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The Central Serotonergic And Cholinergic Systems As Activators Of The Electrocorticogram And Behavior

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The Central Serotonergic and Cholinergic Systems as Activators of the Electrocorticogram and Behavior

by

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Abstract

Experiments were performed on the role of ascending cholinergic and serotonergic electrocortical activating systems and their role in normal physiology and behavior. In urethane-anesthetized rats, electrocortical activation could be obtained by electrical stimulation of the amygdala, the dorsal raphe, the locus coeruleus area, the superior colliculus, and the orbitofrontal cortex. Activation elicited from the amygdala, the locus coeruleus area, and most orbitofrontal sites was abolished by systemic administration of the cholinergic-muscarinic antagonists scopolamine or atropine. Activation elicited from the dorsal raphe or superior colliculus was largely resistant to anti-muscarinic treatment but was abolished by methiothepin, a serotonergic antagonist. Infusions of the local anesthetic lidocaine into the basal forebrain abolished activation elicited from the amygdala. Basal forebrain cells which increased their firing during cortical activation (putative cholinergic cortically-projecting cells) could be excited by single pulse stimulation of the amygdala or locus coeruleus area.

In unanesthetized rats treated with reserpine (a monoamine depletor) and scopolamine, all cortical activation was abolished. Subsequent treatment with pargyline (a monoamine oxidase inhibitor that restores cerebral serotonin levels after reserpine treatment) restored normal activation. However, various serotonergic receptor agonists produced only partial or no activation in rats treated with reserpine and scopolamine.

Differences between the effects of serotonergic antagonists in freely moving and urethane-anesthetized rats suggest that urethane produces anti-serotonergic effects. This hypothesis was confirmed in experiments using rat aortic rings in an organ bath; urethane antagonized the effects of serotonin and strongly enhanced the action of a serotonergic antagonist without altering the action of a noradrenergic antagonist.

Behavioral experiments with p-chlorophenylalanine (an inhibitor of serotonin synthesis) and buspirone (an agonist at serotonin autoreceptors) were consistent with the hypothesis that ascending ser.onergic pathways play a role in the generation of spontaneous locomotion.

Together, these results indicate that (a) ascending cholinergic and serotonergic pathways provide final common pathways for cortical activation through which other brain systems can act; (b) serotonergic activation is linked to certain types of motor activity; and (c) experiments on serotonergic transmission in anesthetized animals may produce results that are irrelevant to the unanesthetized state.

Dedication

To the memory of my father, Rolf Dringenberg.

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Table of Contents

viii

List of Figures

PAPER 2

PAPER 3

List of Tables

Nomenclature

ACh   acetylcholine

ARAS   ascending reticular activating system

CRC   concentration-response curve

EAA   excitatory amino acid(s)

ECoG   electrocorticogram

EEG   electroencephalogram

EPSP   excitatory postsynaptic potential

5-HT   5-hydroxytryptamine (serotonin)

Hz   Hertz

IPSP   inhibitory postsynaptic potential

LISA   large irregular slow activity

LVFA   low voltage fast activity

MUA   multiunit activity

NA   noradrenaline

ms   millisecond

PCPA   *para*-chlorophenylalanine

General Introduction

In 1913, Pravdich-Neminsky published the first depiction of the "activation" of the mammalian electroencephalogram (EEG) (Pravdich-Neminsky, 1913). Commonly, "activation" of the EEG or electrocorticogram (ECoG) refers to the change from larger amplitude-low frequency activity to low amplitude-high frequency activity. Berger (1929) was the first to observe these EEG patterns in humans. In addition, he noted that there appeared to be a relation between EEG patterns and the concurrent behavior of his subjects: large amplitude slow activity was present during sleep, whereas an "activated" EEG pattern of higher frequencies and low amplitude was present in the waking state (Berger, 1929).

Following these initial descriptions of the dominant patterns of the neocortical EEG, subsequent work has attempted to provide a detailed analysis of the neuroanatomical, neurochemical, and physiological systems and mechanisms that produce different EEG patterns. Further, much work has focused on the significance of different EEG patterns in relation to the behavior or "state of consciousness" of an organism (see reviews by Purpura, 1959; Magoun, 1963; Moruzzi, 1972; Vanderwolf and Robinson, 1981; Steriade and Llinas, 1988; Vanderwolf, 1988; Pedley and Traub, 1990; Lopes da Silva, 1991; Semba, 1991). However, much of the neural organization of central activating systems and of their influence over behavior remain to be described. The specific objectives of the experiments contained in this thesis are concerned with: (a) providing a detailed physiological and pharmacological characterization of central pathways involved in ECoG activation; (b) determining if specific neurotransmitter receptor types can be identified that mediate neurochemical activating inputs in the neocortex; and (c) describing some behavioral effects of blocking activating inputs to the neocortex.

Since much of the previous work has been reviewed extensively elsewhere (see

above), I will provide only a brief summary of the mechanisms underlying EEG activation and the other dominant patterns of neocortical slow wave activity, and of the relation between neocortical slow wave activity and behavior.

Mechanisms of neocortical slow wave generation

A considerable amount of research has led to the conclusion that neocortical slow waves are generated by the summation of postsynaptic dendritic and somatic potentials in the neocortex (for reviews see Eccles, 1951; Purpura, 1959; Creutzfeldt and Houchin, 1974; Vanderwolf, 1988; Pedley and Traub, 1990). Synaptic inputs to the dendrites and somata of cortical pyramidal cells result in the generation of extracellular current flows between the soma in the deep neocortical layers and the apical dendrites in the shallow layers of the neocortex (Hubbard et al., 1969; Creutzfeldt and Houchin, 1974; Llinas and Nicholson, 1974). If such extracellular currents summate over a sufficiently large area of cortex, electrodes placed inside the cortex or even outside the skull can detect the voltage created by current flow through the resistance imposed by the extracellular medium.

Several conditions favor the summation of extracellular currents: (a) the principal cells involved in slow wave generation must be elongated since such cells generally generate large field potentials; in cells with spherically arranged processes, extracellular currents are likely to cancel one another; (b) cells should be arranged in parallel to one another; and (c) a large number of cells have to be synchronized with regard to their synaptic inputs and, consequently, postsynaptic electrical activity (Bullock and Basar, 1988). If these conditions are met, as in the neocortex and hippocampal formation, the summed extracellular field potentials that result from synaptic inputs to these cells may be sufficiently large to be measured by an electrode that is rather far removed from the site of the actual extracellular current flows (as is the case in typical encephalographic recordings

from humans with electrodes placed on the scalp).

Both empirical observations and theoretical considerations have supported the hypothesis that neoc..tical slow waves are generated by postsynaptic potentials in cortical pyramidal cells, rather than by action potentials. The short duration of action potentials (usually about 1 ms) makes it unlikely that a large number of action potentials can summate in time to generate a sufficiently large voltage that can be measured by a distant electrode (Humphrey, 1968). In contrast, synaptic potentials can last up to several hundred milliseconds, thus enhancing temporal summation of extracellular currents associated with synaptic activity (e.g., Pedley and Traub, 1990). Further, the area of membrane involved in the generation and propagation of an action potential is much smaller relative to that involved in generation of postsynaptic potentials. As a consequence, extracellular currents associated with action potentials are confined to a small area, a condition that does not favor the spatial summation of extracellular currents necessary for the detection of a voltage by a distant electrode (Humphrey, 1968; Pedley and Traub, 1990). These considerations are confirmed by experimental data. It has been shown that the voltage changes of neocortical slow waves reflect concurrent summated postsynaptic potentials better than concurrent discharge of cortical units (Creutzfeldt et al., 1966). Thus, it appears that action potentials are not an essential component in the generation of cerebral slow waves (see Eccles, 1951).

Precisely how do synaptic inputs to neocortical pyramidal cells generate extracellular current flow and, consequently, voltages in the extracellular medium ? A synaptic input to a cell may depolarize or hyperpolarize the postsynaptic cell membrane, depending on the specific type of synaptic input. An excitatory input leads to a net movement of positive ions from the extracellular space into the cell, thereby depolarizing the postsynaptic membrane; an inhibitory input results in a net movement of positive charge from the cell to the extracellular medium, hyperpolarizing the membrane. An area

of membrane where a net movement of current into the cell occurs is referred to as "sink", whereas an area of membrane where current leaves the cell and enters the extracellular medium is referred to as "source" (Lorente de No, 1947; Pedley and Traub, 1990). The current movement against the resistance of the extracellular medium creates a voltage that can be registered by an extracellular electrode. If the predominant synaptic event in the region of the electrode is an excitatory postsynaptic potential (EPSP), that is, net current is moving from the extracellular space into cells, the voltage change detected by an extracellular electrode will be negative (positive ions are moving away from the electrode and into the cell); if the predominant synaptic event is an inhibitory postsynaptic potential (IPSP), the voltage change will be in the positive direction (positive ions leave the cell and move toward the electrode).

This idealized, simplified scenario of how an extracellular voltage results from synaptic activity is complicated by the fact that current flow across the cell membrane is not limited to the local site of the synaptic interaction between two cells (e.g., presynaptic axon terminal and postsynaptic dendrite). Any synaptic activity will set up "loops" of currents flowing between a sink-source pair, i. e., a dipole (Lorente de No, 1947; Creutzfeldt and Houchin, 1974; Pedley and Traub, 1990; Vanderwolf, 1990). For example, positive ions entering the apical dendrite of a pyramidal cell have a tendency to exit the cell at the level of the soma and, driven by the extracellular negativity at the site of synaptic interaction and concentration gradients for positive ions, move back through the extracellular space toward to site of the synaptic input. The facts that currents move to and from sites of synaptic activity, and that synaptic inputs occur simultaneously at many parts of a neuron (e.g., apical and basal dendrites, soma) complicate the interpretation regarding the predominant synaptic events underlying slow wave activity. Clearly, a change in extracellular voltage is not necessarily an unequivocal indicator of the predominant synaptic events in the region close to the recording electrode.

In the rat neocortex, three dominant patterns of neocortical slow wave activity have been identified: (a) low voltage fast activity (LVFA); (b) large irregular slow activity (LISA); and (c) spindle activity.

LVFA: LVFA (or cortical activation) refers to a pattern characterized by small amplitude, often less than 0.5 mV, and mixed frequencies including frequencies above 10 Hz. If the activity of the deep pyramidal cells of cortical layer V is recorded concurrently with the ECoG during the presence of LVFA, multiunit activity (MUA) is continuous and single units discharge at a relatively stable and regular rate without the presence of long periods of inhibition or quiescence (Hubel, 1959; Creutzfeldt and Jung, 1961; Vanderwolf, 1988).

LISA: LISA is characterized by lower frequency activity, often in the range of 2-6 Hz, and by a large amplitude of up to 3 mV in transcortical, differential recordings. During LISA, MUA and single unit discharge are no longer continuous but occur in an irregular burst-suppression pattern; the bursting phase in MUA recordings may last up to 200-300 ms (Creutzfeldt and Jung, 1961; Calvet et al., 1973; Vanderwolf, 1988).

Spindle activity: Spindles constitute a pattern of rhythmical oscillatory activity in the 6-10 Hz range (up to 12-14 Hz in the case of sleep spindles) and with an amplitude of 1-2 mV. They are associated with a highly rhythmical burst-suppression pattern of MUA, the burst-phase of which does not exceed 50 ms in duration. Generally, spindles occur in bursts lasting no longer than a few seconds at a time (Calvet et al., 1964; Steriade and Llinas, 1988; Vanderwolf, 1988).

Neurophysiological and neurochemical bases of different slow wave patterns

What are the neurochemical inputs and network mechanisms that induce these different types of slow wave patterns (LVFA, LISA, spindles) in the neocortex ? Neocortical LISA is sustained when the neocortex is isolated from the rest of the brain (Kellaway et al., 1966). Thus, it appears that the generation of spontaneous, deactivated slow waves is dependent on networks intrinsic to the cortex and does not require external, subcortical inputs to the neocortex. Presumably, the synaptic interactions between cortical cells are capable of maintaining the membrane depolarizations and hyperpolarizations that underlie slow wave generation and LISA.

Highly synchronized, rhythmical activity such as spindles appears to be maintained by an interplay between neocortical and thalamic neurons (Morison and Bassett, 1945; Andersen and Andersson, 1968; Steriade and Deschenes, 1984; Steriade and Llinas, 1988). The membranes of some thalamo-cortical relay neurons appear to have intrinsic 'pacemaker' properties that produce rhythmical oscillations in the range between 6-10 Hz that are maintained even after all synaptic inputs to such cells are eliminated (Jahnsen and Llinas, 1984 a, b). Many thalamo-cortical relay neurons receive inhibitory inputs from GABAergic cells in the reticular nucleus of the thalamus, which in turn are activated by thalamo-cortical cells. Thus, discharge of thalamo-cortical neurons results in excitation of these GABAergic cells and, consequently, inhibition of thalamo-cortical neurons. The recurrent activation of this inhibitory feedback loop appears to underlie the rhythmical transmission from thalamus to cortex that generates neocortical spindle activity (reviews by Steriade and Llinas, 1988; Buzsaki and Gage, 1989; Steriade et al., 1990; Lopes da Silva, 1991). Consistent with the model, both large thalamic lesions and selective lesions of the reticular nucleus of the thalamus abolish spontaneous or drug-induced neocortical spindles but not LISA or LVFA (Villablanca and Salinas-Zeballos, 1972; Steriade et al.,

1985; Buzsaki et al., 1988; Vanderwolf and Stewart, 1988).

Traditionally, it was thought that neocortical LVFA depends on an ascending pathway to neocortex originating in the midbrain reticular formation and involving the midline thalamic nuclei, i.e., the ascending reticular activating system (ARA^c. Moruzzi and Magoun, 1949; Starzl et al., 1951; Jasper, 1960; Steriade and Llinas, 1988; this view is discussed in more detail in Section 1 and the General Discussion). More recent investigations, however, have suggested that the generation of neocortical LVFA depends on two parallel ascending systems, originating in the basal forebrain and the midbrain raphe nuclei, that provide the cholinergic and a serotonergic innervation of the neocortex (Vanderwolf, 1988). Early evidence for a role of central acetylcholine (ACh) in neocortical LVFA was obtained by the observation that the release of endogenous ACh is higher during periods of LVFA than LISA (Kanai and Szerb, 1965; Celesia and Jasper, 1966). Further, administration of cholinergic agonists by a variety of routes can produce LVFA (Bremer and Chatonnet, 1949; Cuculic et al., 1968), whereas centrally acting cholinergic-muscarinic receptor antagonists can block it (Funderburk and Case, 1951; Wikler, 1952; Cuculic et al., 1968; Spehlmann and Norcross, 1982; nicotinic ACh receptors do not appear to play a critical role in ACh-dependent LVFA since they cannot maintain it after anti-muscarinic treatment e.g., with atropine; Vanderwolf, 1975). The cholinergic innervation of the entire neocortex arises from the cholinergic cell groups of the basal forebrain (Divac, 1975; Bigl et al., 1982; Mesulam et al., 1983; Saper, 1984; Butcher and Woolf, 1986; Semba and Fibiger, 1989; Woolf, 1991). Electrical stimulation of the basal forebrain induced cortical release of ACh and concurrent LVFA (Belardetti et al., 1977; Casamenti et al., 1986; Metherate and Ashe, 1991); destruction of it results in a reduction of LVFA in the neocortex ipsilateral to the lesion (Stewart et al., 1984; Buzsaki et al., 1988). The effects of basal forebrain damage are reversed by some cholinergic agonists (Vanderwolf et al., 1993) or cortical grafts of cholinergic tissue

(Vanderwolf et al., 1990 b).

The mechanisms underlying the activating effect of ACh on the ECoG and cortical unit activity are not completely understood. Acetylcholine, acting at muscarinic receptors on cortical cells, produces membrane depolarizations, possibly by decreasing K+ conductance (Krnjevic et al., 1971; Cole and Nicoll; 1984 a,b; McCormick and Prince, 1986). Further, and perhaps more importantly for LVFA generation, it appears that ACh release in neocortex can modulate the membrane potentials of cortical pyramidal cells from slow oscillations in the 1-5 Hz range to faster oscillations between 20-40 Hz. The consequence of this modulation of intrinsic membrane fluctuations is a shift in the discharge pattern from phasic bursts of action potentials to a more sustained, continuous single spike discharge pattern (Metherate et al., 1992). As pointed out earlier, this is the discharge pattern of deep neocortical cells typically associated with the presence of LVFA. It is likely that by blocking the slow, simultaneous membrane fluctuations of many cells and the subsequent summation of extracellular currents, the large irregular slow waves associated with this current summation are also abolished, resulting in the appearance of desynchronized, activated ECoG patterns (i.e., LVFA).

A slightly different interpretation regarding the cholinergic mechanisms of cortical excitation has been proposed by Buzsaki and Gage (1989). According to this model, the summation of long-lasting after-hyperpolarizations that occurs following excitation of cortical pyramidal cells (Conners et al., 1982) may result in the appearance of large extracellular field potentials that may underlie large amplitude slow waves (Buzsaki et al., 1988). Acetylcholine release, but also other transmitters such as serotonin, may block these long-lasting after-hyperpolarizations and, consequently, the generation of the extracellular deep positive potentials such as those associated with the cessation of MUA during LISA, as outlined above (Buzsaki and Gage, 1989).

The two models outlined above are compatible in that a cholinergic action to block

long-lasting after-hyperpolarizations and a blockade of spc :..aneous slow, large amplitude membrane oscillations occurring simultaneously in many cortical pyramidal cells both would block the generation of large, summed extracellular field potentials. This, in turn, would be expected to reduce the occurrence of large amplitude slow waves in the EEG.

It is clear that the cholinergic input from the basal forebrain to the neocortex constitutes an important system involved in neocortical activation. However, not all LVFA is abolished e.g., by anti-cholinergic/muscarinic drugs or destruction of the basal forebrain. In awake, freely-moving rats, such anti-cholinergic treatments abolish only the LVFA that normally occurs during behaviors such as immobility, or more automatic movements such as grooming, chewing, or drinking (Type 2 behaviors). During behaviors such as walking, rearing, running, or any postural adjustment (Type 1 behaviors), LVFA occurs even after anti-cholinergic treatment e.g., with atropine or scopolamine (Vanderwolf, 1975, 1988). This atropine-resistant (i.e., non-cholinergic) LVFA can be abolished by various treatments that reduce or eliminate central serotonergic transmission. For example, systemic administration of the amine-depletor reserpine or the selective serotonergic synthesis inhibitor para-chlorophenylalanine (PCPA) both abolish the LVFA that is resistant to anti-cholinergic drugs (Vanderwolf and Pappas, 1980; Vanderwolf and Baker, 1986); the same effect occurs after intracerebral injection of the selective serotonergic neurotoxin 5,7-dihydroxytryptamine into the serotonin-containing cell groups of the midbrain (Vanderwolf et al., 1989; Vanderwolf et al., 1990a). Thus, it now appears that serotonin (5-hydroxytryptamine, 5-HT), in addition to ACh, plays an essential role in LVFA generation and maintains the LVFA that is not abolished by anti-muscarinic agents. This hypothesis is confirmed by the finding that in rats treated with both reserpine and atropine, atropine-resistant LVFA can be restored by administration of some serotonergic agonists such as phenylethylamine or inhibitors of monoamine oxidase (Vanderwolf et al., 1980; Vanderwolf, 1984). Also, electrical stimulation of the 5-HT-

containing dorsal or median raphe nuclei in the midbrain, which provide the largest component of serotonergic fibers to the neocortex (Steinbusch and Nieuwenhuys, 1983; Törk, 1990), is very effective in inducing neocortical LVFA (Robinson and Vanderwolf, 1978; Peck and Vanderwolf, 1991).

As outlined above, several hypotheses regarding the mechanisms of cholinergic excitation of neocortical activity have been proposed. It appears that no such hypotheses exist for the generation of 5-HT-dependent LVFA. However, it is noteworthy to mention that 5-HT can have excitatory effects on neocortical neurons (Jones, 1982). For example, 5-HT has been shown to depolarize cortical cells, presumably by suppressing a potassium current (Davies et al., 1987), and to reduce the membrane after-hyperpolarization that follows cell discharge (Araneda and Andrade, 1991). As already reviewed, Buzsaki and Gage (1989) have suggested that the latter effect may be a critical factor in the suppression of LISA and generation of LVFA (see above). Serotonin also reduces spike frequency accommodation, resulting in an increase in firing rate in response to an excitatory input (Araneda and Andrade, 1991). Thus, 5-HT appears to have a number of excitatory actions on cortical cells and it is possible that some of these may play a role in the generalized excitation of neuronal activity that underlies the generation of neocortical LVFA. Inhibitory responses of cortical cells to 5-HT have also been reported, however (Davies et al., 1987; Araneda and Andrade, 1991).

In conclusion, there now is much evidence to suggest that cholinergic and serotonergic inputs to the neocortex together maintain the activated state (i.e., LVFA) of the neocortex. After a simultaneous blockade of both systems, all LVFA and continuous MUA of deep cortical cells are abolished, and neocortical activity consists entirely of LISA associated with burst-suppression MUA. This observation suggests that in the absence of ACh- and 5-HT inputs to the cortex, no other neurochemical system is sufficient to maintain the activated state of neocortical electrical activity (Vanderwolf, 1988;

Vanderwolf et al., 1990a).

Behavioral correlates of central cholinergic and serotonergic transmission

A comprehensive review of the many diverse studies dealing with the functions of the cholinergic and serotonergic systems in the control of behavior clearly is beyond the scope of this section. Thus, only a brief overview of some behavioral studies that link cholinergic and serotonergic control over behavior to the activation of the electrocorticogram will be provided.

Blockade of the cholinergic innervation of the cerebrum, either by lesions of the cholinergic cell groups or by administration of cholinergic-muscarinic receptor antagonists (e.g., atropine, scopolamine), produces impairments of various behavioral capacities in rats. Much of the work on the behavioral effects of cholinergic blockade has focused on learned behaviors and deficits in various learning paradigms after anti-cholinergic treatments have been reported (e.g., Buresova et al., 1964; Pazzagli and Pepeu, 1964; Sutherland et al., 1982; Whishaw et al., 1985; Vanderwolf, 1991; review by Collerton, 1986). For example, it appears that the cholinergic system plays an important role in the performance of spatial learning tasks such as the Morris water maze. In this test, rats naive to the task are severely impaired in learning to locate and swim to the hidden platform in a water pool when treated with atropine (Whishaw, 1985; Whishaw et al., 1985), even though procedures to familiarize rats with the task can eliminate performance deficits associated with anti-muscarinic treatment (Saucier et al., 1996).. Of importance for the purpose of the present review is the observation that there is a good correspondence between the dose-response relationships for the ability of atropine: (a) to abolish neocortical LVFA; and (b) to produce impairments in a spatial navigation test in a swimming pool. Thus, it is possible that these behavioral impairments are, in some way,

related to the loss of ACh-dependent neocortical (and hippocampal) activation (Vanderwolf, 1991).

Behavioral deficits due to cholinergic blockade are not limited to spatial and other learning tasks. In fact, a number of unlearned 'species-typical' behaviors are affected by cholinergic blockade. Administration of atropine or scopolamine changes the circadian activity rhythm in rats and produces a pronounced hyperactivity (Longo, 1966; Whishaw et al., 1985). This effect is mimicked by excitotoxic lesions of the basal forebrain (Whishaw et al., 1985). Other species typical behaviors affected by lesions of the basal forebrain or anti-muscarinic drugs include feeding and food hoarding, placing reactions and limb posture, and sexual behaviors (Singer, 1968; Dubois et al., 1985; Whishaw et al., 1985). Thus, it appears that disruption of central cholinergic transmission produces a rather generalized behavioral deficit that involves impairments of both unlearned and acquired behaviors in rats.

Similar to ACh, 5-HT may also have a rather generalized role in the control of behavior. Early hypotheses suggested that 5-HT has a generalized inhibitory influence over behavior and 'arousal', and that it may promote sleep. For example, raphe lesions or blocking 5-HT synthesis by inhibiting the enzyme tryptophan hydroxylase with a single injection of PCPA has been shown to reduce sleep, suggesting that normally, 5-HT may have a sleep-promoting effect (Mouret et al., 1968; Jouvet, 1968). However, if PCPA is administered chronically to maintain a depletion of 5-HT for several days, sleep recovers (Dement et al., 1972). Thus, it is clear that intact 5-HT transmission is not an essential factor in sleep generation. More recently, Soubrie (1986) suggested that instead of inhibiting behavior by inducing sleep, 5-HT may produce behavioral inhibition by suppressing active behavior or responses to sensory stimuli. According to this view, reducing 5-HT transmission produces a shift from passivity to active responding or even 'impulsivity'.

Clearly, these views of 5-HT as an inhibitor of active behavior do not correspond well with the observation that 5-HT produces neocortical ECoG activation, and that this occurs in relation to the performance of behaviors such as walking, running, swimming, or rearing, among others (see above). In fact, the view that 5-HT inhibits behavior, reduces activity, or promotes sleep has been questioned by a number of investigators. Several lines of evidence suggest that, rather than inhibit behavior, 5-HT transmission may be positively correlated with the occurrence of a number of motoric activities. An early report indicated that serotonergic systems are more active in rats selected for higher activity levels relative to those in less active rats (Rosecrans, 1970). Further, decreasing 5-HT levels by lesioning raphe cells or inhibiting 5-HT synthesis may decrease activity levels in rats (Marsden & Curzon, 1976; Lips!·⁊ et al., 1992). Conversely, elevation of central 5-HT release, either pharmacologically or by electrical stimulation of the raphe nuclei, increases locomotion (Peck and Vanderwolf, 1991; Robertson et al., 1991). Serotonergic neurons in the dorsal raphe discharge at higher rates during active waking than during quiet waking or sleep (for a review, see Jacobs & Fornal, 1993) and, consequently, extracellular forebrain ⁴ ⟘ release is elevated during more active behavioral states (Wilkinson et al., 1991). Finally, as already discussed, 5-HT release produces activation of the neocortical and hippocampal slow wave activity in conjunction with concurrent movement but not during immobility (Vanderwolf and Baker, 1986; Vanderwolf, 1988; Vanderwolf et al., 1990a). Evidence for a stimulatory role of 5-HT on behavior is also found in invertebrates such as the medicinal leech in which 5-HT stimulates locomotion/swimming (Willard, 1981).

In summary, even though the precise nature of 5-HT's influence over behavior is still controversial, it now appears that earlier hypotheses of a general inhibitory role cannot account for a large amount of recent experimental data on the behavioral consequences of manipulations of the central 5-HT system, or on the correlation of endogenous 5-HT

activity with concurrent behavior. In light of these studies, one may suggest that 5-HT can have a direct, stimulating effect on the performance of a number of active behaviors (e.g., running or walking) which is incompatible with concepts of behavioral inhibition or promotion of sleep.

As discussed earlier, cholinergic and serotonergic inputs appear to function in concert to produce neocortical and hippocampal electrocorticographic activation. Thus, it is possible that these two transmitter systems may, in some way, also interact in the control of behavior. Consequently, several recent studies have attempted to characterize the behavioral deficits associated with a combined blockade of cholinergic and serotonergic systems in rats. Vanderwolf and coworkers (Vanderwolf and Baker, 1986; Vanderwolf et al., 1990a) noted that administration of the 5-HT depletor PCPA alone did not produce any gross abnormalities in the behavior of rats. For example, rats treated with PCPA can swim normally and their performance in a simple water maze was impaired only slightly relative to vehicle-treated rats. However, when PCPA-treated rats were given additional atropine or scopolamine, a severe behavioral deficit became apparent. For example, these rats often walked off the edge of an elevated platform without hesitation, i.e., their behavior no longer was controlled by visual or other sensory inputs. Further, they were severely impaired in locating the (visible) escape platform in the water maze. Often, when accidentally touching the platform while swimming past it, they failed to orient to it and climb on it as normal rats do, again suggesting that sensory inputs failed to produce normal, adaptive behavioral responses. Similarly, in the Morris water maze, low doses of only PCPA or atropine produced either no impairment or only a moderate behavioral impairment; a combination of the same low doses of PCPA and atropine, however, produced a severe acquisition and retention deficit (Richter-Levin and Segal, 1989). Shock avoidance learning also is severely impaired by combined 5-HT and cholinergic blockade, but not by PCPA alone (Vanderwolf, 1987).

Based on the observations summarized above, it appears that cholinergic and serotonergic systems can, to a significant extent, compensate for one another and maintain a high level of performance and behavioral organization even when one of these systems is non-functional. If both systems are blocked concurrently, however, the behavioral impairments exceed those present after only cholinergic or serotonergic blockade and a severe disorganization of behavior becomes apparent (see Vanderwolf et al., 1990a). The severity and general nature of this behavioral disorganization suggest that a combined cholinergic and serotonergic blockade may provide an animal model of global human dementia and Alzheimer's disease (Vanderwolf et al., 1990a).

It is possible that there is a direct relation between the deactivation of neocortical (and hippocampal) electrocortical activity and the behavioral impairment apparent after combined cholinergic-muscarinic and serotonergic blockade. As already mentioned, there is a good correlation between the potency of the anti-muscarinic drugs atropine and scopolamine to abolish cholinergic electrocorticographic activation and produce impairments in a swim-to-platform test (Vanderwolf, 1988; 1991). Also, to some extent, the behavioral effects of 5-HT and ACh blockade, alone and in combination, parallel those on the electrocorticogram: blockade of only one system may have very little or only a moderate effect on neocortical activity and behavior, whereas a combined blockade produces effects that may exceed the sum of those of a single blockade, both on behavior and the electrocorticogram (see above). In fact, some of the behavioral impairments after combined muscarinic and serotonergic blockade are reminiscent of those apparent after decortication (see Vanderwolf, 1988). Thus, it is possible that the blockade of all electrocorticographic activation produces a sort of "functional decortication". That is, in the presence of continuous neocortical LISA with concurrent burst-suppression MUA during which the activity of large numbers of neocortical neurons is suppressed for prolonged time periods, the normal functioning of the neocortex is largely abolished,

resulting in the pronounced behavioral disorganization outlined above (see Vanderwolf, 1988).

Interestingly, patients suffering from dementia of the Alzheimer's type show signs of significant cholinergic (Bartus, 1982; Collerton, 1986) and serotonergic (Rossor and Iversen, 1986; Whitford 1986) deficits, progressive deactivation of the neocortical EEG (Prinz et al., 1982; Coben et al., 1985; Penttilae et al., 1985; Soininen et al., 1991), and a global decline in intellectual and behavioral functioning (e.g., Bartus et al., 1982; Prinz et al., 1982; Collerton, 1986). These observations are in good agreement with the animal studies in suggesting a close relationship between central serotonergic and cholinergic transmission, electrocorticographic activation, and intact behavior and intellect.

Summary and general objectives of the following experiments

Based on the experimental findings summarized above, it appears reasonable to suggest that the serotonergic and cholinergic systems play a critical role in: (a) the maintenance of electrocorticographic activation of the neocortex and hippocampal formation; and (b) the maintenance of many or all adaptive behavioral capacities. The disruption of cholinergic and serotonergic systems produces both deactivation of the ECoG and severe behavioral deficits in animals including humans. Much of the neural organization of these activating systems, and of their control over behavior remains to be described, however. Especially with regards to the 5-HT system, only limited information is available to suggest how 5-HT maintains the activated state of the ECoG. For example, the role played by different 5-HT receptor types in 5-HT-dependent LVFA is not understood. Also, little research has been done that would allow one to relate the behavioral changes after 5-HT manipulations to the role of 5-HT in maintaining

electrocorticographic activation during the performance of Type 1 behaviors (see above).

To address some of these issues, I chose a multi-disciplinary approach for the present experiments to meet the following objectives: (a) to provide a more detailed description of central systems involved in stimulating cholinergic and serotonergic inputs to the neocortex; (b) to determine if the role played by different 5-HT receptor types in mediating 5-HT-dependent neocortical activation can be determined pharmacologically; and (c) to study some behavioral consequences of suppression of serotonergic transmission with reference to the role of 5-HT in maintaining movement (Type 1)-related cerebral activation.

Section I:

The Essential Role of Cholinergic and Serotonergic Inputs to the Neocortex in Electrocorticographic Activation: Electrophysiological and Pharmacological Evidence

Based largely on pharmacological evidence, it appears that serotonergic and cholinergic inputs to the neocortex are essential for the activation of neocortical slow wave activity (see General Introduction); in the absence of these two inputs, activation of the neocortex is completely abolished, suggesting that other neurochemical inputs to the neocortex are not sufficient to sustain neocortical activation.

However, electrical stimulation experiments have identified a variety of cortical and subcortical sites where stimulation can modulate the neocortical ECoG and change the slow wave activity from LISA to LVFA. Many of these sites that can induce LVFA are located in areas of the brain that do not contain cholinergic or serotonergic neurons. One possible explanation for these results is that such sites may constitute activating systems that are independent of the serotonergic and cholinergic activating systems. An alternative hypothesis is that such sites outside the cholinergic and serotonergic systems provide an *indirect* activating input that is mediated by an excitation of the direct cholinergic and/or serotonergic inputs to the neocortex.

In the following two studies, these two alternative hypotheses were tested experimentally. A number of central sites (for which stimulation has been shown to produce neocortical LVFA) were electrically stimulated and the effects of selective blockade (e. g., pharmacological antagonism, focal anesthesia) of the cholinergic or serotonergic systems on stimulation-induced LVFA were tested. Further, the question of whether electrical stimulation of activating sites outside the cholinergic and serotonergic systems results in an excitation of the cholinergic activating system was addressed using extracellular single unit recording methods.

Paper 1:

Cholinergic Activation of the Electrocorticogram:
An Amygdalar Activating System

Summary

In urethane-anesthetized rats, electrical 100 Hz stimulation of the basal amygdala changed neocortical electrical activity from ≤ 6 Hz large irregular slow activity (LISA) to low voltage fast activity (LVFA) including frequencies of above 10 Hz. A similar activating effect was seen in the hippocampus where amygdala stimulation induced the appearance of rhythmical slow activity (RSA) in the 2-6 Hz range. This activation of neocortical and hippocampal activity by amygdala stimulation was blocked by the cholinergic-muscarinic receptor antagonist scopolamine (0.5-5.0 mg/kg, i.p.), but not by the peripheral antagonist methyl-scopolamine, in a concentration-dependent manner. In contrast, a blockade of ascending inputs from the midbrain to the neocortex by treatment with the serotonin-depletor p-chlorophenylalanine or cauterization of the rostral midbrain did not block neocortical LVFA to amygdala stimulation, even though the lesions abolished all LVFA to strong noxious stimuli such as tail pinches. Unilateral infusions of the local anesthetic lidocaine (1%) into the basal forebrain selectively blocked LVFA in the neocortex ipsilateral to the infusion. However, intracerebral or systemic administration of various excitatory amino acid antagonists (2-amino-5-phosphonovaleric acid, kynurenic acid, NPC 12626) was not effective in blocking LVFA to amygdala stimulation. An input from the amygdala to the basal forebrain cholinergic system appears to be one of multiple systems involved in the cholinergic activation of neocortical and hippocampal activity. Further, basal forebrain-cholinergic inputs to the cerebrum alone are sufficient to activate the electrocorticogram as they sustain activation even in the absence of inputs from the mesencephalon.

Introduction

The cholinergic neurons of the basal forebrain provide an extensive innervation of the entire neocortex and hippocampal formation (Lehmann et al., 1980; Johnston et al., 1981; Bigl et al., 1982; Mesulam et al., 1983; Saper, 1984; Amaral and Kurz, 1985; Paxinos and Butcher, 1985; Butcher and Woolf, 1986; Mesulam, 1989; Semba and Fibiger, 1989; Woolf, 1991). Several lines of evidence indicate that the release of acetylcholine from these projections activates the electrocorticogram by inducing low voltage fast activity (LVFA) and changing neocortical unit activity from the burst - suppression pattern, which is characteristic of large amplitude slow waves, to the pattern of irregular continuous discharge which is always associated with LVFA. (1) The release of endogenous acetylcholine in the cortex is high during periods of LVFA, but low during periods of large irregular slow activity (LISA) (Kanai and Szerb, 1965; Celesia and Jasper, 1966; Jasper and Tessier, 1971). (2) The discharge rate of cortically projecting basal forebrain neurons is higher during periods of LVFA than during LISA (Detari and Vanderwolf, 1987). In the rat, such neurons are virtually all cholinergic as indicated by immunohistochemical staining for choline acetyltransferase (Woolf et al., 1983; Wahle et al., 1984). (3) Electrical stimulation of cholinergic cell groups in the basal forebrain produces LVFA and increases cortical release of acetylcholine (Belardetti et al., 1977; Casamenti et al., 1986; Metherate and Ashe, 1991). (4) Neocortical activation is reduced by centrally-acting cholinergic-muscarinic antagonists injected systemically (Funderburk and Case, 1951; Wikler, 1952; Vanderwolf, 1975) or locally in the neocortex (Cuculic et al., 1968; Spehlmann and Norcross, 1982), or by damage to basal forebrain cholinergic cell groups (Stewart et al., 1984; Buzsaki et al., 1988). (5) Cholinergic agonists, given by various routes, produce neocortical activation (Bremer and Chatonnet, 1949; Cuculic et al., 1968) and the deactivating effect of basal forebrain damage is

reversed by cholinergic agonists (Vanderwolf et al., 1993) or grafts of cholinergic tissue placed in the neocortex (Vanderwolf et al., 1990).

A component of cerebral activation that survives experimental anti-cholinergic treatment is due to a serotonergic input to the forebrain from the midbrain raphe nuclei (Vanderwolf and Baker, 1986; Vanderwolf et al., 1989; Vanderwolf et al., 1990). If both serotonergic and cholinergic inputs to the neocortex are blocked, neocortical and hippocampal electroencephalograms consist of continuous deactivated patterns, i. e., large irregular slow waves. This observation indicates that in the absence of cholinergic and serotonergic inputs, other inputs to the cerebrum are insufficient to maintain electrocorticographic activation (Vanderwolf, 1988).

As mentioned above, electrocorticographic activation can be induced by stimulation of central cholinergic (or serotonergic; see Peck and Vanderwolf, 1991) systems. However, activation by means of electrical or chemical stimulation is not limited to areas containing cholinergic or serotonergic cell groups (e. g., French, 1958; Berridge and Foote, 1991; Dean et al., 1991). For example, stimulation of the amygdaloid complex produces LVFA in anesthetized preparations (Kaada, 1951; Gloor, 1960), even though the amygdala does not contain cortically projecting cholinergic or serotonergic neurons (De Olmos et al., 1985). However, the emphasis on the central role of the reticular activating system in cortical activation seems to have led to a neglect of the possible activating role of the amygdala: recent discussions of cortical activation do not mention it (Steriade and Llinas, 1988; Vanderwolf, 1988) and nothing is known of the anatomical and neurochemical substrates of amygdaloid-induced cortical activation. In the present study, electrical stimulation of the amygdala was used in combination with selective blockade of the cholinergic and/or serotonergic systems. Thus, we hoped to determine whether amygdaloid-induced LVFA depends on the integrity of one or both of these activating systems, or whether it is independent of cholinergic and serotonergic inputs to the

cerebrum.

Methods

Subjects and surgery

The surgical and electrical recording techniques were similar to those described previously (Dringenberg and Vanderwolf, 1995). Briefly, under sodium pentobarbital anesthesia (60 mg/kg, i. p.) and using conventional stereotaxic techniques (Cooley and Vanderwolf, 1978; Paxinos and Watson, 1986), adult male Long-Evans rats (n=49, 300-450 g) were prepared with chronic bilateral implants of staggered bipolar surface-to - depth recording electrodes in the frontal neocortex (AP between -0.5 and -1.5 mm from bregma; L ± 2-3 mm) or dorsal hippocampus, and monopolar stimulation electrodes in the basal amygdala. All recording and stimulation electrodes were constructed from teflon - coated wire (diameter 125 μm). The vertical tip separation of the bipolar recording electrodes was about 1 mm.

In addition to the standard regime of neocortical and amygdala electrodes, some of the rats were prepared with bilateral implants of either (a) monopolar lesion electrodes (250 μm) in the rostral midbrain at the level of the red nucleus, or (b) guide cannulae (constructed from 18 gauge hypodermic needles) placed in the caudate-putamen dorsal to the globus pallidus. All coordinates were derived from the atlas of Paxinos and Watson (1986). Two weeks were allowed for recovery from the surgery. All surgical and experimental procedures were in accordance with the guidelines published by the Canadian Council on Animal Care.

Experimental procedure

The rats were anesthetized with urethane (Sigma Chemicals, dissolved in distilled water, 1250 mg/kg, i. p.. except where noted otherwise) and placed in a stereotaxic apparatus. Rectal temperature was monitored and heat supplied as needed by a 100-W bulb to maintain temperature between 36-37 ° C. The status of corneal and pinna reflexes was also monitored throughout the experiments. Records of neocortical or hippocampal activity were taken during undisturbed periods and during amygdala stimulation. Neocortical and hippocampal activity was recorded differentially with a Grass Model 7B polygraph (half - amplitude points at 1 and 75 Hz), passed through a band-pass filter (2-6 Hz; see Dringenberg and Vanderwolf, 1995), rectified, and integrated over 1 s intervals. The amplitude of this integrated activity was later measured in mm. Monopolar stimulation of the amygdala was provided by a Grass SM6 stimulator and a Grass Photoelectric Stimulus Isolation Unit (PSIU6) providing a constant current output. The stimulus parameters were 100 Hz, 1.0 ms duration negative-going pulses of varying train durations, and currents of 1.5-2 x the threshold for the elicitation of neocortical LVFA or hippocampal rhythmical slow activity.

