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THE ACTIONS OF GENERAL ANAESTHETICS ON SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES

by

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ABSTRACT

General anaesthetics produce concentration-dependent excitatory and depressant effects in the mammalian central nervous system (CNS), and differential actions are apparent for most agents. At the cellular and synaptic level, however, only depressant actions have been described and common effects reported for all anaesthetics studied. The purpose of the present thesis was to demonstrate concentration-dependent excitatory and depressant actions, and differential effects of anaesthetics on mammalian CNS neuronal function. Anaesthetic actions on excitatory synaptic responses in CA 1 and dentate regions of rat hippocampal slices were studied.

Barbiturate Anaesthetics:

Pentobarbital facilitated transmission between stratum radiatum inputs and CA 1 neurons at low concentrations (0.02 to 0.08 mM), and produced postsynaptic depression at higher concentrations. Only depression was observed for stratum oriens inputs to CA 1 and perforant path inputs to dentate granule neurons. The (+) isomer of pentobarbital was approximately 4 times more potent than the (-) isomer or racemic mixture. Phenobarbital (0.04 to 0.12 mM) produced only depression of synaptic responses in CA 1 and dentate pathways. Comparison of effects on field excitatory post synaptic potentials and population spike responses indicated that the barbiturates act at selective and pathway-specific sites.

Inhalation Anaesthetics:

Halothane (0.25 to 1.25 vol %) depressed postsynaptic excitability of CA 1 pyramidal neurons in response to stratum radiatum synaptic

inputs, and concentration-dependent excitatory (0.25 to 1.25 vol%) and depressant (1.5 to 2.0 vol %) actions were observed on dentate granule neuron excitability and perforant path evoked synaptic responses. Isoflurane increased CA 1 neuron excitability (0.25 to 0.75 vol %) and produced postsynaptic depression of dentate neurons (0.5 to 4.0 vol %). Enflurane also increased CA 1 excitability (0.5 to 4.0 vol %), but depressed synaptic responses at equivalent concentrations, and produced mixed excitatory (0.25 to 1.0 vol %) and depressant (1.0 to 4.0 vol %) effects on dentate synaptic responses. Differential actions were also observed on stratum oriens excitatory inputs to CA 1 neurons, and on antidromic responses. A good correlation ($r = 0.992$) exists between membrane/buffer partition coefficients and half-maximal concentrations for depression of stratum radiatum synaptic responses; however, this correlation does not predict the different, anaesthetic-specific, actions observed.

The results of the present thesis support earlier in vivo observations of mixed excitatory/depressant actions on mammalian CNS neurons and demonstrate that anaesthetics produce differential, agent-specific, effects at the cellular and synaptic levels. The effects observed were dependent on the anaesthetic, concentration, synaptic pathway, neuron population and/or the response (EPSP or PS) measured. These results provide evidence for selective anaesthetic recognition sites in neuronal membranes which can discriminate between anaesthetics on the basis of molecular structure.

ACKNOWLEDGEMENTS

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DEDICATION

This thesis is dedicated to my parents Marjorie and Murdo,

and to

Drs. P. Seeman, C.D. Richards and W.D. Winters

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1. INTRODUCTION

It is generally accepted that anaesthesia results from a depression of excitatory synaptic transmission and/or neuronal excitability in the central nervous system (CNS) (Richards, 1983; Nicoll and Madison, 1982; Winter and Miller, 1984). At the cellular and molecular levels, most studies of anaesthesic effects have emphasized a universal action for all agents (Seeman, 1972; Lenaz, 1978; Roth, 1979; Firestone et al, 1986; Ueda et al, 1986). Thus, a 'Unitary Theory', which proposes a common molecular mechanism of action, shared by all anaesthetics, has evolved from investigations of anaesthetic effects on membrane structure, electrophysiology, simple organisms, and protein/lipid model systems.

Although considerable support exists for a universal depressant action of anaesthetics, enhanced cortical responses and activated brain activity have been observed during anaesthesia, and marked differences in effects are apparent for some anaesthetics (Winters, 1982). Anaesthetics are capable of producing excitatory and depressant effects on nervous system function in vivo (Winters et al., 1967; Clark and Rosner, 1978) and drug-specific effects have also been described on electrical properties and synaptic responses in isolated excitable tissue preparations (Judge, 1983) including a single isolated neuron (MacIver et al., 1979; Roth, 1980; Roth et al., 1986). Anaesthetics are capable of producing increased synaptic responses and direct excitation of neurons in relatively simple, isolated inver-

tebrate preparations (Chalazontis, 1967; Kleinhaus and Pritchard, 1979), and differences in action between agents also occur on these preparations. Differential, biphasic (excitation/depression), and selective anaesthetic actions have also been observed with isolated preparations of mammalian peripheral synapses (Quastel and Linder, 1975; Gage and Sah, 1982). Similar studies utilizing *in vitro* preparations of mammalian CNS neurons have not demonstrated differential and selective actions. For example, recent studies using the hippocampal slice preparation reported only depression of responses, and all anaesthetics studied appeared to produce similar effects (Nicoll and Madison, 1982; Yoshimura et al., 1985), possibly via a common mechanism (Carlen et al., 1984). Results from these recent studies on isolated mammalian neurons support a Unitary Theory, and are not compatible with the differential and biphasic (excitatory/depressant) effects observed *in vivo*, and on simpler isolated preparations.

The purpose of the present thesis was to determine whether general anaesthetics can produce more than depression of excitability and synaptic transmission in an isolated preparation of mammalian CNS neurons. This study compared the effects of several anaesthetics (halothane, isoflurane, enflurane, pentobarbital and phenobarbital) on synaptic responses from three well characterized pathways in the rat hippocampal formation, using the *in vitro* brain slice preparation. The results support previous observations from *in vivo* and invertebrate studies; demonstrating mixed excitatory/depressant effects and

differences between actions of the anaesthetics examined.

The following sections of the INTRODUCTION describe the chemical, physical, and biological characteristics of anaesthetics, which led to a 'Unitary Theory' of anaesthetic action. A brief review of the membrane actions of anaesthetics is presented, and the phenomenon of pressure-reversal of anaesthetic actions is discussed. This is followed by a survey of the electrophysiological literature on anaesthetic actions, at the synaptic and membrane level, providing evidence against a Unitary Theory.

1.1 Anaesthetic Characteristics

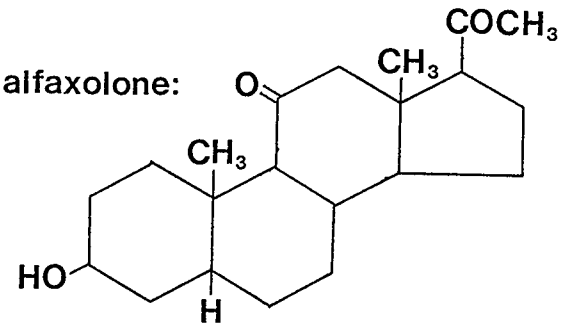
Molecular structures for anaesthetics vary considerably (see Figure 1), and a common chemical feature is not apparent (Seeman, 1972; Wardley-Smith and Halsey, 1984). Structures which range from simple inert gases (eg; xenon and N_2O) to large complex molecules (eg: steriods) are effective anaesthetics. All anaesthetics do not share common atoms much less a critical 'nucleus' for activity, hence, it would appear that molecular size, charge distribution, and/or composition are not major determinants for activity. Clinically used anaesthetics (eg: halothane, isoflurane and enflurane; Figure 1) also lack a structure-activity relationship (Terrell, 1984). Their developement arose from the earlier anaesthetic gases such as diethyl ether and chloroform, and modern structures were empirically arrived at through trial and error, rather than being based on an *a priori* understanding of desired molecular interactions (Terrell, 1984).

Figure 1 - The Chemical structures of some anaesthetics, including the agents used in the present study: phenobarbital, pentobarbital, enflurane, halothane, and isoflurane. Note the optically active carbon in pentobarbital (arrow). Enflurane and isoflurane are structural isomers, differing in the distribution of halogen and hydrogen atoms on the first and second carbons.

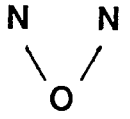
xenon:

Xe

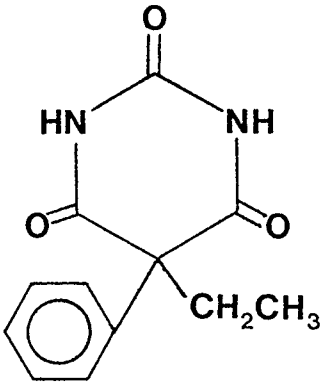
alfaxolone:



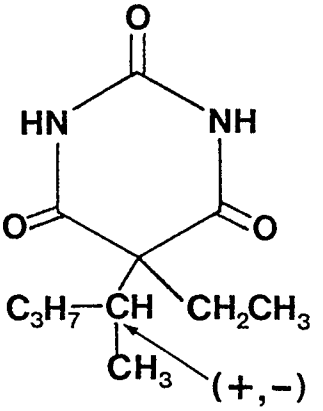
nitrous oxide:



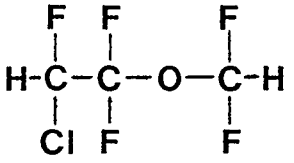
phenobarbital:



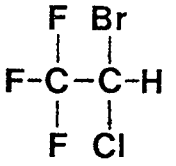
pentobarbital:



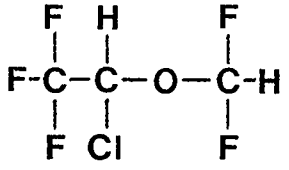
enflurane:



halothane:



isoflurane:



ether:

C_2H_5-O-C_2H_5

Currently used general anaesthetics were developed to eliminate unwanted characteristics (eg: flammability, odour, and toxicity) and virtually no consideration of 'ideal' actions has been possible (Halsey, 1984).

The interaction (binding) of anaesthetics with nervous tissue has been characterized as low affinity - high capacity uptake at effective concentrations (Seeman, 1972; Roth, 1979; Miller, 1985). Drugs which act on specific receptor molecules, in contrast, demonstrate high affinity - low capacity profiles; tightly binding to their target tissues (ie: binding energy of > 1000 kJ/Mol) in a structure-dependent manner, and relatively low concentrations saturate binding sites. Anaesthetic interactions are of low energy (eg: 16 to 56 kJ/Mol) and saturation of sites is not observed, or only seen at concentrations well beyond effective therapeutic ranges (Franks and Lieb, 1982). This suggests that multiple sites are involved in anaesthetic-tissue interactions (Katz and Simon, 1977). A low affinity readily explains why high concentrations of these agents are required for anaesthesia, but little information is provided about the nature of cellular interactions.

Pharmacological agents which are 'receptor-directed' demonstrate a high degree of tissue selectivity, reflecting the specific cellular distribution of receptors for these agents. Anaesthetics are capable of altering the function of all cell types; depressant actions have been demonstrated on simple micro-organisms, plants, and all animal tissues studied (Bruce and Christiansen, 1965; Roth, 1979). This

lack of selectivity contributes to the narrow therapeutic index (safety margin) of these agents. Toxic effects of currently used volatile anaesthetics often involve organs other than the CNS (eg: liver, kidney, and heart), further emphasizing their non-specific nature (Halsey, 1984).

In summary, anaesthetics exibit structural diversity, non-specific binding characteristics, and lack tissue selectivity. Taken together, these properties strongly suggest that 'drug-receptor' interactions are not involved in anaesthetic actions (Roth, 1979; Miller, 1985). The lack of specific anaesthetic 'receptors' might explain why a chemical antagonist for anaesthesia has not been described. Furthermore, the elucidation of a mechanism of action for anaesthetics has been limited by the non-specific nature of their interactions.

1.2 The Mechanism of Action of Anaesthetics

Many theories have been proposed to account for the observed actions and peculiar properties of general anaesthetics. Historical accounts of earlier theories can be found in a number of reviews (eg: Kaufman, 1977; Roth, 1979; Wardley-Smith and Halsey, 1984). Current theories generally fall into one of two groups; the first proposes that all agents share a common molecular mechanism of action (Unitary Theory) while the second group proposes unique structure-dependent actions. The Unitary Theory has received support from several independent experimental approaches and is the generally accepted view

at the present time (Winter and Miller, 1984). Support for selective and differential mechanisms comes almost entirely from electrophysiological studies of anaesthetic effects on excitable membranes, and has been formulated into the "Degenerate Perturbation Hypothesis" by Richards and collegues (1978). Major differences between theories can be attributed to the degree of specificity exhibited by anaesthetics for their site of action. Both theories are in agreement regarding some major features of the site; most important of these are the hydrophobic nature and involvement of the cellular membrane as the primary site of action. The hydrophobic nature of the site is suggested by a striking correlation between the degree of hydrophobicity of anaesthetics, and their effective concentrations (potencies) for producing anaesthesia (Seeman, 1972; Franks and Lieb, 1982).

H.H. Meyer (1899, 1901) and E. Overton (1901) were the first to recognize a correlation between anaesthetic potency and solubility in oil; following the earlier discovery of fat solvent properties of these agents by von Bibra and Harless (c. 1847). Meyer and Overton independently demonstrated that a number of anaesthetics have high oil to water solubility ratios (oil/water partition coefficients) and postulated that any chemically inert substance which preferentially dissolves in fats would produce anaesthesia. Since that time a number of variations in experimental design have confirmed the original correlation; it is now well accepted that an oil-like (hydrophobic) domain is the likely target of anaesthetic action (for review see Seeman, 1972; Roth, 1979; Miller, 1985). At a cellular level, the most likely tar-

get is membranes, ie: the hydrophobic interior region of lipid bilayers, and this was proposed by the early investigators (reviewed by Mullens, 1935). Recent studies have substantiated the idea that cell membranes are the most probable site of anaesthetic action (eg: Braswell et al., 1984). Janoff and coworkers (1981), among others, have shown an excellent correlation between lipid bilayer/buffer partition coefficients and anaesthetic potencies for a wide range of agents. The most convincing evidence for membrane sites of action comes from electrophysiological studies which demonstrate direct anaesthetic effects on transmembrane ionic conductances (see below). The Meyer-Overton 'rule' has proven useful in defining neuronal membranes as the cellular site of action for anaesthesia; but the nature of this correlation does not permit further mechanistic insights based on physicochemical properties (Roth, 1979; Janoff and Miller, 1982; Franks and Lieb, 1982; Miller, 1985).

A more sophisticated approach to determining underlying mechanisms has been to measure anaesthetic-induced changes in lipid bilayers and/or biological membranes. Many investigators, using different experimental techniques, have found that anaesthetics can induce substantial changes in structure of both artificial and biological membranes. Anaesthetics, for example, are capable of producing membrane expansion (Seeman, 1974; Roth and Seeman, 1972; Halsey et al., 1978), and bilayer thinning (Roth and Jay, 1977; Miller, 1974). These structural changes are thought to reflect increases in the degrees of freedom (rotational motion, lateral movement, etc.) of lipid molecules.

