. **Bottom traces:** Only high concentrations of 5HT (30 µM) appreciably affected the rising slope of the e-DPSP evoked with a supramaximal stimulus intensity. APV (10 µM) was present throughout the experiment to reduce the l-DPSP amplitude below action potential threshold.
Figure 17. Frequency and 5HT induced suppression of the IPSP. Stimulus intensities were adjusted above threshold for the e-DPSP to obtain a e-DPSP-IPSP sequence. The holding membrane potentials were above e-IPSP reversal potential. (A) The IPSP amplitude did not change when stimuli were delivered once every 30 seconds (0.03 Hz; top trace). The time scale for recordings in middle and bottom traces are expanded to illustrate the effects of 5HT on different components of the e-DPSP-IPSP sequence. The peak amplitude of the IPSP (arrows) was reversibly attenuated when stimuli were delivered once a second (1 Hz; top and middle traces). 5HT (30 µM) similarly affected both peaks of the IPSP (bottom traces). (B) An expanded time scale was employed to illustrate the effects of 5HT (30 µM) on IPSPs recorded in another neuron. 5HT reversibly reduced both components of the IPSP. Note that a l-DPSP was apparent following suppression of the IPSP. Furthermore, the e-DPSP peak amplitude increased following frequency or 5HT induced suppression of the IPSP in both cells.
Figure 18. 5HT suppressed NMDA and non-NMDA components of the e-DPSP. Stimulus intensities were adjusted to obtain plateau level e-DPSPs in two regular spiking neurons. (A) e-DPSPs evoked in the presence and absence of 5HT (10 µM) are superimposed (top) and subtracted (bottom) to illustrate the marked attenuation of the late component. (B₁) APV was applied to block the e-DPSP late component. Control and APV treated e-DPSPs are superimposed (top) and subtracted (bottom) to illustrate the slow time course of the APV sensitive component. (B₂) The early APV insensitive component was also reduced by 5HT. e-DPSP traces in the presence of APV and APV/5HT (10 µM) are superimposed (top) and subtracted (bottom). The e-DPSP was recorded at rest (-68 mV) in A and 5 mV depolarized to rest (-80 mV) in B.
Figure 19. Suppression of the plateau level e-DPSP was concentration dependent. Cumulative concentrations of 5HT (≤ 100 µM) were bath applied over a 12 minute period. (Left) e-DPSPs recorded prior to (C) and following recovery (R) from four successive 3 minute exposures to 5HT (3, 10, 30, 100 µM) are illustrated. Thirty and 100 µM concentrations of 5HT were equally effective, hence only the former is shown. (Right) Top traces: The same traces shown in A are superimposed to illustrate the concentration dependent reduction in peak amplitude, total area and rising slope. Bottom traces: Traces are amplified to illustrate the effects of 5HT on the rising slope of e-DPSPs observed in control (c) and 5HT (3 and 30 µM) solutions. The e-DPSP fully recovered ≈12 minutes after switching to control solutions.
Figure 20. 5HT attenuated e-DPSPs and matched glutamate potentials in a concentration dependent manner. Cumulative concentrations of 5HT were bath applied over a 12 minute period. e-DPSPs (left) and glutamate depolarizations (right) recorded prior to (C), during four successive 3 minute exposures to 5HT (0.3, 1, 3, 30 μM) and following recovery (R) are illustrated. The 0.3 μM 5HT did not suppress the e-DPSP (not shown). e-DPSPs observed in control and 5HT (1, 3, 30 μM) are superimposed in A (bottom traces). Glutamate potentials observed in control and 5HT (3, 30 μM) solutions are superimposed on right (bottom traces). e-DPSP and glutamate potential traces are averages of 3 and 2 responses respectively.
Figure 21. 5HT reduced the e-DPSP and glutamate potentials in a concentration dependent manner in the presence of APV. Once baseline e-DPSP amplitudes were obtained in the presence of APV (10 µM), cumulative concentrations of 5HT were applied over a 12 minute period. e-DPSPs (left) and glutamate potentials (right) recorded in the presence of APV prior to (C), during and following recovery (R) from cumulative exposures to 5HT (≤30 µM) are illustrated. Following exposure to 5HT, the same stimulus intensity elicited an IPSP in addition to the e-DPSP and the glutamate potential amplitude is larger than control. A similar facilitation was observed in about 10% of the cells.