Muscarinic receptor antagonism: Subsequently, rats received repeated intraperitoneal injections of either scopolamine hydrobromide (n=8) or methyl-scopolamine hydrobromide (n=7; methyl-scopolamine produces the same peripheral effects as scopolamine but does not penetrate the blood-brain barrier; both from Sigma Chemicals and dissolved in saline) to make final cumulative drug concentrations of 5 mg/kg. In the case of hippocampal recordings, rats received only one 5 mg/kg dose of scopolamine hydrobromide (n=8). Neocortical and hippocampal activity was recorded approximately 20 min after each injection. Some rats (n=4) were pretreated with *p*-chlorophenylalanine (PCPA, Sigma

Chemicals, given as a suspension in a 0.5 % solution of gum arabic; 2 or 3 x 500 mg/kg/day, i.p.). Three days after the last PCPA injection, the rats were anesthetized and the effect of amygdala stimulation before and after scopolamine treatment (5 mg/kg, i.p.) was assesred.

Rostral midbrain lesions: Rats (n=7) were anesthetized with 1000 mg/kg urethane (i. p.) After initial neocortical recordings, the rostral midbrain at the level of the red nucleus was cauterized via the implanted electrodes using a Burdick SU/7 Electrosurgical Unit. The effect of the lesion on neocortical activity during amygdala stimulation, strong tail pinches (using a mini alligator clip), and stimulation of the midbrain through the lesion electrodes was monitored and repeated cauterizations were performed until tail pinches and brain stem stimulation failed to produce neocortical activation.

Basal forebrain inactivation: Subsequent to the initial monitoring of neocortical activity, lidocaine hydrochloride (Sigma Chemicals, dissolved in saline to 1%, n=9) was infused unilaterally into the substantia innominata-ventral pallidum complex of the basal forebrain. Infusions were performed using a 50 µl Hamilton microsyringe and a Harvard Apparatus infusion-withdrawal pump. The infusion cannula was constructed from a 20 gauge needle, connected to the microsyringe with Intramedic polyethylene (PE 60) tubing, and lowered through the guide cannula into the ventral pallidum-substantia innominata. Generally, volumes of 5 µl were infused over 6.5 min (occasionally 16.5 min) and the needle was left in place after the infusion. Occasionally, an additional infusion at a second site was performed. Infusions of smaller volumes often produced only a partial effect on neocortical activity and were not included in the final data analysis. Prior to lidocaine infusions, some rats received control infusions of either saline (5-10 µl) into the basal forebrain, or of lidocaine dorsal to the basal forebrain. The effect of amygdala stimulation

on neocortical activity was monitored for up to 3 hours following an infusion.

Excitatory amino acid antagonism: Kynurenic acid (Sigma Chemicals, dissolved in saline, 6 $\mu g/\mu l$) and dl-2-amino-5-phosphonovaleric acid (APV, Cambridge Research Biochemicals, dissolved in saline, 15 $\mu g/\mu l$) were infused directly into the ventral pallidum-substantia innominata, as outlined above. NPC 12626 (dissolved in dimethyl - sulphoxide, 50 mg/ml) was injected intraperitoneally (50 mg/kg). The effect of these treatments on neocortical activity during amygdala stimulation was assessed for up to 2 hrs subsequent to the drug administration (n=6).

Histology

At the conclusion of the experiments, rats were given papaverine (Sigma Chemicals, dissolved in saline, 10 mg/kg, i.p.) to dilate cerebral blood vessels. Next, the brains were perfused with saline followed by 10 % formalin, and fixed in formalin for at least 72 hrs. Finally, a subset of randomly selected brains was cut frozen at 40 μm and stained with gallocyanine to visualize placements of electrodes, cannulae. and lesions.

Data analysis

Data are presented as means ± S.E.M. Analyses of variance, unpaired Student's t tests, and Newman-Keul's follow-up tests were performed using the software package CLR Anova (Version 1.1, Clear Lake Research Inc.) and StatWorks (Version 1.1, Cricket Software Inc.).

Results

Effects of amygdala stimulation on neocortical and hippocampal activity

The tip location of 21 randomly selected stimulation electrodes is shown in Fig. 1 (top). Generally, the electrode tips were located in the basal amygdala, either within or just ventral to the basolateral, basomedial, and ventral basolateral amygdaloid nuclei. Occasionally, electrode tips were located in the lateral amygdaloid nucleus.

In undisturbed, urethane-anesthetized rats, the spontaneous activity of the neocortex consisted of large irregular ¬low activity (LISA) of 1-2 mV and frequencies mainly below 6 Hz. During unilateral 100 Hz stimulation of the basal amygdala (Fig. 2, first trace), LISA was suppressed and replaced by low voltage fact activity (LVFA) of less than 0.5 mV and including frequencies of above 10 Hz (Fig. 2, second trace). This activating effect was also shown by the suppression of integrated neocortical 2-6 Hz activity during stimulation (Fig. 3). The average threshold current required to elicit LVFA was 283 ± 31 μA (n = 35 stimulation sites in 21 rats). LVFA was induced bilaterally by unilateral stimulation and there was no difference between the suppression of integrated 2-6 Hz activity in 18 rats between neocortical recording sites located ipsilateral and contralateral to the stimulation site ($F_{1,16} = 1.63$, p = 0.221). Consequently, except where noted otherwise, data for recording sites located ipsilateral or contralateral to the site of stimulation were combined for the figures shown.

Amygdala stimulation also replaced the large irregular activity (LIA) spontaneously present in the hippocampus of urethane-anesthetized rats, replacing it with bilateral rhythmical slow activity (RSA) in the 2-6 Hz frequency range (Fig. 2, third trace). Detailed analysis of the RSA activity during amygdala stimulation revealed that activity in the 2-6 Hz range increased by approximately 75 % ($F_{4,28} = 9.8$, p < 0.0001) during

Figure 1.

Localization of randomly selected electrode (top) and cannula sites (bottom) on 3 stereotaxic planes (in mm posterior to bregma) modified from Paxinos and Watson (1986). *Top:* The stimulation electrodes generally were located in the basal amygdala. *Bottom:* Infusion sites in the basal forebrain (filled circles) were usually located in the ventral pallidum-substantia innominata; Control injection sites dorsal to the basal forebrain (open circles) were located in the caudate putamen or stria terminalis. Abbreviations: B basal nucleus of Meynert, BL basolateral amygdaloid nucleus, BLV ventral basolateral amygdaloid nucleus, BM basomedial amygdaloid nucleus, CE central amygdaloid nucleus, CP caudate-putamen, GP globus pallidus, LA lateral amygdaloid nucleus, LV lateral ventricle, PCAn posterior cortical amygdaloid nucleus, VP-SI ventral pallidum - substantia innominata, 3V third ventricle.

A: Electrode Sites

-2.3

-3.3

B: Cannula Sites

-1.3
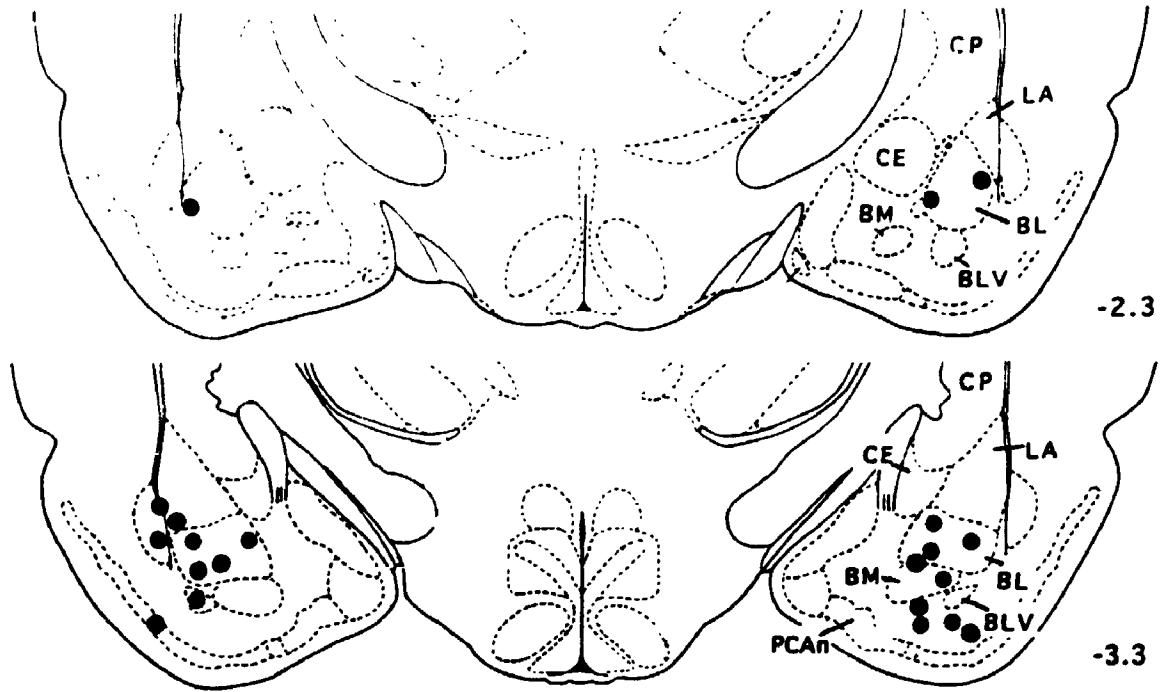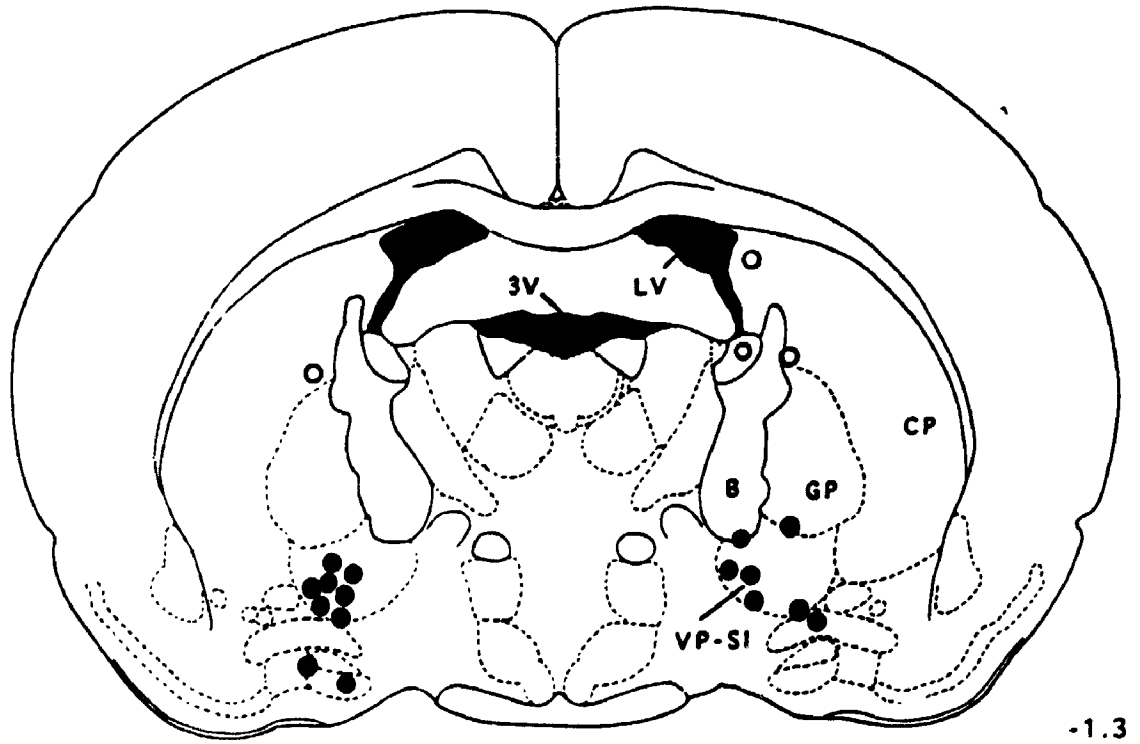
Figure 2.

Neocortical and hippocampal activity before and during amygdala stimulation (300 μA), and the effect of scopolamine administration. Prior to drug treatment, neocortical and hippocampal activity consisted of large irregular activity. During stimulation, LVFA and RSA appeared in the neocortex and hippocampus, respectively. Administration of scopolamine (5 mg/kg, i.p.) completely abolished the responses to amygdala stimulation. Calibrations: 0.5 mV; 1 s. (Note that the calibrations are the same for the following figures except where indicated in the figure caption).

Amygdala Stimulation

OFF	ON
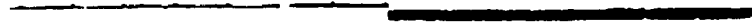
No Drug

Neocortex

Hippocampus
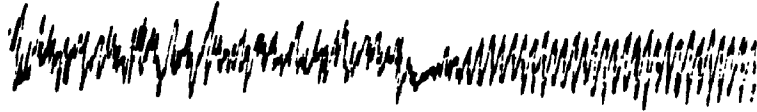
5 mg/kg Scopolamine

Neocortex
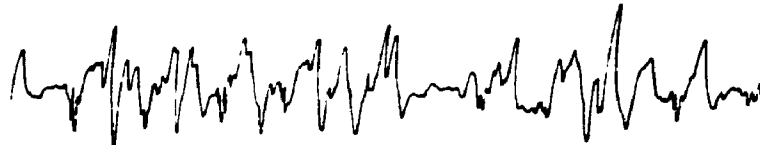
Hippocampus

Figure 3.

Neocortical 2-6 Hz activity during amygdala stimulation (1.5 x threshold), a no - stimulation control (C) period, and the effect of scopolamine (i.p.) or methyl-scopolamine (i.p.) treatment. Prior to scopolamine treatment, stimulation suppressed 2-6 Hz activity. Successive, cumulative administration of scopolamine, but not of methyl-scopolamine, blocked the 2-6 Hz suppression. * different from no-stimulation control (C) at $p \leq 0.003$ (t test) (both groups have n=7; means ± S.E.M. are shown).

Amygdala Stimulation

2-6 Hz
(Arbitrary Units)

Dose [Drug] mg/kg

○ Scopolamine
● Methyl-Scopol.
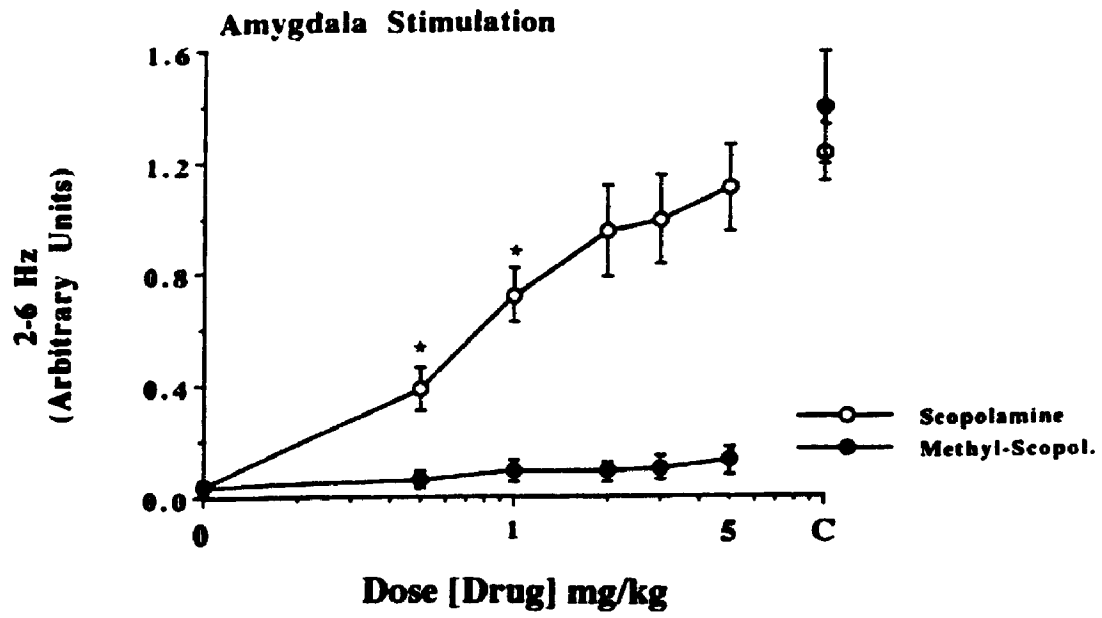
stimulation with threshold or 2 x threshold currents (Fig. 4, top). Mean hippocampal RSA frequency was 4.2 Hz and did not change with increasing stimulation currents ($F_{3,18}$ = 0.3, p = 0.9; Fig. 4, middle). As opposed to frequency, RSA amplitude (measured with a ruler) was modulated by stimulus intensity and decreased slightly with increasing stimulation currents ($F_{3,18}$ = 4.2, p = 0.02; Fig. 4, bottom). Often, at stimulation currents several times higher than those necessary to elicit RSA, hippocampal activity consisted of high frequency, low amplitude activity resembling neocortical LVFA.

Muscarinic receptor antagonism

Administration of the muscarinic receptor antagonist scopolamine blocked the induction of neocortical LVFA and hippocampal RSA by amygdala stimulation (Fig. 2). A single injection of scopolamine (5 mg/kg, i.p.) completely blocked RSA (Fig. 2) and the concurrent increase in 2-6 Hz activity (Fig. 4, top). As shown in Fig. 3, successive, cumulative scopolamine administrations produced a concentration-dependent increase in neocortical integrated 2-6 Hz activity during amygdala stimulation ($F_{5,30}$ = 17.7, p < 0.0001), replacing the LVFA normally present during stimulation with LISA (Fig. 2). At a concentration of 2 mg/kg, stimulation no longer produced a significant suppression of neocortical 2-6 Hz activity relative to a no-stimulation control (C) period (Fig. 3; t_{12} = 1.73, p = 0.11). As shown previously (Dringenberg and Vanderwolf, 1995), scopolamine generally did not abolish neocortical LVFA during tail pinches, but it abolished LVFA that normally outlasts a pinch in urethane-anesthetized rats, replacing it with LISA (data not shown). Administration of the peripheral muscarinic antagonist methyl-scopolamine, which does not readily cross the blood-brain barrier, produced a very slight increase in integrated neocortical 2-6 Hz activity during amygdala stimulation (Fig. 3;

Figure 4.

The effect of amygdala stimulation on hippocampal activity. *Top:* Amygdala stimulation at threshold (TH) and 2 x threshold intensities increased hippocampal 2-6 Hz RSA activity. This effect was abolished by administration of scopolamine (5 mg/kg. i.p.) (* different from no stimulation control and 0.5 x threshold stimulation at $p = 0.01$; ** different from 1 x and 2 x threshold stimulation at $p = 0.01$ and not different from no stimulation control, $p > 0.05$, Newman-Keul's tests). *Middle:* RSA frequency was not affected by increasing stimulation intensities. *Bottom:* With increasing stimulation intensity, RSA amplitude decreased slightly (* different from 1.5 x threshold stimulation at $p = 0.05$. Neuman-Keul's test) (n=7; means ± S.E.M. are shown).

Hippocampus

2-6 Hz Rhythmical Slow Activity
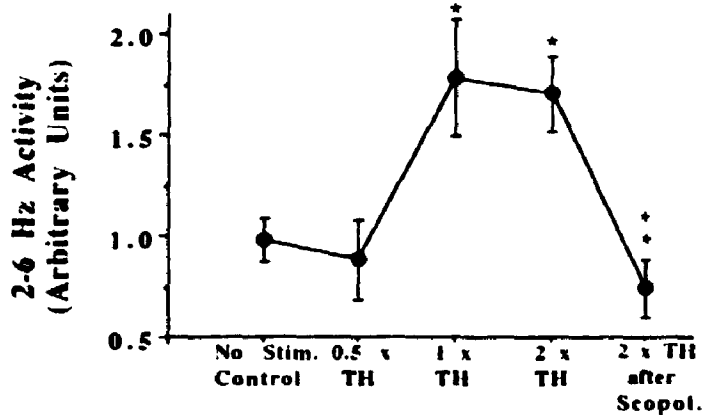
Frequency
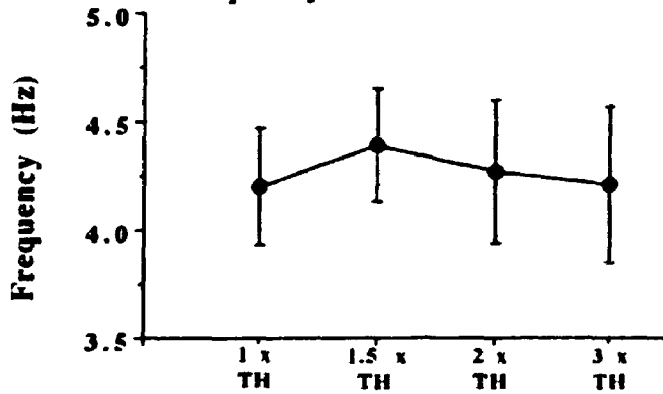
Amplitude
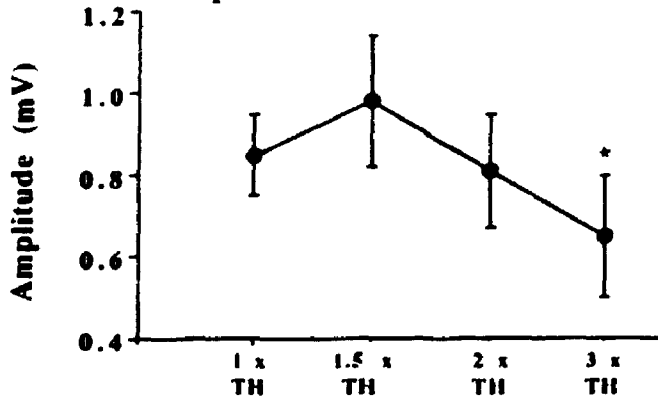
Stimulation

$F_{5,30} = 2.5$, p = 0.051).

Pre-treatment with p-chlorophenylalanine (PCPA, n = 4) had no effect on the induction of neocortical LVFA by amygdala stimulation ($F_{1,16} = 1.71$, p = 0.21). That is, even after very large doses of PCPA (up to 1500 mg/kg over several days), stimulation of the amygdala readily induced normal-appearing LVFA and concurrent 2-6 Hz suppression, an effect that could subsequently be blocked by scopolamine (not shown).

Rostral midbrain lesions

Histological examination revealed that the cauterizing current produced lesions of varying size and hemorrhage in and around the tegmentum and tectum of the midbrain. Often, the cuneiform nucleus, central gray, dorsal raphe, laterodorsal and dorsal tegmental nuclei, pedunculopontine tegmental nucleus, and the superior and inferior colliculi were involved in the lesion effect, being either destroyed or invaded by large amounts of extravasated blood which could be detected as far rostrally as the geniculate nuclei. In some brains, very little actual damage was apparent and the only conspicuous effect was the hemorrhage. Thus, it is possible that some of the effects of the lesions were due to compression of the brainstem, rather than direct damage to the tissue involved. The most extensive lesion produced by the cauterization is shown in Fig. 5.

Prior to the lesion, amygdala stimulation (198 ± 29 μA, n=7), stimulation of the rostral midbrain (151 ± 25 μA, n=5), and tail pinches all induced neocortical LVFA (Fig. 6, top left) and were equally effective in suppressing integrated 2-6 Hz activity relative to a no-stimulation control condition (Fig. 6, bottom). Lesioning the rostral midbrain completely abolished LVFA and 2-6 Hz suppression in response to strong tail pinches or electrical stimulation of the rostral midbrain through the lesion electrodes. However, in response to amygdala stimulation, LVFA and 2-6 Hz suppression could still be elicited,

Figure 5.

Photomicrograph showing the most extensive lesion produced by cauterization of the midbrain. Note the extensive tissue damage in the midbrain core and the presence of a large blood clot. In this rat, tail pinches and stimulation through the lesion electrodes were ineffective in activating the neocortex. However, despite the extensive midbrain damage, amygdala stimulation continued to suppress neocortical LISA, replacing it with LVFA.

Figure 6.

Neocortical activity (top) and integrated 2-6 Hz activity (bottom) during stimulation of the amygdala, stimulation of the rostral midbrain, tail pinching, and a control period without stimulation, all before and after rostral midbrain lesions. Prior to the lesion, all types of stimulation induced LVFA (top) and were equally effective in suppression of 2-6 Hz activity (bottom, $p > 0.05$, Neuman-Keul's test). After midbrain lesions, LVFA (top) and concurrent 2-6 Hz suppression (bottom) during tail pinching and stimulation (2 x threshold) of the midbrain were abolished, whereas stimulation (2 x threshold) of the amygdala continued to induce LVFA (top) and suppress 2-6 Hz activity (bottom, * different from all other post-lesion conditions at $p < 0.01$, but not different from amygdala stimulation prior to the lesion, $p > 0.05$, Neuman-Keul's tests) (n=7; means ± S.E.M. are shown).

Pre-Lesion   Post-Lesion

Tail Pinch

Midbrain

Amygdala

Stimulation   OFF   ON   OFF   ON

Suppression of Neocortical 2-6 Hz Activity

2-6 Hz Activity
(Arbitrary Units)

☐ Control
▨ Tail Pinch
■ Amyg. Stim.
▨ Midbrain Stim.

Pre   Post

Lesion
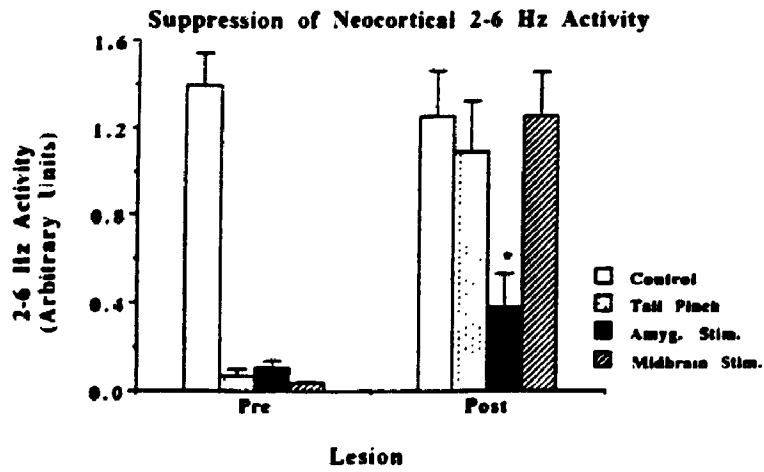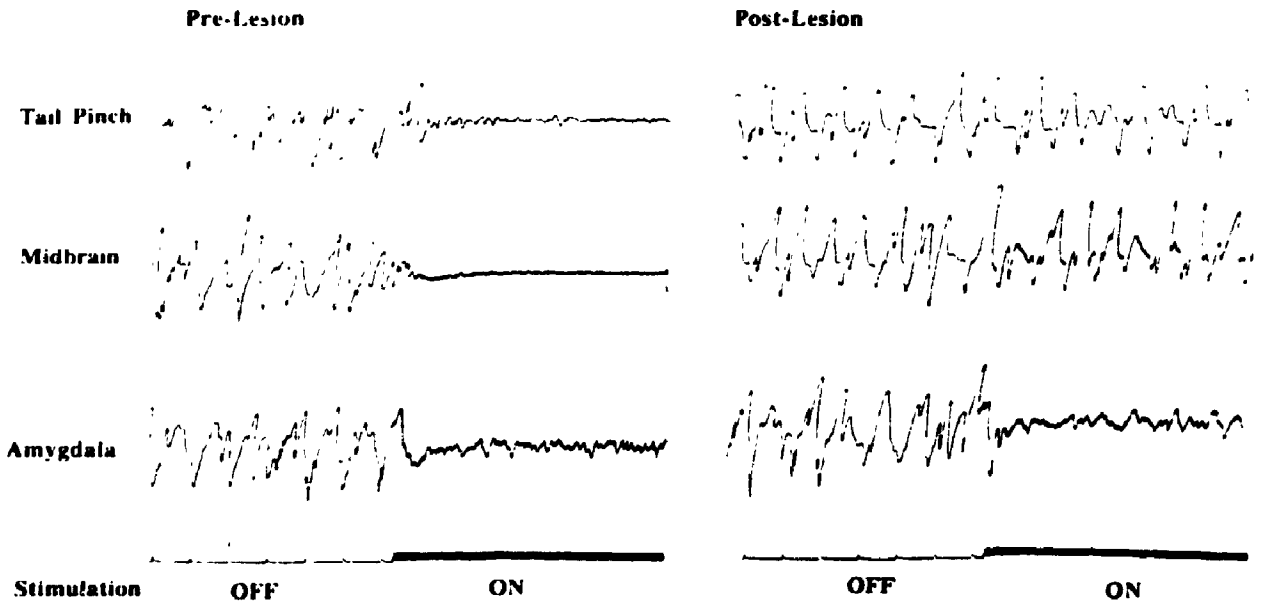
even though the threshold for this effect was raised (Fig. 6). That is, even after disconnection of the forebrain from the ascending activating influences of the midbrain, amygdala stimulation suppressed 2-6 Hz activity and produced good neocortical LVFA in such acutely lesioned rats (lesion by stimulation type interaction, $F_{2,12} = 28.8$, p < 0.0001).

Basal forebrain inactivation

Typical cannula sites of infusions into in the basal forebrain and of control infusions dorsal to the basal forebrain are shown in Fig. 1 (bottom). Generally, basal forebrain infusion sites were located in the substantia innominata-ventral pallidum complex, whereas control infusion sites were located in the caudate-putamen.

Infusions of the local anesthetic lidocaine (1%) into the substantia innominata - ventral pallidum complex produced a selective, unilateral blockade of LVFA induced by amygdala stimulation in the neocortex ipsilateral to the infusion site (Fig. 7, top). Consequently, after lidocaine infusions, the amount of 2-6 Hz activity increased in the neocortex ipsilateral ($t_{27} = 10.8$, p < 0.0001) but not contralateral ($t_{18} = 1.04$, p = 0.31) to the infusion site (Fig. 7, bottom). The amount of 2-6 Hz activity in the ipsilateral neocortex during stimulation after such infusions was equivalent to that during a no - stimulation control period (Fig. 7, bottom; $t_{26} = 0.81$, p = 0.43). In two rats, following an infusion, highly synchronous 4-5 Hz activity resembling the spike-and-wave pattern of neocortical spindle activity in awake, immobile rats appeared during amygdala stimulation in the neocortex ipsilateral to the infusion. Saline infusions did not affect the 2-6 Hz suppression in either the ipsilateral or the contralateral neocortex (Fig. 7, bottom; $t_{20} = 0.28$, p = 0.78), and neither did lidocaine infusions (n=4) into the caudate-putamen dorsal to the globus pallidus ($t(6) = 1.07$, p = 0.43; data not shown).

Figure 7.

Neocortical activity (top) and integrated 2-6 Hz activity (bottom) before and during amygdala stimulation before and after unilateral infusions of lidocaine (1 %) into the basal forebrain. *Top:* Unilateral amygdala stimulation (300 μA) produced bilateral neocortical LVFA. A unilateral basal forebrain infusion of lidocaine blocked the LVFA in the neocortex ipsilateral, but not contralateral to the infusion site. *Bottom:* Amygdala stimulation suppressed neocortical 2-6 Hz activity prior to the infusion (Pre). Infusions of 5 μl saline on either side or infusions of lidocaine contralateral to the recording sites did not affect the 2-6 Hz suppression After an infusion of lidocaine, the stimulation effect was abolished in the neocortex ipsilateral to the infusion site and 2-6 Hz activity during stimulation was equivalent to that during a no-stimulation control period. (N given in figure refer to the number of infused hemispheres; means ± S.E.M. are shown).

Neocortex

Contralateral

Ipsilateral

Lidocaine Infusion (5μl)

Contralateral

Ipsilateral

OFF   ON

Basal Forebrain Inactivation

2-6 Hz Activity
(Arbitrary Units)

1.8
1.5
1.2
0.9
0.6
0.3
0.0

n=14   n=14

n=15   n=7   n=5

Pre   Saline   Lidocaine
Contralat.   Lidocaine
Ipsilat.   No. Stim.
Control
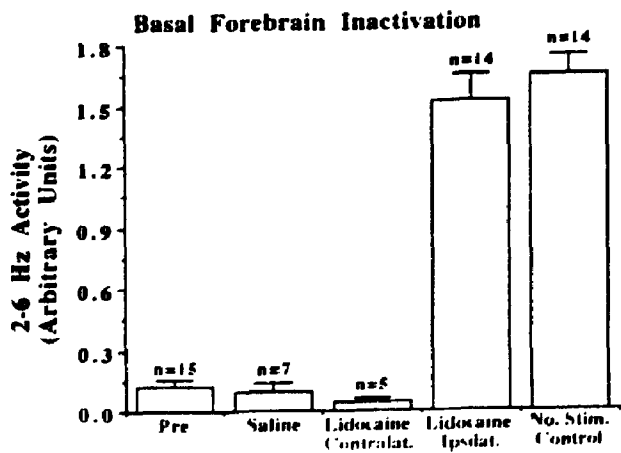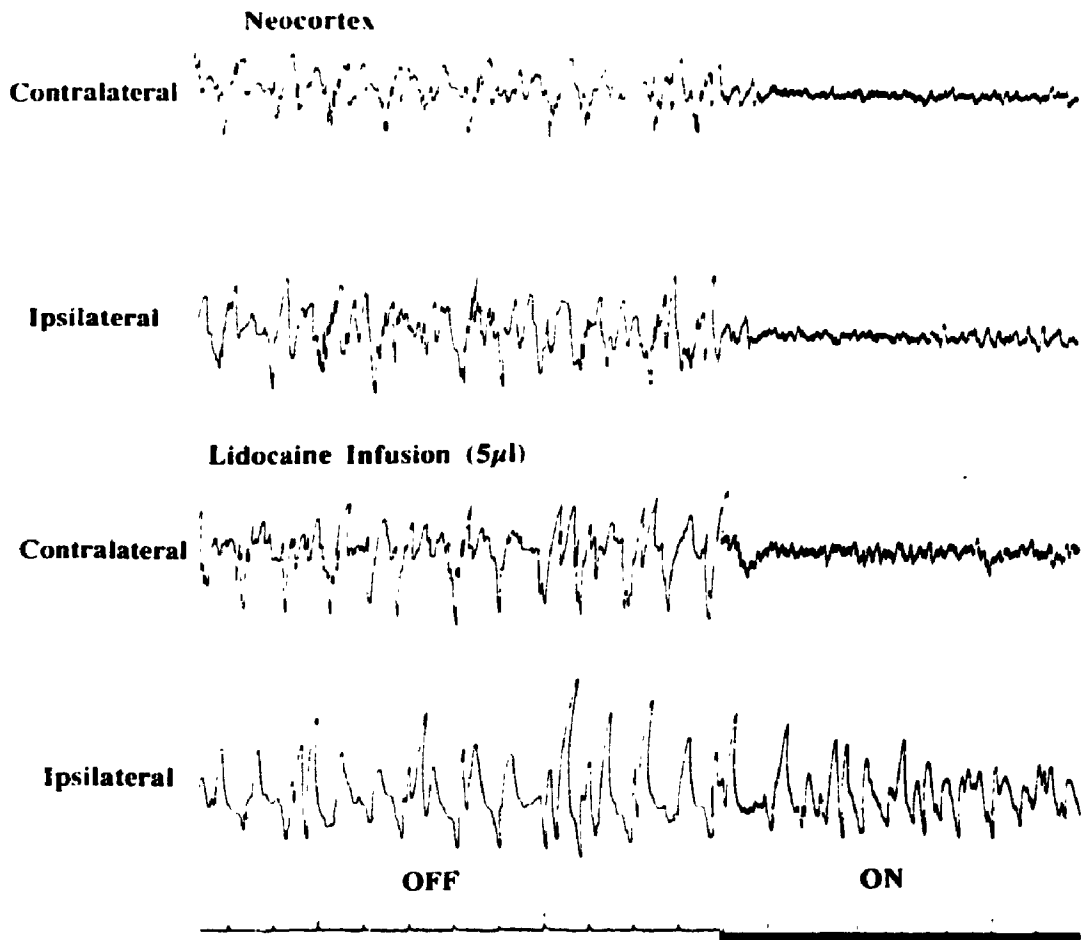
Blockade of excitatory amino acid (EAA) receptors by systemic or intracerebral administration of EAA antagonists did not abolish the LVFA in response to amygdala stimulation. As shown in Fig. 8, neither intraperitoneal administration of the selective NMDA-antagonist NPC 12626 (50 mg/kg), nor a subsequent $5 \mu l$ infusion of the NMDA antagonist APV (15 $\mu g/\mu l$) into the ventral pallidum-substantia innominate blocked the LVFA during stimulation. Further, the amount of neocortical 2-6 Hz activity during stimulation did not increase significantly after systemic NPC treatment (n = 3; $t_4 = 0.6$, p = 0.58) or APV infusions (n = 5; $t_8 = 0.26$, p = 0.8) (data not shown). Infusions of the non-selective EAA antagonist kynurenic acid (5-10 μl, 6 $\mu g/\mu l$) into the ventral pallidum - substantia innominata (n = 3) produced a slight, non-significant increase of integrated 2-6 Hz activity (means of 0.03 and 0.2 before and after the infusion, respectively; $t_4 = 1.23$, p = 0.29), but also failed to produce a blockade of LVFA during stimulation.

Discussion

The present study confirms that electrical stimulation of the amygdaloid complex activates the electrocorticogram and induces low voltage fast activity (LVFA) in the neocortex of anesthetized rats (see Kaada, 1951; Gloor, 1960). In addition, we show that hippocampal activity also is activated by amygdala stimulation, resulting in the appearance of rhythmical slow activity (RSA). Thus, it is possible that the amygdaloid complex may constitute a system involved in the generalized activation of cerebral activity.

As noted by others (Feindel and Gloor, 1954), the effect of amygdala stimulation on the electrocorticogram mimics the classical activating response elicited by stimulation of the midbrain reticular formation, i. e., the 'ascending reticular activating system' (Moruzzi

Figure 8.

Neocortical activity before and during amygdala stimulation (400 μA), and the effects of administration of excitatory amino acid antagonists. Neither systemic administration of NPC 12626 (50 mg/kg, i.p.), nor a subsequent infusion (5 μl) of APV (15 μg/μl) into the basal forebrain of the same rat blocked the LVFA during amygdala stimulation.

Neocortex

No Drug

NPC-12626 (50 mg/kg)

NPC + APV (5μl)

OFF   ON
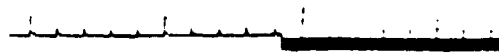
Stimulation

and Magoun, 1949). However, despite these apparent parallels, the activating response to amygdala stimulation appears to be largely independent of systems located in the midbrain. In the present study, damaging the rostral midbrain at the level of the red nucleus did not abolish the activation to ....ygdala stimulation. The activation to cutaneous stimulation such as strong, noxious tail pinches, however, was completely abolished by such lesions. Since neocortical activation to noxious stimulation appears to depend on systems located in the midbrain and specifically on the release of serotonin from the dorsal raphe nucleus in the neocortex (Thompson et al., 1991), it appears that the midbrain systems involved in neocortical activation were fully inactivated by the lesions. The fact that even shortly (15-20 min) after such acute lesions, amygdala stimulation could activate the neocortex strongly suggests that the systems underlying this effect are located outside the mesencephalon. Treatment with large doses of p-chlorophenylalanine to selectively deplete serotonin (Koe and Weissman, 1966) also had no effect on activation to amygdala stimulation. Thus, neither serotonergic nor other midbrain systems appear to be essential for the activation of neocortical activity by amygdala stimulation.

Systemic administration of the centrally acting muscarinic receptor antagonist scopolamine, but not of the peripheral antagonist methyl-scopolamine, produced a concentration-dependent block of neocortical LVFA during stimulation, suggesting an involvement of central muscarinic synapses in the activation response to amygdala stimulation. There is much evidence that the action of such anti-muscarinic treatment on the electrocorticogram is, at least partially, due to a local effect in cortex to synchronize discharge of cortical pyramidal cells (Cuculic et al., 1968; Spehlmann and Norcross, 1982; Metherate et al., 1992) by blocking postsynaptic muscarinic receptors located on pyramidal cells (McKinney and Coyle, 1982; Levey et al., 1991). Hippocampal RSA activity to amygdala stimulation could also be abolished by scopolamine, presumably by blocking the cholinergic innervation of the hippocampal formation originating in the medial

septum to hippocampal granule, pyramidal, and non-pyramidal cells (Frotscher and Leranth, 1985; Semba and Fibiger, 1989) which is directly involved in RSA induction (Stewart and Fox, 1989; 1990; Semba, 1991).

The cholinergic innervation of the entire neocortex arises from the cholinergic cell groups of the basal forebrain (Divac, 1975; F'jl et al., 1982; Mesulam et al., 1983; Saper, 1984; Butcher and Woolf, 1986; Mesulam, 1989; Semba and Fibiger, 1989; Woolf, 1991). In the present study, unilateral infusions of the local anesthetic lidocaine into the substantia innominata-ventral pallidum of the ᵤasal forebrain abolished LVFA to amygdala stimulation in the neocortex ipsilateral to the infusion site, whereas neocortical LVFA persisted in the neocortex contralateral to the infusion. This suggests that the electrocorticographic activation to stimulation of the amygdala is mediated by the basal forebrain cholinergic system, presumably involving cortically-projecting basal forebrain neurons. The observations that (a) unilateral amygdala stimulation induced bilateral LVFA, and (b) that LVFA to amygdala stimulation was abolished only in the neocortex ipsilateral, but not contralateral to the lidocaine infusions, suggest that amygdaloid complex projections to the basal forebrain (Price and Amaral, 1981; Russchen et al., 1985) are bilateral. This view is substantiated by our observation that the activity of single basal forebrain units in either cerebral hemisphere is modulated by unilateral stimulation of the amygdala (Dringenberg and Vanderwolf, unpublished observation). Fibers from the basal forebrain project unilaterally to the ipsilateral cortex (Bigl et al., 1982), thus ruling out the possibility that the bilateral activation of the neocortex we observed was mediated by the basal forebrain of only one cerebral hemisphere. The fact that unilateral anesthesia of the basal forebrain blocked ipsilateral neocortical activation is consistent with these anatomical findings.