Membrane lipids exist in a gel-like ordered phase in equilibrium with lipids in a more disordered liquid-crystalline phase. The phase transition equilibrium is shifted by anaesthetics to favour the more disordered liquid-crystalline phase (Kaufman, 1977; Ueda et al., 1977; Lenaz et al., 1979). It has been suggested that disordering results from a physical disruption of lipid-lipid interactions in a manner analogous to the disordering of water molecules by salts (ie: the melting of ice with NaCl; Franks and Lieb, 1979). Anaesthetic-induced lipid disordering has been termed 'membrane fluidization' and could account for the measured expansion of membranes and bilayer thinning (Roth, 1979; Janoff and Miller, 1982; Ueda et al., 1986). Strong support for membrane fluidization as a mechanism of anaesthetic action has been derived from an independent line of evidence: anaesthetic-induced fluidization of membranes is readily reversed by pressures of approximately 100 atmospheres; similar pressures also antagonize anaesthesia.

1.3 Pressure Reversal of Anaesthesia

Johnson and coworkers (1942) were the first to demonstrate that high pressures could antagonize anaesthetic effects on bacterial luminescence. Since that time a number of investigators have reported similar findings on both isolated preparations and intact animals. Anaesthesia in tadpoles (Johnson et al., 1951), newts (Miller et al., 1971), and mice (Halsey and Eger, 1971) has been shown to be reversed by increases in either hydrostatic or atmospheric pressures (of the order of 100 to 150 atm). Recent studies in mice confirm that the

acute effects of ethanol can be antagonized, and withdrawal symptoms exacerbated by pressures of 100 atm (Alkana et al., 1985). Pressure is usually increased using an inert gas (eg: helium) which produces only minor effects on its own (Miller et al., 1978). In a similar manner, pressure has been shown to antagonize anaesthetic induced neuromuscular blockade (Kendig and Cohen, 1976), nerve conduction failure (Roth et al., 1976), and depression of postsynaptic chemosensitivity (Braswell et al., 1984). At the molecular level, anaesthetic-induced fluidization of membranes is also reversed by increased pressure (Mastrangelo et al., 1978 and 1979; Kamaya et al., 1979).

It is not clear how pressure physically opposes anaesthetic-induced fluidization or, of course, how anaesthesia is reversed (Lever et al., 1971). It has been suggested that mechanical compression reverses anaesthetic-induced changes in membrane volume (expansion), resulting in conformational changes which restore membrane excitability (Miller and Miller, 1975). It is also possible that pressure produces effects which indirectly oppose the actions of anaesthetics; eg: CNS stimulation or altered synaptic transmission (Kendig and Cohen, 1976; Roth, 1979; Wardley-Smith and Halsey, 1984; Kendig and Grossman, 1986). The 'functional antagonism' between anaesthetics and pressure may involve mechanisms which are even more complex than anaesthetic mechanisms alone (Roth, 1979; Kendig and Grossman, 1986). Pressure reversal of anaesthetic effects, at all levels of organization studied, is a phenomenon in need of further explanation.

The Meyer-Overton correlation and pressure reversal phenomenon provide support for theories of anaesthesia which stress effects on membrane lipids. Direct measurement of anaesthetic-induced changes in bilayer structure (eg: lipid fluidization) have been reported (eg: Ueda et al., 1986). Thus, the most favoured explanation of anaesthetic mechanisms involves actions on membrane lipids (Winter and Miller, 1984).

1.4 A Unitary Theory of Anaesthesia

The nonspecific nature of anaesthetics and similar effects produced on membrane lipids have led to a Unitary Theory of anaesthesia. Simply stated, the theory proposes 'that all general anaesthetics ... increase the overall fluidity of lipid bilayers' (Lenaz et al., 1978) and that this results in an alteration of functional membrane protein activity (Miller, 1977; Roth, 1979). Specific functional proteins which are affected by these lipid perturbations have yet to be defined, but it is generally thought that they would regulate transmembrane ionic current flow in neurons (Lee, 1976; Trudell, 1977). The important feature of the Unitary Theory is that effects on membrane function are secondary to anaesthetic-induced perturbation of bilayer structure; and that all general anaesthetics share this common molecular mechanism of action.

1.5 Criticism of the Unitary Theory

If general anaesthetics share a common mechanism of action then similar effects on membrane function would be expected to be produced

by all agents. Studies of anaesthetic effects on membrane excitability have not provided sufficient information to test this prediction. Some studies have described similar effects produced by a number of agents while others have emphasized differences in effects between agents (see below). The Unitary Theory, however, makes three predictions which have been experimently addressed. These predictions are as follows:

a) Any lipid soluble substance which can achieve a critical membrane concentration should produce anaesthesia.

b) A close correlation between anaesthetic potency and the ability to fluidize membrane lipids should be evident.

c) Combinations of anaesthetics should show strict additivity of effects; ie. any anaesthetic should be able to substitute for another.

These predicitons of the Unitary Theory have not received strong experimental support. It has been known for some time that potencies of barbiturate anaesthetics do not correlate well with their lipid solubilities (Roth and Seeman, 1972; Andrews et al., 1979). Furthermore, slight structural changes to the 3' position of the butenyl side chain can change an anaesthetic barbiturate to a convulsant or anticonvulsant (Andrews et al., 1979). Similar changes have been shown to produce either depressant or excitatory responses at the cellular level (Tan et al., 1981; Roth, Tan and MacIver, 1986). It is difficult to explain how a common mechanism can provide the specificity needed to account for anaesthetic, convulsant, and anticonvulsant

actions of structurally related barbiturates, which differ little in solubility (Ho and Harris, 1981). A poor correlation between potency and solubility has also been shown for some alkanols and local anaesthetics (Bradley and Richards, 1984). The ability of an anaesthetic to fluidize lipid bilayers is not solely related to it's lipid solubilty; steric factors and ionic charge help to determine membrane location, which, in turn, appears to be a major factor affecting lipid fluidization (Chaykowski et al., 1979; Franks and Lieb, 1982). The Unitary Theory has further difficulties explaining potency differences between stereoisomers for both barbiturates and steriods (Huang and Barker, 1980; Lawrence and Gill, 1975). Stereoisomers exhibit identical lipid solubilites, yet do not produce the same degree of fluidization nor demonstrate similar abilities to produce anaesthesia.

Although all general anaesthetics share a common physicochemical property (lipid solubility) and can expand membranes, the inverse correlation does not hold. All lipid soluble agents, which are able to expand membranes, do not produce anaesthesia. Tridecanol, tetradecanol, and hexadecanol; for example, expand erythrocyte membranes to the same degree as anaesthetic alkanols (Bull et al., 1982) but do not produce anaesthesia (Franks and Lieb, 1982). Furthermore, recent studies of anaesthetic interactions with bilayers have failed to detect changes in membrane thickness or degree of lipid disorder, even though higher resolution X-ray and neutron scattering techniques were used (Franks and Lieb, 1979).

The Unitary Theory also proposes that anaesthetic effects should be independent of chemical structure; a given agent should be able to substitute for any other (Clarke et al., 1978). If this were the case then mixtures of anaesthetics should produce effects which are strictly additive. Mixtures of steriod and barbiturate agents, however, produced greater than expected effects both *in vivo* and *in vitro*, suggesting that these agents act at independent sites in neuronal membranes (Richards and White, 1981; Roth and Omand, 1982; Coleman and Roth, 1986). Similar, non-additive, effects have been described for inhalation anaesthetics on the discharge activity of crayfish stretch receptor neurons (Coleman and Roth, 1985), and for pressure reversal of anaesthesia (Wardley-Smith and Halsey, 1984). Thus, although the Unitary Theory is based on considerable correlative evidence, it cannot withstand critical tests of its validity. The correlations which led to a Unitary Theory simply describe physicochemical requirements for the anaesthetic site(s), but provide incomplete information to formulate a mechanism of action for anaesthetics (Richards, 1983; Roth, 1979).

1.6 Anaesthetic-Protein Interactions

Lipid soluble regions of membranes are not made up solely of lipids. Proteins embedded in the bilayer can contribute up to 50% of membrane bulk and these proteins also have highly lipophilic regions with which anaesthetics may interact (Seeman, 1972; Franks and Lieb, 1986). Membrane associated proteins are known to be responsible for the selective distribution and gating of ionic currents which underly

membrane excitability (Catteral, 1984). It is reasonable to presume that direct anaesthetic-protein interactions may contribute to the membrane actions of anaesthetics (Franks and Lieb, 1986). Unfortunately, relatively little attention has been given to anaesthetic actions on membrane proteins. Part of the reason why studies have been limited is that it is difficult to isolate membrane proteins from lipid bilayers, and often the function of these proteins is destroyed by isolation procedures. To circumvent this problem, a number of studies have been carried out using pure protein models, or proteins which remain active in aqueous solution (for review see Taussig, 1979).

Anaesthetics can bind to hydrophobic regions of proteins and have been shown to induce conformational alterations of protein structure (Miller and Miller, 1975; Metcalfe et al., 1968). Membrane associated enzymes (eg: Na/K ATPase) are sensitive to anaesthetic actions; both stimulation and depression of activity have been observed (Quastel, 1956; Seeman, 1972). Effects on metabolic enzymes have also been observed, however, it is unlikely that these actions contribute significantly to membrane electrical responses since metabolic poisons and ATPase inhibitors produce responses which differ in both kinetics and dynamics from anaesthetic effects (Seeman, 1972; Vanderkooi et al., 1981).

Recent studies have used the luminescence response of firefly luciferase to provided direct evidence for anaesthetic actions on a purified protein (Franks and Lieb, 1982). Inhibition of luciferase

activity occurs in the presence of relatively low concentrations of anaesthetics (Ueda and Kamaya, 1973). Different molecular mechanisms may be involved in the actions of the agents studied; some appear to directly displace substrate while others may alter enzyme conformation (Franks and Lieb, 1986). These results support earlier theories of anaesthesia which propose that conformational changes in proteins result in altered membrane electrical responses (Eyring et al., 1973; Franks and Lieb, 1978). Conformational changes in membrane proteins can result from direct interactions with anaesthetics and/or indirect effects secondary to alterations of the lipid environment (Lee, 1976; Trudell, 1977; Bangham and Mason, 1980). Further investigation is required to determine precise molecular mechanisms underlying anaesthetic-induced conformational changes of membrane proteins; at present, it is likely that both direct and indirect actions are involved (Roth, 1979; Harris, 1984).

1.7 The Lipid/Protein Problem

Whether anaesthetics act on lipids or proteins is not of paramount importance to the present thesis; clearly, effects on one could affect both. The critical question should be: what membrane functions are altered by these agents? Cell membranes are not static, homogeneous structures, but demonstrate a high degree of molecular segregration and are dynamically active (Severs, 1983; Poo, 1985). Distributions of surface proteins are ever changing, specific funtional elements are localized differentially (eg: sub-synaptic receptor proteins) and interactions between membrane proteins are organized and regulated. If anaesthetics do fluidize lipids, the particular

membrane regions affected should dictate resulting functional changes. The excitable membranes of neurons epitomize this complexity of structure, and posess numerous "micro-heterogeneities" with which anaesthetics may selectively interact (Roth, 1979 and 1980). For this reason, anaesthetic effects on neuronal membranes could be complex, and the opportunity for differential and selective actions certainly exists.

1.8 The Spectrum of Anaesthetic Effects on Nervous Tissue

Anaesthetics have been shown to alter neuronal metabolism (Taussig, 1979), excitability and synaptic transmission (Richards, 1983; Judge, 1983). Effects on each of these inter-dependent processes have been proposed to underly anaesthesia (Marshall and Wollman, 1980). Actions on synaptic transmission and excitability have received recent attention because these occur at concentrations which correspond best with predicted levels during anaesthesia (Richards, 1983).

A serious problem became evident from early studies of the neuronal actions of anaesthetics; Winters (1982) summarizes this problem as follows: 'a search for a unifying single neurochemical or neurophysiological mechanism to explain the basis of anesthetic action is frivolous'. This statement has two connotations, first, observations of neuronal actions have suggested that more than a simple unitary mechanism is involved, second, a great number of neurophysiological effects are produced by anaesthetics and it is not readily obvious which of these contribute most to anaesthesia. Winters (1980) points

out an additional problem: of decreasing CNS irritability leading to depression'; however, he notes that the anaesthetic state 'can be achieved by CNS stimulation or depression' depending on which anaesthetic is used. Although this problem may seem trivial compared to the first two, it has resulted in some confusion in the neurophysiological literature. In the following sections, these difficulties will be addressed by describing anaesthetic effects on various stages of neuronal processing, beginning with presynaptic actions.

1.8.1 Presynaptic Actions of Anaesthetics

Anaesthetics can alter the release of neurotransmitter via several mechanisms (for review see Richards, 1983). One of the earliest mechanisms proposed involved a blockade of impulse conduction in fine preterminal axon fibers (reviewed by Seeman, 1972). That is, general anaesthetics could depress conduction in smaller diameter fibers in the CNS (Frank and Saunders, 1965). Indeed, anaesthetics do appear to depress small diameter axons at concentrations which are lower than required to block larger fibers (Nathan and Sears, 1961; Staiman and Seeman, 1977; Gissen et al., 1980; Fink and Cairns, 1984; Kendig and Grossman, 1986). The critical question is, do general anaesthetics block fiber conduction at concentrations which are relevant to general anaesthesia? Larrabee and Posternak (1952) were the first to demonstrate that synaptic transmission through sympathetic ganglia is depressed by concentrations of general anaesthetics which are five to ten times lower than required for conduction blockage in axons. More recent studies with a number of anaesthetics

have confirmed this observation on CNS synapses (Richards, 1972; Richards, Russell and Smaje, 1975; Richards and White, 1975; Morris, 1978). The best evidence for a selective action of general anaesthetics on synaptic transmission comes from a comparison of pentobarbital and tetrodotoxin actions on CNS transmission (Richards, 1983). Tetrodotoxin is known to block sodium channels of axons with no effect on synaptic channels (Ritchie, 1979). This toxin produced a depression of synaptic responses in olfactory cortex which was accompanied by a decreased amplitude and increased latency of the presynaptic action potential. Pentobarbital, in contrast, produced a depression of transmission with no effect on the presynaptic action potential latency or amplitude. Because these agents produce distinctly different effects on CNS transmission, it is unlikely that depression of preterminal conduction contributes to the general anaesthetic actions of pentobarbital (Richards, 1983).