Figure 22. Concentration effect curves for the 5HT induced suppression of e-DPSPs and glutamate potentials. Decreases (mV) in e-DPSP amplitudes following exposure to cumulative concentrations of 5HT (≤ 100 µM) were averaged for each concentration tested to generate data for concentration-effect curves. **Top graph:** Concentration effect curve for e-DPSP suppression. The mean control e-DPSP amplitude was 10 ± 1 mV (N=11; mean ± sem). The $E_{\text{max}}$ and $EC_{50}$ were 5.22 and 2.67 respectively. **Bottom graph:** Concentration-effect curves for matched e-DPSPs (circles) and glutamate potentials (squares) recorded in 6 neurons. The mean control e-DPSP and glutamate potential were 9.97 and 12.5 mV respectively. The $E_{\text{max}}$s for reduction in e-DPSP and glutamate potential amplitudes were 4.6 and 1.9 mV respectively. $EC_{50}$s for suppression of e-DPSP and glutamate potential amplitudes were 2.6 and 1.8 µM respectively.
Figure 23. Spiperone antagonized the 5HT induced suppression of the e-DPSP. 5HT (30 µM) was bath applied (3 minutes) prior to (A) and following (B) a 45 minute exposure to 1 µM spiperone. 5HT reduced the e-DPSP by 66% and 14% in the absence and presence of spiperone respectively. Control and 5HT treated e-DPSPs are illustrated in the top two traces and superimposed in the bottom traces of A and B.
Figure 24. Spiperone differentially shifts concentration effect curves for the 5HT induced suppression of e-DPSPs and matched glutamate potentials. **Top graphs:** Concentration-effect curves for the 5HT induced decrease (mV) of e-DPSPs recorded in 3 neurons prior to and following a 45 minute exposure to spiperone. Spiperone (1 µM) induced a twofold shift in the EC_{50} from 4.6 to 10.3. The spiperone antagonism was surmounted by higher concentrations of 5HT, hence the E_{max} in the presence or absence of spiperone was 4.1 µM. **Bottom graphs:** Concentration-effect curves for the 5HT induced decrease (mV) of matched glutamate potentials recorded in the same 3 neurons. Spiperone shifted the EC_{50} for the glutamate potential concentration effect curve tenfold from 4.3 to 49.5 µM. The E_{max} in the presence or absence of spiperone was ≈ 3 mV.
Figure 25. Ketanserin did not antagonize 5HT induced attenuation of the I-DPSP. A e-DPSP-I-DPSP sequence was evoked with high intensity stimulation of the callosum. (Top) A e-DPSP-I-DPSP sequence was evoked in the presence (5HT) or absence (control) of 5HT (10 µM). Upper traces: Superimposed control and 5HT treated e-DPSP-I-DPSP. 5HT abolished the I-DPSP and suppressed the e-DPSP. Bottom trace: Recovery. (Bottom) Following recovery, ketanserin was bath applied prior to (≥ 20 minutes) and during a repeat exposure to 5HT (10 µM). Upper traces: Synaptic potentials recorded in the presence of ketanserin alone and ketanserin plus 5HT are superimposed. Note the increased suppression of the e-DPSP in the presence of ketanserin. Control and 5HT treated potentials are superimposed and the recovered e-DPSP-I-DPSP sequence is illustrated below (bottom trace).
Figure 26. Ketanserin did not antagonize e-DPSP suppression by 5HT. (A) The e-DPSP amplitude increased with depolarization from rest, as is typical for the isolated e-DPSP. (B) The membrane potential was depolarized (-65 mV) to maximize the e-DPSP amplitude. The e-DPSPs observed in control and 5HT (1 µM) solutions are superimposed to illustrate the small inhibition induced by 5HT alone (top traces). (B) Following a 20 minute exposure to ketanserin, 5HT (1 µM) was reapplied in the presence of ketanserin. Control and 5HT treated e-DPSPs in the presence of ketanserin are superimposed (bottom traces). Each trace is an average of three responses. The resting potential of this regular spiking neuron was -75 mV.
Figure 27. High concentrations of DPAT (10 µM) antagonized the 5HT induced e-DPSP suppression. In this regular spiking neuron, a ten minute exposure to DPAT (10 µM) reduced callosal EPSPs by less than 10 % (not shown). A e-DPSP-l-DPSP sequence was evoked in the example given. Following a ten minute exposure to DPAT (10 µM), 5HT did not greatly attenuate the e-DPSP-l-DPSP sequence. Similar effects of DPAT were observed in 4 cells.
Table II: Maximal 5HT Suppression of the e-DPSP and the e-DPSP/l-DPSP Sequence

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LITERATURE CITED


Fosse, V.M. and F. Fonnum (1987) Biochemical evidence for glutamate and/or aspartate as neurotransmitters in fibers from the visual cortex to the lateral posterior thalamic nucleus (pulvinar) in rats. Brain Res. 400: 219-224.


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The dissertation is, therefore, accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmacology.

4/17/91  
Date  

[Signature]

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