The volumes of lidocaine solution used here exceed those recommended for chemical stimulation in small animals (Myers, 1971). Such large volumes of a local

anesthetic may be required to inactivate the basal forebrain because cholinergic cortically projecting neurons are scattered throughout a large area of the basal forebrain (e. g., Semba and Fibiger, 1989). For example, Detari and Vanderwolf (1987) encountered cortically-projecting neurons that appeared to contribute to neocortical LVFA throughout the dorsal-ventral extent of the globus pallidus and ventral pallidum-substantia innom....ata. In addition to a direct effect on cholinergic cell bodies, it is likely that large volumes of lidocaine may also have effects on passing axons in the vicinity of the infusion. However, since lidocaine produced only a unilateral block of LVFA to amygdala stimulation, it appears that the infusions did not have any nonspecific effects on the amygdala itself, or on the contralateral basal forebrain, despite the relatively large infusion volumes used.

Fibers from the amygdala to the basal forebrain may terminate on neurons positive for choline acetyltransferase (Zaborszky et al., 1984) and may employ glutamate or aspartate as a transmitter (Fuller et al., 1987). In light of these anatomical data, we were surprised that tne neocortical activation response to amygdala stimulation was not abolished by the excitatory amino acid (EAA) receptor antagonists kynurenic acid, APV, or NPC 12626. The antagonist doses used here are sufficient to severely retard other central electrophysiological phenomena dependent on EAAs such as kindling and long-term potentiation (e. g., Dennison and Cain, 1989; Cain et al., 1992; D. Saucier and D. P. Cain, personal communication). Thus, even though EAAs may modulate excitability of basal forebrain neurons, our results raise the question of whether the EAA input from the amygdala is critically involved in mediating the neocortical activation response. However, it is also possible that other antagonists at EAA receptors (e.g., selective AMPA antagonists) may be more effective in blocking the transmission between amygdala and basal forebrain than the ones tested in the present study. This possibility could be explored with future experiments.

It is noteworthy that systemic administration of the EAA (NMDA) antagonist NPC

12626 did not block neocortical LVFA. Presumably, cortical EAA receptors were blocked by this drug. Thus, our results suggest that neocortical activation is not critically dependent on NMDA systems in the brain.

The amygdala appears to play a role in the control of various autonomic functions and increases in both blood pressure and heart rate have been elicited by amygdala stimulation (Gloor, 1960; Roozendaal et al., 1993). Increased blood pressure may also produce neocortical LVFA in anesthetized animals (Baust et al., 1963), raising the possibility that the amygdaloid activating effect is an indirect one, mediated by an increased blood pressure in response to amygdala stimulation. This is unlikely, however, since LVFA in response to elevated blood pressure requires the integrity of brainstem reticular mechanisms; LVFA to increased blood pressure is abolished after lesions of the reticular formation (Baust et al., 1963). The present experiments suggest that the amygdaloid activating response functions independently of midbrain mechanisms that are involved in blood pressure-mediated LVFA generation (see above). Further, intracerebral lidocaine infusion blocked LVFA in the neocortex ipsilateral to the infusion without affecting LVFA contralateral to the infusion. An activation effec ' e to a rise in blood pressure would be expected to act ωilaterally. Thus, it seems unlikely that the amygdala activating effect is mediated by nonspecific effects on blood pressure.

The traditional view of the anatomical substrate of cortical activation (Moruzzi and Magoun, 1949; Starzl et al., 1951; Jasper, 1960) which continues to be put forward in most modern neuroscience textbooks (e. g., Barr and Kiernan, 1988; Shepherd, 1988; Carlson, 1991) emphasizes the role of the reticular formation and thalamus in activation of the cortical EEG. More recent investigations, however, have shown that the thalamus does not play an essential role in neocortical activation (Vanderwolf and Stewart, 1988). Further, the present experiments indicate that neocortical activation can be maintained even in the absence of ascending activating inputs from the brainstem (i.e., the reticular

formation). Large midbrain lesions blocked all activation to strong noxious stimulation (tail pinches), but amygdala stimulation continued to induce neocortical LVFA. These data are in agreement with early observations that in the chronic cerveau isole preparation in which the cerebrum is disconnected from the brainstem, spontaneous EEG activation occurs within a week after the lesion (Batsel, 1960; 1964). Thus, clearly, inputs from the brainstem reticular formation are not essential for cerebral activation to occur. Rather, current evidence indicates that all electrocortical activation is due to cholinergic-muscarinic and serotonergic inputs to the cerebrum. Treating freely-moving rats with anti-muscarinic drugs (atropine, quinuclidinyl benzilate, scopolamine) abolishes the type of neocortical and hippocampal activation that occurs during concurrent behavioral immobility or more automatic (Type 2) behaviors such as grooming, eating, or licking (Vanderwolf, 1988). However, during Type 1 behaviors such as walking, running, and rearing, neocortical and hippocampal activation persists after anti-muscarinic treatment. This non-muscarinic activation of the neocortex and hippocampus is abolished by treatments that deplete serotonin such as systemic reserpine or p-chlorophenylalanine (PCPA), or intracerebral injections of 5,7-dihydroxytryptamine (Vanderwolf and Pappas, 1980; Vanderwolf and Baker, 1986; Vanderwolf et al., 1989; Vanderwolf et al., 1990). The fact that no activation survives combined anti-muscarinic and anti-serotonergic treatment strongly suggests that other neurochemical inputs to the forebrain are insufficient to maintain activated patterns in the electrocorticogram.

Although systems other than cholinergic and serotonergic do not appear to be essential for the direct activation of the electrocorticogram, both forebrain (present study) and brain stem systems (Jones and Cuello, 1989; Zaborszky, 1989; Losier and Semba, 1993) may indirectly modulate cortical excitability by influencing the activity of cholinergic (or serotonergic) neurons projecting to the cortex. Thus, cerebral activation may be maintained by multiple systems and pathways including the amygdala (present study), all

of which converge on the basal forebrain cholinergic system and/or the raphe serotonergic system. The effects of many lesions e. g., of the midbrain to reduce cortical activation (see Moruzzi, 1972) may largely be due to an interruption of such modulatory inputs to the basal forebrain or raphe, rather than a blockade of directly activating inputs to the cerebrum.

Paper 2:

Neocortical Activation: Modulation by Multiple Pathways Acting on Central Cholinergic and Serotonergic Systems

Summary

In urethane-anesthetized rats, electrical 100 Hz stimulation of the amygdaloid complex or dorsal raphe area changed neocortical electrocorticographic (ECoG) activity from large irregular slow activity (LISA; 1-6 Hz, up to 2 mV) to low voltage fast activity (LVFA; less than 0.5 mV, including frequencies above 10 Hz). The LVFA during amygdala stimulation, but not that during dorsal raphe stimulation, was completely abolished and replaced by LISA after administration of the anti-muscarinic agent scopolamine (5 mg/kg, i.p.), confirming previous work to suggest that neocortical LVFA is maintained by two distinct neurochemical inputs to the cortex that can be dissociated using anti-muscarinic drugs. Electrical stimulation of the locus coeruleus area or of the superior colliculus also suppressed LISA and induced LVFA. The LVFA during locus coeruleus stimulation was abolished by the anti-muscarinic drug atropine (50 mg/kg, i.p.), whereas the LVFA during superior colliculus stimulation was atropine-resistant but could be abolished by the serotonergic antagonists methiothepin or ketanserin. Stimulation of 44 % of electrode sites in the orbitofrontal cortex produced neocortical LVFA which was reduced by atropine and completely abolished by additional administration of methiothepin. Stimulation of the entorhinal or cingulate cortex was ineffective in producing LVFA and often resulted in the appearance of epileptiform activity. Electrical single pulse stimulation of those sites that effectively induced atropine-sensitive LVFA (amygdala, locus coeruleus area) produced excitation of about 60 % of those extracellularly-recorded basal forebrain neurons that fired at higher rates during the presence of neocortical LVFA relative to LISA. About 80 % of basal forebrain cells that were more active during LISA relative to LVFA were inhibited by single pulse stimulation of the amygdala or locus coeruleus.

The present results suggest that widely distributed neuronal systems can produce ECoG activation by acting through mechanisms sensitive to anti-muscarinic or anti-

serotonergic drugs, suggesting that these activating influences involve the release of acetylcholine and/or serotonin. Those neural systems that produce atropine-sensitive (i.e., putative cholinergic) LVFA, believed to be dependent on the cholinergic innervation of the cortex arising in the basal forebrain, may produce ECoG activation by exciting basal forebrain cells that appear to contribute to neocortical LVFA, while concurrently inhibiting cells that may contribute to LISA. The observation that generally, LVFA does not survive combined anti-muscarinic and anti-serotonergic treatment suggests that inputs other than acetylcholine and serotonin may be insufficient to sustain the activated state of the ECoG.

Introduction

Several recent investigations have provided electrophysiological, pharmacological, and anatomical evidence to suggest that cholinergic inputs from the basal forebrain and serotonergic inputs from the raphe nuclei to the neocortex, independently or together, produce the pattern of activation or low voltage fast activity (LVFA) of the electrocorticogram (ECoG) (Vanderwolf, 1988; Buzsaki and Gage, 1989; Semba, 1991; Neuman and Zebrowska, 1992). Support for the cholinergic hypothesis of neocortical activation is provided by two lines of investigation that either have related the normal or endogenous activity of the cholinergic system to the occurrence of neocortical LVFA, or that have determined the effects of stimulation or blockade of the cholinergic input to the cortex on LVFA generation. A close relationship between endogenous cholinergic transmission to cortex and the occurrence of LVFA is suggested by the observation that during periods of LVFA, the cortical release of acetylcholine (ACh) is substantially higher than during periods of large amplitude irregular slow activity (LISA; Kanai and Szerb, 1965; Celesia and Jasper, 1966). Similarly, the discharge frequency of basal forebrain cells that project to cortex (thus largely cholinergic; see Woolf et al., 1983; Wahle et al., 1984) is higher when neocortical activity consists of LVFA than when it consists of LISA (Detari and Vanderwolf, 1987). Further, in senile dementia of the Alzheimer's type, a significant reduction in endogenous cholinergic activity (reviews by Bartus et al., 1982; Collerton, 1986) occurs in close relation to the progressive slowing or deactivation of electroencephalographic activity (Prinz et al., 1982; Coben et al., 1985; Penttilä et al., 1985; Soininen et al., 1991).

Experimental manipulations of the cholinergic system have shown that a blockade of the cholinergic input to cortex by administration of anti-muscarinic drugs, either systemically or locally in the neocortex, reduces LVFA (Funderburk and Case, 1951;

Wikler, 1952; Cuculic et al., 1968; Spehlmann and Norcross, 1982). Similarly, lesions of the basal forebrain containing cholinergic cell groups reduce both cortical markers of ACh activity (e.g., staining for acetylcholinesterase) and cortical LVFA (Stewart et al., 1984; Buzsaki et al., 1988; Ray and Jackson, 1991). Increasing cortical ACh release by electrical stimulation of the basal forebrain (Belardetti et al., 1977; Casamenti et al., 1986; Metherate and Ashe, 1991), or the administration of direct-acting cholinergic agonists (Bremer and Chatonnet, 1949; Cuculic et al., 1968) produces neocortical LVFA. Cholinergic agonists also can reverse the effect of basal forebrain damage on the ECoG (Vanderwolf et al., 1993), as can tissue grafts of embryonic basal forebrain tissue placed in the neocortex (Vanderwolf et al., 1990).

The data summarized above provide support for the hypothesis that ACh release in cortex plays an important role in activation of the ECoG. However, in non-anesthetized, freely moving rats, cholinergic blockade does not eliminate all neocortical LVFA (e.g., Vanderwolf, 1975; Stewart et al., 1984). Some LVFA survives anti-cholinergic treatment, but this LVFA can be abolished by a blockade of the ascending serotonergic input from the raphe nuclei to cortex. For example, systemic administration of the amine - depletor reserpine, or of the more selective serotonin (5-hydroxytryptamine; 5-HT) depletor p-chlorophenylalanine, or intracerebral injections of the selective serotonergic neurotoxin 5,7-dihydroxytryptamine all eliminate the LVFA that is resistant to cholinergic blockade (Vanderwolf and Pappas, 1980; Vanderwolf and Baker, 1986; Vanderwolf et al., 1989; Vanderwolf et al., 1990a). The monoamine oxidase inhibitors pargyline and tranylcypromine, or direct-acting serotonergic receptor agonists such as quipazine and DOI hydrochloride can fully or partially reverse the effect of combined treatment with anti - muscarinic drugs and reserpine (Vanderwolf, 1984; Paper 3 this volume); 5 - hydroxytryptophan (the immediate precursor of 5-HT) is also effective, but L - dihydroxyphenylalanine (the precursor of catecholamines) is not (Vanderwolf, 1984).

Stimulation of the dorsal and median raphe nuclei is very effective in inducing neocortical LVFA (Robinson and Vanderwolf, 1978; Peck and Vanderwolf, 1991), and this effect is blocked by several 5-HT antagonists, at least in anesthetized rats (Dringenberg and Vanderwolf, 1995). Thus, it is probable that the non-cholinergic component of ECoG activation is dependent on a serotonergic input to the neocortex (see Vanderwolf, 1988).

It is of interest to note that after combined cholinergic and serotonergic blockade, all LVFA is abolished and the ECoG consists of continuous LISA (e.g., Vanderwolf and Baker, 1986; Vanderwolf et al., 1990). This observation may suggest that non - cholinergic and non-serotonergic pathways alone are insufficient to maintain ECoG activation. However, stimulation of brain areas outside those containing cortically - projecting serotonergic and cholinergic neurons can be very effective in inducing neocortical LVFA (e.g., Kaada, 1951; French, 1958; Dean et al., 1991). For example, stimulation of the amygdala, locus coeruleus, cingulate cortex, orbitofrontal cortex, entorhinal cortex, and superior colliculus has been shown to produce neocortical LVFA (Kaada, 1951; Gloor, 1960; Segundo et al., 1955; French, 1958; Kaada and Johannessen, 1960; Neuman, 1986; Berridge and Foote, 1991; Dean et al., 1991) and, consequently, the possibility has been raised that noradrenergic or glutamergic pathways, or other non-cholinergic and non-serotonergic pathways may also have an activating influence on the ECoG (e.g., Bernardi, 1982; Neuman, 1986; Berridge and Foote, 1991).

The present set of experiments was designed to firstly examine the effect of stimulation of a number of cortical and subcortical sites that are not part of the ascending cholinergic and serotonergic pathways on the ECoG of urethane-anesthetized rats. Secondly, we asked whether LVFA produced by stimulation of some of these sites is sensitive to either muscarinic or serotonergic antagonists, suggestive of whether release of ACh or 5-HT may or may not play a role in these activating effects. Thirdly, to investigate

further a possible role of ACh in mediating cortical activation, we examined whether cells in the cholinergic basal forebrain are modulated by stimulation of sites that induce neocortical LVFA.

Methods

Subjects and surgery

Adult male Long-Evans rats (300-500 g) were used. Under sodium pentobarbital anesthesia (60 mg/kg, i.p.) and using conventional stereotaxic techniques (Cooley and Vanderwolf, 1978; Paxinos and Watson, 1986), all rats received chronic bilateral implants of bipolar transcortical neocortical recording electrodes (staggered, approximately 1 mm difference in length) in the sensory-motor cortex, and of 2 or 3 ground connections in the bone over the cerebellum. In addition, rats received bilateral implants of monopolar stimulation electrodes in either: (a) amygdala and dorsal raphe area (N=7); (b) orbitofrontal and posterior entorhinal cortex (N=8); (c) cingulate cortex and locus coeruleus area (N=10); and (d) superior colliculus (N=8). Two further groups of rats were chronically prepared with neocortical recording electrodes and bilateral monopolar stimulation electrodes in either the amygdala (N=23) or the locus coeruleus area (N=14). In addition, in these rats, holes of approximately 2 mm in diameter, drilled in the skull over the basal forebrain of both hemispheres, were covered with sterile gel-foam prior to closing the incision. All chronically implanted electrodes were constructed from teflon - coated wire (125 μm diameter; only the cross-section of the tip exposed) and all stereotaxic coordinates were derived from the atlas by Paxinos and Watson (1986). The rats were given at least 10 days of recovery from the surgery. The surgical and experimental

procedures performed were in accordance with the guidelines published by the Canadian Council on Animal Care.

Electrical recording and stimulation

Neocortical ECoG activity was recorded differentially with a Grass Model 7 P polygraph (half-amplitude at 0.3 Hz and 10 kHz). Additionally, neocortical activity was passed through a band-pass filter (1-6 Hz), rectified, and integrated over 1 s intervals. This integrated 1-6 Hz activity was used to analyze neocortical activity quantitatively. Extracellular single unit activity in the basal forebrain was recorded with parylene-insulated tungsten microelectrodes (resistance 3.5-5.0 MΩ, Micro Probe Inc.), amplified with a Grass P 15 pre-amplifier and a Grass 7 P 511 amplifier (half-amplitude for both at 300 Hz and 10 kHz), and led to a Tektronix 5103 N storage oscilloscope and a window discriminator (Mentor N-750 Spike Analyzer). The output from the window discriminator was fed back to the storage oscilloscope and the polygraph for continuous display and storage of unit activity which was later used for the quantitative analyses. The output pulses to the polygraph (1.0 V, 10 ms duration) permitted an accurate count of unit firing up to 100 Hz.

Electrical monopolar stimulation was provided by a Grass S 48 stimulator and a Grass Photoelectric Stimulus Isolation Unit (PSIU6) providing a constant current output. The stimulus parameters for train stimulation were 100 Hz frequency, 1.0 ms duration negative-going pulses of varying train durations, and current intensities of 1.5-2 x threshold current to elicit neocortical LVFA (stimulation currents of higher than 1000 μA were not used). For single pulse stimulation, single 1.0 ms negative-going pulses between 50 - 1000 μA were used. A Scope Raster Stepper Model 140 A (W-P Instruments Co.) was used to display the output of the window discriminator (0.5 ms output pulses) on

the oscilloscope for up to 60 individual single pulse stimulation sweeps which were later used for quantitative data analyses.

Experimental procedure

The rats were anesthetized with urethane (Sigma Chemical Co., dissolved in distilled water, 1250 mg/kg, i.p., supplemented as necessary) and placed in a stereotaxic apparatus. Throughout the experiments, corneal and pinna reflexes, and rectal temperature were monitored and temperature was maintained between 36-37 ° C by periodically warming the rat with a 100-W bulb.

Antagonist experiments: Records of neocortical activity were taken during undisturbed periods and during electrical 100 Hz stimulation. Subsequently, rats received an intraperitoneal injection of atropine sulfate (Sigma Chemical Co., 50 mg/kg, i.p.). Some rats received scopolamine hydrobromide (Sigma Chemical Co., 5 mg/kg, i.p.) instead of atropine. At these doses, selected on the basis of previous dose-response data, atropine and scopolamine have virtually identical effects on the ECoG (Vanderwolf, 1988). Approximately 20 min after the injection, neocortical records were again taken during undisturbed periods and stimulation. In those cases where immediate visual inspection of these records indicated that atropine r scopolamine had not completely abolished the changes in neocortical activity induced by stimulation prior to anti-muscarinic treatment, an additional injection of methiothepin maleate (Hoffman-LaRoche, 5 mg/kg, i.p.) or, in some rats, of ketanserin tartrate (Research Biochemicals Inc., 5 mg/kg, i.p.) was given. These doses were chosen on the basis of previous dose-response data (Dringenberg and Vanderwolf, 1995). After another 20 min, neocortical activity was recorded, as outlined above.

Single unit experiments: Following the initial recordings of neocortical activity

during undisturbed periods and 100 Hz stimulation, the dura over the basal forebrain was opened and a microelectrode was lowered into the brain with a stereotaxic reduction drive. Starting at a depth of approximately 4000 μm below the brain surface, cells were isolated and the spontaneous discharge rate was displayed (via the output generated by the window discriminator) on the polygraph record, together with the concurrent neocortical activity. Cell discharge was monitored during the presence of both LISA and LVFA (induced by tail pinches and/or electrical 100 Hz stimulation of the amygdala or locus coeruleus area). Subsequently, the effect of single-pulse stimulation of the amygdala or locus coeruleus area on the discharge rate of a cell was assessed by repeatedly stimulating and displaying sweeps of unit activity and the window discriminator output before and after the stimulus on the oscilloscope. Photographs of the oscilloscope screen were taken and used for subsequent quantitative data analyses. After all data was collected, the locations of some cells were marked by passing a negative DC current of 10 μA for 2-15 s. Generally, between 3 and 7 penetrations were performed in one rat.

For the subsequent data analyses, the discharge rate of each cell was counted during a 20 s period of concurrent LVFA and 20 s period of concurrent LISA. Based on the relation between discharge rate and concurrent cortical activity, cells then were divided into 3 categories: (a) *A+ Cells:* the discharge of A+ cells showed a positive correlation with neocortical activation, i.e., it was higher ($\geq$ 50 %) during LVFA than LISA (note that cells of this type appear to be cortically-projecting cells since they can be activated antic   ly by cortical stimulation; Detari and Vanderwolf, 1987); (b) *A- Cells:* discharge of A- cells showed a negative correlation with neocortical activation, i.e., discharge was higher ($\geq$ 50 %) during LISA than LVFA; and (c) *A0 Cells:* discharge of A0 cells showed no correlation with neocortical activity, i.e., it differed less than 50 % between neocortical LVFA and LISA. (Alternative criteria to classify cells such as a difference in rate between LVFA and LISA of at least 2 standard deviations or a 30 % cut-

off point produced results very similar to those obtained using a 50 % cut-off point: H. C. Dringenberg, unpublished observations).

Histology

At the conclusion of all experiments, rats were given papaverine (10 mg/kg, i.p.) to dilate cerebral blood vessels and perfused through the heart with saline followed by 10 % formalin. The brain was removed and fixed in formalin for at least 72 hrs. Finally, a subset of randomly selected brains were frozen, sectioned at 40 μm, and stained with gallocyanine to visualize placements of electrodes and microelectrode lesions.

Data analysis

Data are presented as means $\pm$ S.E.M. Analyses of variance, unpaired Student's t tests, and Tukey's tests were performed using the software package CLR Anova (Version 1.1, Clear Lake Research Inc.) and StatWorks (Version 1.1, Cricket Software Inc.).

Results

Antagonist experiments

Amygdala and dorsal raphe stimulation: Histological analysis of the location of stimulation electrodes aimed at the amygdala showed that most electrode tips were located in the central, lateral, and basolateral amygdaloid nuclei (Fig. 1 A). Raphe electrodes were located in or immediately adjacent to the dorsal raphe nucleus (Fig. 1 B).

Spontaneous neocortical activity of urethane-anesthetized rats consisted of

Figure 1.

Localization of randomly selected stimulation electrodes on stereotaxic planes (in mm to bregma) modified from Paxinos and Watson (1986; A, B, C, F, and G) or Zilles (1985; D and E). See text for details. *Abbreviations for A:* BL basolateral amygdaloid nucleus, BM basomedial amygdaloid nucleus, CE central amygdaloid nucleus, LA lateral amygdaloid nucleus Me medial amygdaloid nucleus, st stria terminalis; *for B:* CG central grey, DR dor:- raphe, xscp decussation of the superior cerebellar peduncle; *for C:* Cer cerebellum, CG cen'ral grey, DTg dorsal tegmental nucleus, IC inferior colliculus, LC locus coeruleus, Me5 mesencephalic nucleus of the trigeminal nerve, 4V fourth ventricle; *for D:* AID agranular insular cortex (dorsal part), Cl claustrum, LO lateral orbital area, Pir prepiriform cortex, VLO ventrolateral orbital area; *for E:* Ent entorhinal area, Oc occipital cortex; *for F:* CC corpus callosum, Cg cingulum, PCg posterior cingulate cortex; *for G:* CG central grey, DpG deep grey layer of the superior colliculus, InG intermediate grey layer of the superior colliculus, InWh intermediate white layer of the superior colliculus, Op optic nerve layer of the superior colliculus, SuG superficial grey layer of the superior colliculus.

A: Amygdala
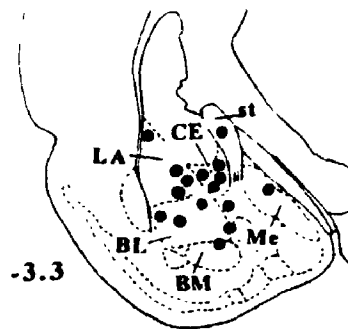
D: Orbitofrontal Cortex
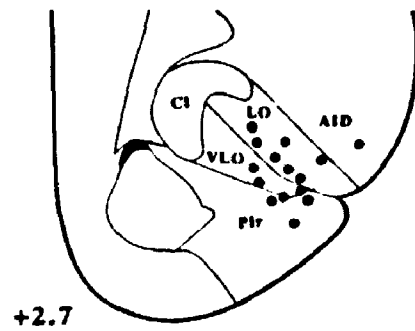
B: Dorsal Raphe
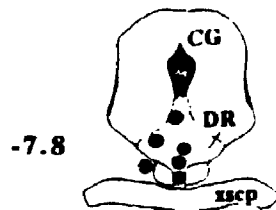
E: Entorhinal Cortex
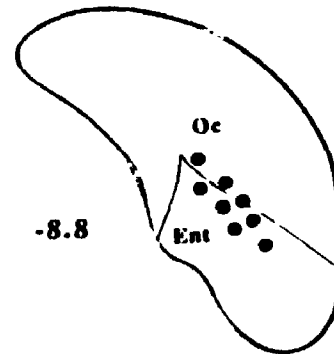
C: Locus Coeruleus Area
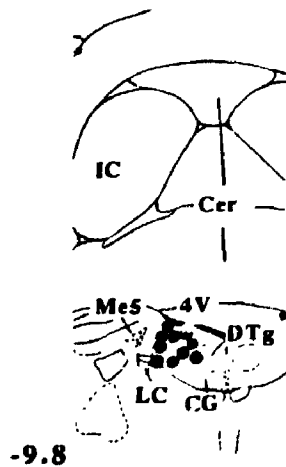
F: Cingulate Cortex
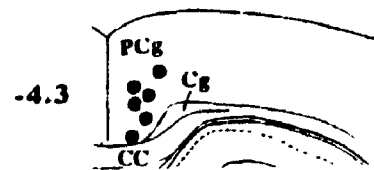
G: Superior Colliculus
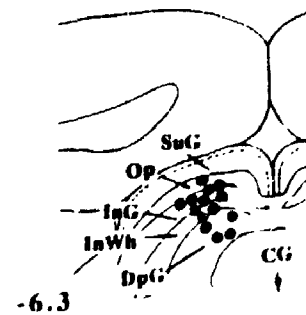
continuous LISA in the 1-6 Hz range. As shown previously (Peck and Vanderwolf, 1991; Paper 1 this thesis), stimulation of the amygdala or-dorsal raphe changed neocortical activity from 1-6 Hz LISA to LVFA (Fig. 2, top) with little activity in the 1-6 Hz range but including frequencies above 10 Hz. Consequently, integrated 1-6 Hz activity was suppressed during amygdala or raphe stimulation (Fig. 2, bottom). These effects were elicited with stimulation of all amygdala and raphe electrode sites tested (14 and 7 sites, respectively). The threshold currents required to elicit LVFA and 1-6 Hz suppression were 357 ± 58 μA and 99 ± 40 μA for amygdala and raphe stimulation, respectively (c .erent according to a t-test, p = 0.003). Midline-raphe stimulation and unilateral amygdala stimulation always induced bilateral neocortical LVFA and 1-6 Hz suppression. Since this was also the case for all other stimulation effects reported here, data obtained from all neocortical recordings sites were combined for the figures and statistical analyses, regardless of laterality.

Administration of the muscarinic receptor antagonist scopolamine (5 mg/kg, i.p.) completely abolished the LVFA (Fig. 2, top) and 1-6 Hz suppression during amygdala stimulation; integrated 1-6 Hz activity was no longer significantly suppressed relative to that during a no-stimulation period (Fig. 2, bottom). Thus, scopolamine completely abolished the effect of amygdala stimulation on neocortical activity. In contrast, after scopolamine administration, raphe stimulation continued to elicit LVFA (Fig. 2, top) and produced a significant suppression of 1-6 Hz activity (Fig. 2, bottom).

Stimulation of locus coeruleus area: Most stimulation electrodes aimed at the locus coeruleus were located just medial (less than 500 μm) to the locus coeruleus in the pontine central grey/laterodorsal tegmental nucleus. Several electrodes were in direct contact with the medial aspect of the locus coeruleus (Fig. 1 C).

Unilateral stimulation of the locus coeruleus area produced bilateral neocortical

Figure 2.

The effect of stimulation of the amygdala or dorsal raphe (1.5 x and 2 x threshold before and after scopolamine administration, respecti·ely; n=7) on neocortical activity. Prior to drug treatment, amygdala and raphe stimulation both induced LVFA (top) and suppressed integrated 1-6 Hz activity (bottom) relative to a no-stimulation period. Following scopolamine administration (5 mg/kg, i.p.), LVFA during amygdala stimulation was completely abolished but, in this rat, LVFA during raphe stimulation was completely unaffected (top). After scopolamine, there was a significant increase in 1-6 Hz activity across all three stimulation conditions ($F_{1,6} = 16.6$, $p = 0.007$). The 1-6 Hz suppressant response to amygdala stimulation was completely abolished by scopolamine and 1-6 Hz activity during amygdala stimulation was not different from that durin_; a no - stimulation period (No Stim.; $p > 0.05$, Tukey's test). Raphe stimulation, however, continued to suppress 1-6 Hz activity after scopolamine administration relative to the no - stimulation period ($p = 0.01$, Tukey's test). Note that during raphe stimulation, 1-6 Hz activity after scopolamine treatment was not significantly higher than that in undrugged rats ($p > 0.05$, Tukey's test). Also note the significant drug x stimulation interaction, $F_{2,12} = 20.2$, $p = 0.0001$. (Means ± S.E.M. are shown).
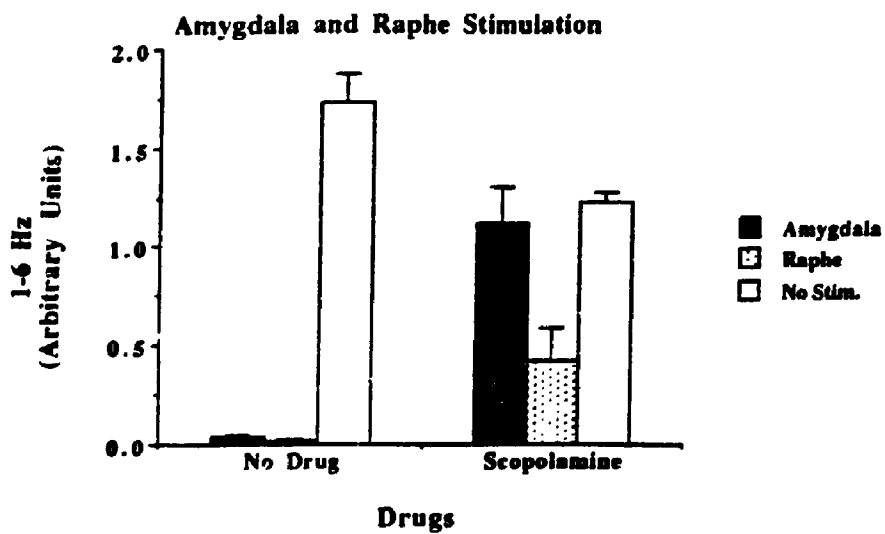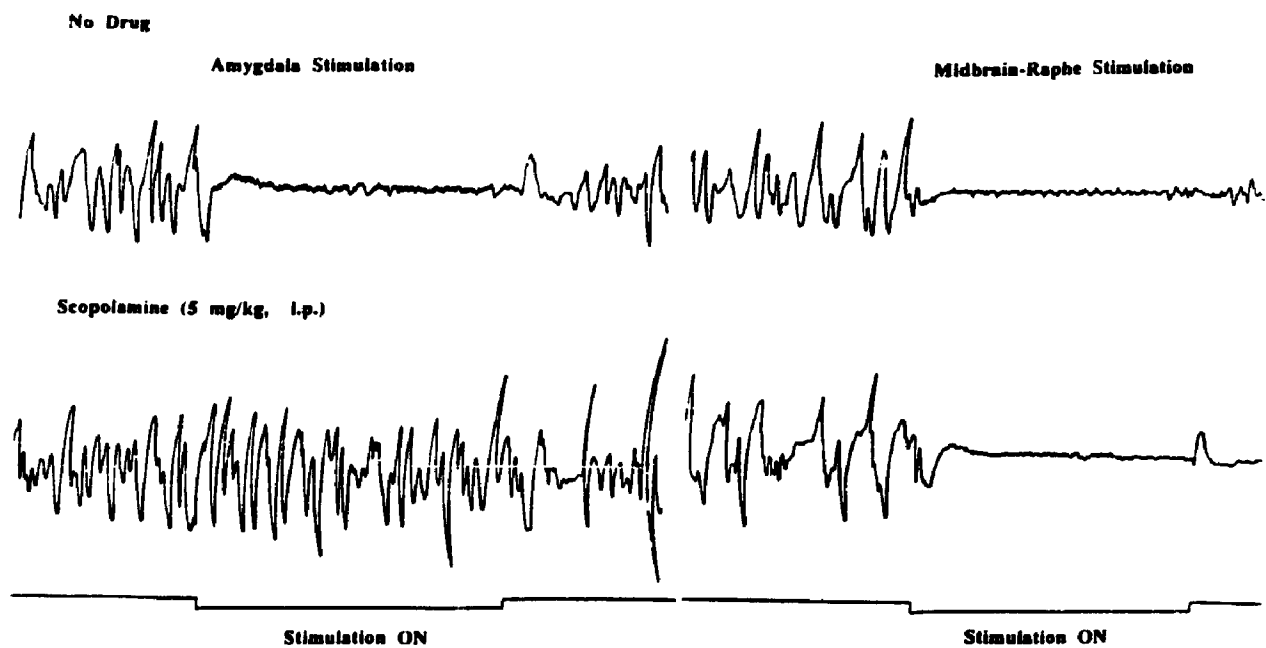
LVFA (Fig. 3, top) and suppressed LISA and integrated 1-6 Hz activity relative to a no -stimulation period (Fig. 3, bottom). This effect was observed with stimulation at 19 of 20 electrode sites (95 %) and the mean thres.o.d current to elicit LVFA was 75 ± 12 μA. This threshold current was lower than that for amygdala stimulation (p < 0.001, t test) but did not differ from that for stimulation of the raphe area (p = 0.52, t test).

Administration of atropine (50 mg/kg, i.p.) complotely blocked the LVFA and LISA suppression during stimulation of the locus coeruleus area (Fig. 3, top). Thus, after atropine treatment, the amount of 1-6 Hz activity during stimulation was no longer different from that during a no-stimulation period (Fig. 3, bottom). Occasionally, stimulation continued to produce an increase in frequency of cortical activity, but frequencies above 6 Hz were not produced by locus coeruleus stimulation after atropine treatment (see Fig. 3, top).

Orbitofrontal cortex stimulation: The locations of electrodes aimed at the orbitofrontal cortex are shown in Fig. 1 D. For 7 of 16 (44 %) stimulation sites tested, unilateral stimulation produced bilateral LVFA and suppression of 1-6 Hz LISA (Fig. 4). The threshold current for LVFA generation and 1-6 Hz suppression was 586 ± 112 μA. This threshold current was higher than those for stimulation of the two midbrain regions (p < 0.003, t test) but not higher than that for amygdala stimulation (p = 0.08, t test).

Administration of atropine (50 mg/kg, i.p.) produced a partial block of LVFA and 1-6 Hz suppression during stimulation, but integrated 1-6 Hz activity remained significantly suppressed relative to that during a no-stimulation period (Fig. 4). Additional administration of methiothepin (5 mg/kg, i.p.) produced a further increase in integrated 1-6 Hz activity and after such combined atropine and methiothepin treatment, 1-6 Hz activity was no longer significantly suppressed during stimulation relative to a no stimulation period (Fig. 4).

Figure 3.

The effect of stimulation (1.5 x threshold) of the locus coeruleus area on neocortical activity. Prior to atropine administration, locus coeruleus stimulation induced LVFA and suppressed LISA (top). Further, integrated 1-6 Hz activity during stimulation (Stim.) was suppressed relative to a no-stimulation (No Stim.) period (bottom; $p = 0.01$, Tukey's test). After administration of atropine (50 mg/kg, i.p.), LVFA (top) and 1-6 Hz suppression (bottom) during stimulation were abolished. Thus, after atropine treatment, integrated 1-6 Hz activity during stimulation no longer was significantly suppressed relative to a no-stimulation period ($p > 0.05$, Tukey's test). Note the significant drug x stimulation interaction, $F 1,9 = 36.3$, $p = 0.0002$. (Means ± S.E.M. are shown).

No Drug
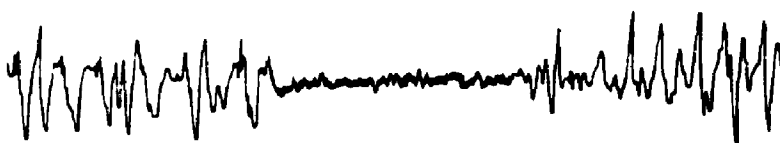
Atropine (50 mg/kg, i.p.)

Stimulation ON

Locus Coeruleus-Area Stimulation

1-6 Hz (Arbitrary Units)

☐ Stim.
☐ No Stim.

No Drug   Atropine

Drugs
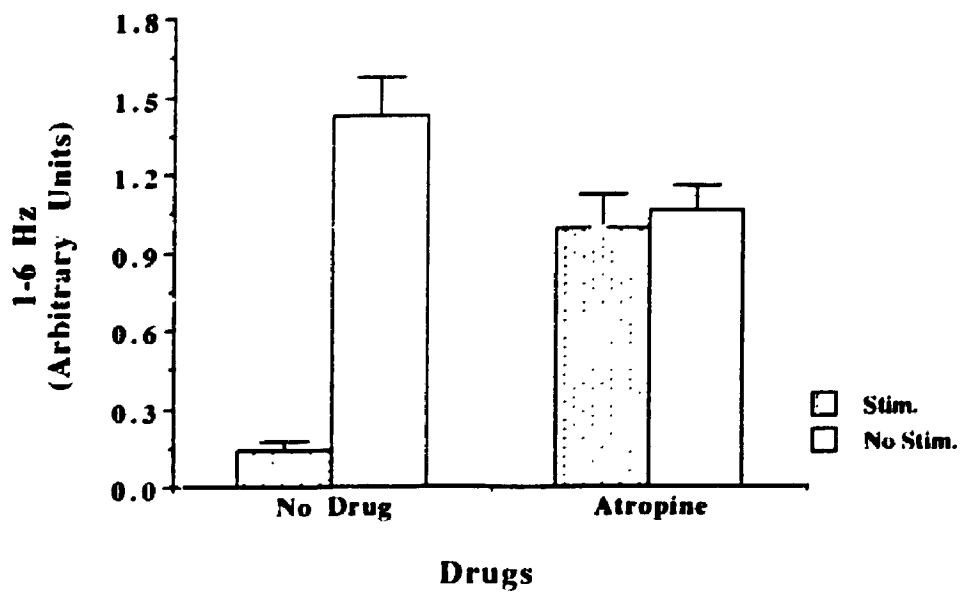
Figure 4.

Stimulation (Stim.; 1.5 x threshold) of 7 orbitofrontal cortex sites decreased neocortical integrated 1-6 Hz activity. Administration of atropine (50 mg/kg, i.p.) and methiothepin (5 mg/kg, i.p.) increased 1-6 Hz activity during stimulation (drug x stimulation interaction, $F_{2,2} = 22.7$, p = 0.042). Student's t tests showed that after only atropine administration, 1-6 Hz activity was still significantly suppressed during stimulation relative to a no-stimulation (No Stim.) period (p < 0.05; but note that a Tukey's test did not show a significant difference, p > 0.05). After additional methiothepin treatment, however, 1-6 Hz activity during stimulation no longer was significantly different from that during a no-stimulation period (p > 0.05, t test and Tukey's test). (Means ± S.E.M. are shown).

Orbitofrontal Cortex Stimulation
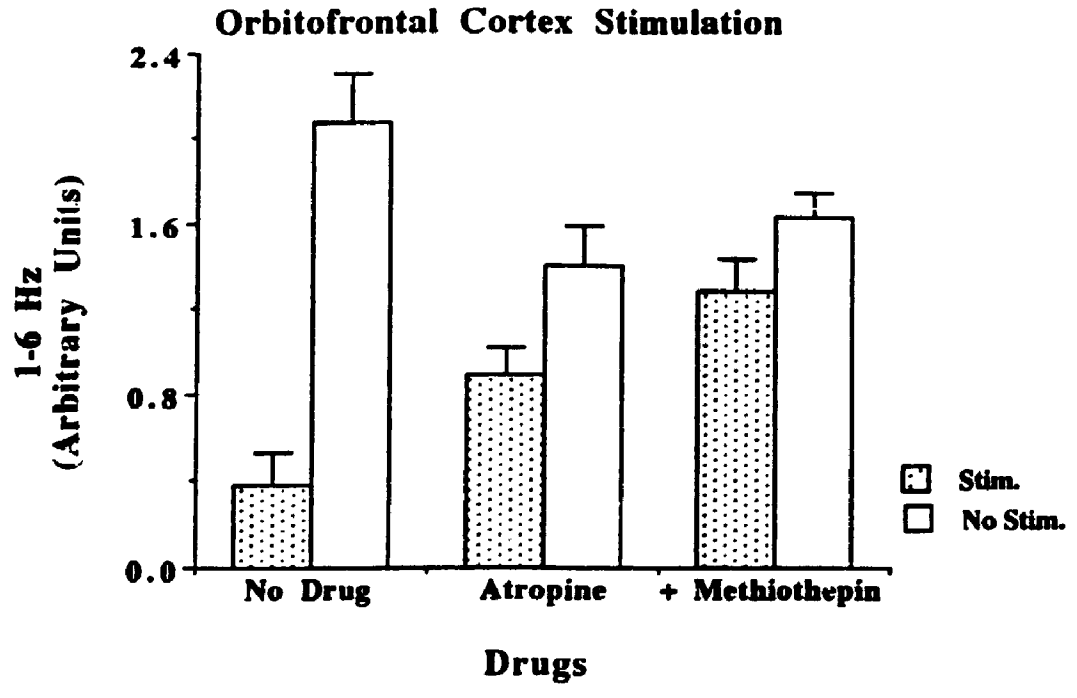
The ability of atropine to reduce LVFA and 1-6 Hz suppression during stimulation was somewhat variable for different stimulation sites. Thus, we analyzed the effects of atropine on 1-6 Hz activity for each stimulation site separately. These analyses showed that for 5 of the 7 stimulation sites that produced LVFA, administration of atropine alone completely blocked LVFA and 1-6 Hz suppression during stimulation, i.e., after atropine, 1-6 Hz activity during stimulation was not different from that during a no-stimulation period ($p \geq 0.08$, t-tests). For the remaining 2 sites, stimulation continued to suppress 1 - 6 Hz activity significantly relative to a no-stimulation period ($p < 0.02$, t-tests). Thus, the remaining mean 1-6 Hz suppression during orbitofrontal stimulation after atropine treatment alone was due to an incomplete block at 2 of the 7 effective stimlation sites.