Depression of synaptic transmission could also result from a decrease in transmitter release. A number of CNS depressants have been shown to reduce acetylcholine release from preganglionic sympathetic nerve fibers (Matthews and Quilliam, 1964). In the CNS, low concentrations of pentobarbital have been shown to reduce the amount of L-glutamate and L-aspartate released from lateral olfactory tract fibers (Collins, 1980). Similar effects have been reported for excitatory amino-acid release from <u>in vitro</u> thalamic slices (Potashner et al., 1980; Minchin, 1981; Kendall and Minchin, 1982). It was interesting, in the context of a 'Unitary Theory', that not all gen-

eral anaesthetics tested produced a depression of transmitter release (Kendall and Minchin, 1982). Anaesthetic-induced depression of transmitter release has been postulated for a number of years on the basis of results from electrophysiological studies (Cutler et al., 1974; Quastel et al., 1972). Weakly (1969) was the first to demonstrate that anaesthetics reduce the quantal content of motorneuron EPSPs but do not depress miniature EPSP amplitudes; indicating that a reduction in evoked transmitter output could occur. It is not clear how anaesthetics alter transmitter release, although some possible mechanisms have been ruled out. Anaesthetics do not appear to alter brain concentrations or turnover of glutamate, aspartate, or acetylcholine (Crossland and Merrick, 1954; Potashner et al., 1980). Nor is the synthesis and storage of transmitters appreciably altered by anaesthetics, evidenced by a lack of effect on post-tetanic and frequency potentiation of CNS synapses (Somjen, 1963; Richards, 1972 and 1973; Richards and White, 1975). Ketamine has been shown to alter post-tetanic potentiation, but this occurs via an antagonism of glutamate (NMDA) receptors (Collinridge, 1985). Although anaesthetics are known to interfer with transmitter release, the mechanisms remain unknown; direct actions on neurosecretory processes appear likely (Richards, 1983; Fung and Fillenz, 1984).

1.8.2 Anaesthetic Effects on Postsynaptic Receptors

Depression of synaptic transmission could be mediated by inhibition of neurotransmitter responses and there is considerable evidence that chemosensitivity can be depressed by some anaesthetics. Richards

and co-workers have studied anaesthetic effects on glutamate evoked excitation of cortical neurons and report either depression of response or no effect. Ether, methohexital, and pentobarbital depressed glutamate responses at concentrations which also depressed synaptic transmission (Richards, Russell, and Smaje, 1975; Richards and Smaje, 1976). Pentobarbital also depressed glutamate-induced depolarization of mouse spinal neurons grown in tissue culture (Ransom and Barker, 1975). Halothane, in contrast, had no effect on glutamate responses at concentrations higher than required to depress transmission (Galindo, 1969; Crawford, 1970; Richards and Smaje, 1976). These results suggest that several anaesthetics may depress synaptic transmission by a predominantly postsynaptic action, however, at least some (eg: halothane) appear to act via a depression of transmitter release (Richards, 1983; Wachtel, 1984). Differences in anaesthetic actions have been demonstrated on other transmitter-receptor systems as well. Pentobarbital, for example, increases the strength of GABA mediated inhibitory transmission in the mammalian CNS (Nicoll et al., 1975; Ransom and Barker, 1976) and on a number of invertebrate and vertebrate neuronal systems (Adams et al., 1982; Cote et al., 1978; Schulz and Macdonald, 1981; Owen et al., 1986). This effect is thought to be mediated by a prolongation of chloride channel conductance and can be antagonized by the chloride ionophore anatagonist picrotoxin (Jackson et al., 1982; Barker and Mathers, 1981). Similar effects are produced by a few other barbiturates (eg: thiopental, secobarbital), but most general anaesthetics and other barbiturates (eg: phenobarbital) do not appear to enhance GABA transmission

(Harrison and Simmonds, 1983; Iadarola et al., 1985; Yoshimura et al., 1985), although this remains controversial (Gage et al., 1986). These observations, together with detailed binding analyses, have provided a model for the GABA receptor-ionophore complex which includes separate binding sites for GABA, barbiturates and benzodiazepines (reviewed by Olsen, 1982; see also Harris and Allan, 1985). Specific 'receptor-like' interactions at this complex are supported by the observation that the stereoisomers of pentobarbital have significantly different actions (Huang and Barker, 1980), and a structure activity relationship for the barbiturates exists for interactions with the GABA receptor-ionophore complex (Ho and Harris, 1981).

Postsynaptic responses to acetylcholine also demonstate selectivity with respect to different anaesthetics. Cortical neuron responses to ionophoretically applied acetylcholine are enhanced by halothane and methoxyflurane; depressed by alphaxalone, and not altered in the presence of pentobarbital (Smaje, 1976). Anaesthetic effects on cholinergic responses at the neuromuscular junction (NMJ) have been extensively investigated (reviewed by Judge, 1983), and opposite effects were produced by different anaesthetics. Ethanol and ether enhance the duration of miniature endplate currents by either increasing the average open-time of channels or via an inhibition of anticholinesterase activity (Quastel and Linder, 1975; Gage et al., 1975; Gage et al., 1979). In contrast, depression of endplate electrical events occurs in the presence of pentobarbital (Adams, 1974), halothane (Gage and Hamill, 1975), enflurane (Kennedy and Galindo,

1975), and long chain alkanols (Gage et al., 1975). These anaesthetics appear to depress responses by accelerating the rate of decay of miniature endplate currents (Gage and Hamill, 1975; Judge, 1983). Ketamine and some barbiturates, however, appear to depress responses by directly blocking open endplate channels (Adams, 1976; Maleque et al., 1981; Gage and Sah, 1982), and demonstrate a use or frequency-dependent effect (see Gage and Hamill, 1981). Use-dependence is generally believed to indicate that anaesthetics gain access to the binding site via the open conformation of a channel protein (Adams, 1976; Koblin and Lester, 1979; Kendig et al., 1979).

The major difficulty with all of the studies described above is that they measured anaesthetic actions on postsynaptic responses which are known to be quite complex and could, therefore, reflect effects on several processes involved in coupling receptor activation to ionic current flow across membranes. Barbiturate interactions with the GABA receptor-ionophore complex appear to involve direct interactions with receptor binding processes (Olsen, 1982; Wong et al., 1984; Miller, 1985). Effects on other transmitter systems could involve channels as well as receptors (Judge, 1983). Further investigation of anaesthetic effects on neurotransmitter-receptor binding is required.

Although the mechanisms underlying anaesthetic-induced alterations of chemosensitivity remain unknown; it is difficult to explain the observed differences by a common mode of action. Richter and coworkers have studied volatile anaesthetic effects on miniature endplate currents at the frog NMJ and conclude: 'the effect of such

structurally non-specific drugs comprises a combination of discrete and distinguishable events which are specific and dose dependent for each of the agents used' (Landau et al., 1979; see also: Diamond et al., 1975; Koblin and Eger, 1980). Differential effects on postsynaptic chemosensitivity provide good evidence for selective anaesthetic actions on different membrane components.

1.8.3 Anaesthetic Actions on Membrane Currents

Alteration of neuronal transmission could be produced by actions on membrane currents which couple synaptic input to action potential discharge of postsynaptic neurons. Numerous investigators have found that anaesthetics are capable of altering ionic currents (reviewed by Judge, 1983; Krnjevic, 1986); however, other studies have found virtually no effect on cell excitability (Richards, 1983) at anaesthetic concentrations which depressed synaptic responses. The question remains whether alteration of postsynaptic currents contributes significantly to anaesthetic-induced depression of neuronal transmission.

The best evidence for direct effects on postsynaptic conductances has come from studies on invertebrate neurons. Chalazonitis (1967) was the first to demonstrate that general anaesthetics (chloroform, ether and halothane) could alter resting membrane conductance in *Aplysia* and *Helix* neurons. Low concentrations of these agents produced increased membrane excitability and depolarization, but membrane resistance was not altered. Increased conductance and hyperpolarization were produced in the presence of higher concentrations, which

also depressed excitatory synaptic responses (Chalanzonitis, 1967). Similar biphasic effects on membrane resistance were produced by pentobarbital and halothane on crayfish stretch receptor neurons (MacIver and Roth, 1980; MacIver and Roth, 1987b). The actions of pentobarbital on Aplysia neurons were studied by Sato et al. (1967) and they demonstrated that hyperpolarization and increased ionic conductances were due to an increased potassium current. Since that time pentobarbital has been shown to alter potassium conductances in a number of neurons, including: CNS cells in Otala (Barker, 1975), burst firing neurons from Aplysia (Cote et al., 1978), and leech Retzius cells (Kleinhaus and Pritchard, 1979). Phenobarbital can also alter potassium currents in Retzius neurons, however, enhancement was produced, whereas pentobarbital produced depression (Pritchard, 1972). These two barbiturates produce opposite effects on the firing activity (Roth, Tan and MacIver, 1986) and membrane electrical properties of crayfish stretch receptor neurons (Tan et al., 1981).

Anaesthetics also appear to alter other ionic conductances which are important determinants of postsynaptic excitability. Urethane, for example, depressed sodium conductance in neurons from Helix and Planorbis (Gierasimov and Janiszewski, 1967), and pentobarbital produced biphasic (enhancement/depression) alterations of calcium currents in Aplysia (Johnston, 1978). Differential responses produced by ethanol, halothane, and pentobarbital on stretch receptor neuron discharge activity could also be ascribed to discrete and biphasic actions on sodium, potassium, and chloride currents (MacIver and Roth,

1987b). Judge (1983) concluded that the spectrum of actions on membrane conductances 'indicate that at this level different anaesthetic agents produce rather different effects. This is not in keeping with a Unitary Theory of Anaesthesia.'

Differential actions on membrane currents have not been described for neurons in the mammalian CNS. Instead, all anaesthetics studied have been shown to produce membrane hyperpolarization (Nicoll and Madison, 1982). In this same study a good correlation between in vivo anaesthetic potencies and minimum effective concentrations for hyperpolarization was shown for a number of anaesthetics (urethane, ether, chloral hydrate, enflurane, phenobarbital, pentobarbital, chloroform, methoxyflurane, halothane, and chloralose). Krnjevic (1972) was the first to propose that general anaesthetics may produce hyperpolarization by activating a calcium dependent potassium conductance, via a release of intracellular calcium. Most recent studies of anaesthetic actions on mammalian neurons are entirely in agreement with this hypothesis (Carlen et al., 1984; Nicoll and Madison, 1982; Morris, 1986). Thus, a unitary mechanism accounts for observed anaesthetic effects on neuronal excitability in the CNS. The contradiction between common actions described in these studies, and the differential actions observed on invertebrate neurons may be due to the limited responses investigated in studies of mammalian neurons (Richards, 1983; see also: PURPOSE and RATIONALE).

1.9 Model Neuronal Systems in Anaesthetic Research

Two factors have limited our understanding of anaesthetic mechanisms of action: 1) the nonspecific nature of anaesthetics and, 2) our incomplete understanding of CNS physiology. The latter factor is made more difficult by the complex organization of the brain. For this reason, model neuronal systems have been utilized for studies of anaesthetic effects. These systems attempt to describe drug effects on relatively small populations of neurons to allow more detailed analysis of sites and mechanisms of action.

In the present thesis, a model system approach was extended to include a comparison of several anaesthetics, more complete dose-response analysis, and comparison of effects on two different types of neurons and three afferent synaptic pathways using an *in vitro* preparation from the mammalian CNS. It was thought that a better understanding of anaesthetic effects would be gained, which might help to explain the complicated effects observed on multineuronal networks and relate these to the continuum of selective effects observed on relatively simple invertebrate preparations.

1.10 The Hippocampal Brain Slice as a Model Neuronal System

Anaesthetic effects were studied on three synaptic pathways in the rat hippocampal formation, using the *in vitro* brain slice preparation. Hippocampal synaptic circuitry has been studied in some detail (see RATIONALE) and consists of the following elements:

1.10.1 Inputs to hippocampal neurons:

The hippocampal formation receives afferent input from a number of sources. Diffuse noradrenergic, serotonergic, and dopaminergic afferents arrive, via the fimbria, from the locus ceruleus, median raphe nuclei, and substantia nigra respectively; and innervate the principle neurons of the dentate and hippocampal cortex (Moore, 1975; Assaf and Miller, 1978). Peptidergic fibers (enkephalin, AVT, Somatostatin, etc.), presumably arising from brainstem and hypothalamic nuclei, also appear to diffusely innervate hippocampal formation neurons (Assaf et al., 1981; Pittman and Siggins, 1981). Cholinergic fibers from the septum (via fimbria) innervate pyramidal and dentate granule neurons in a stratified manner such that medial septal inputs synapse on proximal dendrites while lateral septal inputs synapse on distal dendritic regions(Assaf et al., 1981). An important input to granule cells appears to be perforant path fibers from entorhinal cortex. These fibers are thought to be glutaminergic (Wheal and Miller, 1975; Crunnelli et al., 1983), and also terminate in a proximal to distal stratification. Activation of septal and entorhinal inputs result in graded membrane depolarizations (due to increased Na conductance) which often give rise to single action potentials (Dudek et al., 1976). Activation of other inputs to granule neurons (eg: 5-HT, NA, and various peptides) appear to produce inhibition (Assaf and Miller, 1978; Assaf et al., 1981; Pittman and Siggins, 1981), presumably via an increase in chloride ion permeability (Assaf et al., 1981), or potassium conductance (Carlen et al.,1982).

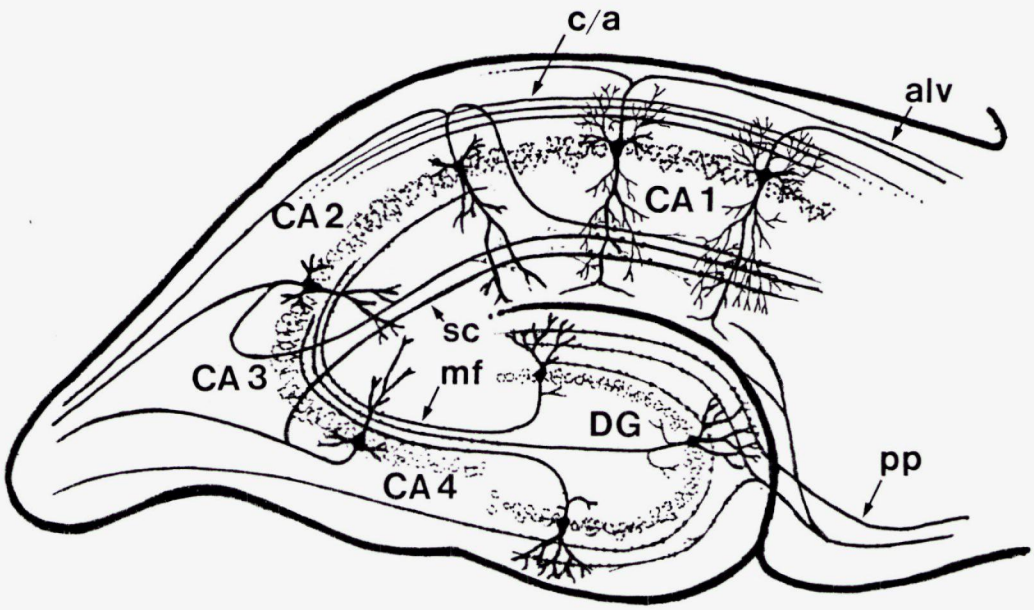
1.10.2 Intrinsic synaptic circuitry of the hippocampal formation:

A schematic diagram of the major fiber systems and neurons of the hippocampal formation is provided in Figure 2. Axons of granule neurons form the mossy fiber pathway which terminates on CA 3 neuron apical dendrites and is excitatory (Andersen, 1975). The mossy fiber synaptic transmitter is not known but ACh, Asp, and peptides have been suggested (Storm-Mathisen, 1977; Siggins and Bloom, 1981). CA 3 pyramidal cell axons bifurcate, one branch leaves the hippocampus via the fimbria and the other branch contributes to the Schaffer collateral fibers which innervate the apical dendrites of CA 1 neurons and are excitatory (Andersen, Bland and Dudar, 1972; Storm-Mathisen, 1977). Axons of CA 1 pyramidal neurons project to the subiculum via the alveus, and to other brain areas via the fimbria. Granule and pyramidal neurons all receive recurrent lateral inhibition via axon collaterals which synapse onto basket cell inhibitory interneurons which, in turn, form inhibitory synapses (GABA) with the cortical neurons (Assaf et al., 1981; Schwartzkroin, 1983).