Entorhinal cortex stimulation: Stimulation electrodes were located in the posterior entorhinal cortex and the adjacent ventral occipital cortex (Fig. 1 E). Unilateral stimulation of 2 (12.5 %) of 16 electrodes aimed at the posterior entorhinal cortex of 8 rats produced neocortical LVFA and concurrent suppression of 1-6 Hz LISA. Both effective electrodes were implanted in the same rat. In all rats, LVFA was easily elicited by tail pinches. To produce clear LVFA in the one rat, very high currents (at least 1000 μA) were required. Administration of atropine (50 mg/kg, i.p.) produced a partial block of LVFA and 1-6 Hz suppression, and additional treatment with methiothepin (5 mg/kg, i.p.) completely abolished LVFA and 1-6 Hz suppression during entorhinal stimulation relative to a no - stimulation period. Stimulation of those entorhinal sites that did not produce LVFA often produced epileptiform activity such as high amplitude spiking and spike and wave activity.

Cingulate cortex stimulation: Stimulation of all 11 electrode aimed at the posterior cingulate cortex (see Fig. 1 F) suppressed neocortical 1-6 Hz activity. However, 1-6 Hz activity was never replaced by LVFA. Instead, stimulation produced bilateral, highly

synchronous, small amplitude activity (0.1-0.2 mV) between 30-50 Hz. Immediately after the offset of the simulation current, cortical activity often was suppressed completely and consisted of an isoelectric potential. Over the next several seconds, cortical activity recovered and often consisted of an epileptiform spike and wave pattern. Administration of atropine (50 mg/kg, i.p.) or of subsequent methiothepin (5 mg/kg, i.p.) did not block the low amplitude 30-50 Hz activity during stimulation of the cingulate cortex.

Superior colliculus stimulation: Electrodes aimed at the supx-ior colliclulus w·re located in the intermediate and deep collicular layers (Fig. 1 G). Unilateral stim· ·lation at 14 of 16 (88 %) sites located in the superior colliculus produced bilateral neocortical LVFA (Fig. 5, top) and concurrent suppression of 1-6 Hz LISA (Fig. 5, bottom). The threshold current required to produce neocortical LVFA was 37.9 ± 1.9 μA. This threshold current was lower than those for stimulation of the amygdala, orbitofrontal cortex, and midbrain - locus coeruleus area ($p < 0.02$, t tests) but did not differ from that for midbrain-raphe stimulation ($p = 0.13$, t tes·).

Administration of atropine (50 mg/kg, i.p.) did not abolish LVFA during colliculus stimulation, even though it abolished the LVFA that normally outlasted the stimulus duration (Fig. 5, top). Further, 1-6 Hz suppression during colliculus stimulation was not significantly affected by atropine (Fig. 5, bottom). Subsequent administration of methiothepin (5 mg/kg, i.p.), however, produced a complete block of LVFA and integrated 1-6 Hz activity no longer was suppressed during colliculus stimulation relative to a no-stimulation period (Fig. 5, top and bottom). Administration of ketanserin (5 mg/kg, i.p.; N=4 stimulation sites in 2 rats) subsequent to atropine treatment had an effect much like that observed with methiothepin and completely blocked LVFA and 1-6 Hz suppression during stimulation (data not shown; comparison of 1-6 Hz activity after atropine + ketanserin treatment before and during stimulation: $t_4 = 0.67$, $p = 0.54$).

Figure 5.

Stimulation (Stim.) of the superior colliculus (2 x threshold) induced neocortical LVFA (top) and suppressed integrated 1-6 Hz activity relative to a no-stimulation (No Stim.) period (bottom). Drug treatment produced a significant overall increase in 1-6 Hz activity ($F_{2,10} = 87.9$, $p < 0.0001$). However, after only atropine administration (50 mg/kg, i.p.), stimulation continued to produce LVFA (top) and a significant suppression of 1-6 Hz activity relative to a no-stimulation period (bottom; $p < 0.01$, Tukey's test). Subsequent methiothepin administration (5 mg/kg, i.p.) completely blocked LVFA and 1-6 Hz suppression during stimulation (top and bottom) and 1-6 Hz activity during stimulation no longer differed from that during a no-stimulation period ($p > 0.05$, Tukey's test). Note the significant drug x stimulation interaction, $F_{2,10} = 150.8$, $p < 0.0001$. (Means ± S.E.M. are shown).

No Drug
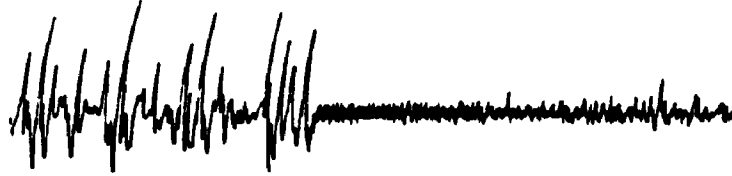
Atropine (50 mg/kg, i.p.)
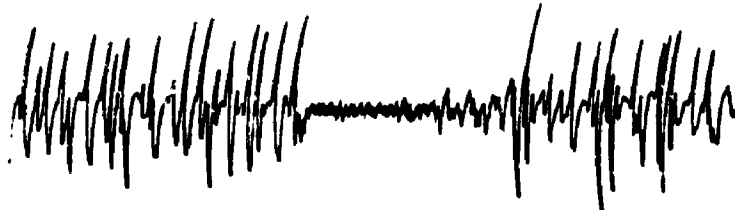
+ Methiothepin (5 mg/kg, i.p.)

Stimulation ON

Superior Colliculus Stimulation

1-6 Hz
(Arbitrary Units)

1.8

1.2

0.6

0.0

No Drug   Atropine   + Methiothepin

☒ Stim.
☐ No Stim.

Drugs
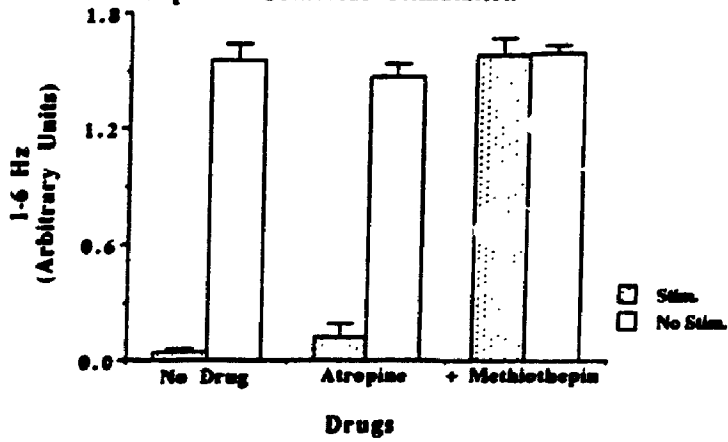
Single unit experiments

Spontaneous activity of basal forebrain cells in relation to the ECoG: The activity of 70 basal forebrain neurons was recorded. As shown in Fig. 6, in a sample of these cells, the majority of cells was located in the globus pallidus, in the ventral pallidum - substantia innominata complex, or in the magnocellular basal nucleus. Some cells were located in the internal capsule. Cells recorded in the lateral thalamus were excluded from the data analysis.

Based on the discharge rates of these cells in relation to concurrently recorded neocortical activity, 3 types of cells were identified: (a) neurons that had a higher discharge rate during LVFA relative to that during LISA, i.e., discharge rate had a positive correlation with neocortical activation (A+ cells); (b) neurons that had a higher discharge rate during LISA relative to LVFA, i.e., discharge rate had a negative correlation with neocortical activation (A- cells); and (c) neurons with discharge frequencies that showed no consistent correlatic : with neocortical activity (A0 cells).

Thirty-one (44 %) of the 70 recorded cells were of the A+ type, that is, these neurons fired at higher rates during the presence of LVFA in the neocortex than during LISA (Fig. 7, top). The mean discharge rate of A+ cells during LVFA and LISA were 12.7 ± 1.8 Hz (range 0.5 to 40.4 Hz) and 4.1 ± 0.9 Hz (range 0.1 to 24.3 Hz), respectively. Fourteen cells (20 %) were of the A- type that discharged at higher rates during LISA than LVFA (Fig. 7, bottom). The mean discharge rates for these cells during LVFA and LISA were 3.5 ±1.2 Hz (range 0 to 15.1 Hz) and 7.8 ± 2.1 Hz (range 0.5 to 24.8 Hz), respectively. Finally, the discharge of 25 cells (36 %) did not show any consistent relation to concurrent neocortical activity (A0 cells). The mean discharge rates of these neurons during LVFA and LISA were 15.1 ± 2.7 Hz (range 0.4 to 41.1 Hz) and 14.2 ± 2.4 Hz (range 0.3 to 36.0 Hz), respectively.

Figure 6.

The location of a subset of neurons recorded in the basal forebrain on three stereotaxic planes adapted from Paxinos and Watson (1986). The majority of cells were located in the globus pallidus (GP), ventral pallidum-substantia innominata complex (VP - SI), ventral to VP-SI, or in the internal capsule (IC). Thalamic cells were excluded from the data analysis. Other abbreviations: CPu caudate putamen, 3V third ventricle. Closed circles, A+ cells; open circles, A-cells; x, AO cells.

From Bregma   -0.8   -1.3   -1.8
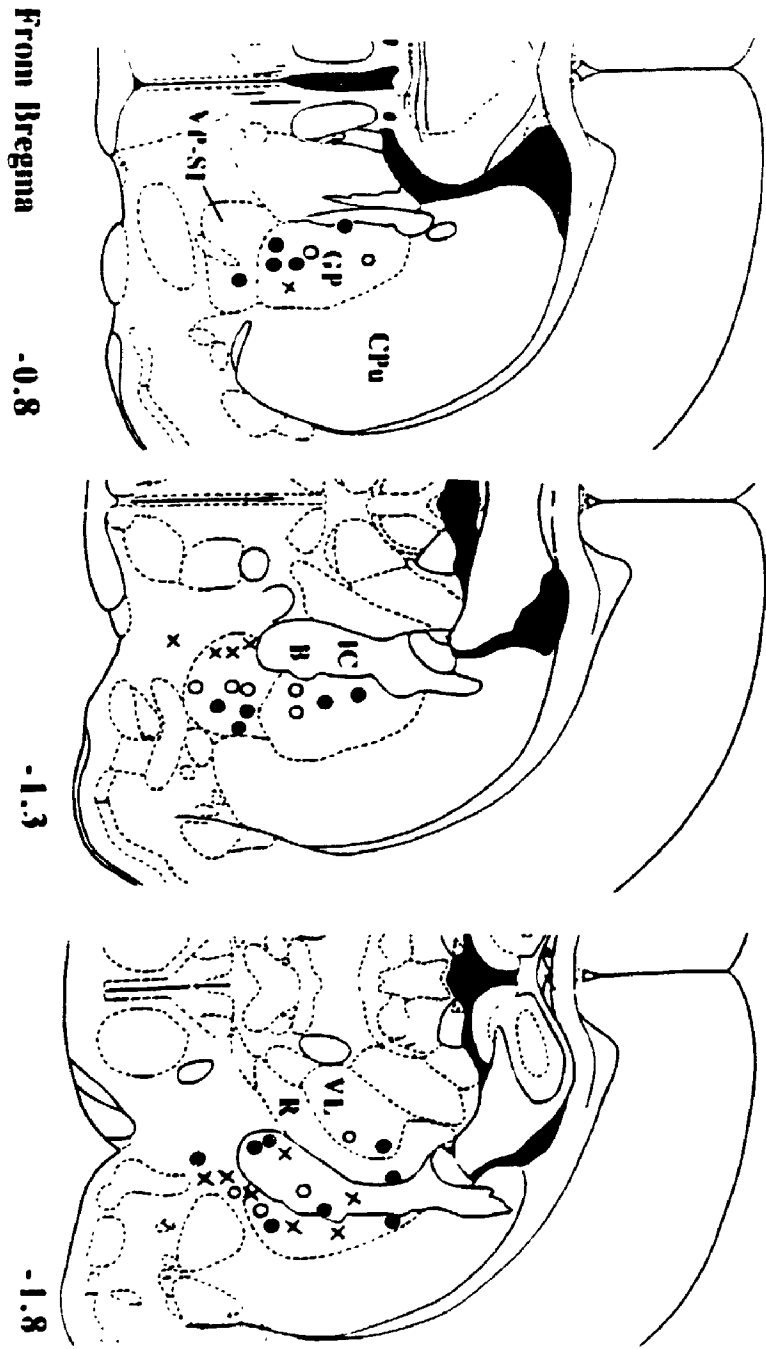
Figure 7.

Examples of the discharge rates of A+ and A- cells in relation to concurrent neocortical activity. A+ cells (top) fired at higher rates during the presence of LVFA in the ı ·ocortex relative to LISA. The mean discharge frequencies of the A+ cell depicted here during LVFA and LISA were 2.3 Hz and 0.65 Hz, respectively. Further, the discharge of this cell was facilitated by stimulation of the locus coeruleus area. A- cells (bottom) fired at higher rates during LISA than during LVFA. The mean discharge frequencies of this A- cell during LVFA and LISA were 0.38 Hz and 5.35 Hz, respectively. This cell was inhibited by stimulation of the locus coeruleus area. Calibration: 0.5 mV; 1 s.

Neocortex: LVFA

Neocortex: LISA

A

B

C

D

A+ Cell

A- Cell

84
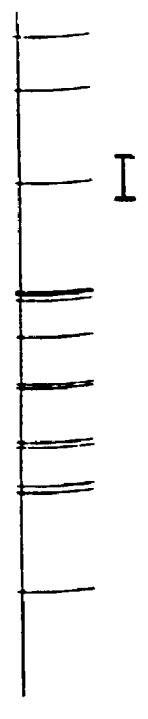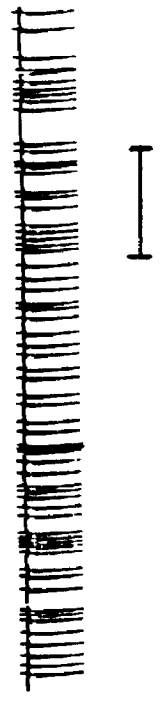
Most action potentials recorded were biphasic, consisting of an initial negative component followed by a positive component; some action potentials, however, were triphasic (positive-negative-positive). The duration of the negative phase of action potentials (recorded with filters set at 0.3 Hz and 10 kHz) ranged between approximately 0.5 and 1 ms. There did not appear to be consistent differences in action potential duration between the different types of basal forebrain cells.

Amygdala stimulation: The effect of single pulse stimulation of the amygdala on the discharge rate of basal forebrain cells was tested on 38 neurons. Amygdala stimulation modulated the activity of 27 of these cells located either ipsilateral or contralateral to the stimulation site. Interestingly, the type of modulation exerted by amygdala stimulation was strongly influenced by the specific type of basal forebrain cell being tested, i.e., by the relation of the spontaneous discharge rate to neocortical activity (see above). As summarized in Table 1 (top), 10 of 15 neurons (67 %) of the A+ type were excited by amygdala stimulation. That is, stimulation had an excitatory effect on the majority of cells that fired at higher frequencies during the presence of neocortical LVFA relative to LISA. One (7 %) A+ cell was not affected, and 2 (13 %) A+ cells were inhibited by amygdala stimulation (Table 1, top). The excitatory effect of amygdala stimulation on the discharge frequency of A+ cells was probably multisynaptic since most cells were not excited each time the amygdala was stimulated, and there was a considerable variability in the latency of the excitatory response for each cell (see below). Two further A+ cells, however, always responded with an action potential of constant latency (4.5 and 7 ms) to stimulation of the ipsilateral amygdala. It is likely that these cells were activated antidromically, rather than synaptically.

In contrast to the excitatory modulation of the majority of A+ cells, A- cells which fired at higher rates during LISA than LVFA were almost always inhibited by stimulation

Table 1.

Summary of the effects of stimulation of the amygdala (top) or of the locus coeruleus area (bottom) on the activity of single neurons in the basal forebrain. Stimulation of either site had a predominantly excitatory effect on A+ cells (cells that fired at higher rates during the presence of LVFA in the neocortex relative to LISA). Most A- cells (cells that fired at higher rates during LISA relative to LVFA) were inhibited by amygdala or locus coeruleus area stimulation. The majority of A0 cells (cells that did not show a consistent relation to neocortical activity) were not affected by stimulation of either amygdala or locus coeruleus. The effects of stimulation on different types of basal forebrain were significantly different according to Chi-Square tests (top: x^2 (4) = 23.5, p < 0.001; bottom: x^2 (4) = 16.3, p < 0.01).

	Cell Type		
	A+ (LVFA>LISA)	A- (LVFA<LISA)	A0 (LVFA=LISA)
Effect of Amygdala Stimulation			
Excitatory (19%)	10 (67%)	0 (0%)	3
Inhibitory (25%)	2 (13%)	6 (86%)	4
No Effect (56%)	1 (7%)	1 (14%)	9
Antidromic Activation	2 (13%)	---	---
Total	15	7	16
Effect of Locus Coeruleus Area Stimulation			
Excitatory (33%)	11 (69%)	1 (14%)	3
Inhibitory (11%)	1 (6%)	5 (71%)	1
No Effect (56%)	4 (25%)	1 (14%)	5
Total	16	7	9

of the amygdala. Of the 7 A- cells, 6 (86 %) decreased their discharge rates or were completely silenced after amygdala stimulation (Fig. 8, top). One A- cell was not affected by the stimulation and we did not observe an A- cell that was excited by amygdala stimulation (Table 1, top).

Most of those cells that did not show a correlation between their discharge rates and neocortical activity (AO cells) were not affected by stimulation of the amygdala. Of 16 neurons of the AO type, 9 cells (56 %) were not affected, 3 (19 %) cells were excited, and 4 cells (25 %) were inhibited by amygdala stimulation (Table 1, top).

It is noteworthy that for some cells, biphasic or even triphasic responses to amygdala stimulation were observed. These could consist of either an initial period of excitation followed by inhibition, or of a period of inhibition followed by excitation. While we used only the initial change in discharge rate that was observed in order to classify the response as excitatory or inhibitory, it is clear that some cells were modulated in a complex way by amygdala stimulation.

The latencies for excitatory responses of A+ cells to amygdala stimulation ranged between 5 and 200 ms, and latencies of approximately 10 ms were most common for both ipsilateral and contralateral stimulation. Due to the large variance in response latencies, there were no consistent, statistically significant differences in response latencies to ipsilateral versus contralateral stimulation. However, for some A+ cells, latencies of less than 10 ms for responses to ipsilateral stimulation were observed. For contralateral stimulations, response latencies of less than 10 ms were not observed in the present study. The duration of the excitatory effect of stimulation on the discharge rate of A+ cells ranged between 4 and 400 ms (mean 122 ms)

The latencies for an inhibitory effect of amygdala stimulation on A- cells ranged between 5 and 400 ms, with the most common latencies between 5-10 ms. The duration of the inhibitory effects ranged between 20 and 800 ms (mean 260 ms).

Figure 8.

Examples of an inhibitory response of an A- cell (top) and of an excitatory response of an A+ cell (bottom) recorded in the basal forebrain. (A) Stimulation (S) of the contralateral amygdala (25 sweeps, 400 μA) resulted in an immediate cessation of firing for about 300 ms of this A- cell located in the ventral pallidum-substantia innominata. The spontaneous discharge rates of this cell during LVFA and LISA were 1.4 Hz and 5.0 Hz, respectively. Calibration: 400 ms. (B) Twenty-five superimposed spontaneous action potentials of this cell. Calibration: 0.2 ms; 200 μV; positivity upwards. (C) Stimulation (S) of the locus coeruleus area (60 sweeps; 100 μA) excited this A+ cell located in the globus pallidus. The spontaneous firing rates of this cell during LVFA and LISA were 7.1 Hz and 1.1 Hz, respectively. Calibration: 40 ms. (D) Twenty superimposed spontaneous action potentials of this cell. (Two sweeps were triggered accidentally.) Calibration: 1 ms; 1000 μV. Stimulations were given during concurrent LISA in the neocortex. Spike count refers to the output of the window discriminator.

90

A — Spike Count — A- Cell

B
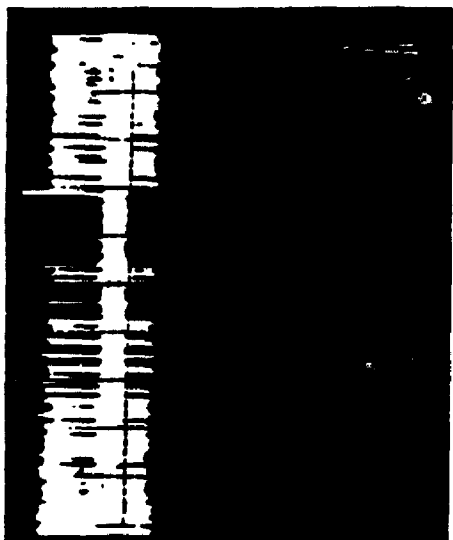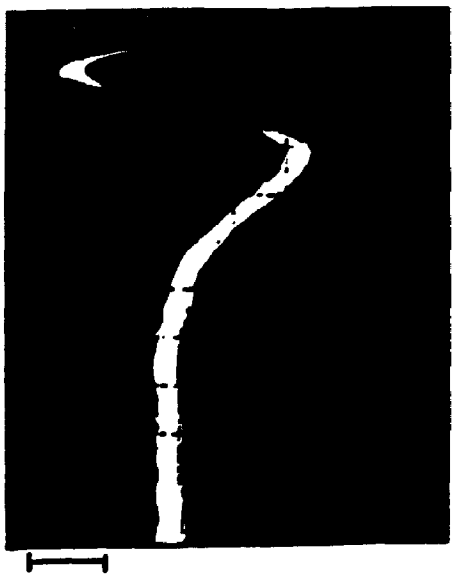
C — Spike Count — A+ Cell

D — Spike Count
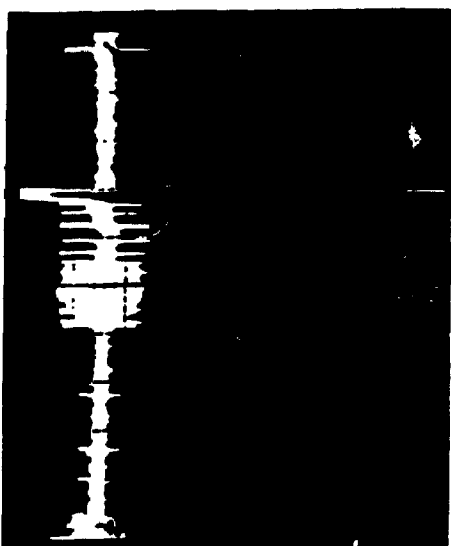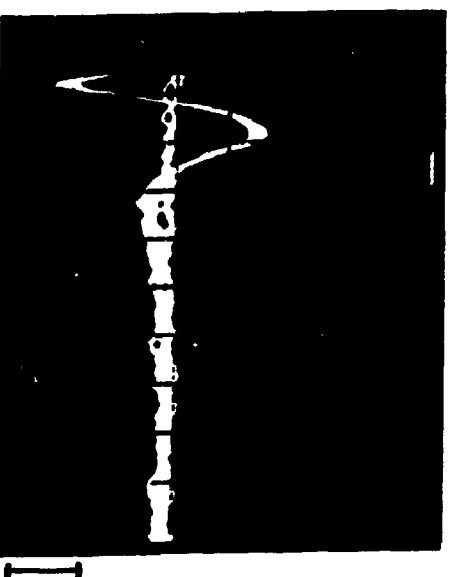
Stimulation of locus coeruleus area: The effect of single pulse stimulation of the locus coeruleus area was tested on 32 cells (16 A+ cell, 7 A- cells, 9 A0 cells). Eleven (69 %) of the 16 A+ cells were excited (Fig. 8, bottom), 4 (25 %) A+ cells were not affected, and 1 A+ cell (6 %) was inhibited by locus coeruleus stimulation (Table 1, bottom). Of the 7 A- cells, 5 (71 %) were inhibited, 1 (14 %) was excited, and 1 (14 %) was not affected by locus coeruleus stimulation. Of the 9 A0 cells, 3 (33 %) were excited, 1 (11 %) was inhibited, and 5 (56 %) were not affected by locus coeruleus stimulation (Table 1, bottom).

The latencies for excitatory responses of A+ cells ranged between 6 and 200 ms with the most common latencies between 10-40 ms for responses to both ipsilateral and contralateral stimulation. The duration of excitation of A+ cells ranged between 5 and 200 ms (mean 63 ms). For the inhibitory responses of A- cells to locus coeruleus area stimulation, latencies ranged between 20 and 200 ms and durations ranged between 30 and 500 ms (mean 178 ms). Interestingly, while excitatory and inhibitory responses tended to have similar latencies for amygdala stimulation, for stimulation of the locus coeruleus, inhibitory responses tended to have somewhat longer latencies than excitatory responses.

Discussion

In the present study, we show that in urethane-anesthetized rats, electrical stimulation of a number of forebrain and midbrain areas can influence neocortical ECoG activity by suppressing LISA in the 1-6 Hz range and replacing it with LVFA. Thus, our findings confirm previous work showing that stimulation of the amygdala, the dorsal raphe, locus coeruleus, superior colliculus, and orbitofrontal cortex can produce neocortical activation (Kaada, 1951; Segundo et al., 1955; Berridge and Foote, 1991; Dean et al., 1991; Peck and Vanderwolf, 1991; Kapp et al., 1994).

In the initial experiment, LVFA induced by stimulation of the amygdala was completely abolished by treatment with the anti-muscarinic drug scopolamine, whereas LVFA during stimulation of the dorsal raphe was largely unaffected. This observation confirms previous work indicating that neocortical LVFA is maintained by two distinct inputs to the cortex that can be dissociated pharmacologically; LVFA that is abolished by atropine or scopolamine appears to be dependent on the release of ACh in the neocortex, whereas LVFA that is resistant to these anti-muscarinic drugs appears to depend on ascending serotonergic fibers from the midbrain raphe nuclei to cortex (Vanderwolf, 1988; 1992).

As shown previously, stimulation of the amygdala can produce neocortical LVFA (Kaada, 1951; Gloor, 1960). This activation effect may be mediated by the release of ACh from the cholinergic cells of the basal forebrain since it can be blocked by anti - muscarinic drugs (present study; Kapp et al., 1994) or infusions of the local anesthetic lidocaine into the basal forebrain (Paper 1 this thesis). The basal forebrain provides the diffuse cholinergic innervation of the entire neocortex (Divac, 1975; Bigl et al., 1982; Saper, 1984; Butcher and Woolf, 1986; Mesulam, 1989; Semba and Fibiger, 1989; Woolf, 1991) and appears to be essential for the maintenance of cholinergic electrocorticographic activation (Stewart et al., 1984; Buzsaki et al., 1988; Vanderwolf, 1988; Buzsaki and Gage, 1989; Ray and Jackson, 1991; Semba, 1991). The present experiment confirms and extends these data by demonstrating that the activity of neurons in the basal forebrain is modulated by single pulse stimulation of the amygdala: cells which fired at higher rates during the presence of LVFA than during LISA (i.e., A+ cells that had a positive correlation with cortical activation) often were excited by amygdala stimulation. Interestingly, cells that fired at higher rates during periods of LISA than during LVFA (i.e., A- cells that showed a negative correlation with activation) almost always were inhibited by amygdala stimulation. For cells that showed no obvious relation to neocortical

activity, no predominant stimulation effects were noted. Effects on basal forebrain neurons almost identical to those produced by amygdala stimulation were observed with stimulation of the area of the locus coeruleus, another site where stimulation can produce LVFA sensitive to anti-muscarinic drugs (present results). Thus, stimulation of the locus coeruleus area excited the majority of A+ cells, inhibited most A- cells, and did not affect AO cells in any consistent way.

Basal forebrain cells of the types just outlined have been described previously (Detari and Vanderwolf, 1987; Buzsaki et al., 1988; Szymusiak and McGinty, 1989; Whalen et al., 1994). As of yet, the neurochemical identity of these different cell types present in the basal forebrain has not been established with certainty. Detari and Vanderwolf (1987) showed that many of those cells that fire at higher rates during the presence of LVFA (i.e., A+ cells) are cortically-projecting since they can be activated antidromically by cortical stimulation. The large majority of cortical projection neurons in the basal forebrain, at least in rats, appear to contain ACh (Woolf et al., 1983; Rye et al., 1984; Wahle et al., 1984). Thus, it is possible that many of the A+ cells recorded here, and that were excited by stimulation of the amygdala and locus coeruleus, also were cholinergic projection neurons. For future investigations, it would be desirable to test this hypothesis with combined electrophysiological and neurochemical techniques.

Cells of the A- type that fired at higher rates during neocortical LISA generally cannot be activated antidromically by cortical stimulation (Detari and Vanderwolf, 1987; but also Szymusiak and McGinty, 1989) and may be inhibitory interneurons, as has been suggested (Detari and Vanderwolf, 1987). The large majority of these cells (86 % and 72 %, respectively) were inhibited by stimulation of the amygdala and locus coeruleus area, i.e., of those sites that produced atropine-sensitive (putative cholinergic) LVFA. There is evidence that ACh-containing neurons are subject to GABAergic inhibition (Wood and Richard, 1982; Zaborszky, 1989) and that A- interneurons may inhibit cortically-

projecting A+ cells (Detari et al., 1990). Further, there is some evidence that modulatory inputs to basal forebrain cholinergic cells from both the amygdala and locus coeruleus may, at least partially, be mediated by GAB.'.ergic interneurons (see Zaborszky, 1989). Based on these considerations and the present results, it may be that the inhibition of A- cells (putative inhibitory interneurons) and the possible subsequent disinhibition of A+ cells, in addition to a more direct excitation of A+ cells, may be an important mechanisms underlying the stimulation of the cholinergic input to the cortex. In fact, in our experiments, inhibition of A- cells was more common than excitation of A+ cells. Thus, despite the relatively small sample size of cells included here, these observations may suggest that inhibition of specific cells in the basal forebrain may contribute significantly to an overall stimulation of cholinergic transmission between basal forebrain and cortex.

Recently, Semba et al. (1995) have shown that the activity of EEG-related basal forebrain neurons can be modulated by stimulation of the p dunculopontine tegmental nucleus and the dorsal raphe. Similar to our findings, most cells that were more active during LVFA than during LISA could be excited by single pulse stimulation of these nuclei. However, the majority of cells more active during LISA also were excited by single pulse stimulation of these midbrain nuclei (even though these cells were inhibited by 100 Hz train stimulation). At present, it is not clear how to explain these results. It is possible that various brainstem nuclei and forebrain structures exert differential influences over basal forebrain neurons. Alternatively, different stimulation parameters used may exert different physiological effects due to variations in the volume of tissue excited and the types of fibers activated by a stimulus (see Ranck, 1975). Clearly, the different results obtained by Semba et al. (1995) and us require clarification.

Previous work has shown that excitation of the locus coeruleus can produce neocortical LVFA and it has been suggested that this effect may be mediated by the release of noradrenaline (Neuman, 1986; Berridge and Foote, 1991). The present experiment

confirms that electrical stimulation of the area surrounding the locus coeruleus is very effective in inducing neocortical LVFA. The placements of stimulation electrode in the present study were just medial to the locus coeruleus in the central grey and laterodorsal tegmental nucleus. Thus, it is possible that non-noradrenergic (e.g., cholinergic) cells were among those that were excited by the stimulation. However, several electrodes were in direct contact with the medial aspect of the coeruleus and given the stimulus parameters used, it is probable that the stimulation excited cell bodies and axons several hundred μm away from the electrode tip (Ranck, 1975). Thus, it is likely that noradrenergic cells in the locus coeruleus were among those neurons that were excited by the stimulation. It is not clear, however, whether the release of noradrenaline is *directly* responsible for the ECoG activation in response to locus coeruleus stimulation observed here and previ .sly (see above). Depletions of noradrenaline and dopamine of up to 99 % after intraventricular injections of 6-hydroxydopamine do not abolish either atropine-sensitive or atropine - resistant LVFA (Whishaw et al., 1978), suggesting that noradrenaline may not be critical for the *direct* activation of the electrocorticogram. Here, administration of atropine completely abolished LVFA to stimulation of the locus coeruleus area, implying that the release of ACh may play an important role in this activating effect. The basal forebrain cholinergic system receives a significant fiber input from the locus coeruleus (Russchen et al., 1985; Fuller et al., 1987; Jones and Cuello, 1989; Zaborszky, 1989; Losier and Semba, 1993) and this anatomical evidence is supported by our observation that locus coeruleus stimulation could excite about 60 % of basal forebrain A+ cells (possibly cholinergic cells; see above). Most A- cells (possibly inhibitory interneurons; see above) were inhibited by stimulation of the locus coeruleus area. Thus, our findings raise the possibility that stimulation of the locus coeruleus area stimulates the cholinergic input to the cortex which, in turn, would result in the appearance of neocortical LVFA. Consequently, the role of the locus coeruleus anu noradrenergic system in ECoG

activation may be an *indirect* one that is mediated by the cholinergic system. There is ample evidence to suggest that ACh, but not noradrenaline (see Baust et al., 1963), produces LVFA by a direct action in cortex (Kanai and Szerb, 1965; Cuculic et al., 1968; Spehlmann and Norcross, 1982; Casamenti et al., 1986; Metherate and Ashe, 1991; Metherate et al., 1992), supporting the distinction of *direct* and *indirect* cortical activation just outlined.

In contrast to stimulation of sites in the amygdala or midbrain, stimulation of cortical areas was not very effective in suppressing LISA and inducing neocortical LVFA. Stimulation of the orbitofrontal cortex was effective for 44 % of the stimulation sites tested, and stimulation of the posterior entorhinal and cingulate cortex was generally ineffective in activating the neocortex. In contrast, in cats and monkeys, stimulation of cortical areas including cingulate and entorhinal cortex appears to be more effective in producing cortical activation (Segundo et al., 1955; French, 1958; Kaada and Johannessen, 1960). It is not clear as to what extent the discrepancy between the experiments using cats and monkeys and the present one using rats may reflect differences in the anatomical organization of corticofugal fiber systems, i.e., if corticofugal fibers in the rat exert a smaller excitatory influence over the ascending activating systems than in cats or dogs.

Generally, it was assumed that inputs from cortex to the reticular formation are involved in ECoG activation to cortical stimulation (for review, see French, 1958). However, more recently, it has become clear that the cortical mantle, including cortical areas that have been shown to produce cortical activation (e.g., orbitofrontal and cingulate cortex), has a significant fiber input to the basal forebrain (Mesulam and Mufson, 1984; Saper, 1984; Russchen et al., 1985; Semba and Fibiger, 1989; Zaborszky, 1989; Butcher, 1992), as well as to the reticular formation. It is not known what the relative contributions of corticofugal fibers to midbrain and basal forebrain are to the induction of LVFA. However, in the present study, LVFA during stimulation of the majority of sites

in the orbitofrontal cortex was abolished by atropine, suggesting that this activating effect involves, at least in part, the cholinergic input to the cortex from the basal forebrain.

Of all sites tested, only stimulation of the dorsal raphe and of the superior colliculus produced LVFA that was largely resistant to anti-muscarinic treatment. We have shown previously that LVFA during raphe stimulation can be reduced or abolished by serotonergic receptor antagonists such as ketanserin and methiothepin, or by serotonergic depletion with p-chlorophenylalanine, indicating a role of 5-HT release in this activation effect (Dringenberg and Vanderwolf, 1995). Similarly, LVFA induced by noxious stimulation such as tail pinches, an effect which involves serotonergic cells in the dorsal raphe (Thompson et al., 1991), is abolished by serotonergic antagonists (Neuman, 1986; Neuman and Thompson, 1989, Neuman and Zebrowska, 1992). Thus, it seems that 5 - HT is involved in producing LVFA to noxious stimulation or to electrical stimulation of the midbrain-raphe nuclei. As already outlined (see Introduction), there is considerable evidence that 5-HT can maintain neocortical LVFA even after all cholinergic inputs to the cortex have been eliminated (e.g., Vanderwolf et al., 1990), suggesting that the cortical activation produced by 5-HT can function largely independent of the cholinergic systems, as opposed to the action of noradrenaline which may be mediated by ACh, as outlined above. In addition, the fact that direct, intracortical application of a number of serotonergic receptor antagonists (e.g., ketanserin, ritanserin) can block neocortical LVFA to noxious stimulation in anesthetized rats (Neuman and Zebrowska, 1992) also supports the hyp' thesis that 5-HT can exert a *direct* activating effect in cortex, independent of cholinergic systems.

Stimulation of the superior colliculus also produced atropine-resistant LVFA. We have confirmed this result in non-anesthetized, freely-moving rats given atropine: LISA present during immobility was suppressed by colliculus stimulation despite the anti - muscarinic treatment, confirming that the activating effect is not critically dependent on the

cholinergic input to the cortex (H. C. Dringenberg and C. H. Vanderwolf, unpublished observation). The fact that LVFA to colliculus stimulation could be abolished by the 5-HT receptor antagonists methiothepin and ketanserin may suggest that this activating effect depends on the release of 5-HT and, therefore, may be mediated by the 5-HT-raphe activating system described above. To our knowledge, these are the first data to suggest that the 5-HT system may be involved in mediating the cortical activation to stimulation of the superior colliculus (see Dean et al., 1991). The projections from the superior colliculus to areas of the reticular formation such as the cuneiform nucleus (Mitchell et al., 1988; Redgrave et al., 1988), which in turn has inputs to the raphe nuclei (Edwards, 1975), could be involved in mediating the stimulation of the 5-HT input to the cortex and subsequent LVFA generation during colliculus stimulation. It would be interesting to test these hypotheses, e.g., by examining whether serotonergic cells in the dorsal raphe are excited by stimulation of the superior colliculus, or whether LVFA during colliculus stimulation is abolished by lesions of the raphe nuclei.

In summary, the results of the present experiments confirm previous data in suggesting that neocortical LVFA is *directly* maintained by two ascending inputs to the cortex originating in the basal forebrain and midbrain raphe nuclei and utilizing the cortical release of ACh and 5-HT, respectively (see above; Vanderwolf, 1988). A combined blockade of the cholinergic and serotonergic inputs in awake, freely moving rats can abolish all spontaneously-occurring ECoG activation (present results; Vanderwolf and Baker, 1986; Vanderwolf, 1988; Vanderwolf et al., 1990). Similarly, in the present study using urethane-anesthetized rats, combined cholinergic and serotonergic blockade eliminated all LVFA induced by electrical stimulation of a variety of forebrain- and midbrain structures. Thus, even though systems outside those containing cholinergic or serotonergic neurons can induce cortical LVFA, these systems appear to produce LVFA *indirectly* by stimulating the *direct-acting* cholinergic and serotonergic inputs to the cortex.

The observation that spontaneous or stimulation-induced LVFA does not appear to survive a combined cholinergic and serotonergic blockade strongly suggests that inputs to the cortex other than cholinergic and serotonergic, or intracortical systems, are insufficient to maintain the activated state of the electrocorticogram.

Section II:

Can the 5-HT Receptors Involved in Mediating 5-HT-dependent Neocortical Activation Be Identified ?   Electrophysiological and Pharmacological Studies

The release of 5-HT in the neocortex can activate the neocortical ECoG and induce the appearance of LVFA. The specific mechanisms that mediate this action of 5-HT over neocortical activity are not well understood. For example. it is not clear which of the multiple 5-HT receptor types found in the central nervous system are involved in mediating neocortical LVFA.

One way to investigate the role played by different 5-HT receptors in LVFA generation is the use of selective 5-HT receptor agonists to restore LVFA in rats in which the endogenous 5-HT and cholinergic inputs to the cortex have been eliminated. Possibly, a relatively selective stimulation of some, but not other types of 5-HT receptors may produce LVFA in the neocortex, thus suggesting what 5-HT receptor types may be involved in neocortical activation. This approach was used in Paper 3.

An alternative approach to this question is the use of selective 5-HT receptor antagonists in an attempt to block naturally-occurring LVFA. Such experiments have been carried out but have produced inconsistent results. For example, $5-HT_2$ receptor antagonists such as ketanserin appear to block LVFA in rats anesthetized with urethane, but not in unanesthetized, freely moving rats (see Paper 4 for details). One hypothesis derived from this observation is that anesthetics such as urethane may affect 5-HT systems in the brain and alter the action of 5-HT antagonists. These hypotheses were tested with the experiments outlined in Paper 4.

Paper 3:

5-Hydroxytryptamine (5-HT) Agonists: Effects on Neocortical Slow Wave Activity after Combined Muscarinic and Serotonergic Blockade

Summary

In freely-moving rats treated with a combination of reserpine (10 mg/kg, i.p.) and scopolamine (5 mg/kg, i.p.), neocortical low voltage fast activity (LVFA) associated with continuous multiunit activity (MUA) was abolished and replaced by 2-6 Hz large irregular slow activity (LISA) above 1.5 mV associated with a burst-suppression pattern of MUA. Administration of the monoamine oxidase inhibitor pargyline (50-100 mg/kg, i.p.) completely suppressed 2-6 Hz LISA and restored normal appearing LVFA and continuous MUA. The 5-hydroxytryptamine (5-HT) receptor agonists quipazine (0.5-20 mg/kg, i.p.), (±)-DOI (0.1-5 mg/kg, s.c.), and buspirone (0.1-10 mg/kg, i.p.), but not 8-hydroxy-2 - (di-n-propylamine) tetraline (8-OH-DPAT, 0.05-0.8 mg/kg, s.c.) and RU 24969 (1-30 mg/kg, i.p.), produced a partial suppression of 2-6 Hz LISA and restored some lower voltage activity (< 1 mV) above 6 Hz associated with continuous MUA. However, as opposed to pargyline, no receptor agonist tested restored continuous, normal- appearing LVFA. Even though agonists at 5-HT receptors can produce some activation of neocortical slow wave activity after combined cholinergic and serotonergic blockade, this effect is not equivalent to that observed after restoration of endogenous 5-HT transmission.

Introduction

The activation of neocortical slow wave activity (low voltage fast activity, LVFA) appears to depend on two partially independent cortical afferent systems. a cholinergic input from the basal forebrain and a serotonergic input from the midbrain. The neocortical LVFA that occurs during Type 2 behaviors such as immobility, face washing, or licking is abolished after treatment with centrally-acting cholinergic-muscarinic receptor antagonists such as atropine or scopolamine (Vanderwolf, 1975; 1988) or lesions of the basal forebrain cholinergic system (Stewart et al., 1984; Buzsaki et al., 1988). LVFA during Type 1 behaviors such as walking or rearing is resistant to anti-muscarinic treatments but such atropine-resistant LVFA can be abolished by depletions of central serotonin (5 - hydroxytryptamine, 5-HT) with systemic reserpine or p-chlorophenylalanine, or with intracerebral 5,7-dihydroxytryptamine (Vanderwolf and Pappas, 1980; Vanderwolf et al., 1989; 1990). If both cholinergic and serotonergic inputs are blocked concurrently, all LVFA is abolished and neocortical slow wave activity consists of continuous 2-6 Hz large irregular slow activity (LISA). In reserpinized rats, atropine-resistant LVFA can be restored by some drugs that restore central 5-HT levels such as the 5-HT precursor 5 - hydroxytryptophan or inhibitors of monoamine oxidase (Vanderwolf, 1984).

The role of different types of 5-HT receptors (Humphrey et al., 1993) in mediating 5-HT-dependent neocortical LVFA is not understood. In urethane-anesthetized rats, antagonists at $5-HT_2$ receptors (e. g., ketanserin, ritanserin) appear to block the activating effects of 5-HT on neocortical slow wave and unit activity (Neuman and Thompson, 1989; Neuman and Zebrowska, 1992). However, in unanesthetized freely-moving rats, selective $5-HT_2$ antagonists are ineffective in antagonizing LVFA; only the non-selective antagonist methiothepin produces a small but significant reduction of LVFA in unanesthetized rats (Watson et al., 1992). Recent evidence indicates that urethane and

other general anesthetics appear to alter the action of 5-HT antagonists significantly, and that results obtained with such antagonists under urethane anesthesia may not be applicable to the unanesthetized state (Dringenberg and Vanderwolf, 1995; Dringenberg et al., 1995). Thus, the use of receptor antagonists has not yet clarified the role played by different 5-HT receptor types in mediating neocortical LVFA.

In the present study, we administered 5-HT receptor agonists to freely-moving rats pretreated with reserpine and scopolamine to eliminate serotonergic and cholinergic inputs to the neocortex. The question was, can some 5-HT receptor agonists restore neocortical LVFA after blockade of all endogenous activating ir_ .ts ?

Methods

Subjects and surgery

Using conventional stereotaxic techniques (Cooley and Vanderwolf, 1978) and sodium pentobarbital anesthesia (60 mg/kg, i.p.), adult male Long-Evans rats (350-500 g) were chronically implanted with bipolar surface-to-depth electrodes in the sensori-motor cortex and with a ground connection in the bone over the cerebellum. The rats were given 2 weeks of recovery time before commencement of the experiments.

Recording procedure

Neocortical slow wave activity was recorded differentially with a Grass 7B polygraph (half-amplitude points set at 0.3 Hz and 10 kHz), passed through a band-pass filter (half-amplitude points set at 2-6 Hz; roll-off of 24 dB/octave; Dytronics Model 724), rectified, and integrated over 1 s intervals. Multiunit activity (300 Hz-10 kHz) was also

recorded and displayed on a Tektronix storage oscilloscope.

Recordings were taken (a) from undrugged rats; (b) 14-18 hrs after pretreatment with reserpine crystalline (Sigma, dissolved in polyethylene-glycol-500, 10 mg/kg, i.p.; rats were warmed to 36-37 ° C prior to the recording); (c) 20 min after additional scopolamine hydrobromide treatment (Sigma, 5 mg/kg, i.p.); and (d) 10 min after every injection of the agonist being tested (for pargyline, recordings were taken every 20 min until 1 hr after an injection). For each agonist, cumulative concentration-response curves were established by administering successive agonist injections to each rat at 15 min intervals (1 hr interval between the pargyline injections). The agonists tested were: buspirone hydrochloride (Sigma, 0.1-10 mg/kg, i.p.); (±)-1-(4-Iodo-2,5 - dimethoxyphenyl)-2-aminopropane hydrochloride (DOI; Research Biochemicals Inc., 0.1 - 5.0 mg/kg, s.c.); (±)-8-hydroxy-2 (dl-n-propyl-amine)tetraline (8-OH-DPAT, Research Biochemicals Inc., 0.05-0.8 mg/kg, s.c.); pargyline hydrochloride (Sigma, 50-100 mg/kg, i.p.); RU 24969 (Roussel-Uclaf, 1-30 mg/kg, i.p.); quipazine dimaleate (Research Biochemicals, 0.5-20 mg/kg, i.p.). All drugs were dissolved in saline except where noted otherwise.

Data analysis

For each rat, one 10 s epoch of slow wave activity from each treatment condition was used to measure peak amplitude (defined as the mean amplitude of the two largest nonartifactual waves in the epoch from each treatment condition) and the amount of integrated 2-6 Hz activity, and to determine the presence of LVFA (defined as activity of < 0.5 mV and suppressed 2-6 Hz activity). The epochs selected for analysis were taken during behavioral immobility for all agonists except pargyline where they were taken during periods of sensory/cutaneous stimulation (picking up the rat, placing a hand on the

rat's dorsal body surface, tail pinching). Data are presented as mean ± S.E.M. and were statistically analvzed using the software package CLR Anova (Version 1.1, Clear Lake Research Inc.).

Results

In undrugged rats, neocortical activity consisted of continuous LVFA including frequencies of above 10 Hz (Fig. 1) Concurrent multiunit activity (MUA) was characterized by continuous discharge of units (Fig. 2 a). Integrated 2-6 Hz activity was almost completely suppressed (4.2 % of maximum, Fig. 3, top) and LVFA mean amplitude was 0.27 mV (17 % of max.; Fig 3, bottom). Occasionally, spindle activity associated with a burst-suppression pattern of MUA was present during periods of immobility. Fourteen to eighteen hours after reserpine administration (10 mg/kg, i.p.), rats were akinetic and neocortical slow wave activity consisted of a mixture of LVFA and large irregular slow activity (LISA) of 1-2 mV associated with a burst-suppression pattern of MUA. Generally, if the rat was undisturbed, LISA was present, but LVFA could be elicited by touching or pinching the rat. After additional scopolamine treatment, all LVFA was abolished and 1-2 mV LISA with frequencies largely in the 2-6 Hz range (Fig. 1) and concurrent burst-suppression MUA (Fig. 2 b) were present continuously and could not be suppressed by handling or pinching the rat. Integrated 2-6 Hz activity was at a maximum, taken as 100 % for all comparisons with other treatment conditions (Fig. 3, top), and peak amplitude was 1.6 mV (100 %; Fig. 3, bottom).

As shown previously (Vanderwolf, 1984), administration of pargyline (50-100 mg/kg, i.p., n=6) resulted in a complete suppression of LISA and restored normal - appearing LVFA (Fig. 1). Further, the burst-suppression pattern of MUA associated with LISA was abolished and continuous MUA reappeared after pargyline treatment (Fig 2 c).

Figure 1.

Typical examples of neocortical slow wave activity in awake rats before drug treatment, after administration of reserpine (10 mg/kg) + scopolamine (5 mg/kg), and after additional treatment with one of 6 serotonergic agonists. See text for further details. HS = headshake. Calibrations: 1 s: 0.5 mV.

No Drug

Reserpine (10 mg/kg) + Scopolamine (5 mg/kg)

+ Pargyline (100 mg/kg)

+ Quipazine (20 mg/kg)

HS
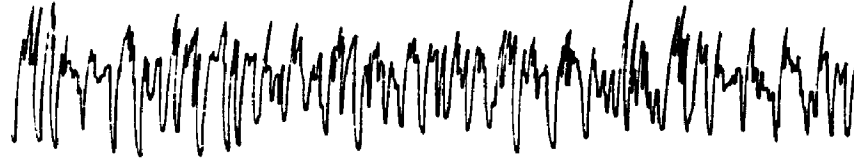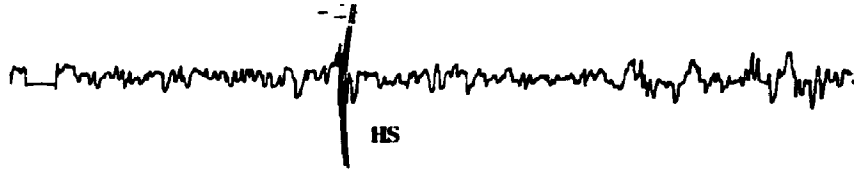
+ DOI (5 mg/kg)

+ Buspirone (10 mg/kg)

+ RU 24969 (30 mg/kg)
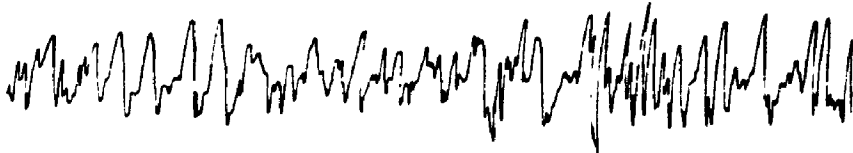
+ 8-OH-DPAT (0.8 mg/kg)
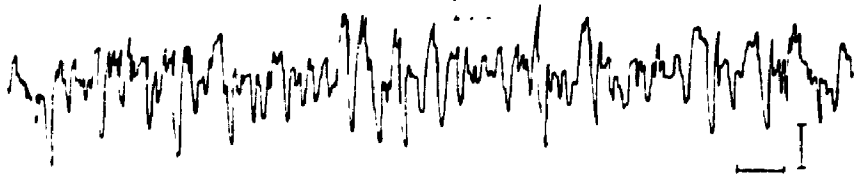
Figure 2.

Neocortical slow wave and concurrent multiunit activity (MUA) in an awake,
undrugged rat (A), after administration of reserpine (10 mg/kg, i.p.) + scopolamine (5
mg/kg, i.p.) (B), and after additional treatment with either pargyline (100 mg/kg, i.p.)
(C), quipazine (10 mg/kg, i.p.) (D) or DOI (2 mg/kg, s.c.) (E). A, B, and D were
obtained from the same rat. See text for further details. Calibrations: 0.5 mV for EEG;
0.05 mV for MUA; 1 s.

111

A: No Drug
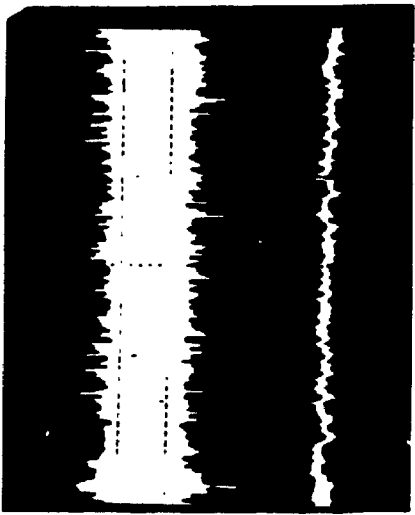
B: Reserpine (10 mg/kg) + Scopolamine (5 mg/kg)
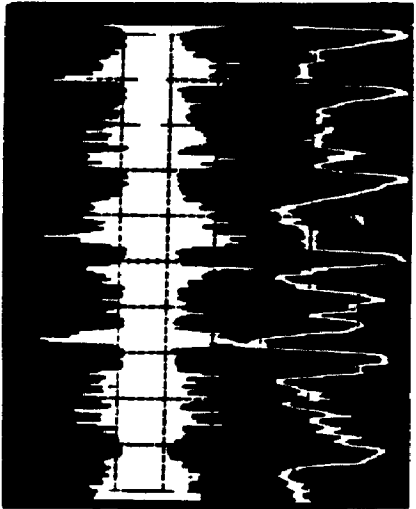
C: + Pargyline (100 mg/kg)
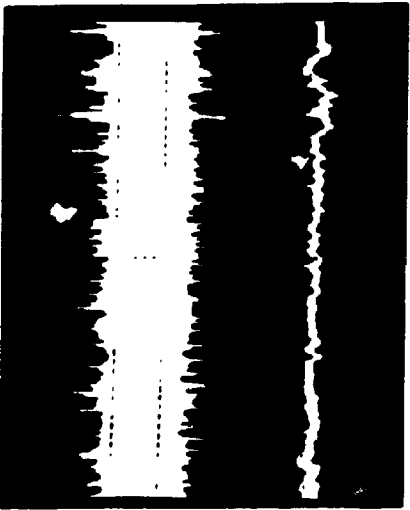
D: + Quipazine (10 mg/kg)
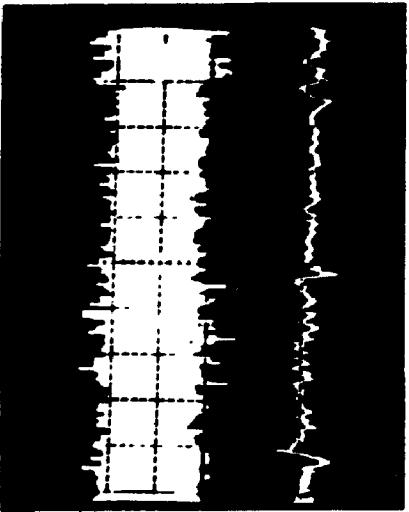
E: + DOI (2 mg/kg)

Figure 3.

Amount of integrated neocortical 2-6 Hz activity (top) and peak amplitude (bottom) in awake, undrugged rats, after reserpine (10 mg/kg, i.p.) + scopolamine (5 mg/kg, i.p.) administration, and after additional treatment with one of 6 serotonergic agonists. *Top:* ANOVAs across all drug treatments revealed significant drug effects on 2-6 Hz activity for all 6 treatment groups (p < 0.0001). Newman-Keul's test showed that pargyline (i.p.), RU 24969 (i.p.), DOI (s.c.), quipazine (i.p.), and buspirone (i.p.), but not 8-OH-DPAT (s.c.), reduced 2-6 Hz activity relative to that after reserpine + scopolamine treatment (p < 0.05). However, only pargyline reduced 2-6 Hz activity to the level present in undrugged rats (p > 0.05, Newman-Keul's test). *Bottom:* ANOVAs across all drug treatments revealed significant drug effects on peak amplitude for all 6 treatment groups (p < 0.0001). Newman-Keul's test showed that pargyline, RU 24969, DOI, quipazine, but not buspirone and 8-OH-DPAT, reduced peak amplitude relative to peak amplitude after only reserpine + scopolamine treatment (p < 0.05). However, only pargyline reduced peak amplitude to a level equivalent to that in undrugged rats (p > 0.05, Newman-Keul's test).

2-6 Hz Activity

Peak Amplitude

8-OH-DPAT (n=5)
Buspirone (n=7)
RU 24969 (n=6)
Quipazine (n=7)
DOI (n=7)
Pargyline (n=6)

Dose [Agonist] mg/kg
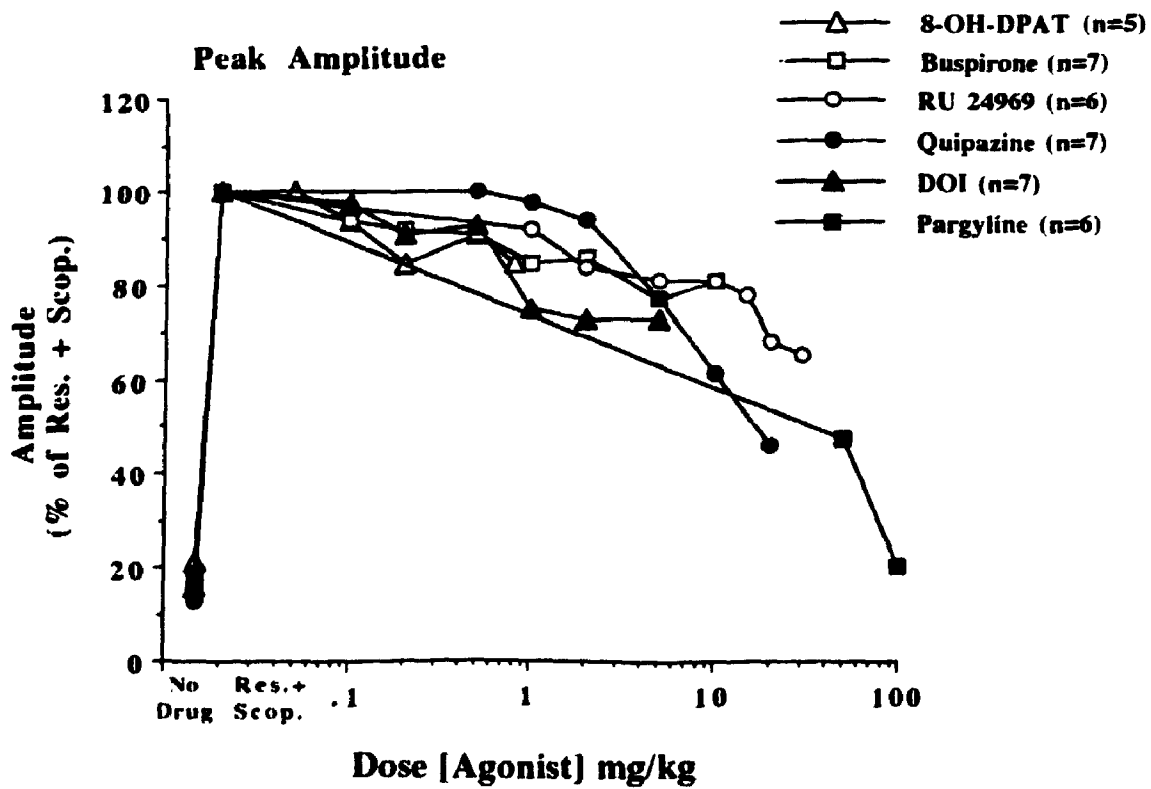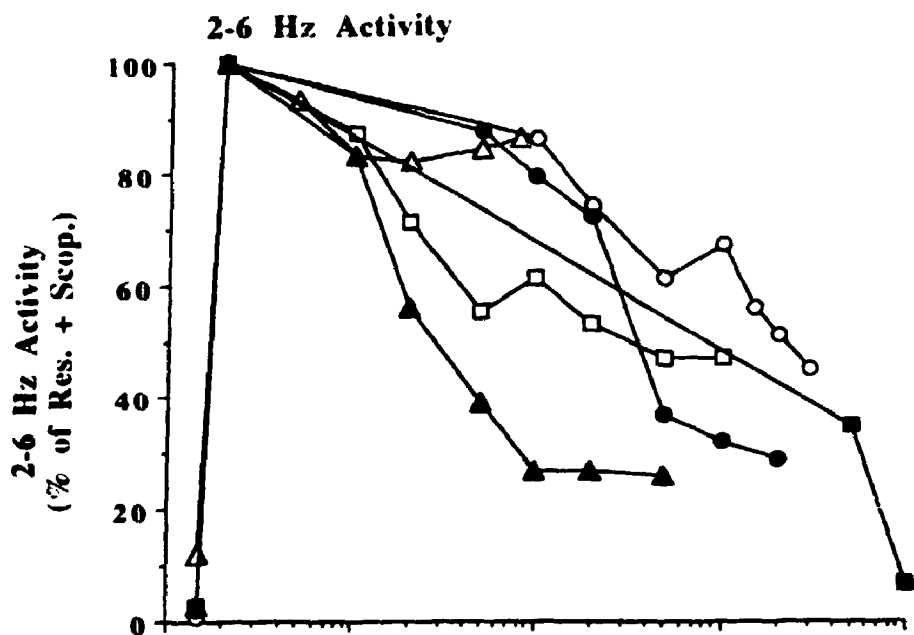
As shown in Fig. 3, after a cumulative dose of 100 mg/kg pargyline, integrated 2-6 Hz activity and peak amplitude were suppressed to 7 % and 20 % (0.3 mV), respectively, of that in reserpine + scopolamine treated rats. These values did not differ from those in the same rats before drug treatment (3 % and 18 % for 2-6 Hz activity and peak amplitude, respectively; Neuman-Keuls tests p > 0.05). After 50 mg/kg pargyline, LISA was generally present during immobility but LVFA could be induced by pinching or picking up the rat. Thus, the typical relationship between behavior and LVFA normally apparent after anti-muscarinic treatment (Vanderwolf, 1975) was largely restored by pargyline. At higher pargyline concentrations, however, LVFA often appeared independently of concurrent movement or sensory stimulation.

Quipazine treatment (0.5-20 mg/kg, i.p., n=7) produced a general, continuous suppression of LISA in the 2-6 Hz range (Fig. 1). Generally, large amplitude 1-2 mV waves were completely abolished by quipazine. However, normal-appearing LVFA was not restored. The LISA was replaced by lower amplitude activity (peak amplitude 0.9 mV; 46 % of max.; Fig. 3, bottom) with frequencies mostly above 6 Hz. Integrated 2-6 Hz activity was suppressed to 29 % relative to that after combined reserpine + scopolamine administration (Fig. 3, top). The ED $_{50}$ to suppress 2-6 Hz activity to 50 % of max. was 3.88 mg/kg (8.71 μmol/kg). Quipazine reversed the effect of reserpine + scopolamine treatment on MUA. During the lower amplitude activity present after quipazine injections, the burst-suppression pattern of MUA present after reserpine + scopolamine administration was abolished and replaced by continuous MUA as is normally associated with LVFA (Fig. 2 d).

Administration of DOI (0.1-5.0 mg/kg, s.c., n=7) led to a significant suppression of neocortical LISA. Neocortical activity after DOI administration consisted of a mixture of large amplitude (> 1 mV) waves in the 2-6 Hz frequency range and higher frequency lower amplitude activity (Fig. 1). Multiunit activity was continuous during low amplitude

activity, but reverted to a burst-suppression pattern during bursts of LISA (Fig. 2 e). Integrated 2-6 Hz activity was reduced to 26 % (Fig. 3, top; ED $_{50}$ = 0.31 mg/kg or 0.86 μmol/kg), and peak amplitude to 1.2 mV (73 % of maximum; Fig. 3, bottom) following DOI treatment. In addition, DOI frequently induced the appearance of 1-2 s bursts of spindle-like oscillations of approximately 10 Hz.

Neocortical activity after buspirone administration (0.1-10 mg/kg, i.p., n=7) also consisted of a mixture of LISA and higher frequency-lower amplitude activity (Fig. 1) and the typically associated patterns of MUA (i. e., burst-suppression and continuous, respectively). Integrated 2-6 Hz activity was reduced to 47 % (Fig. 3, top; ED $_{50}$ = 3.5 mg/kg or 8.29 μmol/kg) and peak amplitude to about 1 mV or 81 % (Fig. 3, bottom).

Administration of RU 24969 (1-30 mg/kg, i.p., n=6) produced a decrease in the amount of integrated 2-6 Hz activity to 45 %, and in the peak amplitude to 65 % (Fig. 3). However, these effects were not accompanied by the reappearance of periodic higher frequency-lower amplitude activity (Fig. 1). Thus, MUA continued in the burst -suppression pattern associated with LISA (not shown). The decrease in integrated 2-6 Hz activity after RU 24969 treatment appeared to be due to the appearance of some very low frequency activity below 2 Hz (see Fig. 1).

Administration of 8-OH-DPAT (0.05-0.8 mg/kg, s.c., n=5) did not produce any significant changes in the slow wave activity of rats treated with reserpine and scopolamine. Thus, even at the highest concentration of 8-OH-DPAT tested, neocortical slow wave activity consisted of continuous LISA (Fig. 1) and burst-suppression MUA (not shown). Peak amplitude and 2-6 Hz activity were reduced to 85 % and 86 %, respectively (Fig. 3).

All agonists tested had a stimulatory effect on motor activity. Rats treated with reserpine + scopolamine were akinetic and spontaneous movements were absent even when rats were held up. Treatment with all 5-HT agonists resulted in the re-appearance of

spontaneous activity that included forelimb treading, head movements and head shakes, sniffing, and locomotion/crawling. Further, during handling after agonist treatment, rats often vocalized and struggled violently.

Discussion

A series of pharmacological and lesion experiments has suggested that a serotonergic (5-HT) input from the midbrain raphe nuclei to the neocortex (Azmitia and Segal, 1978) is involved in maintaining the neocortical activation or LVFA that is resistant to anti-cholinergic treatment. If cholinergic and serotonergic inputs to the rat neocortex are blocked concurrently, all LVFA is abolished and neocortical activity consists of continuous 2-6 Hz LISA (Vanderwolf and Baker, 1986; Vanderwolf et al., 1989; 1990). Thus, the integrity of the serotonergic and cholinergic systems is essential for LVFA to occur; other inputs to the ^ortex are insufficient to maintain the activated state of the electrocorticogram.

Consistent with previous work (Vanderwolf, 1984), we show that in rats pretreated with reserpine and scopolamine to block the endogenous serotonergic and cholinergic activating inputs to the neocortex, administration of the monoamine oxidase inhibitor pargyline restores (scopolamine-resistant) LVFA and continuous multiunit activity (MUA). Pargyline completely reversed the effects of reserpine + scopolamine on both peak amplitude and amount of integrated 2-6 Hz activity, i. e., both measures returned to levels equivalent to those in undrugged rats. Thus, it seems that the LVFA produced by pargyline may be equivalent to spontaneously occurring LVFA in normal, undrugged rats. A similar effect has been reported for the monoamine oxidase inhibitor tranylcypromine (Vanderwolf, 1984). It is likely that these effects of monoamine oxidase inhibitors are due to the restoration of central 5-HT levels since these drugs produce a rapid, pronounced increase in brain 5-HT when given after treatment with reserpine, but only slight and

slower changes of dopamine or noradrenaline levels (Fuxe, 1965). The fact that treatment with the 5-HT precursor 5-hydroxytryptophan also restores LVFA after combined reserpine + atropine treatment (Vanderwolf, 1984) further supports the hypothesis that 5 - HT is critically involved in this restoration of LVFA.

Several of the direct acting 5-HT receptor agonists tested here had significant activating effects on neocortical slow wave activity in reserpine + scopolamine treated rats. Treatment with quipazine, DOI, or buspirone reduced 2-6 Hz large amplitude activity associated with intermittent multiunit activity (MUA) and resulted in the re-appearance of periods of lower amplitude activity with frequencies above 6 Hz and concurrent continuous MUA. However, none of the agonists tested completely restored normal-appearing, continuous LVFA equivalent to that in undrugged rats or in rats treated with reserpine, scopolamine, and pargyline (see above).

The agonists tested have relatively high selectivity for several types of 5-HT receptors. Buspirone and 8-OH-DPAT both act as agonists at 5-HT $_{1A}$ receptors (Glaser and Traber, 1983; Pazos and Palacios, 1985; Peroutka, 1986; Vandermaelen et al., 1986; Pompeiano et al., 1992); RU 24969 appears to interact with both 5-HT $_{1A}$ and $_{1B}$ binding sites (Peroutka, 1986; 1990; Green and Backus, 1990); and DOI has a high selectivity for 5-HT $_2$ receptors (Shannon et al., 1984; Glennon et al., 1986). Of the agonists tested here, quipazine exhibits the least selectivity for central 5-HT binding sites as it has affinities for all subtypes of 5-H $_1$ and $_2$ receptors (K $_i$ values between 10-1000 nM; see Peroutka, 1990). Quipazine also acts as an antagonist at 5-HT $_3$ binding sites (K $_i$ < 10 nM) (Kilpatrick et al., 1987; Peroutka, 1990). Thus, it appears that the relatively selective stimulation of either 5-HT $_1$ or 5-HT $_2$ receptors, or non-selective stimulation of 5 - HT $_1$ and $_2$ receptors concurrently is not sufficient to fully reverse the effects of combined serotonergic and cholinergic blockade and produce normal-appearing LVFA in the

neocortex of freely-moving rats.

At present, it is not clear why buspirone, but not 8-OH-DPAT, produced a partial activation of neocortical activity. Both drugs act as agonists at 5-HT $_{1A}$ receptors (see above). The buspirone and 8-OH-DPAT doses used here are in the range that is effective in other electrophysiological assays of 5-HT $_{1A}$ receptor stimulation in freely-moving rats (Kasamo et al., 1994). However, in these doses, buspirone also can be expected to bind to dopamine and, possibly, 5-HT $_2$ receptors (Riblet et al., 1982; McMillen, 1991), and its metabolite 1-(2-pyrimidinyl)-piperazine blocks alpha 2-adrenoreceptors (Hjorth and Carlsson, 1982; Bianchi and Garattini, 1988). Whether the ability of buspirone to activate the neocortex involves an action at other than 5-HT $_{1A}$ receptors remains to be determined.

One of the quantitative measures used to analyze neocortical activity was peak amplitude, i.e., the mean amplitude of the two largest non-artifactual waves present in each 10 s epoch selected for data analyses. We used this measure (rather than alternative ones such as mean amplitude of the entire epoch) because it better reflects the occurrence of isolated large amplitude low frequency (< 6 Hz) waves that are always associated with MUA suppression. Such waves and the concurrent MUA suppression are almost continuous after combined reserpine + scopolamine treatment but never occur in undrugged, awake rats. Thus, the suppression of this activity provides a useful measure of the degree to which drugs reverse the effects of combined reserpine + scopolamine treatment.

As pointed out, in contrast to these receptor agonists, in rats treated with reserpine and atropine, LVFA can be restored by compounds that enhance endogenous 5-HT transmission by stimulating 5-HT synthesis or release, or by preventing 5-HT breakdown (Vanderwolf, 1984; 1988). These observations may suggest that stimulation of 5-HT receptors with relatively selective ligands may not always mimic the action of stimulating

endogenous 5-HT transmission. Several hypotheses can be proposed to account for this difference between stimulating endogenous 5-HT transmission and administration of receptor agonists. Release of endogenous 5-HT should, in varying degrees, stimulate many pre- and postsynaptic 5-HT receptors simultaneously. Also, the specific pattern of release and receptor activation may be important in determining the action of a transmitter at the network level. Relatively selective agonists may not have the same effect as a release of 5-HT by serotonergic neurons. Thus, even though a receptor agonist may mimic some of 5-HT's actions on the biochemical or cellular level, it may not have an action that mimics that of endogenous 5-HT release on widespread neuronal networks.

It is interesting to note that the least selective receptor agonist used here, quipazine, had the most pronounced activating effect of all receptor agonists tested. A possible hypothesis derived from this observation may be that 5-HT-dependent neocortical LVFA may involve the concurrent stimul $_i$ of several types of 5-HT receptors. This hypothesis is compatible with the observations that: (a) a restoration of endogenous 5-HT levels with pargyline completely reversed the effects of reserpine + scopolamine treatment and produced normal-appearing LVFA, whereas a selective receptor stimulation with agonists (quipazine, DOI, buspirone) produced only partial effects (present results, see above); and (b) only the non-selective 5-HT antagonist methiothepin, but not selective antagonists such as ketanserin or ritanserin, can reduce 5-HT-dependent LVFA in freely - moving rats (Watson et al., 1992).

In contrast to the findings in freely-moving rats, selective 5-HT $_2$ antagonists such as ketanserin and ritanserin block serotonergic neocortical LVFA in rats anesthetized with urethane (Neuman and Thompson, 1989; Neuman and Zebrowska, 1992). Consequently, Neuman and coworkers have suggested that the 5-HT $_2$ receptor mediates the activating effect of 5-HT on neocortical slow wave and unit activity (Neuman and Zebrowska, 1992). The interpretation of these findings is complicated, however, by the

fact that urethane appears to exert strong anti-serotonergic effec s and to alter the action of some 5-HT antagonists including ketanserin (Dringenberg and Vanderwolf, 1995; Dringenberg et al., 1995). Here, the potent and highly selective 5-HT_2 agonist DOI had a clear activating effect. However, continuous, normal-appearing LVFA was not restored. Thus, it is unlikely that this receptor type alone mediates 5-HT- dependant LVFA in unanesthetized rats.

The hypothesis that multiple receptor stimulation may mediate 5-HT-dependent neocortical activation is a tentative one. For future work in this field, attempts to restore LVFA with a mixture of agonists with selectivity for different types of 5-HT receptors would be desirable.

Paper 4:

**Urethane Reduces Contraction to 5-Hydroxytryptamine (5-HT)
and Enhances the Action of the 5-HT Antagonist Ketanserin
on the Rat Thoracic Aortic Ring**

Summary

The general anesthetic urethane (ethyl carbamate) is widely used in electrophysiological *in vivo* experiments. However, its pharmacological effects are poorly understood. Here, the effects of urethane on *in vitro* contractile responses of the rat thoracic aortic ring preparation were investigated. Bath application of 5-HT produced a concentration-dependent contractile response (EC_{50} = 4.3 x 10^{-6} M). Urethane (11.2 mM = 1 mg/ml) shifted the concentration-response curve (CRC) for 5-HT to the right (EC_{50} = 1.7 x 10^{-5} M) and decreased the maximal contraction by 30.8 %. The CRC for NA (EC_{50} = 7.2 x 10^{-9} M) was also shifted to the right by urethane (EC_{50} = 1.4 x 10^{-8} M), but the shift of the 5-HT-CRC was twice that of the NA-CRC (3.95 vs. 1.95). The CRC to KCl was shifted rightwards only slightly by urethane (ratio 1.27) and the maximal contraction to KCl was not affected. The CRC to replacement of $CaCl_2$ (0.1-10 mM) to KCl-depolarized vessels in a Ca^{2+}-free Krebs solution was unaffected by urethane. Ketanserin (10^{-9} M) antagonized the contraction to 5-HT, and a combination of ketanserin and urethane was markedly more effective than either drug alone, decreasing the maximal contraction by 58 %. Antagonism of NA contraction by prazosin (5 x 10^{-8} M) was not increased by addition of urethane.

The urethane dose used here approximates blood and brain concentrations required to produce anesthetic effects in mammals. It is possible that reductions in 5-HT transmission and, to a lesser extent, in NA transmission, but not blockade of Ca^{2+} or K^+ channels, may contribute to the anesthetic effect of urethane. In addition, the action of the selective 5-HT $_2$ antagonist ketanserin is clearly altered by urethane. These findings are important to consider when urethane is used for *in vivo* neurophysiological investigations, particularly when 5-HT mechanisms are involved.

Introduction

The anesthetic urethane (ethyl carbamate) is widely used in *in vivo* physiological experiments on a number of body systems (Maggi and Meli, 1986). However, despite its extensive use, urethane's pharmacological actions are poorly understood. Recently, it has been suggested that urethane may have some interaction with endogenous serotonergic transmission, and may substantially influence the action of serotonergic receptor antagonists in the rat neocortex *in vivo* (Dringenberg and Vanderwolf, 1995).

Serotonin (5-hydroxy tryptamine, 5-HT) plays an important role in activation of the electrocorticogram (ECoG) (Vanderwolf, 1984; Vanderwolf and Baker, 1986; Vanderwolf, 1988; Vanderwolf et al., 1989). The serotonergic nature of neocortical activation was suggested by the fact that it could be abolished by reserpine, p-chlorophenylalanine, or intracerebral injections of 5,7-dihydroxytryptamine. These treatments all cause depletions of central 5-HT of varying selectivity. Further, after depletion of brain amines by reserpine, neocortical activation can be restored by the 5-HT precursor 5-hydroxytryptophan and some 5-HT agonists, but not by L-DOPA or other catecholaminergic agonists (Vanderwolf, 1988).

Selective 5-HT $_2$ receptor antagonists such as ketanserin, mianserin, and ritanserin have also been used to block serotonergic neocortical activation (Neuman and Thompson, 1989; Neuman and Zebrowska, 1992). However, these drugs appear to block neocortical serotonergic activation only in rats anesthetized with urethane, as was the case in the experiments of Neuman and his co-workers. In non-anesthetized rats, large doses of ketanserin, mianserin, ritanserin, and nine other 5-HT receptor antagonists were not effective in blocking serotonergic neocortical activation (Vanderwolf and Pappas, 1980; Vanderwolf, 1984; Robertson et al., 1991; Watson et al., 1992). . Only the non-selective 5-HT antagonist methiothepin produced a small but significant reduction of

neocortical activation in non-anesthetized rats (Watson et al., 1992).

The discrepancy regarding the action, or lack thereof, of 5-HT antagonists to block serotonergic neocortical activation respectively in urethane-anesthetized and in behaving rats may suggest that urethane alters some aspect of endogenous serotonergic transmission, the action of 5-HT receptor antagonists, or both. General anesthetics appear to alter central serotonergic systems significantly (Johnson et al., 1969; McGinty, 1973; Urca and Liebeskind, 1979; Heym et al., 1984; Dringenberg and Vanderwolf, in press). However, often it is not clear whether such alterations are due to a direct interaction of the anesthetic with some (pre- or postsynaptic) aspect of the 5-HT system, or whether they are secondary to interactions of the anesthetic with one or several other neurotransmitter systems. Also, to date, nothing is known with regard to urethane's effects on the action of 5-HT antagonists.

Contractions of the isolated rat aorta to 5-HT are mediated by serotonergic 5-HT $_2$ receptors and these contractile responses are blocked by 5-HT $_2$ antagonists such as ketanserin (for a detailed review of 5-HT actions in vascular smooth muscle preparations, see Cohen, 1988). This is the same receptor type that was blocked by 5-HT $_2$ antagonists in urethane-anesthetized rats (Neuman and Thompson, 1989) but not in non-anesthetized, freely moving rats (Watson et al., 1992). Thus, in the present study, we employed the rat aortic ring preparation to determine if urethane has a direct effect on aortic contractile responses to 5-HT, and if it interacts with the selective 5-HT $_2$ receptor antagonist ketanserin. Further, as a control, we also determined if urethane would affect contractile responses to noradrenaline or interact with a noradrenergic antagonist, prazosin. Finally, we tested whether some of the effects of urethane may be mediated by a blockade of potassium (K^+) or calcium (Ca^{2+}) channels.

Methods

Aortic ring preparation

Adult male Sprague-Dawley rats (250-350 g) were used. The rats were stunned with a blow to the neck, the cervical spinal cord was rapidly cut, and the thoracic aorta was removed and placed in cold modified Krebs solution. After dissecting the aorta free of fat and connective tissue, rings of approximately 4 mm width were cut with sharp scissors and suspended in a 10 ml organ bath containing Krebs solution (composition in mM: KCl 4.7; KH_2PO_4 1.19; $MgSO_4$ 1.2; $CaCl_2$ 2.5; NaCl 115; $NaHCO_3$ 25; glucose 10.5; aerated with a gas mixture of 95 % O_2 and 5 % CO_2, aintained at 37° C). The aortic rings were equilibrated for 1 h. Subsequently, a resting tension of 1 g was applied and adjusted until a stable baseline of this tension was maintained. Isometric tension was recorded with a Statham FT-03 transducer and displayed on a four channel Grass Model P7 polygraph. Typically, for each experiment, 4 aortic rings were used simultaneously in parallel baths.

Concentration-response curves (CRC)

Following equilibration, all aortic rings were contracted with KCl (15 to 85 mM cumulative concentrations). After washing the bath, rings were contracted with 35 mM KCl and acetylcholine (5 x 10^{-6} M) was added to test whether a functional endothelium was present on the tissues. Lack of a relaxation response to acetylcholine indicates that functional endothelium is not present (Furchgott and Zawadzki, 1980).

Initial CRC's to the test compound were established by adding successive (cumulative) doses of the agonist to the bath. The maximum response observed during this

first CRC was taken as 100 % contraction for subsequent comparisons. After a wash-out, a second CRC to the agonist was established from which the experimental data were obtained. Generally, for this CRC, tissue baths were randomly assigned to contain (1) urethane (11.2 mM = 1 mg/ml); (2) antagonist; (3) urethane + antagonist; and (4) a time - control that did not contain any additional drug. Maximum tension values (in grams) given are those obtained during the second CRC. Similarly, contraction and EC_{50} values given were obtained during the second CRC and are expressed as % of the maximal contraction during the first CRC to the test drug.

For the experiments using noradrenaline (NA), sotolol (10^{6} M) was added to the bath prior to contraction with NA. Sotolol prevents beta adrenoreceptor-mediated relaxation of the aorta to high concentrations of NA (J. T. Hamilton, unpublished observations).

For Ca^{2+} contractions, Ca^{2+}-free Krebs solution was used. $CaCl_2$ was deleted from the normal Krebs solution. 80 mM KCl was added to depolarize the rings, and an equimolar amount of NaCl was deleted to maintain isoosmolarity. Successive additions of $CaCl_2$ to the bath were used to obtain the CRCs. At the end of each experiment, the baths were filled with normal Krebs solution and the preparations were tested for the presence of functional endothelium, as outlined above.