2. PURPOSE OF THE PRESENT STUDY

The loss of consciousness associated with anaesthesia has been attributed to a generalized depression of synaptic transmission (Richards, 1980; 1983), or to decreased neuronal responsiveness (Nicoll and Madison, 1982; Krnjevic, 1986). Some anaesthetics, however, appear to produce unique patterns of excitatory CNS activity during anaesthesia, in animals and man (Stockard and Bickford, 1975;

Figure 2 - Diagram of a transverse section through the hippocampal formation showing the location of pyramidal neurons (CA 1 to 4) and dentate granule (DG) neurons, together with some fiber pathways: sc - Schaffer-collaterals, mf - mossy fibers, c/a - commissural/associational, alv - alveus, pp - perforant path.


c/a
alv
CA2
CA1
sc
mf
CA3
DG
pp
CA4

Winters, 1982; Stevens et al., 1984). At the cellular level, anaesthetics appear to act at multiple synaptic sites and these sites are probably different for some anaesthetics (MacIver and Roth, 1987b). The popular belief that 'general anaesthetics work by blocking synaptic transmission' (Richards, 1980) does not provide a good explanation for the neuronal excitation observed during anaesthesia; or for the different effects produced by various anaesthetics. The present study attempted to determine whether anaesthetics produce excitatory and depressant actions on CNS neurons, by examining effects on synaptic transmission and postsynaptic excitability.

The purpose of the present study was to examine anaesthetic-induced alteration of synaptic transmission in mammalian CNS circuits and to demonstrate, selective, differential actions between agents. An attempt was made to correlate excitatory and depressant actions with concentration, sites of action and drug structure. The results may help to unify in vivo and in vitro investigations of anaesthetic mechanisms of action, and may provide useful direction in defining subcellular sites of action. The in vitro hippocampal brain slice preparation was used, because it is one of the best characterized mammalian CNS model systems (Schwartzkroin, 1983; Teyler and DiScenna, 1984 see also RATIONALE) and allowed for precise control of drug concentration and electrode placement.

3. WORKING HYPOTHESIS

The working hypothesis was: EXCITATORY AND DEPRESSANT ACTIONS ON NEURONAL FUNCTION ARE RELATED TO STRUCTURAL DIFFERENCES BETWEEN ANAESTHETICS. It is unlikely that a single, common, effect (ie: depression) is produced by all anaesthetics, and more likely that different profiles of excitatory and depressant actions are produced.

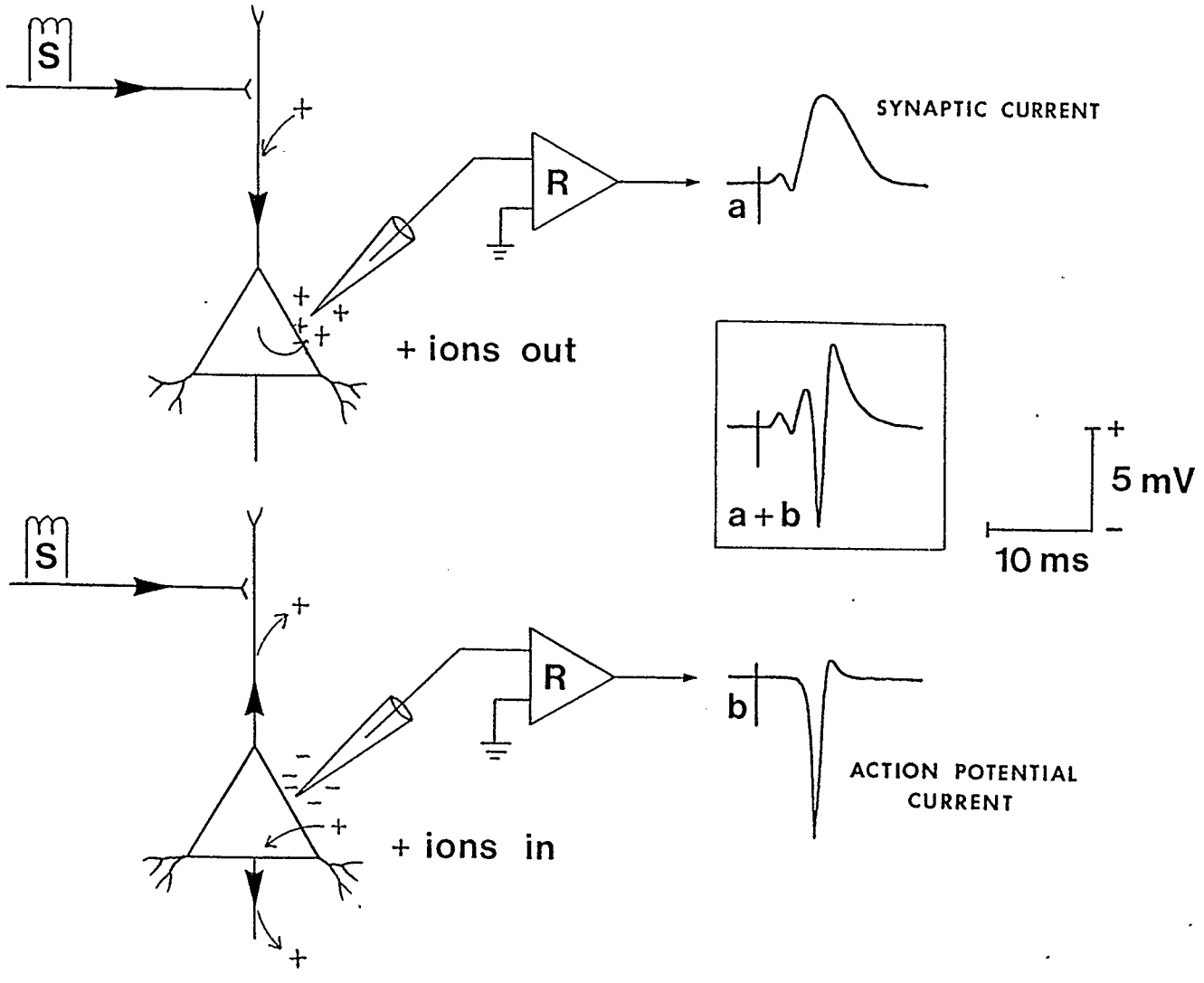
4. SIGNIFICANCE

The results will help to determine the cellular basis for the continuum of excitatory and depressant actions produced *in vivo*; and may provide evidence for multiple sites and mechanisms of action for anaesthetics in the mammalian CNS.

5. PROCEDURE

This study involved an electrophysiological investigation of anaesthetic agents on synaptic transmission in the rat hippocampal formation. Dose-response profiles for pentobarbital, phenobarbital, halothane, enflurane and isoflurane were determined on three synpatic pathways and two neuron types. Synaptic responses were measured by analysing field potential recordings (see Figure 3) following electrical stimulation of well characterized afferent pathways in the hippocampal formation (see METHODS). Particular interest was given to excitatory and depressant anaesthetic actions, in the context of a concentration-dependent continuum of effects.

Figure 3 - The generation of field potentials by CA 1 neurons results from ionic current flow across dendritic and somal membranes. Stimulation (S) of afferent fibers causes positive ions (Na and Ca) to move into dendrites, and out again across the somal membrane, where it produces a local positive field potential (field EPSP, a - upper), recorded (R) in the cell body layer of the CA 1 area. If the resulting depolarization reaches threshold, then CA 1 discharge results in the rapid flow of positive ions into cell bodies (lower), producing a local negative field 'action' potential (b). The temporal sum of these two potentials (a + b) exhibit components of both synaptic input and postsynaptic discharge (see Figure 5).


S
R
SYNAPTIC CURRENT
a
+ ions out
a+b
+
5 mV
–
10 ms
S
R
b
ACTION POTENTIAL
CURRENT
+ ions in

6. SPECIFIC OBJECTIVES

The following objectives were undertaken to demonstrate concentration dependent excitatory and depressant anaesthetic actions on CNS neurons:

6.1 To determine whether anaesthetics produce concentration dependent excitatory and depressant effects on synaptic transmission.

a) Anaesthetic actions on synaptically evoked field potentials from three pathways were studied.

i) Log dose-response curves for field potential amplitudes (see DATA ANALYSIS) were generated, to determine whether increases and/or decreases in evoked responses were concentration dependent.

ii) Stimulus-response relationships were used to determine whether anaesthetic actions were dependent on level of activity, and to provide data for subsequent input/output analysis.

iii) Input/output analysis of synaptic systems was studied by examining the relative amplitudes of the field excitatory postsynaptic potential (EPSP) and population spike (PS) at different stimulus intensities. This provided an indication of anaesthetic sites of action (synaptic vs.

postsynaptic) and mechanisms (see DATA ANALYSIS).

b) Equi-effective concentrations and membrane/buffer partition coefficients were compared to determine whether a 'Meyer-Overton' correlation exists for mammalian CNS neuronal responses to anaesthetics.

6.2 Anaesthetic actions on antidromic excitability were studied, to provide a control for drug-induced alteration of electrical excitability (postsynaptic), and to determine whether volume conduction characteristics of the preparation (or medium) contributed to anaesthetic-induced changes in synaptically evoked field potentials.

a) Threshold for antidromic field excitation was studied in the absence and presence of anaesthetics. These data, together with results from the input/output analysis described above, provided evidence for direct anaesthetic actions on electrical excitability.

b) Log dose-response curves for antidromic field potential amplitudes were compared with profiles for synaptically evoked responses to determine whether non-synaptic alterations contributed to anaesthetic actions on synaptic transmission.

6.3 Concentration-response curves for each anaesthetic on the three synaptic pathways were compared to determine if:

a) the pathways demonstrated different sensitivities to the drugs, and b) whether the anaesthetics shared common profiles of action. Concentrations which included the estimated clinical range for producing anaesthesia were studied for comparison with *in vivo* effects.

The experimental objectives described above should provide an adequate basis to determine whether more than a simple blockade of synaptic transmission is involved in anaesthetic actions in the hippocampal formation.

7. RATIONALE

7.1 Hippocampal brain slice as a model system

The hippocampal formation was chosen for this study because of its well characterized anatomy and physiology (Storm-Mathisen, 1977; Andersen, 1975; Teyler and DiScenna, 1984; see Figure 2), and previously described sensitivity to anaesthetics (Richards, 1978). This preparation also permitted a comparison of anaesthetic actions on different cell types (ie: pyramidal and granule neurons). The hippocampal formation is one of the most extensively studied regions in the mammalian CNS; so normal physiological responses were more easily characterized (Schwartzkroin and Knowles, 1983). The synaptic organization appears to be representative of other cortical regions; particularly with respect to input/output stratification, lateral inhibition, and neurotransmitters used by the various afferent systems and

interneurons in both neocortex and hippocampus (Storm-Matheson, 1977; Schwartzkroin, 1983). The highly organized laminar structure of the hippocampus allows preservation of input/output fibers and synaptic integrity in 200 to 400 μm thin slices of tissue. Thin slices are necessary for maintaining the viability of the tissue *in vitro* and also permits free access of anaesthetics to neurons.

7.2 Choice of anaesthetics for this study:

Different anaesthetics produce markedly dissimilar profiles of effects on animal behaviour and nervous system activity. Even the clinical end point produced by these drugs (ie: loss of response and unconsciousness) may be quite different (Winters, 1982; Deady et al., 1986). It is not clear, however, whether these differences are due to differential drug distribution and kinetics or to dissimilar actions at the cellular level. In the current literature, there appears to be a common bias towards a universal depressant action for all anaesthetics at the cellular level. Futhermore, anaesthetic actions on different types of neurons and synaptic pathways in the CNS have not been systematically investigated.

The five anaesthetics chosen for this study should provide a good representative sample of agents, which are of therapeutic interest, from the two major classes of currently used general anaesthetics. Pentobarbital and phenobarbital produce different clinical profiles, and opposite effects have been described on isolated preparations (Judge, 1983). Enflurane has been shown to produce excitation and

firing pattern alterations on invertebrate neurons and produces burst firing and seizure activity in the CNS (Stevens et al., 1984; Weston and Roth, 1986). Halothane and isoflurane are the most commonly used volatile agents for clinical anaesthesia and have been studied in a number of investigations on both invertebrate and vertebrate neurons (eg: Yoshimura et al., 1985).

In addition, the stereoisomers of pentobarbital were also studied and together with the data from the two structural isomers (enflurane and isoflurane) allow more detailed analysis of differential site selectivity. Structural and stereoisomers of anaesthetics would be least likely to demonstrate differential actions according to the Unitary Theory, and, therefore, provide the most sensitive test of their validity.