Drugs

Drugs used were: acetylcholine iodide (BDH); arterenol (noradrenaline; Sigma); 5-hydroxytryptamine (Sigma); ketanserin tartrate (ICN Biochemicals); prazosin (Sigma); sotolol (Sigma); urethane (ethyl carbamate) (Sigma). All drugs were dissolved in distilled water.

Data analysis

Data are presented as mean ± S.E.M. ANOVAs and Newman-Keul's follow- up tests were performed using the software package CLR Anova (Version 1.1: Clear Lake Research Inc.).

Results

5-Hydroxytryptamine (5-HT)

The concentration-response curve (CRC) for 5-HT application to aortic rings was biphasic. As shown in Fig. 1 (top), successive increases in the bath concentration of 5 - HT produced a concentration-dependent contraction of the aortic rings with a maximum tension of 2.04 ± 0.2 g (EC_{50} = 4.3 x 10^6 M). At concentrations of 5 x 10^{-5} M and higher, however, a relaxation response was elicited. (Generally, such high 5-HT concentrations were not used so that 5-HT-induced relaxation did not interfere with the effects of the antagonists used).

Application of urethane (1 mg/ml) to the bath shifted the CRC to 5-HT to the right (EC_{50} = 1.7 x 10^{-5}M). Further, urethane decreased the maximal contraction (E_{max}) to 5 - HT by 30.8 % (Fig. 1, top). Urethane's effect of blocking contractions to 5-HT was not related to the amount of endothelium present on the preparations. A Spearman's rank order correlation for relaxation responses to 5 x 10^{6}M ACh, indicative of functional endothelium (Furchgott and Zawadzki, 1980), and contractions to 5-HT in the presence of urethane was not significant (r_s = -0.168, p = 0.691). That is, urethane's effect of blocking contractions to 5-HT occurred in tissues with and without significant amounts of functional

Figure 1.

Contractile responses of the isolated aortic ring preparation to successive, cumulative bath applications (range: 5×10^{-7} to 2×10^{-4} M) of 5-hydroxytryptamine (5 - HT). *Top:* Application of 5-HT produced a concentration-dependent contractile response. At higher 5-HT concentrations, a relaxation response was elicited. Urethane (1 mg/ml) shifted the concentration-response curve (CRC) to the right and suppressed maximal contraction. The CRCs obtained in the absence and presence of urethane were significantly different ($F_{1,14} = 11.4$, $p = 0.0046$). Simple effects of urethane were significant for 5-HT concentrations of $\geq 5 \times 10^{-6}$ M (p's < 0.03). *Bottom:* Ketanserin (10^{-9} M) shifted the CRC to 5-HT to the right ($F_{1,14} = 12.0$, $p = 0.0038$). Simple effects of ketanserin were significant for 5-HT concentrations of $2 \times 10^{-5} - 2 \times 10^{-5}$ M (p's < 0.04). Note that at a 5 - HT concentration of 5×10^{-5} M maximal contraction was no longer significantly different between the control and ketanserin CRCs ($p = 0.095$). Urethane (Ure.) and ketanserin (Ketan.) together had a much more profound effect than ketanserin alone ($F_{1,14} = 35.0$, $p < 0.0001$). Simple effects for the ure. + ketan. CRC relative to the ketanserin CRC were significant for 5-HT concentration of $\geq 2 \times 10^{-5}$ M (p's < 0.001). (Each CRC has n = 8; means $\pm$ S.E.M. are shown).

5-HT Contraction
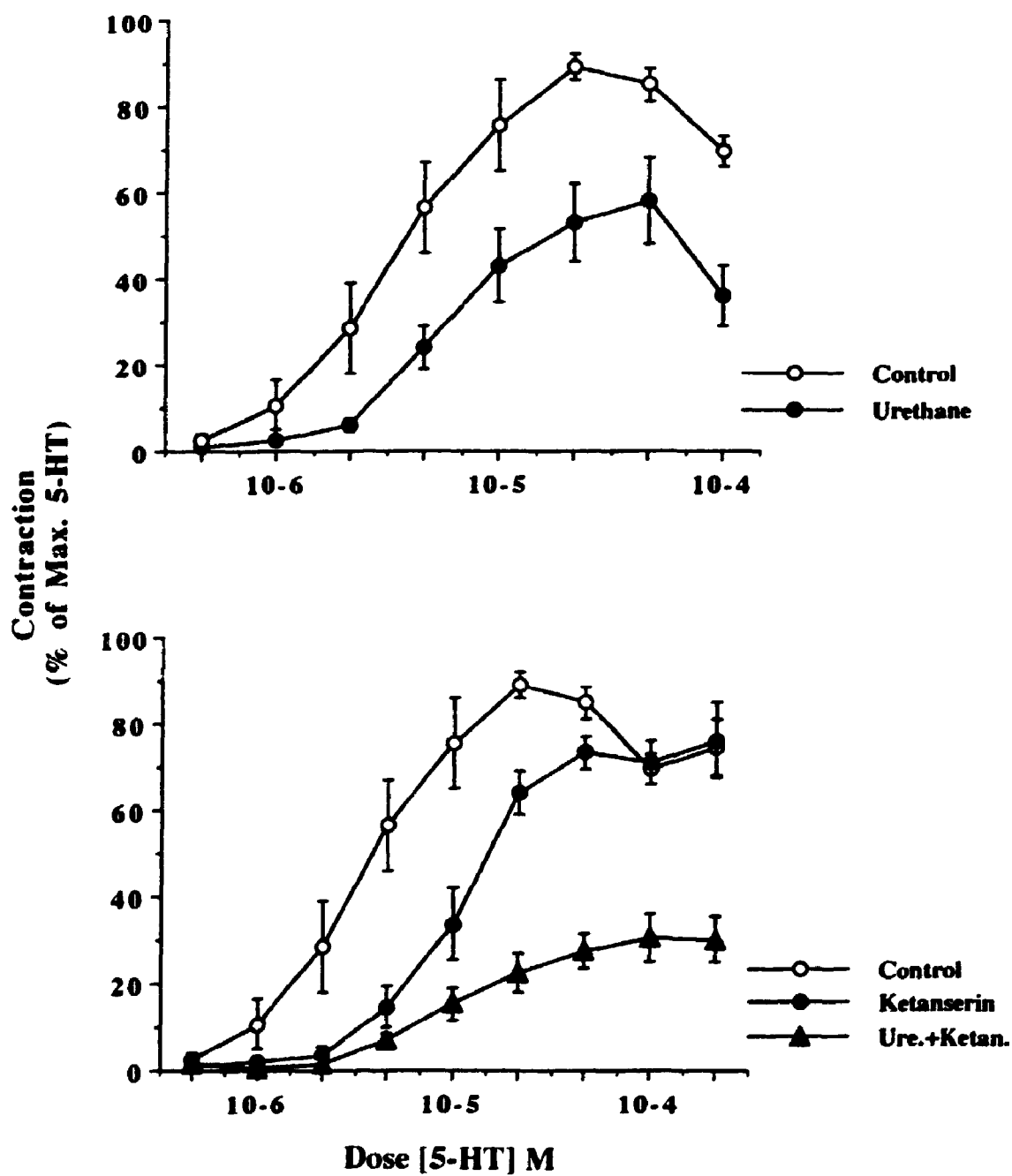
endothelium. Even though not studied systematically, it appeared that urethane did not block the relaxation responses to high concentrations of 5-HT.

As shown in Fig. 1 (bottom), the 5-HT receptor antagonist ketanserin (10^{-9} M) also produced a rightward shift of the CRC to 5-HT ($EC_{50} = 1.54$ x 10^{-5} M). At a 5-HT concentration of 5 x 10^{-5} M, the maximal response to 5-HT in the presence of ketanserin was obtained and this response was not significantly depressed relative to that of the control tissue. Also, ketanserin appeared to block the relaxation response to high concentrations of 5-HT (Fig. 1, bottom).

A combination of urethane (1 mg/ml) and ketanserin (10^{-9} M) was markedly more effective in depressing contractions to 5-HT than either drug alone (Fig. 1, bottom), suppressing the maximal response to 5-HT by 58 % (relative to the control tissue). As noted earlier, ketanserin alone did not significantly suppress the maximal contraction to 5 - HT, and urethane produced only a 30.8 % suppression.

Noradrenaline (NA)

As shown in Fig. 2 (top), bath application of NA produced a CRC with an EC_{50} of 7.2 x 10^{-9} M. Maximum tension developed was 2.34 ± 0.38 g. Urethane (1 mg/ml) produced a slight rightward shift of the CRC to NA ($EC_{50} = 1.4$ x 10^{-8} M), but this shift was only half of that of the CRC to 5-HT by urethane (1.95 vs. 3.95 fold).

Prazosin shifted the CRC to NA to the right ($EC_{50} = 3.2$ x 10^{-6} M) and, at a NA concentration of 2 x 10^{-5} M, suppressed maximal contraction by 28 % (Fig. 2, bottom). In contrast to the effect of combining urethane and ketanserin, a combination of urethane and prazosin was not more effective than prazosin alone in antagonizing contractions to NA. As shown in Fig 2 (bottom), NA application produced virtually identical CRCs in the

Figure 2.

Aortic concentration-response curves (CRCs) to successive increases in the bath concentration of noradrenaline (NA). *Top:* NA at concentrations between 10^{-9}-2×10^{-6} M produced a contractile response which was slightly suppressed by urethane (1 mg/ml). The CRCs in the absence and presence of urethane were significantly different from one another ($F_{1,12} = 5.1$, p = 0.0443). Simple effects of urethane for every NA concentration were significant between 5×10^{-8} M and 2×10^{-6} M (p's $\leq$ 0.046). *Bottom:* Prazosin (5×10^{-8} M) shifted the CRC to the right and depressed the maximal contraction to NA ($F_{1,12} = 303.6$, p < 0.0001; simple effects of prazosin were significant for NA concentrations of $\geq$ 5×10^{-9} M, p's < 0.003). A combination of urethane (Ure.) and prazosin (Pra.) was equi - effective to prazosin alone ($F_{1,12} = 0.2$, p = 0.6368). That is, urethane did not enhance the action of prazosin. (CRCs have n = 7; means ± S.E.M. are shown).

NA Contration

Dose [NA] M
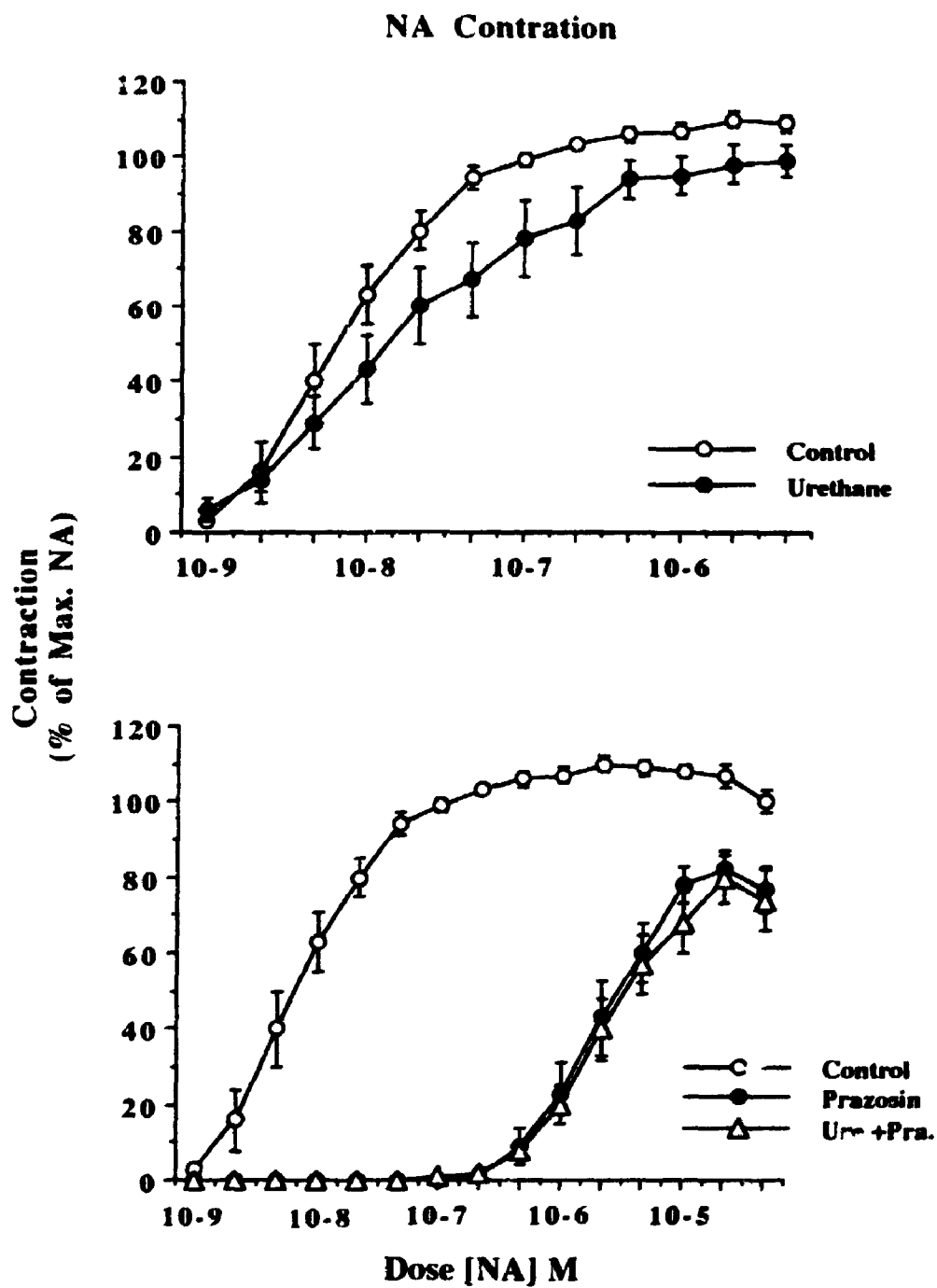
presence of prazosin alone and urethane + prazosin.

Potassium chloride (KCl)

Bath application of KCl contracted aortic rings to a maximum tension of 2.12 $\pm$ 0.21 g (EC_{50} = 20.5 mM) (Fig. 3, top). Addition of urethane (1 mg/ml) to the bath resulted in a slight rightward shift of the CRC to KCl (EC_{50} = 26.1 mM: 1.27-fold shift). At concentrations of 35 mM and higher, however, urethane did not produce a significant reduction of the contractile responses to KCl (Fig. 3, top).

Ketanserin (10^{-9} M) also produced a slight, non-significant rightward shift of the CRC to KCl application (Fig. 3, bottom). However, a combination of urethane and ketanserin was not more effective than either drug alone in reducing contractile responses to KCl (Fig. 3, bottom).

Calcium chloride ($CaCl_2$)

As shown in Fig. 4, in Krebs solution from which $CaCl_2$ had been deleted, successive addition of $CaCl_2$ (0.1-10 mM) produced contractions of aortic rings depolarized with 85 mM KCl (EC_{50} = 0.19 mM). Maximum tension developed was 2.04 $\pm$ 0.4 g. Urethane (1 mg/ml) had no effect on contractions to $CaCl_2$ application (Fig. 4).

Discussion

In urethane-anesthetized rats, ketanserin, mianserin, ritanserin, and other antagonists at the 5-HT$_2$ receptor are effective in blocking the activating effect of serotonin

Figure 3.

Concentration-response curves (CRCs) to application of potassium chloride (KCl).
Top: KCl (15-85 mM) contracted aortic rings and urethane (1 mg/mg) produced a
depression of the contractile responses to low KCl concentrations ($F_{1,8}$ = 5.5, p = 0.0468;
simple effects of urethane significant only for a KCl concentration of 25 mM, p = 0.02).
Bottom: Ketanserin (10^{-9} M) produced a slight. non-significant suppression of
contractions to KCl ($F_{1,7}$) = 2.2, p = 0.1778). A combination of urethane (Ure.) and
ketanserin (Ketan.) did not potentiate the effects of ketanserin alone on KCl contractions
($F_{1,6}$ = 0.007, p = 0.9371). (Control and urethane CRCs: n = 5; ketanserin and Ure. +
Ketan.: n = 4; means ± S.E.M. are shown).
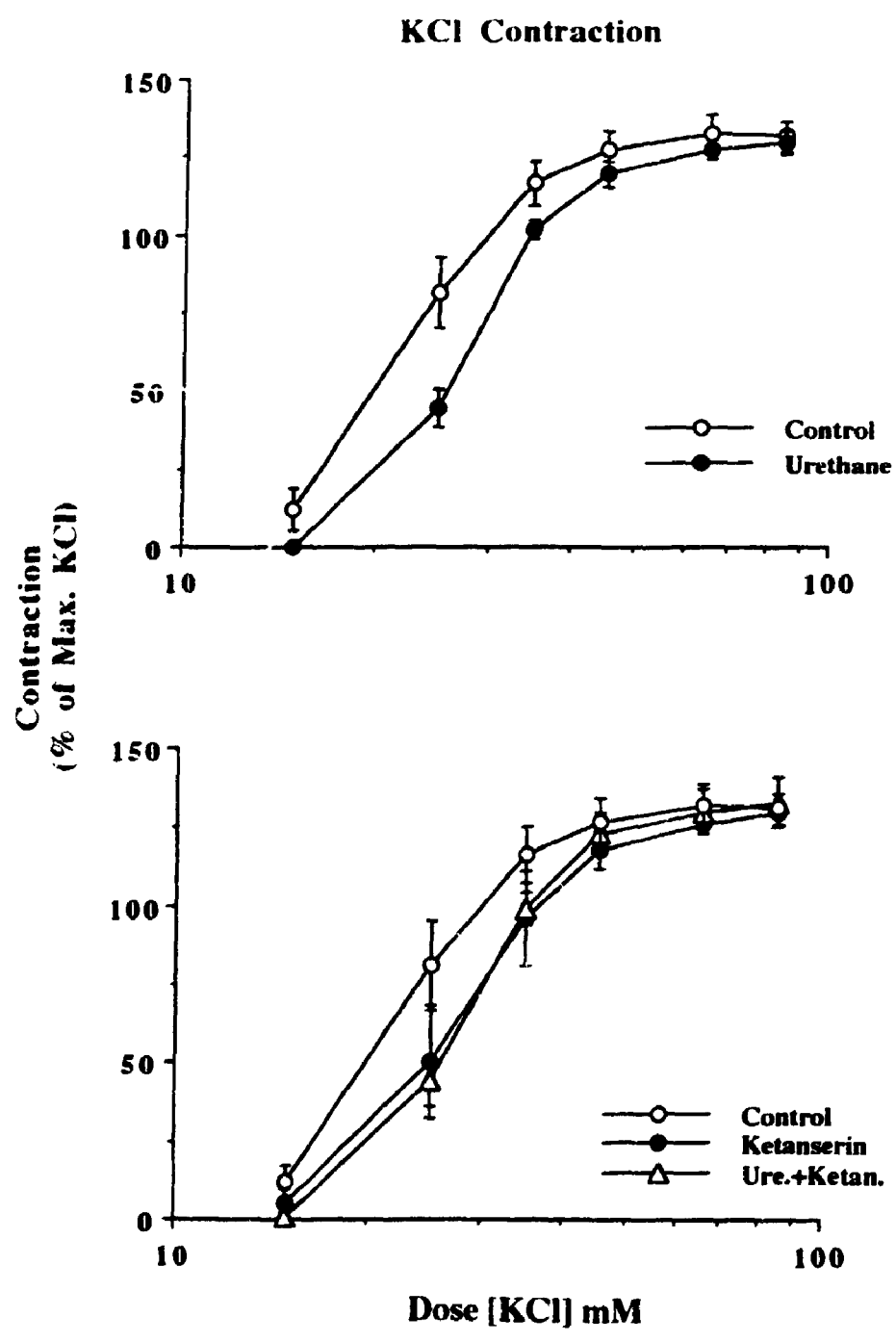
KCl Contraction

Figure 4.

Concentration-response curves (CRCs) for KCl-depolarized (85 mM) aortic rings in Ca^{2+}-deficient medium to successive applications of $CaCl_2$ (0.1-10 mM). $CaCl_2$ produced contractile responses of aortic rings that were not affected by addition of urethane (1 mg/ml) ($F_{1,6} = 0.1$, $p = 0.7782$). (CRCs: $n = 4$; means ± S.E.M. are shown).

CaCl$_2$ Contraction

Dose [CaCl$_2$] mM
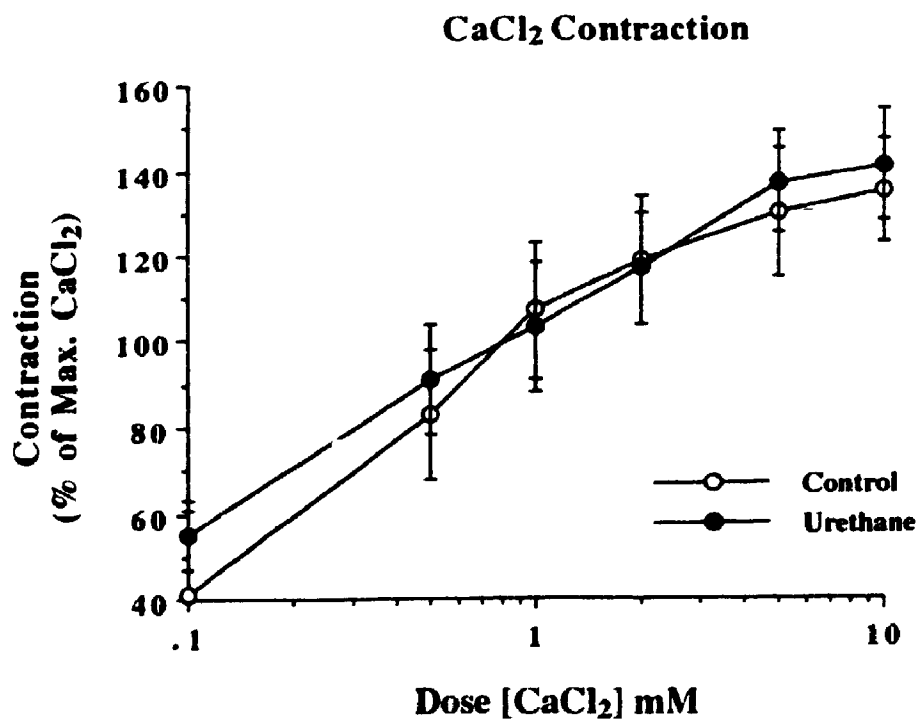
on neocortical slow wave (i. e., ECoG) activity and concurrent pyramidal cell activity (Neuman and Thompson, 1989; Neuman and Zebrowska, 1992). In non-anesthetized, freely moving rats, however, these and a number of other 5-HT antagonists are ineffective in blocking serotonergic effects on neocortical slow wave and concurrent multiunit activity (Vanderwolf and Pappas, 1980; Vanderwolf, 1984; Robertson et al., 1991; Watson et al., 1992). Both Neuman and Vanderwolf and their coworkers used blockade of 5-HT - dependent neocortical activation as a measure of antagonist action (see Neuman, 1986; Vanderwolf, 1988). The principal difference between their experiments appears to be the use of urethane in Neuman's experiments.

In the present study, we demonstrate that urethane itself appears to interfere with 5 - HT effects *in vitro*, and that a combination of urethane and ketanserin produces a much greater blockade of the action of 5-HT than that produced by either drug alone. Further, contractile responses to NA and KCl were reduced by urethane, but these effects were of much lesser magnitude than those observed in the experiments using 5-HT as the spasmogenic agent. Thus, it appears that urethane and other general anesthetics have profound depressant effects on 5-HT transmission with some selectivity (see also Johnson et al., 1969; McGinty, 1973; Urca and Liebeskind, 1979; Heym et al., 1984), and that urethane alters the action of 5-HT antagonists (present results; also Dringenberg and Vanderwolf, in press). Consequently, it is questionable whether results on serotonergic transmission obtained in anesthetized preparations can be unequivocally applied to the non - anesthetized state.

The doses of urethane used for anesthetic effects generally range between 1.2-1.5 g/kg (Maggi and Meli, 1986) and a blood concentration of at least 80 mg urethane/100 ml is required to anesthetize rats (Boyland and Rhoden, 1949). Further, the concentration of urethane in rat brain is 138 and 85 mg/100 g tissue one and two hours after a 1 g/kg injection of urethane, respectively (Boyland и .d Rhoden, 1949). Thus, the urethane

dose used in this study (1 mg/ml) is approximately equivalent to doses used as an anesthetic in the rat and it is possible that some of the effects observed here may also be relevant to urethane's anesthetic action. In addition to the reduction of serotonergic and noradrenergic transmission noted here, urethane may also block excitatory amino acid transmission (Dalo and Larson, 1990; Pina-Crespo and Dalo, 1992), and it hyperpolarizes neurons, possibly by increasing potassium conductance (Nicoll and Madison, 1982).

Interestingly, there appear to be structural similarities between NA and 5-HT receptors (Fargin et al., 1988) and a number of ligands such as ketanserin, spiperone, propranolol, and cyanopindolol have affinities for both 5-HT and NA receptors (e.g., Richardson and Engel, 1986; Fargin et al., 1988; Peroutka et al., 1990). Since urethane reduced contractile responses to both 5-HT and NA, it is tempting to speculate that urethane's anesthetic action may, at least partially, involve a direct action on 5-HT and NA binding sites. We are not aware of prior evidence for such a direct receptor action of urethane. It is interesting to note, however, that neither urethane alone, nor a combination of ketanserin and urethane significantly interfered with contractions to KCl. Further, both 5-HT $_2$ and adrenergic alpha 1 receptors are linked to the same second messenger system, phosphoinositide hydrolysis (see Putney, 1987; Sanders Bush, 1988 for reviews), but urethane produced only a small reduction of contractions to NA presumably mediated by alpha 1 receptors. Thus, it appears that non-specific effects on intracellular events or contractile mechanisms cannot account for the effects of urethane observed here.

Neither calcium nor potassium channels appeared to be significantly affected by urethane. Potassium channels may be blocked partially by urethane at low extracellular K+ concentration. However, this effect was overcome by increasing extracellular K+

concentration to 35 mM or higher. Blockade of Ca^{2+}-channels interferes with contractions to Ca^{2+} application of KCl-depolarized smooth muscle preparations kept in Ca^{2+}-deficient medium (Spedding and Cavero, 1984; Godfraind et al., 1986). Urethane had no effects on contractile responses to replacement of $CaCl_2$, indicating that Ca^{2+}-channels were fully functional. The fact that Ca^{2+} channels do not appear to be affected by urethane clearly distinguishes urethane from other general anesthetics which may act by blocking Ca^{2+} influx into cells (Gelb et al., 1992; Chang and Davies, 1993). Also, in contrast to a number of other general anesthetics, urethane produces only small or no enhancement of GABAergic transmission (Scholfield, 1980; Minchin, 1981; rev. by Maggi and Meli, 1986). The question whether urethane may act partially by blocking certain (especially serotonergic) central binding sites deserves the attention of future investigations.

The antagonist ketanserin may block some central effects of 5-HT release only in conjunction with urethane anesthesia (see above). In the aorta preparation used here, ketanserin's ability to block contractions to 5-HT was strongly enhanced when used in conjunction with urethane. The action of the alpha 1 adrenoreceptor antagonist prazosin, however, was not enhanced by urethane. These observations may suggest that the enhancement of antagonist action does not appear to be a general, non-specific action of urethane on aminergic receptors. Instead, the potentiation of antagonist activity by urethane appears to be, at least somewhat, selective for those antagonists acting at serotonergic binding sites. The action of the 5-HT antagonist methiothepin in blocking activation of the rat neocortex also is strongly enhanced by urethane (Dringenberg and Vanderwolf, in press).

At present, it is not clear how urethane may act to alter 5-HT antagonist action, and antagonize the effects of 5-HT and NA. Future work may provide further insight into the pharmacological properties of urethane and its interaction with other pharmacological

agents. Also, to evaluate the generality of the phenomenon described here, it would be desirable to test whether urethane reduces serotonergic actions in other tissues and experimental species. Given that urethane anesthesia is frequently used for *in vivo* investigations, such knowledge would be desirable for the interpretation of data obtained in urethane-anesthetized preparations.

Section III:

Serotonin as an Activator Substance: Behavioral Evidence

The following experiments were designed to investigate whether 5-HT transmission, in addition to activating neocortical activity, may play a role in behavioral activation. As pointed out earlier, 5-HT produces neocortical LVFA during behaviors such as walking, rearing, or running (Type 1 behaviors), but not during Type 2 behaviors such as immobility, grooming, or chewing. It may be that there is a link between the effects of 5-HT release on the ECoG and on concurrent behavior.

The effects of reducing 5-HT release on behavior were examined by using two behavioral paradigms. In the first, spontaneous exploratory locomotor activity (which can be argued to consist largely of Type 1 behaviors such as walking, head movements, and rearing) was measured in normal rats and rats that had varying levels of 5-HT depletions induced by PCPA- treatment. In the second behavioral test, undrugged rats and rats treated with the 5-HT-autoreceptor agonist buspirone that produces a reduction of forebrain 5-HT release were given the choice of carrying a food item from an exposed food source to a shelter for consumption, or of immediately consuming the pellet at the food source; these two behavioral outputs can be taken as consisting predominantly of behaviors of the Type 1 and Type 2 categories, respectively, and thus may constitute a suitable and sensitive behavioral assay for examining the effects of small changes in central 5-HT transmission. The question of whether some of the behavioral changes apparent after 5-HT manipulations are related to the effect of 5-HT on the ECoG or some other effects such as reduced "anxiety" levels is discussed.

Figure 2.

The effects of treatment with gum arabic (i.p.) or PCPA (i.p.) on spontaneous exploratory locomotor activity during 60 min in a digitized open field activity monitor (n=8/group; No-injct. no injection; Gum A, gum arabic injections). PCPA treatment in doses of 150, 500, and 1000 mg/kg produced a dose-dependent decrease of general locomotor activity characteristics (A) (omnibus test $F_{44,112} = 7.0$, p < 0.0005), horizontal movement characteristics (B) (omnibus test $F_{16,140} = 4.4$, p < 0.0005), and vertical movement characteristics (C) (omnibus test $F_{16,140} = 2.6$, p < 0.001). Univariate F-tests revealed significant effects of drug administration on every movement characteristic across all groups (p < 0.001) and across the three PCPA groups only (p < 0.013). (Means ± S.E.M. are shown).

Summary

Para-chlorophenylalanine (PCPA) depletes central serotonin (5-hydroxytryptamine. 5-HT) by inhibiting tryptophan hydroxylase. an enzyme necessary for the synthesis of 5 - HT. The effects of a wide range of PCPA doses (150-1000 mg/kg) on spontaneous exploratory locomotor activity in a novel environment, activity in running wheels, and a number of sensory-motor capacities were examined. Administration of 1000 mg/kg PCPA reduced whole brain levels of 5-HT and its metabolite 5-hydroxyindoleacetic acid to 9.4 and 8.2 % of control levels, respectively. Treatment with PCPA produced a dose - dependent decrease in exploratory locomotion in an unfamiliar automated open field relative to vehicle- treated animals. Further, all measures of general, horizontal, and vertical activity were suppressed by PCPA treatment. In contrast to previous work, hyperactivity of rats chronically housed in cages with running wheel access was not observed. In their home cages, some PCPA-treated rats exhibited hyper-reactivity to cutaneous stimulation. No other sensory-motor deficits were apparent. Previous theories of 5-HT function state that its action may be to inhibit motor activity or promote sleep. The present results challenge this view and suggest that 5-HT, at least in certain environments, may stimulate locomotor activity without directly controlling various sensory-motor capacities in rats.

Introduction

Serotonergic neurons in the midbrain raphe nuclei project both caudally and rostrally along the entire neuroaxis (Anden et al., 1966; Törk, 1990). The extent of serotonin (5-hydroxytryptamine, 5-HT) innervation of the forebrain has led to the assumption that, rather than controlling specific ·· ͺ ͺcts of behavior, 5-HT may exert a more general effect over behavior such as control of activity levels, behavioral inhibition, arousal mechanisms, and the sleep-waking cycle (Jouvet, 1972; Soubrie, 1986).

The role of 5-HT in the control of locomotor activity has been investigated by depleting 5-HT using *para*-chlorophenylalanine (PCPA), an inhibitor of the 5-HT-synthesizing enzyme tryptophan hydroxylase (Koe and Weissman, 1966). Despite extensive investigations, however, there is no consensus regarding the effects of PCPA administration on locomotion. Using various PCPA doses and procedures to assess locomotor activity, both decreases and increases in locomotor activity have been reported. Generally, increases in activity after 150-400 mg/kg PCPA have been reported when locomotion was assessed in a familiar environment (e.g., a home cage with running wheel access) (Fibiger and Campbell, 1971; Borbely et al., 1973; Jacobs et al., 1975; Marsden and Curzon, 1976). The enhancement of locomotor activity in familiar environments after PCPA administration is mimicked by lesions of the median raphe (Jacobs et al., 1975).

Activity of rats placed in an unfamiliar environment may differ from locomotion in home cages or running wheels in that it includes behaviors such as frequent walking, sniffing, climbing, and rearing which have been regarded as "novelty reactions" and "exploratory" responses (Bindra and Spinner, 1958; Claus and Bindra, 1960). Detailed analyses of such exploratory activity have shown that in an unfamiliar open field, rats establish a home base from which they explore the surrounding environment (Eilam and

Golani, 1989). For such exploratory locomotor activity in unfamiliar environments, both increases (Pirch, 1969: Fibiger and Campbell, 1971) and decreases (Tenen, 1967: Marsden and Curzon, 1976), as well as no change in locomotor activity (Köhler and Lorens, 1978) have been reported after PCPA treatment. Similarly, both reduced (Hole et al., 1976; Lipska et al., 1992) or unchanged (Lorens et al., 1976) open field activity has been observed following intraventricular infusions of the 5-HT neurotoxin 5.7 - dihydroxytryptamine.

Although studies of the effect of altering brain serotonergic function have not yielded any simple conclusion, other data suggest that serotonin may play a role in the central control of motor activity. A series of mainly pharmacological investigations have indicated that ascending serotonergic pathways exert an activating effect on the neocortex and hippocampal formation that is closely coupled to the performance of certain motor activities (Vanderwolf, 1988). Recordings of the activity of raphe neurons (Fornal and Jacobs, 1988) or measurement of 5-HT release (Wilkinson et al., 1991) are consistent with this in suggesting a correlation between serotonergic function and motor activity. Despite the large amount of data available relating 5-HT transmission to locomotion, however, very conflicting views exist with regard to the precise nature of this serotonergic control over activity and behavior (Soubrie, 1986).

In the present experiment, we characterize the changes in open field exploratory locomotor activity of rats after PCPA treatment. Using a digitized activity monitor, we measured changes in a variety of movement patterns separately over a one hour period. Further, we studied the effect of a wider range of doses than was done in previous work, and also re-investigated the effect of PCPA on running wheel activity. Finally, we subjected rats to a number of additional behavioral tests to assess the general sensory-motor capacities of rats treated with PCPA. Thus, we hoped to determine whether sensory - motor deficits, especially after high doses, contribute to alterations in locomotor activity

after PCPA administration.

Methods

Open field exploratory activity and sensory-motor tests

Subjects: Forty male Long-Evens rats (2 0-300 g) were used. The rats were housed individually in wire-mesh cages. Food and water were freely available. In addition to the regular lab chow diet, the rats were provided with a mash consisting of lab chow pellets, water, and sucrose. In previous work, we have found that this diet reduces the weight loss that is associated with PCPA treatment at high doses by approximately 50 % (unpublished observation). Prior to any behavioral tests, all rats were removed from their home cage, individually handled, and placed on a large open field arena in groups of twelve for approximately 30 min every days for five days prior to drug treatments. Thirty-two rats were used for the additional running wheel experiments and twenty-four rats were used for the parallel biochemical analyses.

Drug treatment: PCPA (Sigma Chemical Company) was suspended in gum arabic solution. For the open field and sensory-motor tests, five groups of rats (n=8) were used, receiving the following injections over two consecutive days: (1) 2 x 500 mg/kg PCPA (i.p.); (2) gum arabic (i.p.) and 500 mg/kg PCPA (i.p.); (3) gum arabic (i.p.) and 150 mg/kg PCPA (i.p.); (4) 2 x gum arabic (i.p.); (5) no injections. Behavioral testing occurred between 8:00 and 16:00 three days following the last injection.

Open field exploratory locomotor activity: The apparatus consisted of 6 Digiscan Animal Activity Monitors (Omnitech). Each monitor included a 40 x 40 x 30.5 cm open

field. a grid of infrared beams mounted every 2.54 cm horizontally, and one vertical grid 11.5 cm above the floor. Beam interruptions were recorded by means of a microcomputer. The following movement characteristics were measured: *General:* total distance travelled, average distance travelled per movement, average speed per movement: *Horizontal:* number of horizontal movements, time in horizontal movement, time per horizontal movement: *Vertical:* number of vertical movements, time in vertical movement, time per vertical movement. This is largely a measure of rearing activity. Rats were individually placed in an activity monitor and the locomotor activity was monitored for twelve consecutive 5 min samples (60 min total).

Sensory-motor tests: Sensory-motor function was evaluated using tests outlined below: (1) *Reactivity to stimuli applied to the feet:* Using a Q-Tip (a thin wooden stick with one end tightly wrapped in cotton fibers), the feet of rats in their home cage were lightly touched. Responses were recorded manually, including withdrawal of the foot. orienting responses of the head, whole body turns. and severity of the response. (2) *Hanging duration:* A rat was suspended by the forepaws on a horizontal wire 83 cm above a box filled with sawdust. The latency to fall off the wire was determined with a stopwatch. Each rat received three trials with 15 s intervals between trials. (3) *Limb strength:* A rat was placed on a 16 x 13 cm wire grid which was connected to a spring scale by a 23 cm long wire. Once a rat had a firm grip on the wire grid with all four paws, it was pulled backward by the base of the tail. The spring scale indicated the force of pull a rat tolerated before letting go of the wire grid. Each rat received three successive trials. (4) *Platform climb-down test:* A rat was placed on an elevated, exposed platform (9 cm diameter, 21 cm above ground), and the time a rat took before climbing down and placing all four paws on the ground was measured with a stopwatch. Each rat received three trials. Any movement abnormalities during climbing were noted by the experimenter. (5) *Swim*

test; The apparatus used was a circular water pool in which two planks were inserted parallel to each other and 30 cm apart to create a straight swim alley (1.5 m length). At one end of the alley, wire mesh suspended on the pool wall provided a rat with a 30 cm high vertical climb out of the pool to a horizontal escape platform attached to the pool wall. A trial consisted of placing a rat in the swim alley opposite to the wire mesh, allowing the rat to swim down the alley, and climb out of the pool onto the horizontal platform. The time required to swim from the start point to the alley end was measured with a stopwatch. Further, the experimenter noted whether a rat climbed out of the pool and onto the escape platform. Each rat received a total of ten trials in two blocks of 5 trials separated by a 20 min interval.

Running wheel activity

Four groups (n=8) of rats were used. The rats were housed in cages with access to a running wheel. The rats were habituated to the apparatus for 10 days. On day 10 and 11, the groups received the following injection schedule: (1) 500 + 500 mg/kg PCPA (i.p.); (2) gum arabic (i.p.) + 150 mg/kg PCPA (i.p.); (3) gum arabic (i.p.) + gum arabic (i.p.); and (4) no injections. Activity was monitored until day 15, i. e., 4 days after PCPA treatment. Wheel revolutions were displayed by a counter and recorded throughout the entire habituation and test period every day at 15:00 hrs.

Biochemical assay

Four groups of rats (n=6) were used. The groups received the following drug treatments over two days: (1) 2 x 500 mg/kg PCPA (i.p.); (2) gum arabic (i p.) and 150 mg/kg PCPA (i.p.); (3) 2 x gum arabic (i.p.); and (4) no injections. Three days after the

last injection, rats were decapitated, the brain was rapidly removed, frozen in liquid isopentane kept in frozen carbon dioxide, and stored at -70 ° C. After homogenization in 0.1 N perchloric acid, centrifugation and retention of the supernatant, whole brain biochemical assays were performed using high-pressure liquid chromatography with electrochemical detection as described previously (Baker et al., 1987).

Statistics

All data are presented as mean ± S.E.M. Analyses of variance and, where appropriate, Newman-Keul's follow-up tests were performed using the software packages SPSS/PC+ and CLR Anova (Version 1.1, Clear Lake Research Incorporated). A Fisher exact probability test was also used (Siegel, 1956).

I · ults

Biochemical assay

As shown in Fig. 1, 1000 mg/kg PCPA treatment resulted in a profound depletion of whole brain 5-HT and 5 hydroxyindoleacetic acid (5-HIAA), which were reduced to 9.4 % and 8.2 % of control (no injection) levels, respectively. Even at the 150 mg/kg dose, PCPA still produced a significant reduction in 5-HT and 5-HIAA. Dopamine and noradrenaline levels were affected only slightly at the 1000 mg/kg PCPA dose.

Open field exploratory locomotor activity

PCPA treatment at all three doses, but especially at the 500 and 1000 mg/kg doses,

Figure 1.

Effect of PCPA treatment (i.p.) on whole brain levels of 5-hydroxytryptamine (5 - HT), 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NA), and dopamine (DA) (group size n=6). There were significant effects of drug treatment on all compounds, $p <$ 0.007 or better. 5-HT and 5-HIAA were both significantly reduced at both PCPA doses (p < 0.01) and the 1000 mg/kg dose reduced both more severely than the 150 mg/kg dose (p < 0.05). NA and DA were reduced only after treatment with 1000 mg/kg PCPA (p < 0.05). (Means ± S.E.M. are shown).