8. METHODS

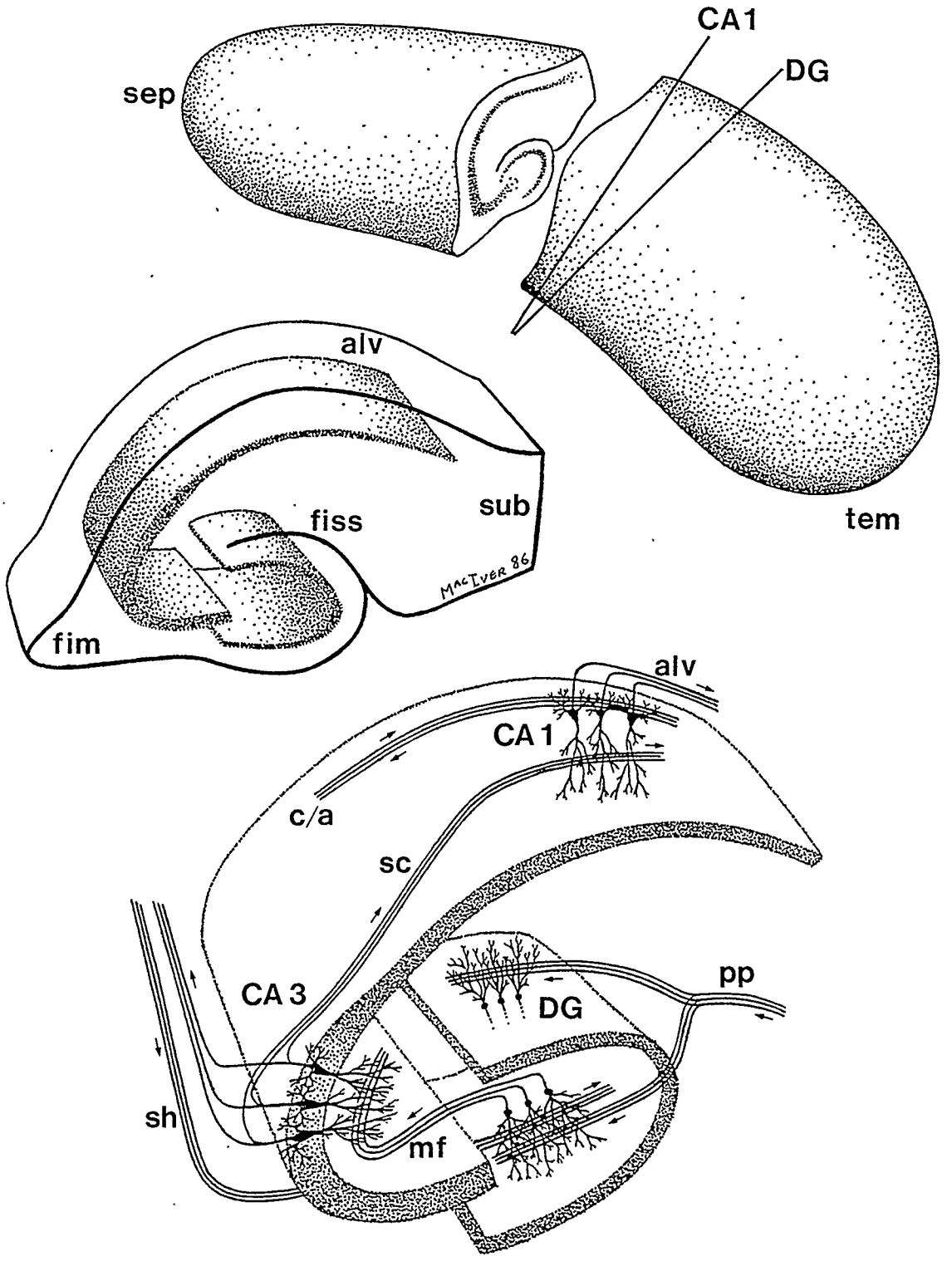
The hippocampal brain slice preparation provides an ideal model system for investigating synaptic actions of anaesthestics on mammalian CNS neurons. This preparation has been well characterized and electrophysiological responses recorded from the *in vitro* hippocampus are similar to responses seen *in vivo* (Teyler, 1984; Schwartzkroin, 1982). The major advantage of the *in vitro* preparation is that it permits accurate control of drug concentrations and limits possible sites of action. Techniques for the preparation of brain slices and maintainance of this tissue *in vitro* have been recently reviewed (Kerkut and Wheal, 1982); details of the specific methods used in the present study are provided below.

8.1 Dissection

Rats were anaesthetized with diethyl ether and the heart was stopped by a blow to the back of the thorax, to minimize bleeding in subsequent stages of the dissection. Scissors were used to remove the overlying scalp and the cranium was fractured along the midline by inserting one blade of the scissors through the foramen magnum. A small pair of Rongeurs was used to remove the two halves of the cranium, and the dura was carefully removed with a scapel. The olfactory nerves were severed and a sharpened stainless steel spatula was inserted between the cerebellum and striate cortex and used to gently lift and separate the brain from the medulla oblongata and spinal cord. The brain was rapidly removed and placed in a petri dish

containing oxygenated artificial cerebral spinal fluid (ACSF) solution (approximately 25 ml; at 10 °C; see: Materials for ACSF composition), the solution was pre-cooled to slow metabolism and increase oxygen solubility. The brain was then transected at the midline and the right hemisphere placed on moistened filter paper. The hippocampal-dentate formation was dissected by separating the alveus from the overlying neo-cortex, along the lateral ventricle, and cutting attachments to entorhinal cortex and the basal ganglia. Two sharpened stainless steel spatulas were used to gently separate the sides of the lateral ventricle, and care was taken not to touch the hippocampal formation during this procedure. A small block of tissue from the middle third of the hippocampus was isolated using a pre-cleaned (degreased) scapel blade, and mounted on a teflon stage with a thin layer of cyanoacrylate adhesive. Care was taken to ensure that orientation of the isolated block of tissue was parallel with fibers on the alveus surface, to perserve the "on beam" alignment of synaptic pathways. The angles used to prepare slices for studies of CA 1 vs. DG pathways are shown in Figure 4; and were approximately 5 to 10 degrees on either side of the striation pattern of alveus fibers (Andersen et al., 1971). Transverse slices of hippocampus (250 to 600 μM) were made using a vibratome (Campden Instruments, U.K.) while the tissue was submerged in pre-cooled and oxygenated ACSF solution. Slices of 400 μM thickness provided the most consistent and stable responses and were used for all studies of anaesthetic effects. Five or six slices were transfered to the recording chamber using a wide mouth glass pipette to minimize mechanical disturbance of the tissue. Brain

Figure 4 - Diagram of the rat hippocampal formation showing the angle used to prepare slices for CA 1 pyramidal and dentate granule (DG) synaptic pathways. Stimulating electrodes were placed in stratum oriens, to activate commissural/associational (c/a), or stratum radiatum, to activate Schaffer-collateral (sc) inputs to CA 1 pyramidal neurons. Dentate granule evoked field potentials were produced by stimulating perforant path (pp) fibers. Antidromic responses were produced by stimulating the alveus (alv) or mossy fiber (mf) pathways, for CA 1 or DG responses, respectively. Abbreviations: sep - septal, tem - temporal, fim - fimbria, fiss - hippocampal fissure, sub - subiculum, sh - septo-hippocampal fibers.


CA1
DG
sep
tem
alv
sub
fiss
MacIver 86
fim
alv
CA1
c/a
sc
pp
CA3
DG
sh
mf

slices were maintained at the gas/liquid interface on nylon mesh screens in a McIlwain tissue chamber at 35 °C (Richards and Tegg, 1977). This chamber was kindly provided by Dr. C.D. Richards (Royal Free Hospital, London, U.K.). It was modified by incorporating a second connecting chamber for removal of perfusate, to stabilize the flow rate. In addition, the internal electronic heating system was replaced by a circulating water bath (Haake) controlled by a Yellow Springs, Inc. thermistor feedback system.

The entire dissection procedure required less than 10 minutes, and the tissue was in pre-cooled ACSF for most (> 8 min) of this time. It was generally found that careful dissection procedures and gentle treatment of the slices was prerequisite to optimal survival; the time required to complete the procedure was of secondary importance. Slices were allowed to equilibrate for at least 1 hour prior to recording, and during this time period they were perfused for 2 or 3 minutes every 20 minutes. ACSF solution levels were adjusted so that the entire upper surface of the slices were exposed to warmed, humidified oxygen and carbon-dioxide (95/5 %; carbogen). The flow rate of carbogen was accurately controlled with a pressure regulator and flowmeter and maintained at 3 to 4 L/min. Following the equilibration period, oxygenated, prewarmed, ACSF or drug containing solutions were continuously perfused through the bath at a flow rate of 0.5 to 1 ml/min. At this time, slices appeared translucent and the mesh screen below them was readily visualized; unfortunately, the boundaries between cell bodies and fiber tract regions were less apparent than

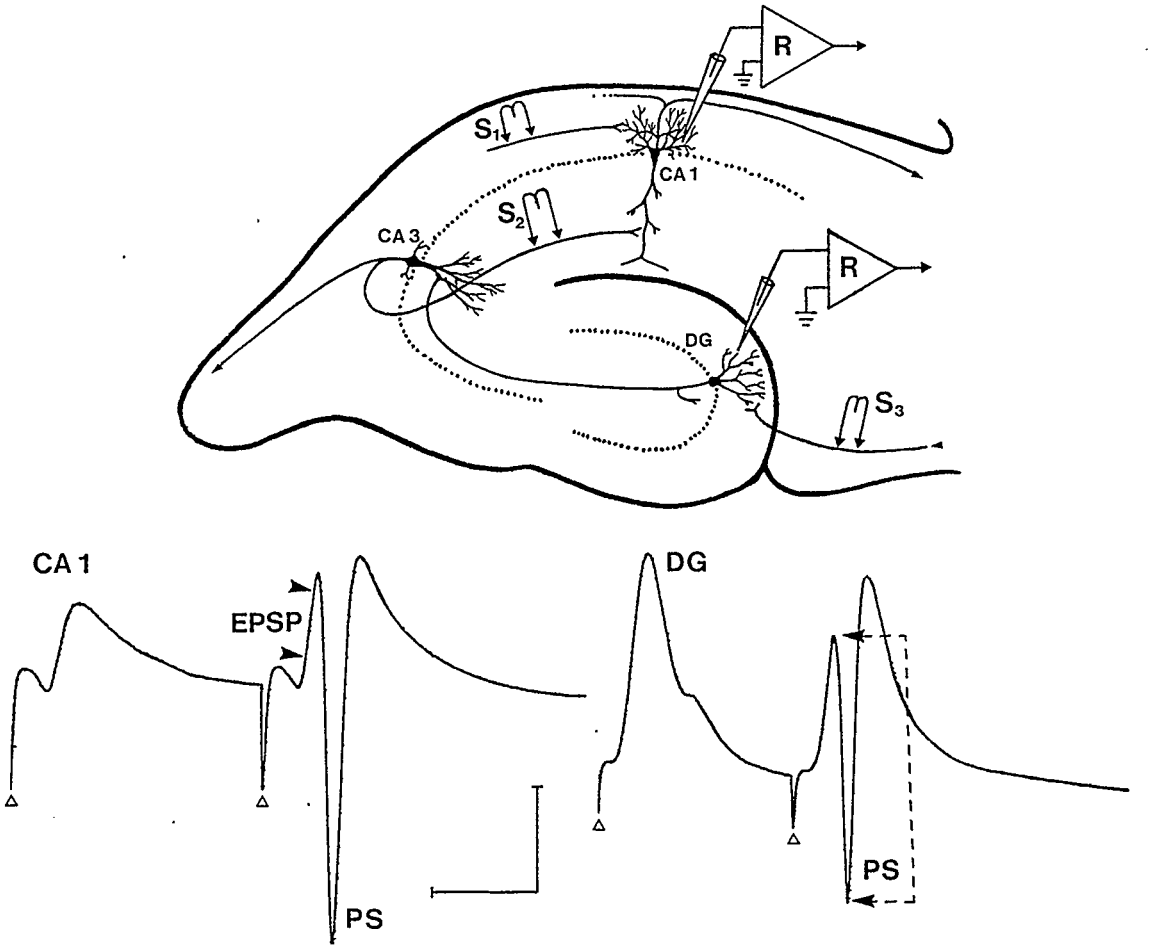
when the slices were first placed in the chamber. At optimal solution levels, the surface of the tissue glistened with a thin layer of moisture, and this was monitored during the course of the experiment to ensure that the surface did not dry out.

8.2 Electrode Placement

Bipolar metal stimulating electrodes were kindly provided by Dr. Q. Pittman (Univ. of Calgary), and made from parallel nichrome wires (22 guage) insulated to within 0.5 mm of the tips. These electrodes were placed on perforant path fibers, or in Str. Radiatum or Oriens and used to activate excitatory synaptic inputs to dentate granule and CA 1 pyramidal neurons respectively (see Figure 5). Antidromic responses were produced by stimulation of the alveus or mossy-fiber pathways. Recording electrodes were made from 'Kwik-Fil' glass capillaries (W-P Instruments, Inc., New Haven, Conn.). These were pulled using a Narashige (Japan) model 5 pipette puller and backfilled with 2 M NaCl; tips were broken off to provide resistances of 2 to 10 Mohm. Electrodes were placed close to the cell body or dendritic regions of granule and CA 1 neurons and used to record synaptically evoked field potentials (FP). Only one stimulating and recording configuration was used for a given experiment, unless specifically noted. All electrodes were mounted on Narishige (Japan) micromanipulators and positioned under visual control. Recording electrodes were coupled to the micromanipulator by a pizoelectric driven Inchworm System (Burleigh Instruments Inc., Fishers, N.Y.) which allowed fine control of electrode depth (2 μ steps).

Figure 5 - Upper - Diagram showing the placement of recording (R) and stimulating electrodes on stratum oriens (OR; S1), stratum radiatum (RAD; S2) and perforant path (PP; S3) fibers. Recording electrodes were placed either in the dendritic layers (radiatum, moleculare; eg: DG) or cell body layers (pyramidal, granulare; eg: CA 1) to record dendritic field EPSPs or somal EPSP and PS responses.

Lower - Slope of EPSP (arrowheads) and amplitude of population spike (PS; dashed arrows) were measured on field potentials recorded from somal layers of CA 1 and DG. Open arrowheads indicate paired pulse stimulus artifacts; note that potentiation occurs in response to the second stimulus. Calibration: 5.0 mV and 10 ms.


R
S1
CA1
S2
CA3
R
DG
S3
CA1
EPSP
PS
DG
PS

8.3 Stimulation and Response Parameters

Paired stimulus pulses of 0.01-0.05 ms duration (0.5 to 20 V; 25 to 80 μA) were delivered at 0.1 Hz, using a Grass S-88 stimulator and stimulus isolation unit. Stimulus intensity was varied to determine input/output relationships between field EPSP and PS. These relationships have been termed E-S (EPSP-Spike) curves in previous studies (Andersen et al., 1980; Abraham, et al., 1985). In some experiments, stimulation currents were measured using a Grass constant current unit in series with the stimulator and isolation unit. Interpulse interval delays were varied between 5 and 120 ms, in 5 ms increments, to examine the time course of short-term potentiation as a measure of synaptic facilitation and postsynaptic inhibition. Recorded signals were amplified (x 100) and filtered (1 Hz to 10 KHz, bandpass) using a Grass P-15 preamplifier and further amplified (x 20) and conditioned for DC offset to optimize the input requirements of a Digital Equipement Corp. PDP 11/23 minicomputer. Signals were digitized, using a 12 bit A/D converter with a resolution of 40 μs, and stored for later analysis. Data were viewed on oscilloscopes (Tektronix, 565 and 5113 series) immediately after preamplification, and also after digital conversion to insure minimal attentuation and alteration of stored recordings.

8.4 Field Potential Analysis

When a localized population of neurons is synchronously activated by a common synaptic pathway, the summed charge movement across their

membranes produces a local voltage change which can be recorded with extracellular electrodes placed near the membrane regions generating the current(s) (Andersen et al., 1971; see Figure 3). These local voltage changes are known as 'field potentials' and have been used extensively to characterize synaptic responses in both the hippocampal formation (Teyler, 1984) and other cortical regions (Richards, 1982).

Field potential recordings offer several advantages over traditional single unit and/or intracellular recordings; the most important advantage is that the response is graded, in contrast to the 'all or none' discharge of an individual neuron. As more afferent fibers are activated, field EPSP size gradually increases and an increasing number of postsynaptic neurons discharge, contributing to a larger population spike. The relationship between the field EPSP and PS amplitude, at various stimulation intensities, defines an input/output or transfer function for the given synaptic pathway. This relationship provides a direct measure of discharge threshold and also allows discrimination of drug effects on synaptic input vs. postsynaptic excitability of the neuronal population. Thus, drug effects on the PS can be determined relative to alterations of the EPSP; if the PS is depressed and no change in EPSP amplitude occurs, then the drug must act to depress postsynaptic excitability. Combinations of effects on synaptic input and neuronal excitability can be readily discriminated (see: RESULTS), an advantage not shared by the other electrophysiological approaches mentioned above. In addition, field potential responses are stable for long time periods and reflect the averaged

activity of many neurons, thus reducing experimental variability. One final advantage of field potential recordings is they provide a measure of brain slice viability when compared with in vivo responses (Teyler, 1984); single unit and intracellular responses have not been as well characterized on both intact and isolated preparations of the hippocampal formation.

Field potentials were recorded from the cell body layers (stratum pyramidal, stratum granulare) or dendritic zones (stratum radiatum, stratum moleculare) to allow better discrimination between field EPSPs and population spikes. Population spike amplitudes were measured from threshold (first positive peak) to the peak negativity (Figure 5) and expressed in mV. EPSP slopes were determined by linear regression through data points collected between 20 and 80 percent of the initial positive going wave form (see Figure 5). A minimum of 8 and mean of 12 to 15 points contributed to the regression analysis and data expressed as dV/dt in Volts/second.

Data were stored and analyzed under UNIX on a PDP 11/23 computer. Most programs for data acquistion and analysis were developed in this laboratory with the exception of some standard UNIX utilities for numerical management and statistics. Input/output curves were generated by plotting EPSP slopes vs. PS amplitudes to compare drug effects on synaptic and postsynaptic response (see RESULTS). Paired pulse latency curves were generated by plotting interstimulus intervals vs. PS amplitudes to determine anaesthetic effects on presynaptic facilitation and postsynaptic inhibition. Concentration-response

curves were plotted as a percent change of control PS amplitudes for both orthodromic and antidromic responses. A minimum of 3, and often 4 to 6, determinations at each concentration were averaged and each point represents the mean +/- standard error of the mean (SEM). Half-maximal (ED 50) concentrations were determined from concentration-response curves and used to compare potencies of the anaesthetics and relative sensitivity of synaptic and antidromic responses for each pathway.

8.5 Experimental Procedure

Initially, field potential responses were recorded from the exposed surface of slices. Recording position, stimulus intensity and interpulse intervals were varied to produce a response showing good paired pulse potentiation, maximum amplitude, and reproducibility. Once a suitable position was found, the recording electrode was slowly advanced (2 μ steps/min) to optimize response amplitude; a recording depth of 80 to 120 microns from the surface produced optimal responses. Slice preparations usually required 1 to 2 hours to stabilize, following placement in the recording chamber. At this time, FPs of up to 30 mV (peak to peak) could be repeatedly elicited using current intensities of approximately 60 to 100 μA. A FP amplitude of 5 mV was arbitrarily chosen as the minimum acceptable response for all drug experiments. Control data for input/output curves and latency plots were acquired by varying stimulus parameters as previously described. Stimulus intensity was set just below threshold for a PS on the first of the two pulses for latency and concentration-response

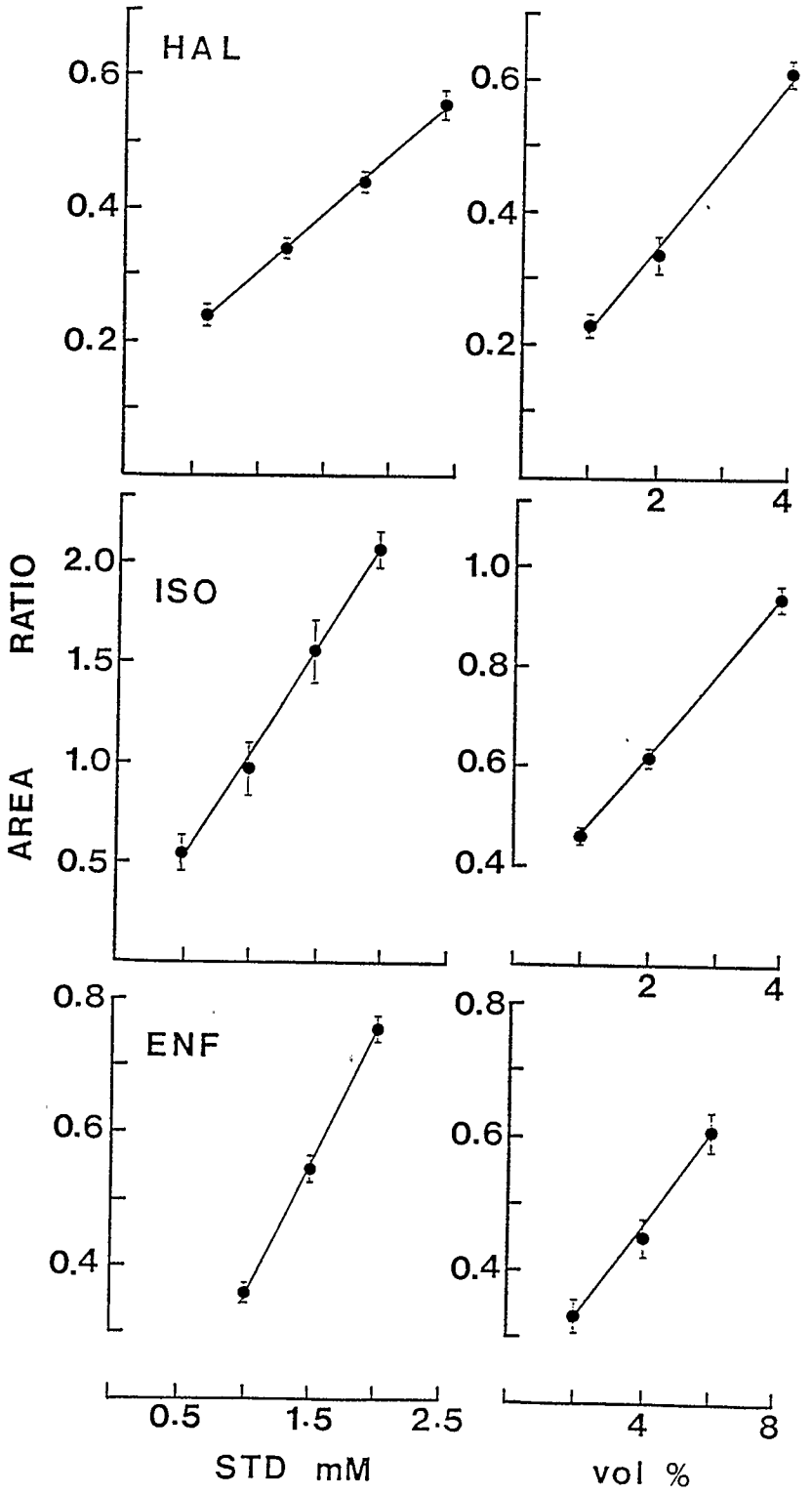
data. Control ACSF was replaced by solutions containing anaesthetic (usually beginning with low concentrations) and the recording protocol repeated. For most experiments with the volatile anaesthetics, however, control ACSF was continually perfused and the volatile agent was administered via calibrated commercial vaporizers and mixed with the O_2/CO_2 gas stream above the slices. Drug actions were reversed by washing with control solution until the responses returned to pre-drug values. Anaesthetic effects which could not be reversed were not included in subsequent analyses.

8.6 Analysis of Volatile Anaesthetic Concentrations

Inhalation agents were applied as vapours via the prewarmed and humidified O_2/CO_2 gas stream above the brain slices, using calibrated commercial vaporizers. Concentrations, expressed as volume percent (vol %), refer to settings on the vaporizer dials. To reach equilibrium, anaesthetics were applied for a minimum of 30 minutes. Onset times for anaesthetic effects were often less than 1 minute and maximum effects reached a plateau within 5 to 10 minutes. Concentrations of volatile anaesthetics in the perfusate and gas mixture above the slices were determined using gas chromatography. Temperatures for the injector, oven and detector were 155, 140 and 170 °C, respectively. A flame-ionization detector was used and the carrier gas was nitrogen at a flow rate of approximately 40 ml/min.

Aqueous test samples (20 µl) were taken from the recording chamber with a gas tight Hamilton syringe and injected directly onto

Figure 6 - Graphs showing the relationship between anaesthetic concentration and peak area ratios measured using GC analysis. Perfusate concentrations of halothane (HAL), isoflurane (ISO), and enflurane (ENF) were determined by comparing peak area ratios (AREA) for bath samples (vol %) and known standards (STD). Each point represents the mean +/- SEM for at least 7 determinations, and linear regression lines are shown.


HAL
ISO
ENF
AREA RATIO
0.6
0.4
0.2
2.0
1.5
1.0
0.5
0.8
0.6
0.4
0.5
1.5
2.5
STD mM
2
4
8
vol %

the column. To determine volatile anaesthetic concentrations the peak areas associated with recording chamber samples were compared with areas determined from known standards. Values were determined as peak area ratios relative to dextrose, which was used as an internal standard in the ACSF. Column retention times were approximately 3 to 5 min for the inhalation anaesthetics, and 7.5 min for dextrose. Plots showing the relationship between vaporizer setting and determined concentrations are provided in Figure 6. The scales on each calibrated vaporizer appeared to be ideal for the effective concentration range to produce effects in the present study, including complete depression of synaptic transmission.

8.7 MATERIALS

Adult male Sprague-Dawley rats (250 to 300 gm) were obtained from the University of Calgary Medical Vivarium. The drugs used in this study were obtained from the following suppliers. Laboratory grade diethyl-ether was obtained from Fisher Scientific Ltd. (Calgary, Alta.). Pentobarbital was obtained from BDH Chemicals (Ont.); the R(+) and S(-) stereoisomers of pentobarbital were synthesized by Dr. M.H. Benn from the Department of Chemistry at the University of Calgary. Phenobarbital was obtained from BDH Chemicals (Ontario), halothane from Halocarbon (Ontario) Ltd. (Mississauga, Ont.), enflurane and isoflurane were obtained from Ohio Medical Anaesthetics (Pointe Claire, Que.). The vaporizers used to deliver volatile agents were provided by the Foothills Hospital Anaesthesia Department;

manufactured by Ohio Medical Products (Madison, Wis.).

The ACSF physiological solution had the following composition (in mM): NaCl - 134, KCl - 3.5, $CaCl_2$ - 1.2 KH_2PO_3 - 1.25, $MgSO_4$ - 2, $NaHCO_3$ - 16, Glucose - 10. The original formulation was that of Richards et al. (1974) and modifications of Ca and K levels were made to conform more closely to measured levels in vivo (Krnjevic et al., 1982). All chemicals were reagent grade or better and solutions were made from water which was pre-filtered to 18 Mohm using a Millipore Super Q system. ACSF and drug solutions were made fresh before each experiment and were stored at approximately 10 $^{\circ}$ C in an ice bath. Solutions were saturated with 95% oxygen 5% CO_2 and prewarmed to 35 $^{\circ}$ C immediately before entering the recording chamber; pH was approximately 7.0 to 7.4 at 35 $^{\circ}$ C.

Carbogen (O_2/CO_2) and the specialty gases for GC analysis were obtained from Medigas Ltd. (Calgary, Alta.) and were manufactured by Union Carbide Canada Ltd. (Edmonton, Alta.).

9.0 RESULTS

9.1.1 Control Field Potential Responses

A characteristic field potential was recorded from the cell body layers when input fibers to either DG or CA 1 cells were electrically stimulated. Weak stimuli (10 to 15 μA) produced a positive waveform (field excitatory postsynaptic potential; EPSP) which originates from the synchronous depolarization of postsynaptic neurons in response to transmitter release from input fibers (Andersen, Blackstad and Lomo, 1966; Langmoen and Andersen, 1983). When stimulation intensity was increased and/or paired pulse potentiation occurred, the positive waveform increased in amplitude and a negative spike appeared superimposed on the EPSP (compare responses to 1st and 2nd stimulus pulses in Figure 5). This negative waveform reflects the synchronous discharge of postsynaptic neurons and has been termed the population spike (PS: Andersen, Bliss and Skrede, 1971; Bliss and Richards, 1971).

Stratum radiatum to CA 1 responses usually generated the largest PS amplitudes and interstimulus intervals of 22 to 25 ms produced maximal paired pulse potentiation. Stratum oriens to CA 1 responses were always smaller in amplitude and an optimal interpulse interval of 20 to 22 ms was observed for paired pulse potentiation. Stimulation of perforant path inputs to dentate granule neurons generated the largest EPSP amplitudes, although PS responses were seldom as large as CA 1

responses (eg: Figure 5). Dentate EPSP responses demonstrated faster rise times and a shorter half-time for decay, compared with CA 1 responses, and interstimulus intervals of 16 to 20 ms produced maximal paired pulse potentiation.

9.1.2 Input/Output Analysis

The relationship between the positive synaptic potential (EPSP) and negative population spike (PS) amplitudes describes an input/output function for a synaptic pathway (Richards and White, 1975; Andersen et al., 1980; Abraham, Bliss and Goddard, 1985). The intercept on the EPSP axis provides a measure of postsynaptic discharge threshold, and the slope of the curve describes depolarization-excitation coupling (see also: Lomo, 1971; Richards, Russell and Smaje, 1975; Balestrino, Aitken and Somjen, 1986). To determine whether the anaesthetics altered postsynaptic excitability to a greater extent than synaptic function, input/output relationships for stratum radiatum and perforant path responses were studied. This method of analysis involves a comparison of the strength of synaptic input (EPSP) with the amplitude of postsynaptic response (PS; output), termed E-S curve analysis (Andersen et al., 1980). Rate of rise (dV/dt) of the field EPSP was used as a measure of synaptic strength for two reasons. First, responses are measured prior to possible alteration by postsynaptic currents contributing to discharge and polysynaptic drive (Turner, Richardson and Miller, 1985). Second, the rate of rise of the EPSP correlates better with discharge probability than EPSP amplitude and is commonly used for this type of analysis

(Abraham, Bliss and Goddard, 1985).

9.1.3 Paired Pulse Potentiation

Paired pulse responses at various interpulse intervals were studied to characterize barbiturate effects on short-term potentiation in the CA 1 and DG pathways. Stratum radiatum inputs to CA 1 neurons demonstrated marked (greater than 250 % of control) increases in field potential amplitudes produced by the second (test) pulse relative to the first (conditioning) stimulus. This degree of potentiation can result in near-maximal discharge of the CA 1 neurons in response to a subthreshold stimulus, and may contribute to information processing in hippocampal synaptic pathways (Assaf and Miller, 1981; Abraham et al., 1985). The degree of potentiation was dependent on the interstimulus interval, and an optimal interval of 25 to 30 ms was observed. A similar degree of potentiation was observed for perforant path inputs to dentate neurons with an optimal interstimulus interval of 18 to 22 ms.