PCPA Treatment
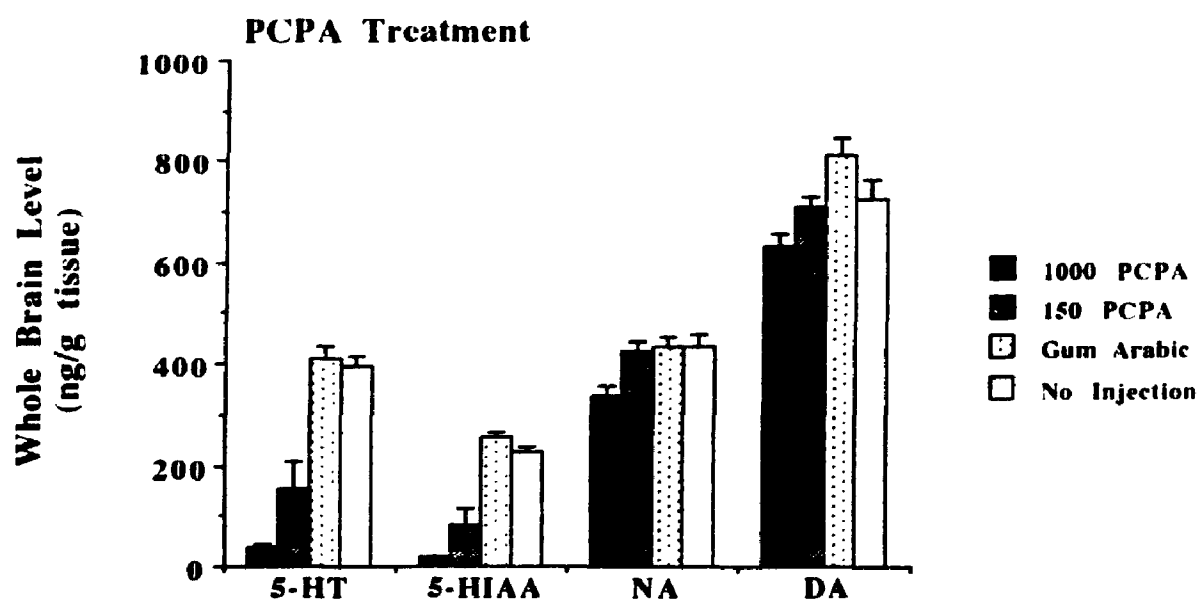
resulted in a clear reduction of all measures of locomotor activity (Fig. 2). All general activity measures (total distance travelled, distance and speed per movement) were decreased at a PCPA dose of 1000 mg/kg (Fig 2 a). At lower PCPA doses, total distance travelled was decreased, whereas speed and distance per movement were not decreased relative to the control groups. Interestingly, there was a tendency for the 150 mg/kg PCPA group to show increased speed and distance travelled per movement, whereas the 1000 mg/kg PCPA group showed a clear decrease in these activity measures.

All measures of horizontal activity were decreased by PCPA treatment (Fig. 2 b). Both the number of horizontal movements and the time spent in horizontal movement were decreased at all three PCPA doses and the decrease was maximal at the 500 and 1000 mg/kg doses. The time per horizontal movement was decreased only after 500 and 1000 mg/kg PCPA.

Administration of PCPA also decreased all measures of vertical activity (Fig. 2 c). Again, all three doses reduced the number of vertical movements and the time spent in vertical movement. As in the case of horizontal activity, the effect of PCPA on vertical movements was saturated at a dose of 500 mg/kg. The time per vertical movement was reduced only after 500 and 1000 mg/kg PCPA.

Inspection of activity levels over consecutive 5 min samples for a total of 60 min revealed that the total distance travelled, the number of horizontal and vertical movements (Fig. 3), and the time spent in horizontal and vertical movement were reduced by the two highest PCPA doses already at the onset of the test period. Further, the 500 and 1000 mg/kg PCPA groups tended to remain at reduced activity levels relative to the control groups throughout the entire 60 min. test period. The overall increase in the distance and speed per movement after 150 mg/kg PCPA was caused by a increase of these activity measures relative to the control groups during the second 30 min of testing (data not shown).

Figure 2.

The effects of treatment with gum arabic (i.p.) or PCPA (i.p.) on spontaneous exploratory locomotor activity during 60 min in a digitized open field activity monitor (n=8/group; No-injct. no injection; Gum A, gum arabic injections). PCPA treatment in doses of 150, 500, and 1000 mg/kg produced a dose-dependent decrease of general locomotor activity characteristics (A) (omnibus test $F_{44,112} = 7.0$, $p < 0.0005$), horizontal movement characteristics (B) (omnibus test $F_{16,140} = 4.4$, $p < 0.0005$), and vertical movement characteristics (C) (omnibus test $F_{16,140} = 2.6$, $p < 0.001$). Univariate F-tests revealed significant effects of drug administration on every movement characteristic across all groups ($p < 0.001$) and across the three PCPA groups only ($p < 0.013$). (Means ± S.E.M. are shown).

Figure 3.

Spontaneous exploratory locomotor activity in an open field during 12 consecutive 5 min. samples for a total of 60 min. Both horizontal ($F_{11,385} = 59.6$, $p < 0.0005$) and vertical ($F_{11,385} = 102.7$, $p < 0.0005$) movements decreased over the test period. There also were significant interactions between drug treatment and sample period ($F_{44,385} = 2.0$, $p < 0.0005$ for horizontal movements; $F_{44,385} = 2.1$, $p < 0.0005$ for vertical movements). Note that relative to the control (gum arabic and no inject) groups, the 500 and 1000 mg/kg PCPA (i.p.) groups displayed reduced activity levels already at the onset of the test period. Students' t tests comparing the combined activity levels of the two high dose PCPA groups to the combined control group activity levels indicated that during the first 20 min of testing, both horizontal ($p = 0.007$) and vertical ($p = 0.037$) activity were reduced in PCPA-treated rats relative to controls. Thus, immediate exploration of the unfamiliar open field was particularly sensitive to PCPA treatment.

Horizontal Movements

Vertical Movements

Activity (Movements/5 min)

Sample Period (5 min)

1000 PCPA
500 PCPA
150 PCPA
Gum Arabic
No Inject
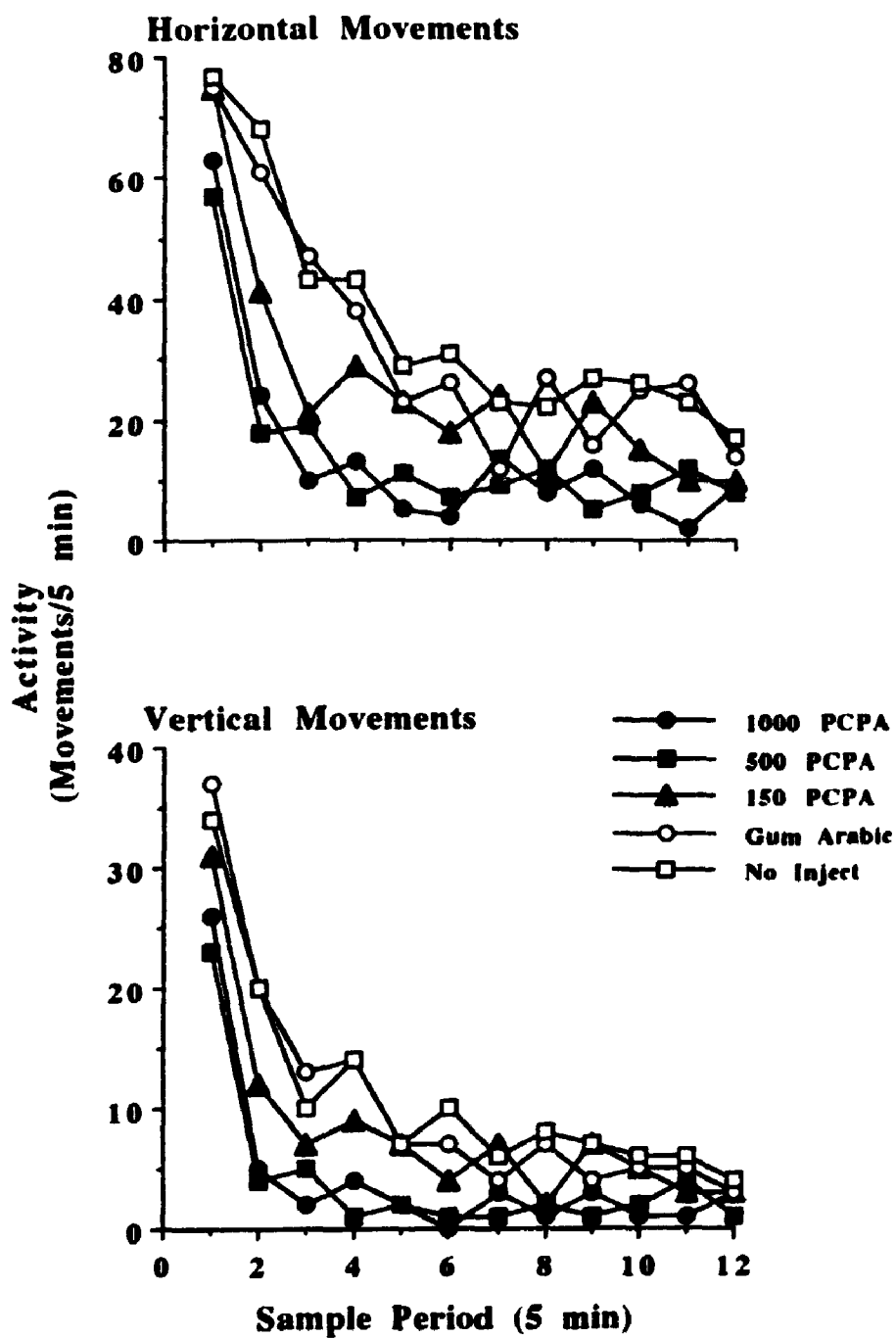
Sensory-motor tests

The sensory-motor tests did not reveal any impairments after PCPA treatment and all three PCPA groups performed at control levels in all the tests. There was, however, a tendency for PCPA-treated rats toward hyper-reactivity in their home cage to cutaneous stimulation of the feet. Nine PCPA-treated rats (three in each PCPA group), or 37.5 %, displayed hyper-reactivity, and frequently these rats would run, jump, or even climb upon the food tray or cage wall when their feet were touched. Only one rat, or 6 %, in the two control groups displayed similar responses. This difference is significant according to a Fisher exact probability test (p = 0.02). Interestingly, hyper-reactivity of PCPA- treated rats was never observed in any other test situation.

As shown in Fig. 4 (top), rats in the two control groups had a hanging duration of approximately 15-20 s and PCPA did not significantly affect this duration. Similarly, limb strength was not affected by PCPA treatment (Fig. 4, middle). When control and PCPA - treated rats were placed on a wire grid and pulled backward, they all tolerated a pull of approximately 1800-2000 g before letting go of the grid.

As depicted in Fig. 4 (bottom), control rats remained on the elevated platform for about 30 s. There was a tendency for PCPA-treated rats to remain on the platform for a shorter time, and this trend was more pronounced after lower PCPA doses. However, tne latencies of PCPA-treated rats did not differ significantly from those of control rats. Also, there were no obvious abnormalities in the motor pattern when rats climbed down from the elevated platform. Generally, rats would position their forelimbs against the side walls of the platform to support their upper body while they slowly lowered themselves in a head - down posture, keeping their hind feet on the platform. When the forelimbs and head were close to the ground, the hind limbs were taken off the platform and rats would perform a small jump to land on the ground.

Figure 4.

The effects of PCPA (i.p.) and gum arabic (i.p.) treatment on hanging duration (top), limb strength (middle), and latency to climb down from an elevated platform . There were no effects of drug treatment on the time rats spent hanging by their forepaws before falling ($F_{4,35} = 0.95$, $p = 0.45$), toleration of force applied to the feet ($F_{4,35} = 1.0$, $p = 0.42$), or escape latency from the platform ($F_{4,35} = 0.3$, $p = 0.87$). For each test, the means and S.E.M. are based on 3 trials/rat, n=8/group.

Hanging

Limb Strength

Platform

Group
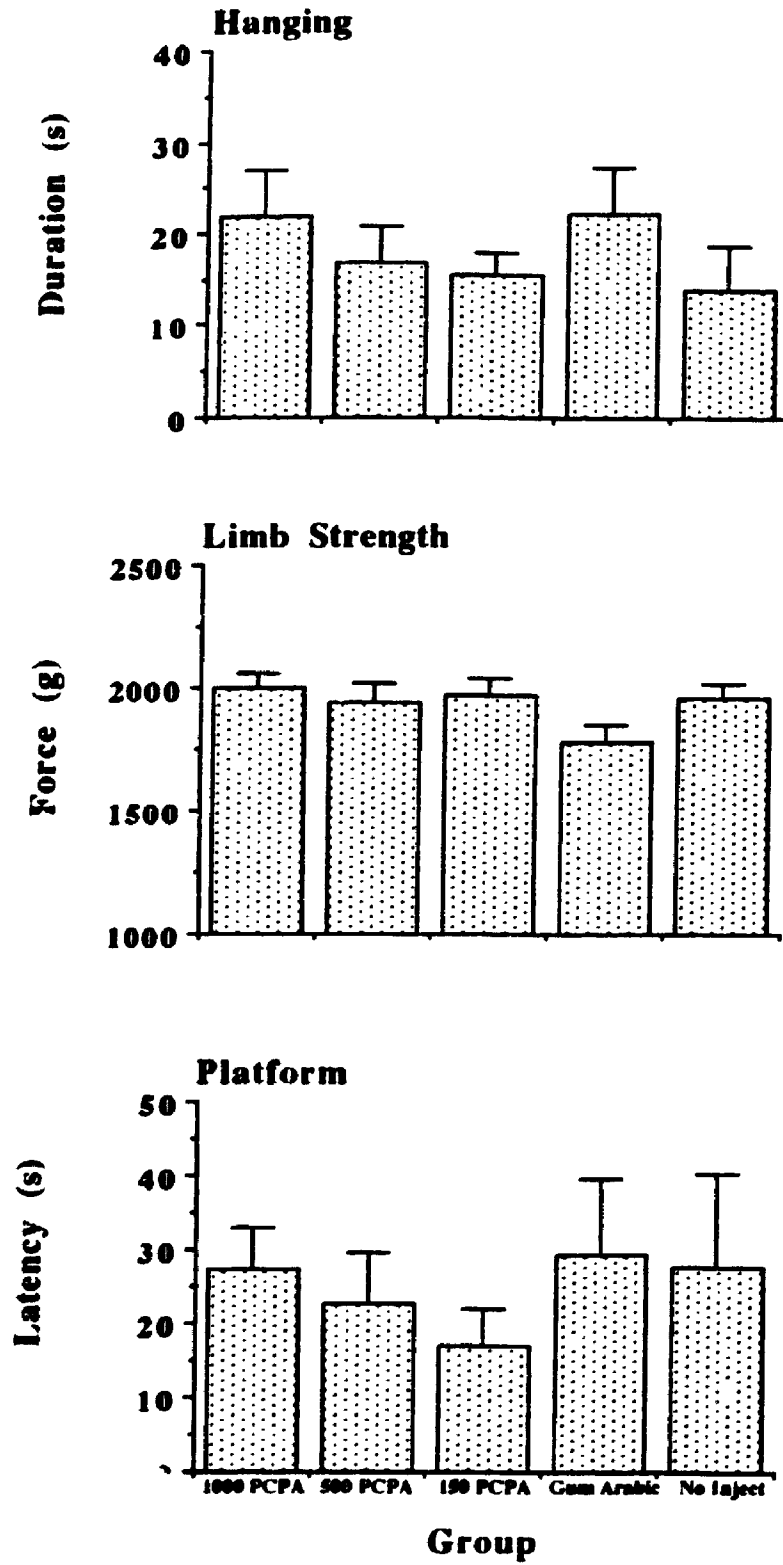
Fig. 5 displays the latency for rats to swim along the alley to a climb escape on the opposite end of the alley. After a single trial, all rats had acquired the necessary motor pattern to direct themselves toward the opposite end of the alley and swim along the alley when placed in the water. There were no differences in the swimming speed between PCPA and control groups. At the far end of the alley, all rats reliably climbed up the wire grid and escaped to the elevated rest platform.

Running wheel activity

PCPA treatment using doses of 150 and 1000 mg/kg was without significant effects on activity levels for rats housed in cages with running-wheel access (Fig. 6). There was a decline in activity levels on the days following either 150 or 500 mg/kg injections, but the effect was not significant. On the subsequent day and throughout the remainder of the test period, activity returned to approximately the same levels as those observed prior to drug treatment. The activity levels of both control groups continued to increase slightly after the treatment period.

Discussion

The fact that there is no consensus regarding the role of 5-HT in the control of behavior is illustrated in a recent review by Soubrie (1986) and the subsequent open peer commentary. Even when clearly defined behaviors such as locomotor activity have been under investigation following the manipulation of central serotonergic transmission, the data are inconsistent. Thus, both increases and decreases of various types of locomotor activity have been reported following depletion of brain 5-HT (Fibiger and Campbell, 1971; Borbely et al., 1973; Marsden and Curzon, 1976; Lipska et al., 1992). In order

Figure 5.

The effects of PCPA (i.p.) and gum arabic (i.p.) treatment on swim speed (n=8/group). The drug treatments had no effect on the time taken to swim along the alley to the escape climb ($F_{4,35} = 1.9$, $p = 0.14$). There was a significant decrease in the time over trials ($F_{9,315} = 47.0$, $p < 0.0001$) which was equivalent for all groups (group by trial interaction $F_{36,315} = 1.3$, $p = 0.12$,

Swim
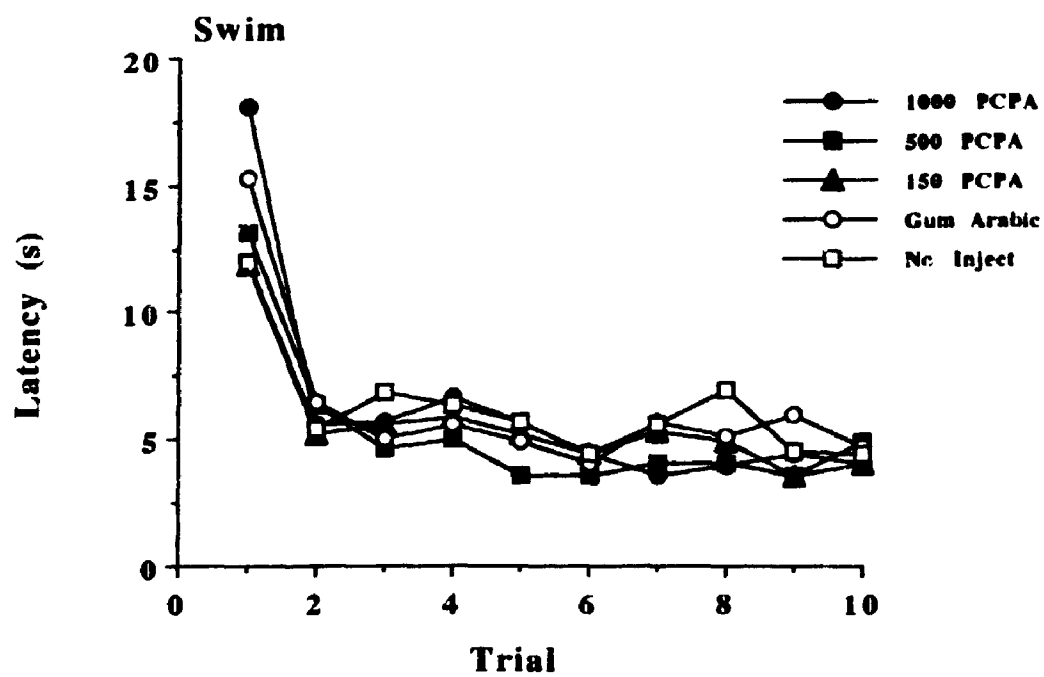
Figure 6.

The effects of PCPA (i.p.) and gum arabic (i.p.) treatment on activity in running wheels (n=8/group). Injections of either 150 or 2 x 500 mg/kg PCPA, but not of gum arabic, produced an acute decrease in activity during the subsequent 24 hrs. However, activity levels returned to pre-treatment levels two days after the last PCPA administration. An ANOVA for the data of the last three days before drug treatment and days two to four after drug treatment did not reveal significant group differences ($F_{3,28} = 0.8$, $p = 0.5$) or pre- and post-treatment by group interaction ($F_{3,28} = 2.0$, $p = 0.13$).

Running Wheel
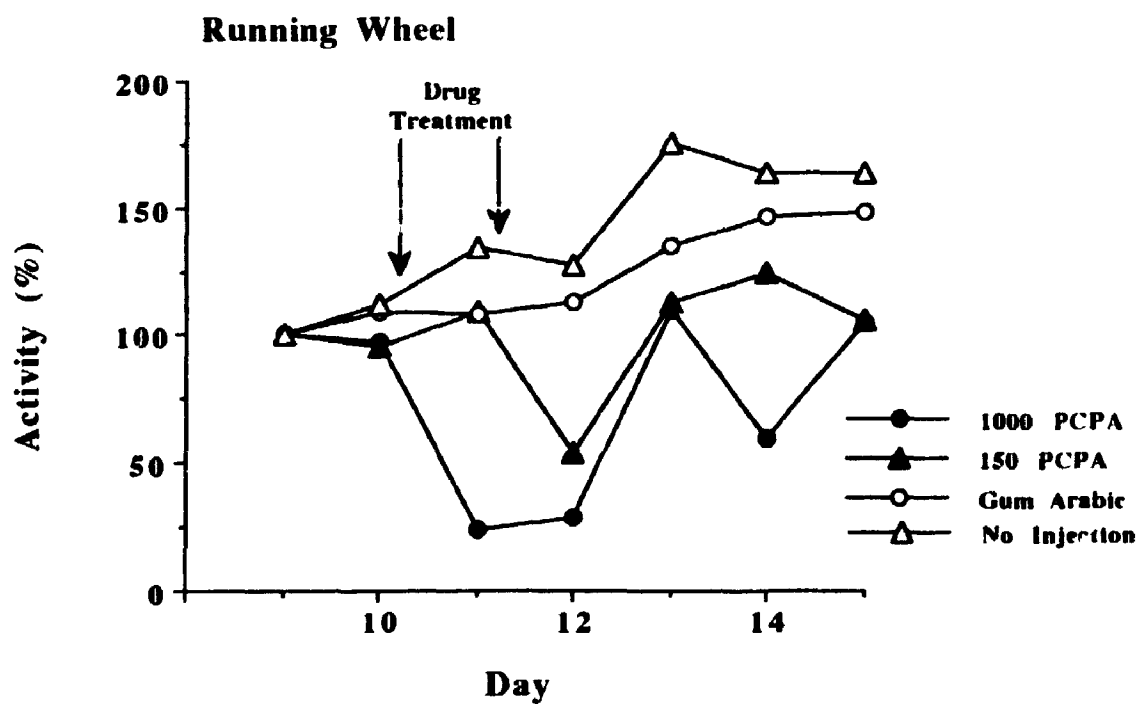
to develop a more coherent understanding of the role of 5-HT in the control of behavior, it is important to develop an understanding why such significant discrepancies exist in the data on behavioral correlates of 5-HT manipulations.

In the present study, we employed a wide range of PCPA doses to deplete 5-HT in order to test whether PCPA may have a biphasic effect on exploratory locomotor activity. However, our results indicate that doses between 150 and 1000 mg/kg all reduce spontaneous exploratory locomotor activity, but some activity measures were affected only at doses larger than 150 mg/kg (average distance per movement, average speed per movement, time per horizontal movement, time per vertical movement). These doses cover and extend the range of those used by other investigators. Thus, the contradictory results regarding the locomotor effects after PCPA treatment may not be due to a simple biphasic relation between PCPA dose and locomotion.

A problem with the interpretation of previous work on the behavioral consequences of 5-HT depletion with PCPA is the fact that most studies have not included biochemical analyses to indicate the level of 5-HT depletion achieved. The present and previous experiments suggest that doses substantially higher than those used in previous studies may be needed to produce sufficiently complete depletions of 5-HT and, consequently, behavioral or electrophysiological consequences of lowered brain 5-HT. In the present study, 1000 mg/kg PCPA produced a significantly higher level of depletion of 5-HT than 150 mg/kg PCPA. Further, as mentioned already, several components (e. g, average speed of movement, time spent in horizontal movement, time per vertical movement) of exploratory locomotion were not suppressed by 150 mg/kg PCPA, whereas higher doses had pronounced effects on all movement characteristics measured. As shown previously, three doses (i. e., 1500 mg/kg) of PCPA, but not one dose of 500 mg/kg, block atropine - resistant hippocampal and neocortical activation (Vanderwolf et al., 1990). Also, depletions of 76 % of cortical 5-HT are without any significant effects on a number of

complex behaviors in rats (Robinson et al., 1993). These data strongly suggest that the use of inadequate doses of PCPA and, consequently, insufficient levels of 5-HT depletions may, in part, account for the fact that some investigators did not observe decreases in activity after doses of up to 300 mg/kg (Tenen, 1967; Brody, 1970; Köhler and Lorens, 1978).

A further concern for the interpretation of behavioral effects following partial 5-HT depletion is the possible development of pre- and postsynaptic compensatory changes. Such changes occur after partial dopaminergic denervation (Hornykiewicz, 1979) and, consequently, significant behavioral abnormalities often are apparent only at very high levels of dopamine depletion (Stricker and Zigmond, 1976; Robinson et al., 1990), even though it is possible that more sensitive behavioral measures may detect lower levels of depletion. It may be that similar compensatory mechanisms obscure the effect of reduced brain 5-HT and may potentiate the action of remaining (e.g., after inadequate PCPA treatment) or recovering 5-HT transmission.

It is not obvious whether manipulation of 5-HT transmission has a direct effect on locomotor activity, or whether such activity changes are secondary to some peripheral effects or alteration in the sensory-motor capacities of animals. PCPA treatment, especially at high doses, has a number of adverse side effects such as reduced body temperature (Borbely et al., 1973), reduced food and water intake (Borbely et al., 1973; Marsden and Curzon, 1976), fur discoloration, and decreased body weight (present study). However, in the present study, rats treated with PCPA did not show deficits in their general sensory-motor capacities. The only behavioral abnormality we could identify was a tendency of PCPA-treated rats to display hyper-reactivity in their home cage to cutaneous stimuli applied to their feet. In all other behavioral capacities examined, the rats appeared normal. Thus, peripheral effects or deficits in the general sensory-motor capacities cannot directly account for the changes in activity levels associated with PCPA treatment.

It is noteworthy to point out that in the present study, even very high doses of PCPA (1000 mg/kg) produced only marginal alterations of central levels of dopamine and noradrenaline. This is also the case for doses of 500 mg/kg PCPA (Vanderwolf et al., 1990). A dose of 150 mg/kg PCPA decreased 5-HT levels without affecting dopamine or noradrenaline, but produced decreases in several of the measures of exploratory locomotor activity. Thus, it appears that nonspecific effects on brain amines probably do not account for the changes in exploratory locomotion observed in the present study.

Surprisingly, we did not see hyperactivity after PCPA treatment in rats chronically housed in cages with access to a running wheel. If anything, PCPA treatment appeared to produce a reduction of activity. This finding is in definite contrast with those of several other reports of PCPA-induced increases in running wheel activity (Fibiger and Campbell, 1971; Jacobs et al., 1975). Dose-differences cannot account for this discrepancy since our low PCPA dose (150 mg/kg) is approximately equivalent to t`at used by others investigators who have observed ` `reased running wheel activity. Neither this dose, nor our 1000 mg/kg PCPA dose, produced any indications of hyperactivity. At present, we cannot account for this discrepancy. However, it seems worth pointing out that running wheel activity and other types of locomotion such as exploratory open field activity or stabilimeter activity appear to have distinct neural substrates that can be dissociated anatomically by means of brain lesions (Dirlam, 1969; Lynch, 1970; Capobianco and Hamilton, 1976; Whishaw, 1990). Thus, it is not surprising that pharmacological manipulations, like brain lesions, may have different effects on different measures of motor activity.

Ideally, laboratory measures of behavior should give results that are applicable to real life situations. Running in a rotating wheel is quite unlike running on a fixed surface since the momentum of the wheel provides cutaneous and proprioceptive stimulation that is not present in running under more natural conditions. When tested with a Q-tip in the

home cage, many PCPA-treated rats reacted to tactile stimulation of the feet with a burst of running and climbing. If a PCPA-treated rat in a running wheel attempts to stop running, the cutaneous stimulation resulting from the inertia of the wheel may trigger further running. Thus, the hyperactivity often seen in running wheels after PCPA treatment may be an artifact of this method of testing. Our results indicate that the true effect of PCPA treatment may be a reduction in spontaneous motor activity, particularly exploratory activity.

Other results also suggest a link between levels of central 5-HT transmission and locomotor activity. Forebrain 5-HT levels are higher in more active relative to less active rats (Rosecrans, 1970). Electrical stimulation of 5-HT-containing cell groups (Peck and Vanderwolf, 1991) or pharmacological elevation of central 5-HT ieveis (Robertson et al., 1991) increase locomotion, as does administration of the direct-acting 5-HT receptor agonist RU 24969 (Green et al., 1984). Further, 5-HT produces activation of the electrocorticogram in conjunction with movement but not with immobility (Vanderwolf, 1988). Serotonergic neurons in the dorsal raphe discharge at higher rates during active waking than quiet waking or sleep (Jacobs and Fornal, 1993), and extracellular 5-HT levels are elevated during active behavioral states (Wilkinson et al., 1991). This serotonergic control over activity and locomotion may be a phylogenetically old control mechanism since it is present even in invertebrates (Willard, 1981). In addition, the present results suggest that not all forms of locomotion are equally dependent on central 5 - HT levels or transmission. Exploratory locomotor activity in an unfamiliar environment (see Eilam and Golani, 1989) may be especially sensitive to PCPA-induced depletions of 5 - HT.

Central 5-HT transmission has been suggested to play a role in "anxiety mechanisms" and there is substantial evidence that drugs that act as agonists at 5-HT $_{1A}$ receptors reduce "anxiety" in humans and animals (Feighner et al., 1982; Eison et al.,

1986; Traber and Glaser, 1987; Charney et al., 1990; Taylor, 1990). Whether changes in locomotor or exploratory activity following PCPA treatment are, in some way, related to decreased "anxiety" levels remains to be established.

It appears that neither dose-differences across studies, nor the presence or absence of sensory-motor deficits following different PCPA doses can satisfactorily account for the inconsistent results of PCPA-induced 5-HT depletions on locomotor activity. In the present study, 5-HT depletions of over 90 % reduced all components of exploratory activity in an unfamiliar open field. This reduction in locomotion was apparent for doses between 150-1000 mg/kg. Thus, our results provide strong support for a stimulatory role of 5-HT in the control of, at the least, exploratory locomotor activity. The specific nature of this locomotor facilitation, as well as its interaction with different environments (i.e., familiar and novel) could be the focus of future research.

Paper 6:

Food Carrying in Rats Is Blocked by the
Putative Anxiolytic Agent Buspirone

Summary

The effects of the putative anxiolytic agent buspirone on food handling behavior of laboratory rats were investigated. Rats trained to travel from a covered shelter to a food source were provided with food pellets of six sizes. Smaller pellets were eaten at the exposed food source, whereas larger pellets were carried back to the shelter for consumption. Subcutaneous administration of buspirone hydrochloride (0.2 - 2.0 mg/kg) reduced carrying of larger food pellets in a dose-dependent manner. Instead, these pellets were also eaten at the exposed food source. Carrying was maximally suppressed one hour after drug administration. Handling of smaller pellets, travel times, and eating times were not affected by buspirone. Similar results have previously been obtained with diazepam. Buspirone appears to exert its effects through 5-HT $_{1A}$ and/or dopamine receptors, whereas diazepam interacts with benzodiazepine receptors. Thus, manipulations of distinct transmitter systems may have similar behavioral consequences on the food carrying responses of rats.

Introduction

The food handling behavior of animals is influenced by a variety of environmental factors that together determine the way an animal responds to a food item encountered during foraging excursions. It has been proposed that foraging animals trade off eating time against exposure and predation risk. The behavior of animals is organized such that eating or food intake is maximized and exposure risk is minimized (Krebs and McCleery, 1984; Vander Wall, 1990).

A series of controlled laboratory experiments has elucidated some of the factors that influence the way rats handle food items encountered in their environm nt. When a foraging rat encounters a small food piece, it is immediately eaten at the location where th r food is found. Larger food pieces, however, are taken to a shelter or home cage (Whishaw et al., 1989; Whishaw and Tomie, 1989). Black-capped chickadees and grey squirrels displayed behaviors sin.ilar to those of laboratory rats when these animals encountered food items of varying sizes in natural foraging settings (Lima, 1985; Lima et al., 1985). Further investigations that dissociated food size and time to eat a food item showed that eating time is inversel / related to the probability of eating in an exposed area (Whishaw, 1990). Also, food availability, travel distance and difficulty, ambient lighting, presence of a predator, and food deprivation all influence the food handling behavior of laboratory rats (Whishaw and Dringenberg, 1991; Whishaw et al., 1991). Thus, it appears that food handling in rats is controlled by a complex array of both environmental and internal stimuli to produce the most adaptive foraging behavior for the animal.

Protective food carrying can be manipulated using pharmacological agents. The anxiolytic drug diazepam (Randall et al., 1961) blocks a number of defensive behaviors in the rat, including food carrying to a shelter (McNamara and Whishaw, 1990). This raises

the question whether food carrying is sensitive to anxiolytics other than diazepam. In the present experiment, we tested whether the putative anxiolytic agent buspirone (Feighner et al., 1982; Goldberg and Finnerty, 1982) resembles diazepam in reducing food carrying, or whether the distinct pharmacological propertie. of diazepam and buspirone, namely interactions with benzodiazepine receptors (Squires and Braestrup, 1977) for diazepam, and serotonergic (Glaser and Traber, 1983; Vandermae!-- et al., 1986) and dopaminergic (McMillen et al., 1983; McMillen, 1991) receptors for buspirone, may differentially affect food handling in laboratory rats.

Methods

Animals and Materials

Male Long-Evans rats (n=12; 250-300 g) were housed individually in hanging wire mesh cages in a colony room under a 12:12 hr light schedule. Prior to training, the rats were placed on a restricted feeding schedule and their body weight was maintained at approximately 90 % of their normal body weight. Water was freely available.

The testing apparatus consisted of a shelter attached to a wooden beam with a food receptacle placed at the end opposite to the shelter. The shelter was a wire mesh cage 25 x 18 x 18 cm with an opening of 5 x 5 cm on one side to which the beam was attached. The cage was covered with dark cardboard except for the side opposite to the one with the opening. Thus, it was possible to observe a rat inside the cage. The wooden beam was 240 cm long, 9 cm wide, and was supported by legs 25 cm high. A small weighing dish in which food could be placed was located at the end of the beam.

Single cereal pellets (Post Honey Comb) were cut into smaller pieces of one of six sizes: 9 ± 1 mg (size 1); 24 ± 1 mg (size 2); 67 ± 4 mg (size 3); 96 ± 3 mg (size 4); 225

± 8 mg (size 5); 405 ± 17 mg (size 6). The weights were determined by weighing 20 randomly chosen pellets of each size (means ± S.E.M. are given).

Buspirone hydrochloride (Sigma Chemical Company) was dissolved in saline. Injections were given subcutaneously.

Procedure

For one week, the rats were habituated to the test apparatus by placing them individually on the beam on which food pellets had been scattered at varying distances from the shelter. During the subsequent week, rats were familiarized to the testing procedure by placing them in the shelter and allowing them to retrieve food pellets of varying sizes from the receptacle at the end of the beam. By the end of the second week of pre-training, all rats would reliably leave the shelter and travel to the food receptacle to obtain food pellets.

The following doses of buspirone hydrochloride were used: 0.0 (i. e., saline vehicle only), 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, and 2.0 mg/kg. Six rats received these doses in ascending and six rats received these doses in descending order. The drug injection and behavioral testing were administered every second day.

Twenty minutes after receiving an injection, a rat was placed in the shelter and a food pellet was placed in the receptacle. The rat would leave the shelter and travel to the food receptacle where it took the food pellet into its mouth. The behavior of the rat subsequent to taking the food pellets was classified as: (1) *Eat*, the rat chewed and swallowed the pellet; (2) *Sit*, the rat sat on its hind feet, transferred the food pellet from its mouth to the forepaws, and ate it from its forepaws; (3) *Carry*, the rat carried the food pellet in its mouth back to the shelter where it was consumed. The behavior classification was recorded manually. Further, the following times were taken with stopwatches and recorded manually: (1) *Eating time*, time to eat a food pellet; (2) *Carry time*, time to carry

hypothesis to be tested experimentally is that olfactory stimulation activates the amygdala - basal forebrain-neocortex pathway, resulting in an enhanced activity of cells in the amygdala, the basal forebrain, and enhanced release of ACh in the neocortex. Conversely, visual stimuli (especially behaviorally relevant stimuli such as images of predators) may activate the superior colliculus-raphe-neocortex pathway, resulting in an enhanced release of 5-HT in the neocortex. A combination of behavioral, electrophysiological, and biochemical techniques (e.g., *in vivo* microdialysis) could easily be used to address these and related questions and add a functional/behavioral dimension to the concept of multiple, ascending activating inputs to the cerebrum.

(3) The mechanisms of anti-serotonergic effects of general anesthetics (urethane, chloral hydrate, ethyl ether) are not known. As discussed in Paper 3, it is possible that 5 - HT dependent neocortical activation may involve an action of 5-HT at multiple receptor types. Thus, anesthetics may reduce or abolish cortical activation by blocking 5-HT effects at several of those binding sites. To date, this possibility has been examined only for one type of 5-HT receptor (the 5-HT$_2$ site; see above) and it will be necessary to perform additional binding and autoradiographic experiments to determine whether anesthetics block other 5-HT receptor types. Along the same lines, it will be necessary to use additional biochemical approaches to examine whether general anesthetics may interfere with intracellular events triggered by 5-HT-receptor activation (e.g., second messenger systems) and whether such effects may exhibit a degree of selectivity for serotonergic transmission.

Figure 1.

Effect of pellet size on probability of eating, sitting, and carrying for saline injected rats (n=12). With increasing pellet size, probability of eating decreased and carrying increased. Sitting occurred for medium sized pellets. (Means ± S.E.M. are shown).

Food Handling
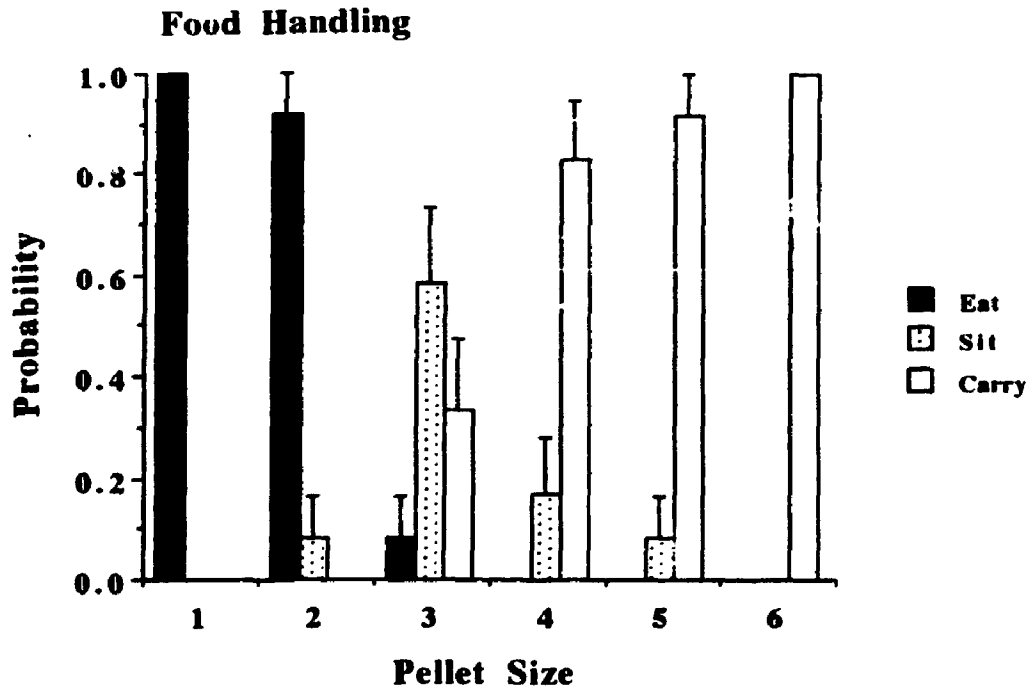
Administration of buspirone resulted in a dose-dependent increase in the probability of pellets being consumed at the exposed food source, and a corresponding decrease in the probability of pellets being carried to the shelter for consumption (Fig. 2). Overall drug effects were significant for all three behaviors, eat $F_{8, 80} = 2.4$, $p = 0.023$, sit $F_{8, 80} = 9.2$, $p < 0.0001$, and carry $F_{8, 80} = 8.4$, $p < 0.0001$. However, for eating, Newman - Keul's tests revealed no significant differences except that the 0.8 mg/kg dose differed from the 1.2 and 1.5 mg/kg doses at $p < 0.05$. There was a pronounced and dose - dependent increase in the incidence of sits with increasing drug dose. Newman-Keul's tests showed that for the probability of sitting, the 1.2, 1.5, and 2.0 mg/kg doses were significantly different from the saline, 0.2, 0.4, and 0.8 mg/kg dose at $p < 0.01$ and from the 0.6 and 1.0 mg/kg doses at $p < 0.05$. Finally, with increasing drug doses, there was a clear, dose-dependent decline in the incidence of carries. Newman-Keul's tests showed that the 1.2, 1.5, and 2.0 mg/kg doses were significantly different from the saline, 0.2, and 0.4 mg/kg doses at $p < 0.01$. In addition, the 1.2 and 2.0 mg/kg doses also differed from the 0.6 and 0.8 mg/kg doses at $p < 0.01$. When the effects of buspirone were analyzed for each pellet size, there were significant drug effects for sizes 3-6 for both sit and carry probability (p's < 0.03). Thus, buspirone increased sits and decreased carries selectively for medium and large pellets, whereas small pellets were always eaten.