In Figure 7, a comparison of optimal interstimulus intervals for orthodromic and antidromic responses is provided for stratum radiatum inputs to CA 1 pyramidal neurons. Antidromic field potential responses did not demonstrate potentiation; however, interstimulus intervals of approximately 10 ms were required to produce equivalent response amplitudes to both the conditioning and test stimuli (Figure 7). Intervals of 15 to 150 ms were found to be optimal for potentiation of EPSP responses recorded at the level of stratum radiatum

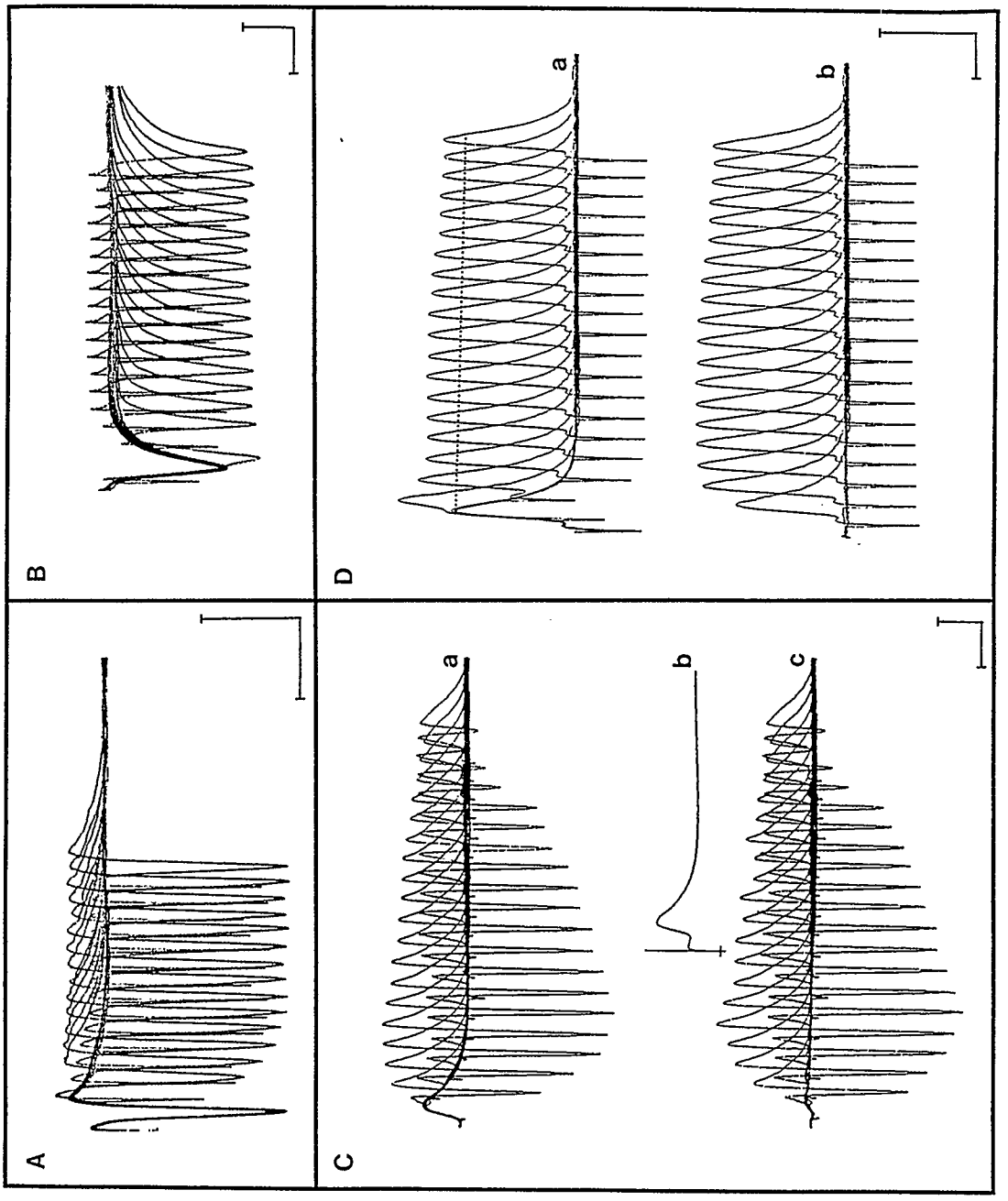
Figure 7 - Superimposed recordings at increasing interpulse intervals for:

A - Antidromic field potentials from CA 1.

B - Field EPSP responses recorded from stratum radiatum.

C - Stratum radiatum to CA 1 evoked field potentials recorded from the cell body layer. The lower plot (c) was generated by digitally subtracting the response (b) to the first stimulus pulse from each paired pulse recording, to correct for the positive deflection of the field EPSP in (a).

D - Field EPSP responses recorded from the CA 1 cell body layer. As above, recordings (b) were generated by digitally subtracting the response to the first stimulus pulse from paired pulse recordings in (a). All calibrations: 2 mV and 20 ms.


A
B
C
a
b
c
D
a
b

inputs to CA 1 apical dendrites (Figure 7), although intervals of 20 to 100 ms appeared to be optimal for EPSP responses recorded at the somal layer (Figure 7). Orthodromic PS responses were maximal at interpulse intervals of 25 to 30 ms (Figure 7). The differences observed between optimal interstimulus intervals for potentiation of EPSP and PS responses are postulated to involve early and late post-synaptic inhibition, described further in the DISCUSSION.

E-S analysis of paired pulse responses demonstrated that potentiation was associated with increased synaptic input and decreased threshold for discharge of CA 1 pyramidal neurons. Field potential responses to a series of stimuli at various intensities is shown in Figure 8, for stratum radiatum inputs to CA 1. In Figure 9, input/output curves for test pulses are compared with curves for conditioning stimuli. Paired pulse potentiation was associated with larger EPSP amplitudes in response to the test (T) pulses when conditioning (C) stimuli intensities were below levels which produced half-maximal discharge (Figure 9). In addition, smaller EPSPs were required to produce large PS responses for test stimuli relative to conditioning stimuli. Potentiation remained stable for several hours; the five series of responses shown were acquired over a 12 hour period. Conditioning stimuli at intensities strong enough to produce large PS responses on the first pulse resulted in test responses of lower amplitude (Figure 9). This inverse relationship between conditioning and test responses at higher stimulus intensities may reflect the activation of inhibitory currents which reduce the probability of

Figure 8 - Hidden-line plot of stratum radiatum to CA 1 evoked field potentials at various intensities of paired pulse stimulua-tion. Low stimulus intensities (approximately 5 to 10 V; 2 to 10 uA) produced only field EPSP responses (eg: first two sweeps). Higher stimulus intensities (10 to 50 V; 10 to 100 μA) result in both EPSP and PS responses. Following paired pulse potentiation, both EPSP and PS responses are increased, and discharge threshold appears to be decreased (compare PS threshold, on first vs. second pulse).

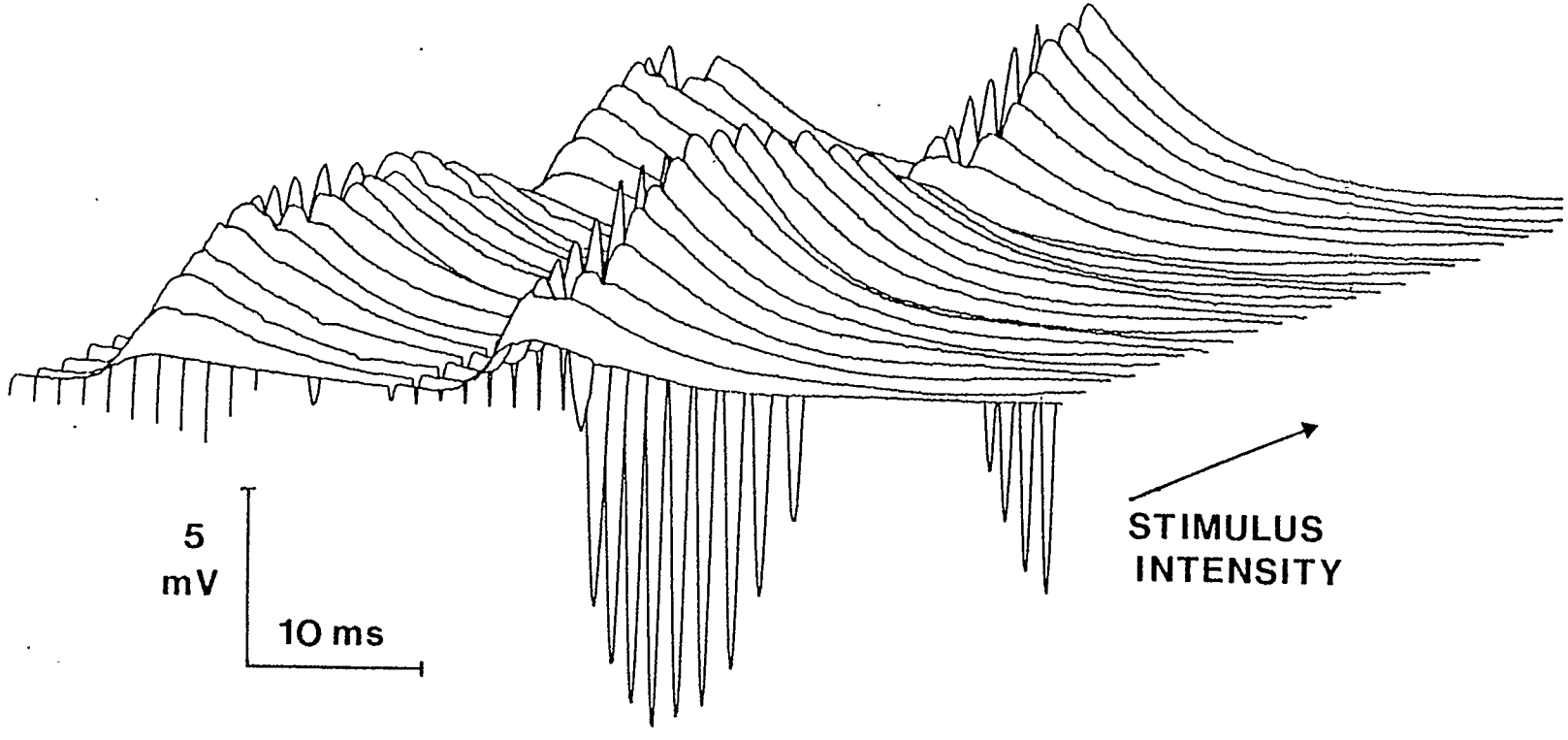
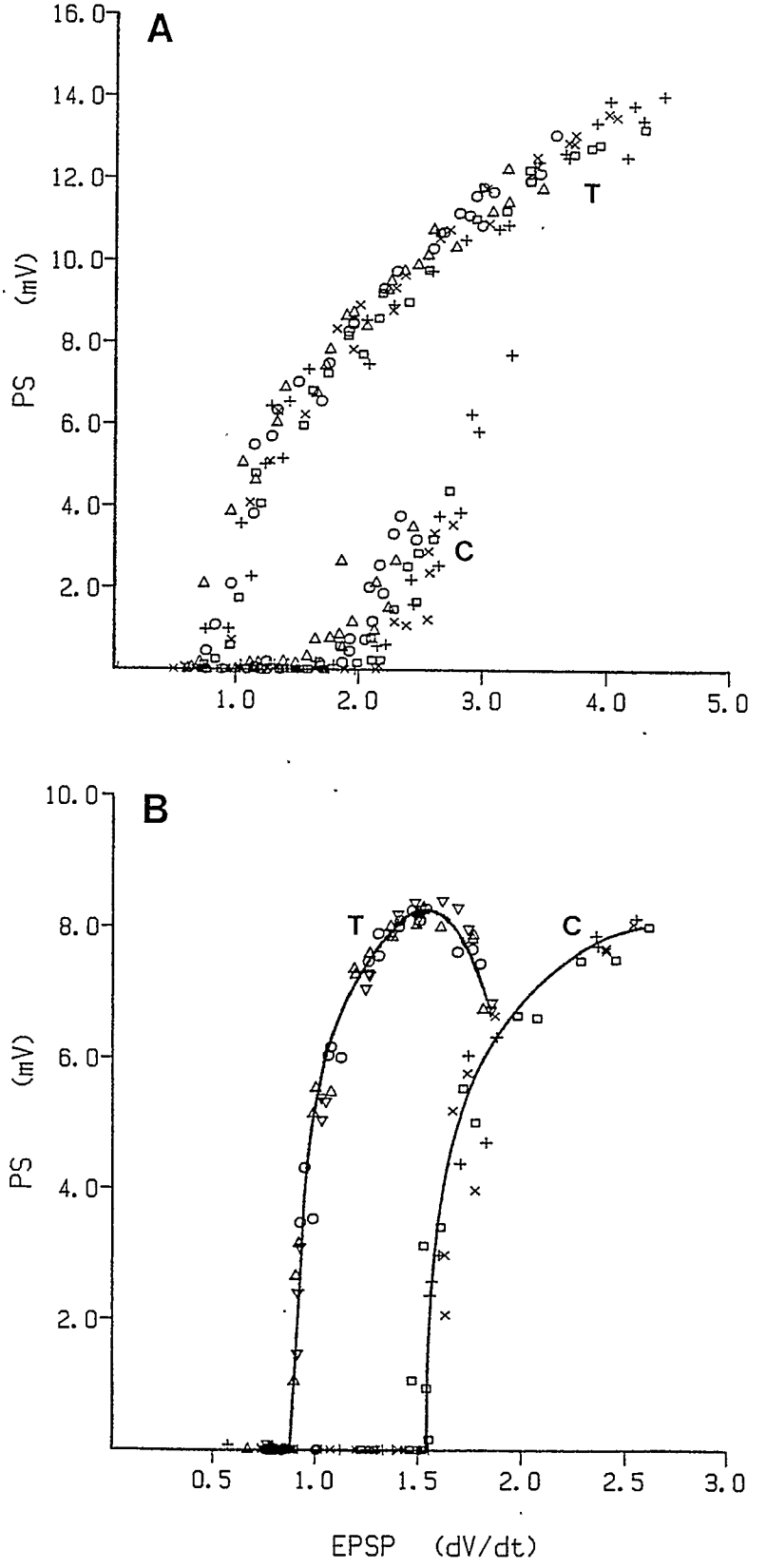

5
mV
10 ms
STIMULUS
INTENSITY

Figure 9 - A - E-S curves showing the relationship between EPSP dV/dt (Volts/second) and population spike amplitudes for the conditioning (C) and test (T) stimuli of stratum radiatum fibers to CA 1 pyramidal neurons. Symbols: (+) 1 hour, (x) 2 hours, (○) 5 hours, (□) 8 hours, and (△) 12 hours after preparation of slices.