Ascending and descending buspirone doses had equivalent effects on all food handling behaviors. Thus, there were no significant effects of injection schedule on either eat, sit, or carry probability (F's $_{1, 10} = 0.24$, 0.15, and 0.47, respectively; p's > 0.5).

The effect of buspirone was most pronounced one hour after administration of the drug. As shown in Fig. 3, a single 1.5 mg/kg injection produced a clear decrease in the carry probability for pellet sizes 3-5 one hour subsequent to the injection. After a further hour, carry probability had returned to saline-control levels. The fact that buspirone, but not saline, reduced food carrying over time was highlighted in the significant drug by time

Figure 2.

Effects of saline (s.c.) and buspirone administration (0.2-2.0. mg/kg, s.c.) on probability of eating, sitting, and carrying across all six pellet sizes. There was no consistent effect of buspirone on eating probability. However, sitting probability was significantly increased and carrying probability significantly decreased with successively higher drug doses (see text). (Means ± S.E.M. are shown).

Eat

Sit

Probability

Carry

Drug Dose (mg/kg)
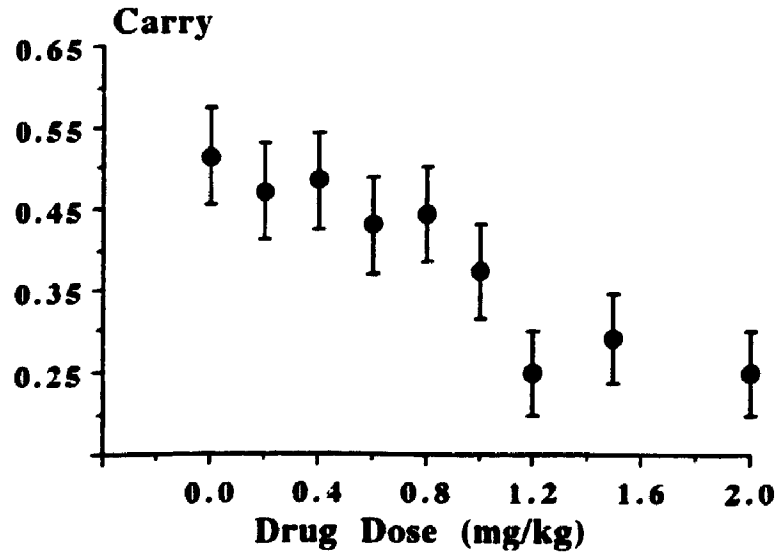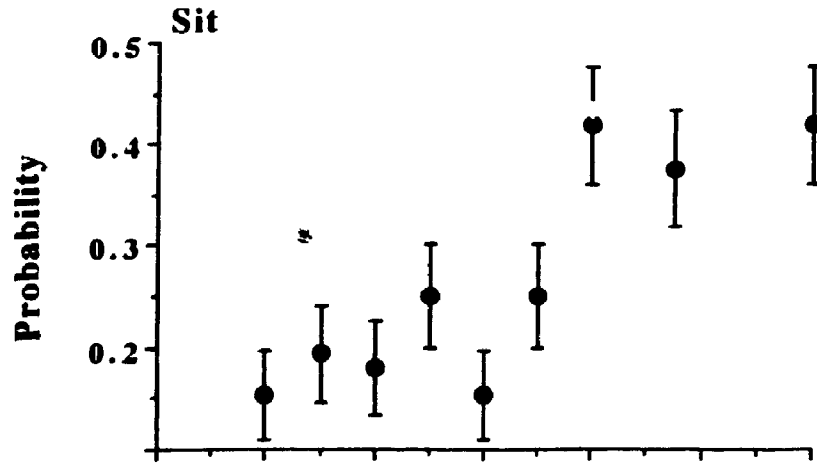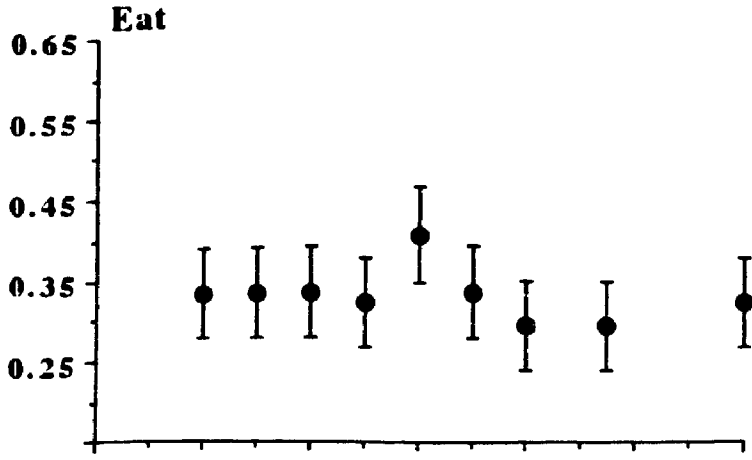
Figure 3.

Effect of a 1.5 mg/kg injection (s.c.) of buspirone (n=6) or saline (n=6) on carrying probability for pellet sizes 3-5. Buspirone decreased carrying probability one hour after the injection. Two hours after drug administration, carrying probability for buspirone injected rats had returned to saline levels.
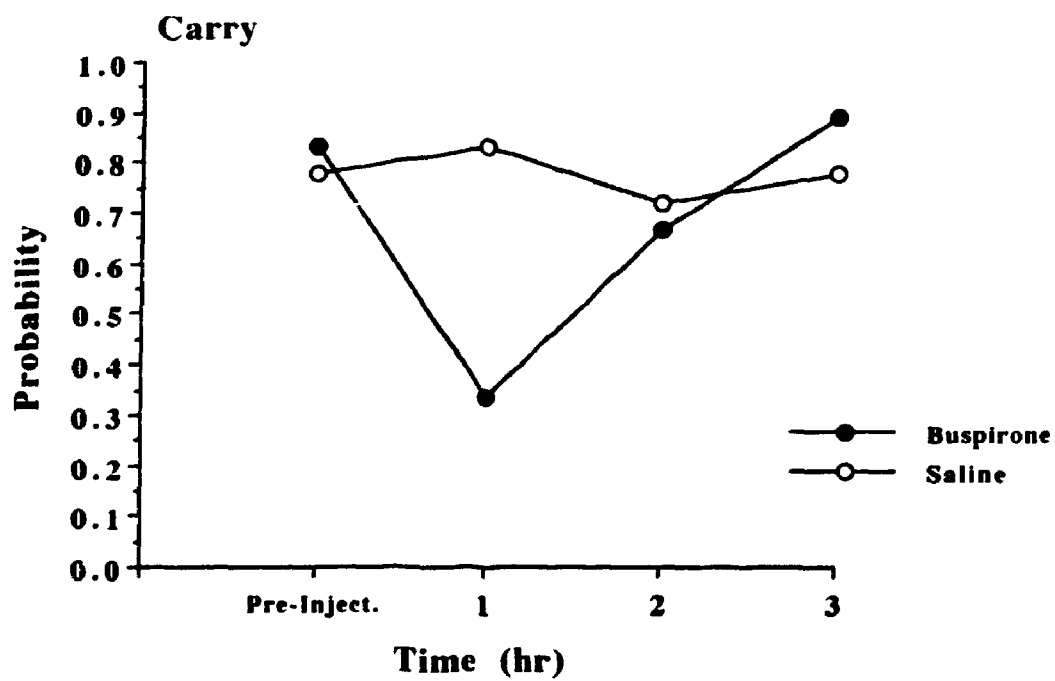
interaction ($F_{3, 30} = 8.54$, $p = 0.0003$). Further, comparisons of carry probability for each test time showed that only at one hour after the injection was there a significant difference between the saline and buspirone treated rats ($F_{1, 10} = 12.27$, $p = 0.006$, all other p's > 0.25).

The effects of buspirone on eat, carry, and return time were analyzed only for pellet 6 since this was the only pellet carried sufficiently across all drug doses to yield sufficient data for reliable analyses. Buspirone administration had no effects on either eat, carry, or return times for this pellet. As shown in Fig. 4, it took a rat an average of approximately 80 s to eat a size 6 pellet, 3 s to carry it to the shelter, and 4 s to make the return trip from the shelter to the food source. None of these times were significantly altered by buspirone and comparisons of eat, carry and return times after all buspirone doses to the saline condition failed to show any significant differences (p's > 0.05, Student's t test). The only exception was a significant difference between the carry times for the saline and 1.5 mg/kg buspirone conditions ($p = 0.017$).

The fact that travel and eat times were not altered by the administration of buspirone highlighted the fact that no sensory-motor impairments were apparent after buspirone administration. There was, however, a pronounced lack of activity in the home cage shortly after buspirone administration in the rage of 0.8-2.0 mg/kg. Within 5 minutes of an injection, the rat would lie down and spontaneous locomotor activity was virtually absent for variable time periods. If such a rat was removed from its home cage and placed on an open surface, however, it would immediately resume a normal upright posture and engage in sniffing, lateral head movements, scanning, and stepping.

Figure 4.

Effects of saline and buspirone administration (0.2-2.0 mg/kg, s.c.) on eating, carrying, and returning times for size 6 pellets. Buspirone had no effects on any of these times. (Means ± S.E.M. are shown).
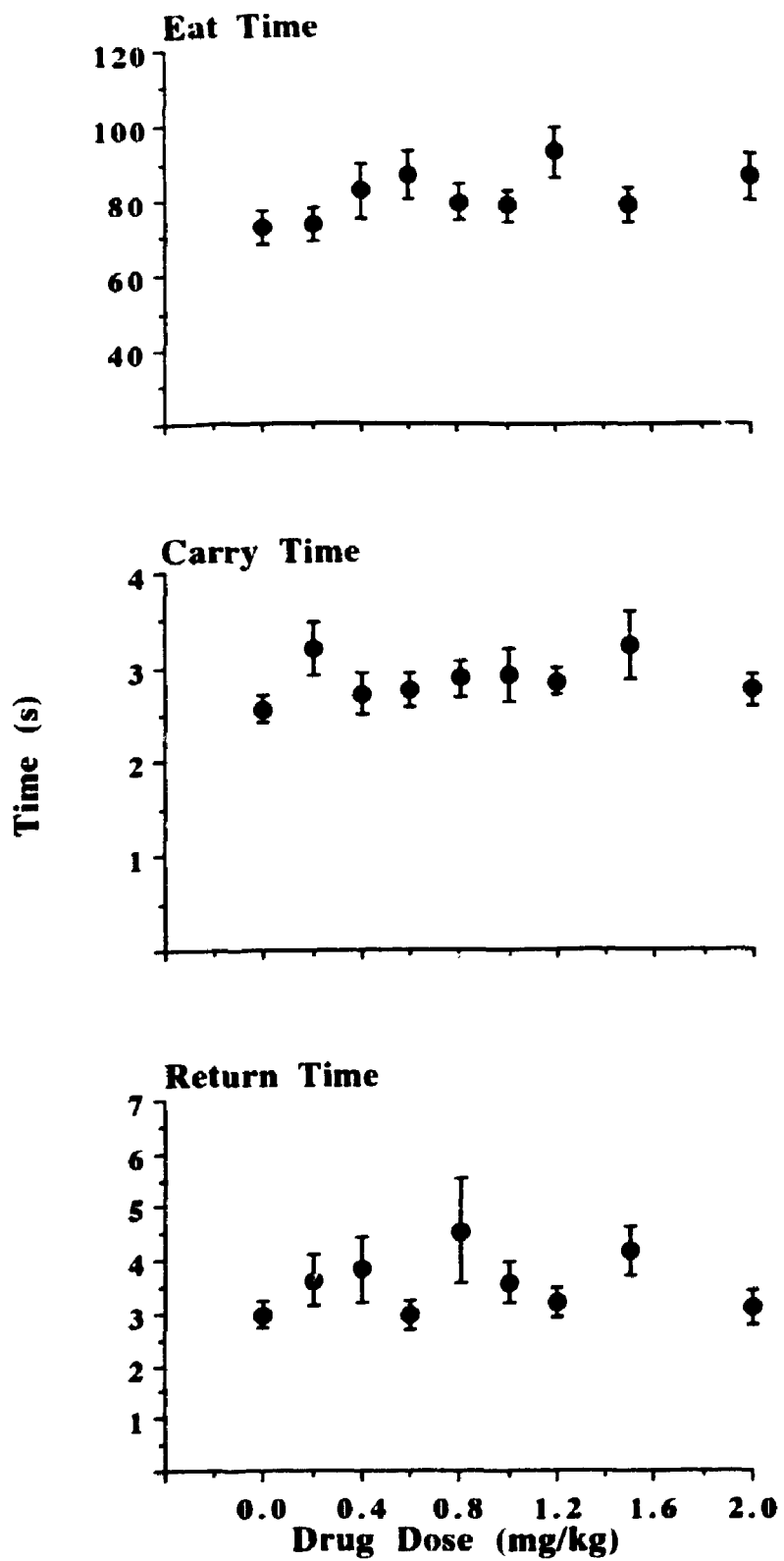
Discussion

Laboratory rats, squirrels, and a number of bird species all consume smaller food items in open, exposed locations, whereas larger items are taken to a sheltered place for consumption. Thus, animals optimize food intake and minimize exposure and predation risk (Lima, 1985; Lima et al., 1985; Valone and Lima, 1987; Vander Wall, 1990; Whishaw and Dringenberg, 1991). In the present study, administration of buspirone significantly reduced the carrying of large food items to a covered shelter for consumption. Instead, these food items were consumed at the food source, that is, in an open, exposed locale. Buspirone did not affect the way rats responded to small food items that were always eaten immediately, or mid-sized items that were always picked up and consumed from the forepaws at the food source. Also, the time to eat a large pellet, carry it to the shelter, and return from the shelter to the food source were not affected by buspirone.

The fact that many aspects of the food carrying behavior were consistent across the saline and all buspirone doses suggests that the present results were not due to some general alteration of the way food items or eating times were perceived which, in turn, could affect the handling of food items (Whishaw, 1990). Also, even though high buspirone doses reduced carrying of the large pellets, carrying of these pellets still occurred. Thus, the drug treatment did not interfere with locomotion or food carrying per se. Instead, the likelihood of rats to perform of a specific motoric response to large food items was reduced.

Although the limited duration of the experiment precludes any firm conclusions, we did not note the development of any tolerance to buspirone. The response to a given dose of buspirone was not significantly affected by previous experience (or no experience) with the drug. This finding is consistent with clinical data indicating that tolerance to buspirone may not develop (Feighner, 1987; Taylor, 1990). The lack of order-of-dose

effects also suggests that possible holdover effects of buspirone treatment were minimal since it may be expected that these would increase the effect of doses that followed large doses.

Buspirone increases eating (Clark and Fletcher, 1986) and cork gnawing (Pollard and Howard, 1991) in the rat and it is possible that this may influence food handling behaviors. However, the reduced food carrying reported here may not be due to a general effect of buspirone on eating. Increasing appetite or eating by decreasing body weight increases food hoarding (McCleary and Morgan, 1946; Fantino and Cabanac, 1980). Also, in the food carrying paradigm used here. food deprivation does not alter the carrying of food items of varying sizes (Whishaw and Tomie, 1989). Thus, an increase in appetite or eating cannot satisfactorily account for the reduction in food carrying apparent after buspirone treatment.

Buspirone interacts with both serotonergic 5-HT $_{1A}$ receptors (Glaser and Traber, 1983; Vandermaelen et al., 1986) and dopamine autoreceptors (McMillen et al., 1983; McMillen, 1991). Serotonergic mechanisms have been proposed to play a role in anxiety (see Soubrie, 1986 for a review) and a case has been made that it may be the serotonergic system that plays a primary role in mediating the anxiolytic action of buspirone (Eison et al., 1986; Traber and Glaser, 1987; Charney et al., 1990; Taylor, 1990). Whether the effect of buspirone to reduce food carrying is related to its anxiolytic action, however, is questionable. Buspirone is effective in reducing "anxiety" only in some animal models of anxiety (Riblet et al., 1982; Eison et al., 1986; Pich and Samanin, 1986) and does not appear to act as an anxiolytic, or has effects much smaller than those obtained with more conventional anxiolytics, in several other animal models (Goldberg et al., 1983; File, 1984; Pich and Samanin, 1986; Howard and Pollard, 1990). Even the effect of the more conventional anxiolytic diazepam to block food carrying has been interpreted as a possible change in the perception of pellet size or time required to consume a food item, or as a

suppression of movement components involved in food carrying, rather than as a consequence of reduced "anxiety" (McNamara and Whishaw, 1990). Also, for buspirone to produce anxiolytic effects in humans, several weeks of buspirone treatment are required (Lickey and Gordon, 1991). Thus, the immediate behavioral change reported here apparent after a only a single dose of buspirone may not be related to the anxiolytic actions of this drug.

Both buspirone (present study) and diazepam (McNamara and Whishaw, 1990) block food carrying, that is, these drugs alter a specific aspect of food handling behavior, namely the motoric response to large food items. The fact that manipulations of both GABA and serotonergic/dopaminergic transmission block food carrying suggests that there may be a common mechanisms through which buspirone and diazepam exert their effects. It has been suggested that the behavioral effects of these drugs are, at least partially, mediated by the hippocampus since they both reduce hippocampal theta frequency (McNaughton and Sedgwick, 1978; McNaughton et al., 1986; Coop and McNaughton, 1991). Consistent with this notion, high densities of 5-HT $_{1A}$ binding sites and mRNA (Pazos and Palacios, 1985; Pompeiano et al., 1992), as well as benzodiazepine receptors (Braestrup et al., 1977) and GABAergic interneurons (Seress and Ribak, 1983) ar found in the hippocampus. Further, the effects of diazepam and buspirone on food carrying are mimicked by hippocampal lesions (Whishaw et al., 1990). Alternatively, buspirone also acts at the level of the dorsal raphe where it inhibits neuronal discharge (Vandermaelen et al., 1986). This reduction in activity of raphe neurons may not be linked to changes in hippocampal theta frequency, however, since theta activity is not substantially altered by inhibition of serotonin synthesis or neurotoxic lesions of the raphe nuclei (Vanderwolf et al., 1990).

As already mentioned, it is questionable whether the results obtained here are related to a reduction in "anxiety" levels. Hippocampal theta frequency and amplitude are

related to size and vigor of movement pattern. as well as to the initiation of movement (Whishaw and Vanderwolf, 1973; Frederickson amd Whishaw, 1977; Whishaw, 1982). Thus, it is tempting to speculate that reduced hippocampal theta frequency after buspirone administration may affect the priming or initiation of the movement sequence involved in carrying a food item back to a shelter. However, in other animal models of "anxiety", disinhibition and increased motor activity such as punished licking have been noted *after* buspirone administration (Eison et al., 1986) It may be some movement types (type 1 behaviors such as walking) depend more heavily on hippocampal circuits than others (type 2 behaviors such as licking, chewing, and grooming) (Vanderwolf, 1988). Clearly, whether the hippocampus is involved in mediating some of the behavioral effects of anxiolytics remains to be established.

General Discussion and Some Hypotheses

1.   Cortical activation is maintained by parallel pathways and not by a single, unitary ascending system

The traditional concept of cerebral activation suggests that activating influences arise in the reticular formation of the brainstem from where they ascend to the non-specific midline thalamic nuclei and subsequently to the neocortex (the ascending reticular activating system, ARAS). The empirical evidence on which this concept is based consists of the observations that damage to the midbrain reticular formation can abolish all cortical activation, whereas stimulation of the reticular formation is very effective in producing it. The notion that a wide variety of, or possibly all, external and internal stimuli act on the reticular system appears to further support the concept of a nonspecific, unitary ascending system believed to be essential for the maintenance of all cerebral activation (e.g., Moruzzi and Magoun, 1949; Magoun, 1963, Moruzzi, 1971).

The present experiments, together with previous work, challenge many of the basic assumptions of this traditional view of the neural substrates of cortical activation. In fact, it is possible to argue that the concept of a single ascending pathway that acts to maintain cortical activation does not accommodate the data presented here or those contained in a number of other recent publications (see Vanderwolf, 1988 for review). It appears that instead of depending on a single, unitary ascending pathway from reticular formation to cortex, cortical activation is maintained by two parallel systems, one cholinergic and the other serotonergic in nature, that are independent of one another in terms of their anatomical organization and neurotransmitter content. Further, as shown in Paper 1, at least the cholinergic system can maintain cortical activation even in the absence of inputs from the midbrain reticular formation. These two *direct-acting* activation systems

appear to receive inputs from a number of other, secondary syste..as that may *indirectly* produce cortical activation by acting through the cholinergic and/or serotonergic systems. Thus, the cholinergic and serotonergic inputs to the cortex can be regarded as a final common pathway in the induction of cerebral activation. On a more speculative note, it may be that these numerous inputs to the cholinergic and serotonergic systems may subserve distinct functions, i.e., they may be brought into play by different sensory stimuli or environmental demands (see below). Thus, it is possible that there are functional specializations among parallel activating systems, rather than an integration of all different types of influences into a single, ascending activating pathway, as was proposed in the initial concept of the ARAS (see above).

As is shown in Papers 1 and 2, electrical stimulation of the amygdala, midbrain core at the level of the locus coeruleus or raphe nuclei, superior colliculus, and orbitofrontal cortex of rats can produce activation of neocortical electroencephalographic activity, confirming previous findings (e. g., Kaada, 1951; Feindel and Gloor, 1954; French, 1958; Kaada and Johannessen, 1960; Berridge and Foote, 1991; Dean et al., 1991). Many of these sites are located outside of regions that can be considered as part of the reticular formation, but also outside of those brain regions that contain cholinergic or serotonergic neurons (amygdala, superior colliculus, orbitofrontal cortex). A possible interpretation of these observations is that neither the traditional concept of the ARAS, nor the view that all activation is maintained by the central cholinergic and serotonergic systems is sufficient to account for all neural systems that can maintain/induce neocortical activation. However, the present experiments show that the LVFA in response to stimulation of all these sites can be abolished by pharmacological blockade of cholinergic-muscarinic and/or serotonergic receptors in urethane anesthetized rats, or by inactivation of the cholinergic innervation of the neocortex in the case of amygdala stimulation. Thus, it appears that LVFA induced by stimulation outside of the cholinergic or serotonergic systems is induced

by a stimulation of release of ACh or 5-HT. In support of this conclusion, the present experiments demonstrate that stimulation of sites that produce putative cholinergic - dependent (i. e., scopolamine-sensitive) LVFA involves the excitation of basal forebrain cells that appear to contribute to cortical LVFA, i.e., cells that fire at higher rates during LVFA than during LISA. Many of these cells appear to project to the neocortex (Detari and Vanderwolf, 1987) and the large majority of projections from basal forebrain to neocortex appear to contain ACh (Woolf et al., 1983; Wahle et al., 1984). Thus, stimulation of sites that produce scopolamine-sensitive LVFA appear to induce LVFA indirectly by exciting the cholinergic input to the neocortex. Stimulation of the superior colliculus produced LVFA that was resistant to anti-muscarinic treatment but could be abolished by 5 - HT receptor antagonists ketanserin or methiothepin. It is possible that this LVFA involves the excitation of serotonergic input to the neocortex. This hypothesis should be tested in future studies.

A possible conclusion drawn from the findings summarized above is that all electrocorticographic activation is dependent on the dual innervation of the neocortex by the serotonergic and cholinergic systems. The fact that generally, no activation survives the concurrent blockade of these inputs suggests that other inputs to the neocortex are insufficient to *directly* activate the neocortex (Papers 1 and 2; Vanderwolf, 1988; Vanderwolf et al., 1990). This view does not, however, suggest that non-cholinergic and non-serotonergic systems such as inputs from the reticular formation do not play an important role in cortical activation. It is likely that the reticular formation or other systems modulate cortical excitability and produce LVFA *indirectly* by acting through the serotonergic and/or cholinergic systems (see Paper 2). For example, the basal forebrain cholinergic system receives projections from numerous brain stem nuclei such as the pedunculopontine and laterodorsal tegmental nuclei, dorsal and median raphe, locus coeruleus, ventral tegmental area, substantia nigra, and the central gray (Russchen et al.,

1985; Fuller et al., 1987; Jones and Cuello. 1989; Zaborszky, 1989; Losier and Semba, 1993). Many of these nuclei can be considered as part of, or closely linked to the reticular formation (Andrezik and Beitz. 1985; Barr and Kiernan, 1988). While these areas may not have direct activating inputs to the neocortex, they are likely to modulate cortical excitability via their projections to the basal forebrain cholinergic system.

The argument that the brainstem reticular formation acts as an indirect, rather than a direct, cortical activator does not agree with the traditional view that it constitutes the principal origin of all central activating impulses and is essential for neocortical activation to occur. However, two of the present results suggest that this more traditional activation concept may be erroneous. Firstly, stimulation of sites outside the brainstem can induce neocortical LVFA as readily as stimulation of the midbrain core. Secondly, and perhaps more importantly, the LVFA induced by amygdala was shown to be largely independent of midbrain structures since large lesions to disconnect the forebrain from all ascending inputs from the midbrain did not abolish the LVFA during amygdala stimulation. Thus, it seems that there may be several parallel, partially independent systems that provide activating inputs to the cortex. These data are in good agreement with the work of Batsel (1960) who showed that in the chronic *cerveau isole* preparation (i. e., the "isolated cerebrum", see Bremer, 1935) in which the forebrain is completely disconnected from the midbrain reticular formation, spontaneous cortical activation re-appeared after a survival time of several weeks. Again, these observations indicate that systems located entirely in the forebrain can maintain neocortical activation independent of inputs from the midbrain.

On a more hypothetical note, it is tempting to speculate that the different pathways that can, directly or indirectly, activate the neocortex may show some functional specialization, i.e., they may be brought into play by different sensory inputs or behavioral demands. For example, it has been suggested that the amygdala plays a role in stress and 'fear' responses, aggression and defense, or attention (Gloor, 1960; Ursin

and Kaada, 1960; Roozendaal et al., 1993). Possibly, the amygdala may play a role in mediating cortical (and behavioral) activation by these conditions or behaviors. Alternatively, the amygdala receives fibers from the olfactory system (Heimer, 1968) and single units in the amygdala are responsive to odors (Cain and Bindra, 1972). Olfactory stimulation (electrical or odorous) is an effective way to produce cortical activation (e.g., Motokizawa and Furuya, 1973) and, consequently, it is possible that this activating response involves pathways that include the amygdala. This hypothesis is currently under investigation in our laboratory. The other activating pathways described here (Paper 2) may produce cortical activation during different stimuli or behavioral outputs. The superior colliculus is believed to be important for defensive behaviors to visual input (Mitchell et al., 1988; Dean, 1991), the locus coeruleus may play a role in vigilance or control of sleep - waking cycles (Aston-Jones and Bloom, 1981: Foote et al., 1980; 1983), and the dorsal raphe nucleus appears to contribute to the performance of specific types of movements and more active behavioral states (Jacobs and Fornal, 1993). It is possible that the relative contributions of these separate activating pathways to cortical activation may vary in relation to such different behavioral states, thereby introducing a functional specialization to central activating systems, rather than an integration of all activating impulses to the cortex in a unitary pathway.

2.   The 5-HT-dependent cerebral activation as a target of general anesthetics

Many of the investigations that demonstrated that ACh and 5-HT produce electrocorticographic activation made use of pharmacological antagonists to block these two activating systems. The cholinergic component of cortical activation is revealed by the partial blockade of LVFA produced by various muscarinic receptor antagonists such as atropine, scopolamine, or quinuclidinyl benzilate (Vanderwolf, 1975; 1988; Stewart et

al., 1984). Interestingly, muscarinic antagonists block cholinergic dependent LVFA both in non-anesthetized, freely-moving rats (Vanderwolf, 1988) and in rats anesthetized with urethane, ethyl ether, or chloral hydrate (Dringenberg and Vanderwolf, 1995). In fact, a comparison of the dose-response curves for scopolamine to block LVFA and induce neocortical large amplitude irregular slow waves obtained in freely-moving rats (see Vanderwolf, 1988, Fig. 11) and rats anesthetized with urethane (see Paper 1, Fig. 3 this volume) shows that the two curves are almost identical. Thus, it appears that the effect of scopolamine (and possibly that of other anti-muscarinic drugs) to block neocortical LVFA and induce LISA is not altered by the presence of a general anesthetic in any obvious way.

While the cholinergic component of electrocorticographic activation can be abolished with muscarinic receptor antagonists in freely moving rats, a large number of serotonergic receptor antagonists do not abolish (in freely moving rats) the LVFA that appears to depend on 5-HT and that can be abolished by 5-HT depletions with systemic reserpine or PCPA, or with intracerebral 5,7-di-hydroxytryptamine (Vanderwolf and Pappas, 1980; Vanderwolf and Baker, 1986; Vanderwolf et al., 1989; 1990). To date, of 12 receptor antagonists tested in freely moving rats, only one (methiothepin) produced a small but significant reduction of LVFA and none abolished 5-HT-dependent LVFA (Vanderwolf and Pappas, 1980; Vanderwolf, 1984; Robertson et al., 1991; Watson et al., 1992).

In contrast to these findings in non-anesthetized rats, in rats anesthetized with urethane, ligands that act as antagonists at 5-HT $_2$ receptors such as ketanserin, ritanserin, or mianserin are effective in blocking LVFA to noxious stimulation such as tail pinches (Neuman and Thompson, 1989; Neuman and Zebrowska, 1992) which appears to depend on 5-HT release in the neocortex (Neuman, 1986; Neuman and Zebrowska, 1992). Similarly, in rats anesthetized with urethane or chloral hydrate, the antagonists ketanserin, ritanserin, and methiothepin block LVFA in response to electrical stimulation

of the median raphe (Dringenberg and Vanderwolf, 1995). Thus, it appears that the effect of serotonergic receptor antagonists to abolish serotonergic LVFA is dependent on the concurrent use of a general anesthetic such as urethane or chloral hydrate.

While the precise nature of the interaction between agents such as urethane and serotonergic antagonists is not known, the results of Paper 4 indicate that there is a direct effect of the presence of urethane on the action of the 5-HT $_2$ antagonist ketanserin to block 5-HT-dependent postsynaptic events; ketanserin was much more potent in inhibiting aortic contractile responses to 5-HT in the presence of urethane than by itself. This observation explains, in part, the contradictory results obtained with ketanserin in the neocortex of freely moving versus urethane anesthetized rats.

In addition to the demonstration of enhanced 5-HT antagonist action, the present experiments also show that urethane itself antagonizes some of the effects of postsynaptic receptor stimulation by 5-HT since urethane alone reduced aortic contractile responses to 5 - HT application. As shown previously, other anesthetics such as ethyl ether or chloral hydrate also can abolish the effects of 5-HT release *in vivo*, i.e., they can completely abolish 5-HT-dependent neocortical LVFA even without the presence of an additional 5 - HT antagonist (Dringenberg and Vanderwolf, 1995). None of these anesthetics abolish cholinergic LVFA. Thus, it is possible that generally, the cholinergic activating system is not substantially affected by anesthetics, whereas the serotonergic activating system is subject to a somewhat selective suppression by general anesthetics. This hypothesis is supported by the observation that general anesthetics have effects similar to that on LVFA on hippocampal rhythmical slow activity (RSA) which also depends on the dual innervation by 5-HT and ACh: the 5-HT-dependent component of RSA is completely abolished by anesthesia e.g., with ethyl ether or urethane (Vanderwolf et al., 1975; also Paper 1 this thesis), whereas the cholinergic component is resistant to treatment with such agents. Thus, it seems that the cerebral activation that depends on 5-HT is suppressed or abolished

in general anesthesia, whereas the cholinergic component of activation is largely spared.

The mechanisms underlying anti-serotonergic effects of general anesthetics are not known. However, the suggestion proposed in Paper 3 that the anti-serotonergic effects of urethane may involve a blockade of 5-HT $_2$ receptors by urethane was not substantiated; neither urethane nor chloral hydrate or its active metabolite 2,2,2,-trichloroethanol interfered significantly with *ex vivo* or *in vitro* binding of the highly selective 5-HT $_2$ ligand [3H]ketanserin (unpublished data). Thus, the mechanisms by which anesthetics reduce or abolish 5-HT-dependent neocortical and hippocampal activation remain to be determined.

3.   5-HT can stimulate those behaviors that occur in relation to 5-HT-dependent cortical activation

As outlined previously (see Introduction and Paper 5), the 5-HT-dependent component of electrocorticographic activation occurs during the performance of Type 1 behaviors such as walking, rearing, swimming, or postural adjustments, but not during Type 2 behaviors such as immobility, chewing, licking, or grooming (see Vanderwolf, 1988).   A possible hypothesis derived from this observation is that central 5-HT transmission may influence or stimulate Type 1 behaviors to a greater extent than Type 2 behaviors. The experiments of Papers 5 and 6 attempted to test this hypothesis by manipulating 5-HT transmission and observing the effects thereof on some unlearned ('species-specific'), spontaneous behaviors of rats. The drugs used to manipulate central 5-HT transmission, PCPA and buspirone, both produce significant reductions in the release of 5-HT. PCPA inhibits 5-HT synthesis (Koe and Weissman, 1966), thus producing a long-term (several days) depletion of 5-HT in excess of 90% relative to vehicle treated rats (see Paper 5); buspirone acts as an agonist at somatodendritic 5-HT $_{1A}$ autoreceptors on 5-HT-containing neurons and inhibits discharge of these cells (e. g.,

Glaser and Traber, 1983; Vandermaelen et al., 1986). Such autoreceptor agonists can decrease forebrain release of 5-HT measured *in vivo* by about 50 % (Wilkinson et al., 1991).

The present experiments show that a suppression of central 5-HT levels with PCPA or buspirone reduces spontaneous exploratory behaviors and carrying of food items to a shelter, respectively. Both behavioral patterns (exploration, food transport) are relatively complex and consist of a large number of distinct, individual movements. For example, general exploration of a novel environment involves walking, rearing, sniffing, and head movements. In the food carrying paradigm used, rats pick up a food item with their mouth, turn on the elevated beam to orient the body toward the home cage, and walk or run to the home cage. Despite the complexity of these behaviors, it is apparent that many of the individual movement components involved consist of Type 1 behaviors (walking, turning, running, head movements).

In both behavioral paradigms, the performance of Type 1 behaviors was reduced by decreasing 5-HT availability in the forebrain. As shown in Paper 5, depleting 5-HT with PCPA reduced exploratory movements such as walking and rearing. In Paper 6, reducing 5-HT release in the forebrain with buspirone decreased the likelihood of rats initiating the food carrying response. Further, in both cases, the reduction in the performance of Type 1 behaviors such as walking, running, and rearing occurred concurrently with an increase in the incidence of Type 2 behaviors such as immobility or immediate eating/chewing after being presented with a food item.

Based on these observations, it is tempting to speculate whether 5-HT may play a dual role in modulation of Type 1 and Type 2 behaviors, normally stimulating the former and concurrently inhibiting the latter. To date, no such comprehensive theory regarding the nature of serotonergic control over behavior has been formulated; most recent reviews on the relation between 5-HT transmission and behavior have focused on relatively specific

types of behavior such as general activity (Jacobs and Fornal, 1993), behavioral inhibition (Soubrie, 1986), anxiety levels (Charney et al., 1990), or feeding (Blundell, 1986; Garattini et al., 1986) without attempting to develop a more comprehensive framework as to how 5-HT modulates complex behaviors in mammals. However, several examples that support this general idea of a dual modulation of Type 1 and Type 2 behaviors by 5-HT can be found. Numerous studies have demonstrated that feeding (e.g., eating rate), a typical Type 2 behavior, is suppressed by increasing the availability of 5-H$_1$ at postsynaptic receptors, e.g., with the 5-HT releaser and uptake inhibitor fenfluramine or 5-HT precursors such as tryptophan, or by administration of 5-HT receptor agonists (Garattini et al., 1986; Blundell, 1986). Further, the autoreceptor agonist 8-OH-DPAT that reduces release of 5-HT in the forebrain (see above) has been shown to increase feeding (Curzon et al., 1984). These studies suggest that 5-HT normally exerts an inhibitory influence over feeding behavior. Similarly, several aspects of sexual activity in rats, another Type 2 behavior, are increased by treatments that reduce 5-HT transmission such as treatment with 8-OH-DPAT or PCPA, and suppressed by enhancing it e.g., with the precursor 5 - hydroxytryptophan (Ahlenius et al., 1971; Malmnas, 1973; Ahlenius and Larsson, 1984; 1987). As already discussed, in the behavioral paradigms used here, suppression of 5-HT availability resulted in a shift in the performance of Type 1 to Type 2 behaviors, also indicative of a dual modulation of these two behavior categories by 5-HT.

One of the behavioral effects of increased central 5-HT activity that has been described in most detail is the appearance of the "5-HT syndrome" which consists of various stereotyped motoric responses such as forelimb treading, head weaving, and hindlimb abduction, many of which may not fall into the category of typical Type 1 behaviors; that is, they are not accompanied by scopolamine-resistant RSA in the hippocampal formation (but note that they are accompanied by scopolamine-resistant LVFA in the neocortex; Robertson et al., 1991). However, it is clear that these behavioral

effects are mediated largely by 5-HT receptors in the brain stem and spinal cord, rather than the forebrain (Jacobs and Klemfuss, 1975). When these spinal receptors are blocked selectively, the most pronounced behavioral effect of increasing 5-HT release is a striking increase in locomotion, i.e., in Type 1 behaviors such as walking or running (e.g., Robertson et al., 1991). Conversely, as shown in Paper 5 and elsewhere, reductions of central 5-HT levels reduces both horizontal locomotion and rearing. Thus, several lines of evidence suggest that 5-HT may normally have a stimulatory influence over Type 1 behaviors (see Paper 5 for details). In this context, it is worth mentioning that destruction of the 5-HT-containing raphe nucl i has been shown to increase locomotion/activity in certain situations (see Paper 5), but that this effect may be mediated by a disruption of GABAergic and, indirectly, of dopaminergic systems, rather by the 5-HT mechanisms (Wirtshafter et al., 1993).

To conclude, some empirical evidence can be found to support the notion of a dual modulation of Type 1 and Type 2 behaviors by 5-HT that corresponds with the serotonergic influence over neocortical activation. Serotonin can have an activating or stimulatory effect on Type 1 behaviors such as locomotion (and on concurrent neocortical activity) without such an effect on various Type 2 behaviors (and concurrent neocortical activity). It is possible that the analysis of the role played by 5-HT in the control of behavior may be aided by adopting a general framework based on these two behavioral categories.

4.   Some directions for future research

As outlined in the Discussion sections of Papers 1-6 contained in this thesis, many questions regarding the anatomical, physiological, pharmacological, and behavioral organization of the central activating systems remain unanswered; further work is

necessary to gain a more complete description of the function of central activating systems on all levels of organization (cellular, network, and behavioral). In this final section, I will list a few, select issues that could be the focus of future research work on the nature of central activating systems in the mammalian nervous sy..em.

(1) As discussed in Paper 2, to date, the neurochemical identity of ECoG-related types of basal forebrain neurons has not been identified. For example, basal forebrain cells that fire at higher rates during neocortical activation are believed to be cholinergic since many of them project to cortex (as determined by antidromic activation; see Detari and Vanderwolf, 1987) and the large majority of cortic·lly-projecting cells in rats appear to contain ACh (as determined histochemically, 'ce Paper 2). However, this hypothesis has not been tested directly. For future work, it would be desirable to record basal forebrain cells intracellularly in order to determine their activity in relation to the ECoG, and subsequently inject an intracellular dye (e.g., Procion yellow) and process the brain immunohistochemically to determine the neurochemical identity (e.g., cholinergic, GABAergic) of these cells. Such data would contribute significantly to the understanding of transmission within the basal forebrain, and of the afferent and efferent connections to and from it.

(2) As hypothesized above, the multiple parallel pathways that mediate neocortical activation may be functionally distinct in that they are brought into play by different sensory inputs or behavioral output demands; again, this hypothesis has not been tested directly. A possible way to approach this question may be to examine whether impulse flow in the parallel activating pathways can be selective stimulated e.g., by sensory stimuli that may have some degree of selectivity for a particular activating pathway. For example, as mentioned, the superior colliculus receives input from the visual system and plays an important role in the performance of movements and defensive behaviors to visual stimuli; the amygdala, on the other hand, is closely linked to the olfactory system. A possible

hypothesis to be tested experimentally is that olfactory stimulation activates the amygdala - basal forebrain-neocortex pathway, resulting in an enhanced activity of cells in the amygdala, the basal forebrain, and enhanced release of ACh in the neocortex. Conversely, visual stimuli (especially behaviorally relevant stimuli such as images of predators) may activate the superior colliculus-raphe-neocortex pathway, resulting in an enhanced release of 5-HT in the neocortex. A combination of behavioral, electrophysiological, and biochemical techniques (e.g., in vivo microdialysis) could easily be used to address these and related questions and add a functional/behavioral dimension to the concept of multiple, ascending activating inputs to the cerebrum.

(3) The mechanisms of anti-serotonergic effects of general anesthetics (urethane, chloral hydrate, ethyl ether) are not known. As discussed in Paper 3, it is possible that 5 - HT dependent neocortical activation may involve an action of 5-HT at multiple receptor types. Thus, anesthetics may reduce or abolish cortical activation by blocking 5-HT effects at several of those binding sites. To date, this possibility has been examined only for one type of 5-HT receptor (the 5-HT$_2$ site; see above) and it will be necessary to perform additional binding and autoradiographic experiments to determine whether anesthetics block other 5-HT receptor types. Along the same lines, it will be necessary to use additional biochemical approaches to examine whether general anesthetics may interfere with intracellular events triggered by 5-HT-receptor activation (e.g., second messenger systems) and whether such effects may exhibit a degree of selectivity for serotonergic transmission.

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