B - PS responses to test (T) stimuli were attenuated when the conditioning (C) stimuli were of sufficient intensity to produce population spikes greater than 5.0 mV in response to the first pulse (C). Data of A and B are from different slices.


A
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
PS (mV)
T
C
1.0
2.0
3.0
4.0
5.0
B
10.0
8.0
6.0
4.0
2.0
PS (mV)
T
C
0.5
1.0
1.5
2.0
2.5
3.0
EPSP (dV/dt)

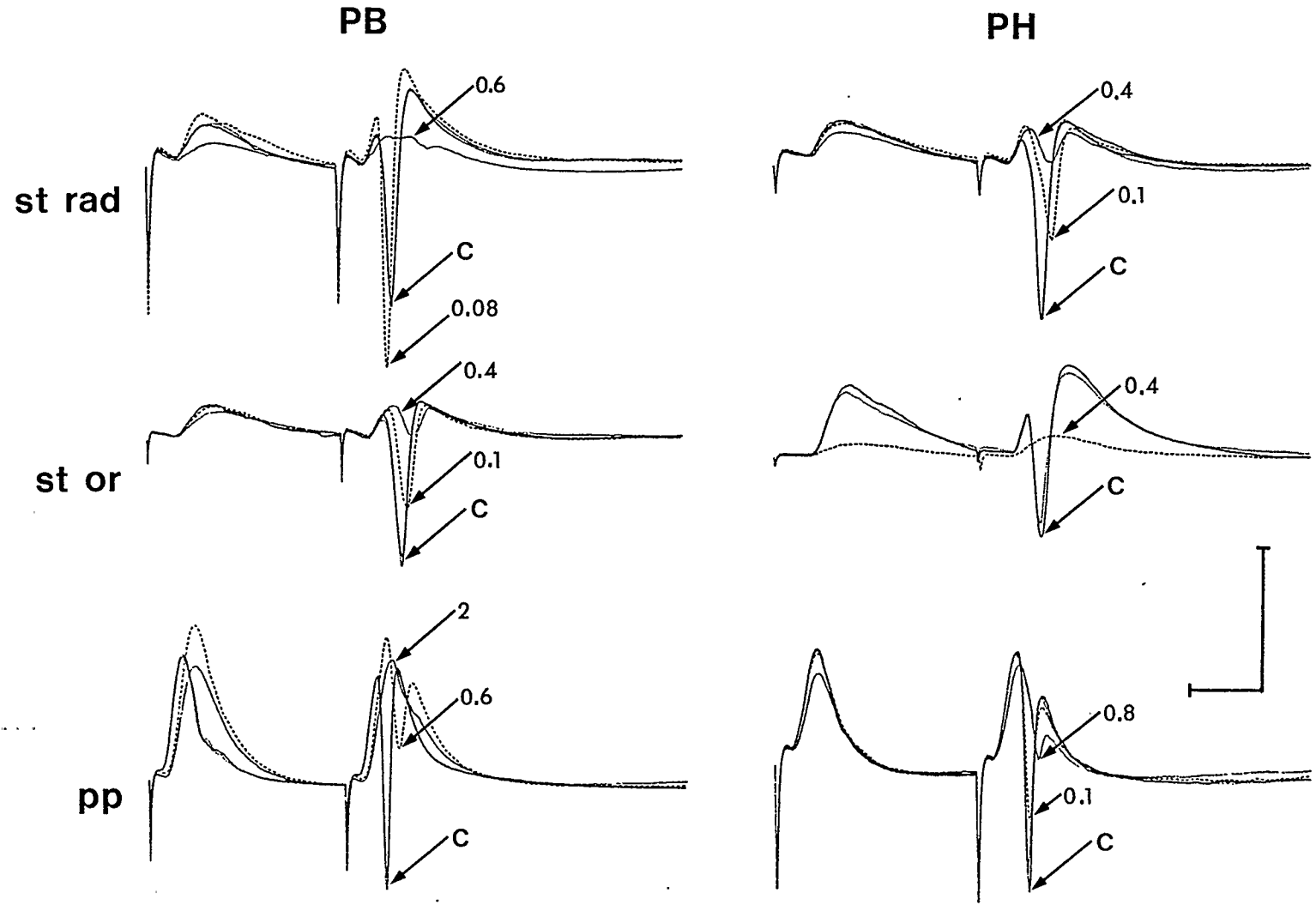
discharge in response to the second pulse (Creager et al., 1980; Gribkoff and Ashe, 1985).

9.2 Barbiturates

9.2.1 Effects on Field Potentials

The effects produced by racemic pentobarbital (PB) on field potential recordings from stratum radiatum and oriens inputs to CA 1 pyramidal neurons, and perforant path inputs to dentate granule neurons are shown on the left side of Figure 10. In the presence of low concentrations of pentobarbital (dashed lines in Figure 10) EPSP amplitudes were increased for both stratum radiatum to CA 1 (0.02 to 0.1 mM) and perforant path to dentate (0.04 to 0.6 mM). Increased synaptic responses to the first (conditioning) stimulus of the paired pulse recordings shown in Figure 10 were clearly evident. The enhanced EPSP amplitude in dentate was associated with an increased latency to peak of the EPSP and a prolonged EPSP duration, but CA 1 responses demonstrated a decreased latency to peak and only a slight increase in EPSP duration. Concentrations less than 0.1 mM did not alter EPSP responses for stratum oriens inputs to CA 1 neurons. Concentrations above 0.2 mM decreased EPSP amplitudes for both inputs to CA 1, in response to the first stimulus pulse. Concentrations of pentobarbital above 1.0 mM also decreased perforant path input to dentate EPSP amplitudes, however, the duration of the EPSP remained prolonged.

Figure 10 - Field potential recordings from stratum radiatum (st rad), oriens (st or) inputs to CA 1 and perforant path (pp) inputs to dentate neurons. Recordings in the presence of pentobarbital (PB) or phenobarbital (PH) were superimposed on control recordings (C; before - solid line and after washout - dotted line). Concentrations shown are in mM. Calibration: 10 mV and 10 ms.


PB
PH
st rad
st or
pp
0.6
C
0.08
0.4
0.1
C
2
0.6
C
0.4
0.1
C
0.4
C
0.8
0.1
C

The pentobarbital-induced enhancement of EPSP responses (0.6 mM), observed on the first pulse, did not result in larger population spike responses from dentate granule neurons following potentiation; instead, discharge was depressed (dotted line in lower recordings of Figure 10). In contrast, larger population spike amplitudes accompanied the synaptic facilitation produced by low concentrations of pentobarbital for radiatum inputs to CA 1 neurons (0.08 mM; Figure 10).

In the presence of higher concentrations of pentobarbital (0.1 to 1.0 mM), depression of population spike amplitudes was observed for all three pathways. Pentobarbital appeared to markedly depress EPSP responses from radiatum inputs to CA 1 neurons at higher concentrations, although oriens and dentate EPSP responses were not depressed to the same degree. At these concentrations, the major effect on each pathway was depression of population spike amplitudes, suggesting that postsynaptic discharge was more sensitive to depression by pentobarbital than synaptic function.

In the presence of phenobarbital (0.02 to 0.1 mM), depression of population spike amplitudes was observed for both radiatum and perforant path inputs. EPSP responses to the first stimulus pulse superimposed on control recordings (dashed lines on right of Figure 10); however, PS responses to the second stimulus pulse were depressed. In contrast, phenobarbital produced a depression of the EPSP from oriens inputs to CA 1 neurons (0.02 to 0.4 mM) and this resulted in smaller PS responses. At higher concentrations (0.1 to 1.0 mM), effects on

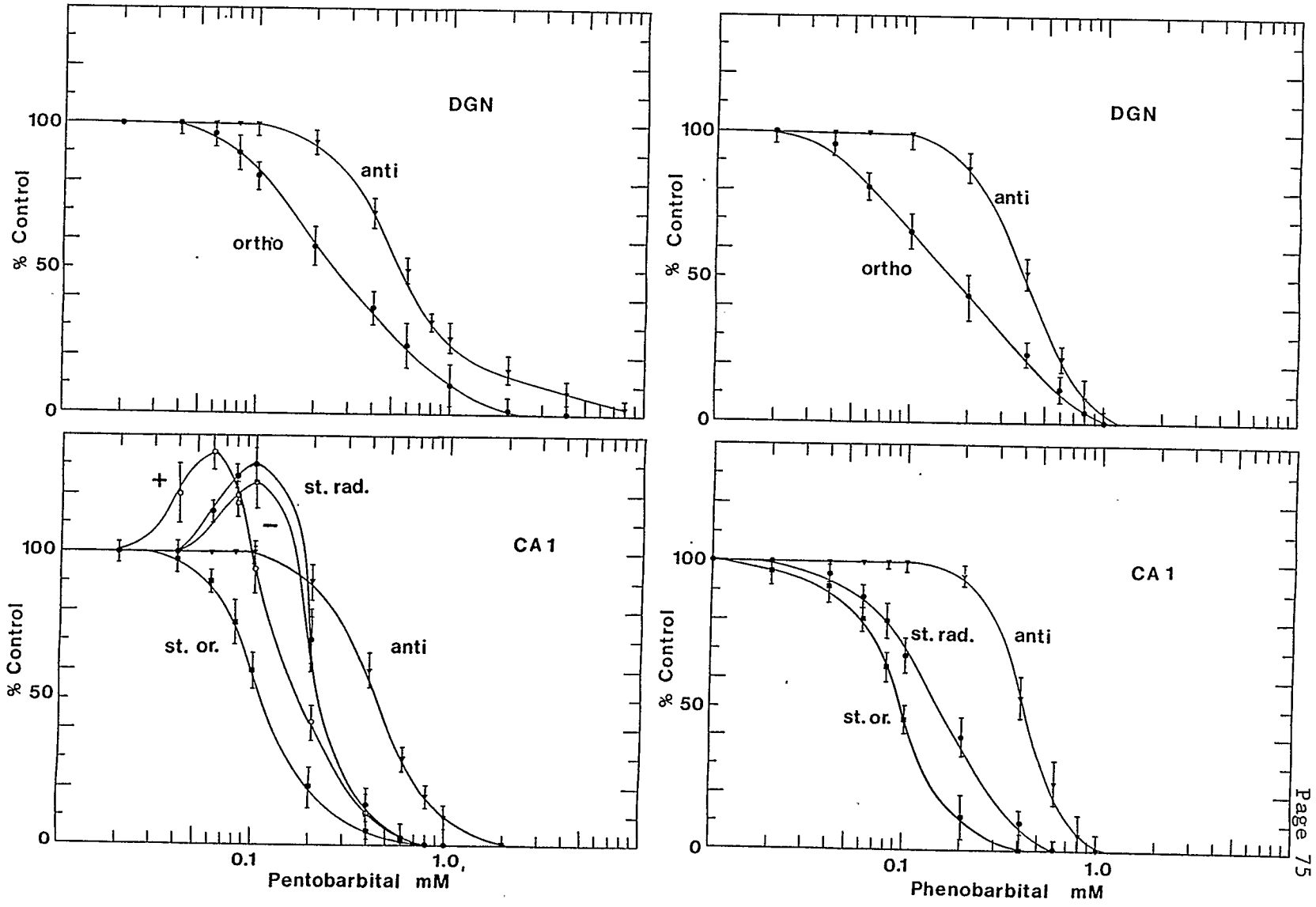
both the EPSP and PS were observed for all three pathways. The selective depression of PS amplitudes for radiatum and perforant path inputs, suggests a predominantly postsynaptic action. Synaptic events of the response from oriens inputs to CA 1, however, appeared to be the most sensitive to phenobarbital.

Both barbiturates produced effects within the time required for complete exchange of bath solutions (less than 10 min.; determined by dye studies). The time to reach maximum effect was 12 to 15 minutes. Recovery, following washout, required at least 30 minutes for the lowest effective concentrations and often 1 to 2 hours for reversal of the higher concentrations (> 0.1 mM). Wash out data are shown (light dotted lines) in Figure 10, superimposed on pre-drug control (C) recordings.

9.2.2 Concentration-Response Relationships

Concentration-response profiles for pentobarbital and phenobarbital on stratum oriens and radiatum inputs to CA 1 and perforant path inputs to dentate are shown in Figure 11. The stratum oriens-evoked population spike in CA 1 appeared to be most sensitive to the depressant actions of both pentobarbital and phenobarbital. Half-maximal depression of the oriens evoked PS responses occurred at approximately 0.1 mM for pentobarbital and phenobarbital (Figure 11). Biphasic effects (enhancement/depression) were only observed on radiatum inputs to CA 1 in the presence of low concentrations of pentobarbital. The (+) and (-) stereoisomers of pentobarbital produced qualitatively

Figure 11 - Concentration-response curves for pentobarbital and phenobarbital on population spike amplitudes (percent control) for the three hippocampal pathways, including the (+) and (-) stereoisomers of pentobarbital on radiatum inputs to CA 1. Abbreviations: ortho - orthodromic, anti - antidromic, st. or. - stratum oriens, st. rad. - stratum radiatum. Each point represents the mean +/- SEM for at least 4 determinations on different preparations.


DGN
anti
ortho
% Control
100
50
0
DGN
anti
ortho
% Control
100
50
0
+
st. rad.
−
CA 1
st. or.
anti
% Control
100
50
0
0.1
1.0
Pentobarbital mM
CA 1
st. rad.
anti
st. or.
% Control
100
50
0
0.1
1.0
Phenobarbital mM

similar effects compared with the racemic mixture, however, differences in potency and magnitude of effect were observed. On a molar basis, the (+) stereoisomer was approximately 4 times more effective in producing synaptic enhancement of radiatum inputs to CA 1 (Figure 11) compared with the (-) stereoisomer and racemic mixture.

Antidromic stimulation of CA 1 and dentate neuron axons was used to measure effects on electrical excitability for comparison with orthodromic (synaptic) responses. Depression of antidromic PS amplitudes required approximately 5 to 10 times higher concentrations compared with synaptic responses (Figure 11). In the presence of concentrations greater than 0.3 mM, effects on the synaptically-evoked PS appeared to include direct depression of electrical excitability or axonal conduction; indicated by the overlap in concentration-response curves for orthodromic and antidromic responses.

9.2.3 Effects on Input/Output (E-S) Relationships

Low concentrations of pentobarbital produced an increase in both the EPSP (CA 1 and dentate) and PS amplitude (CA 1); however, this increase was not dependent on the intensity of stimulation (Figure 12), indicating that recruitment of afferent fibers was not altered. E-S analysis was used to determine if the increase in PS amplitude was due to the observed increase in synaptic drive (EPSP). Relationships between field EPSP (dV/dt; see Methods) and PS amplitudes are shown in Figure 13, for stratum radiatum inputs to CA 1 pyramidal neurons and perforant path inputs to dentate granule cells. Increased synaptic

Figure 12 - Field potential recordings from stratum radiatum to CA 1, at indicated stimulus currents (uA), were enhanced by 0.08 mM pentobarbital. Paired pulse potentiation was not altered by pentobarbital. Calibration: 5 mV and 10 ms.

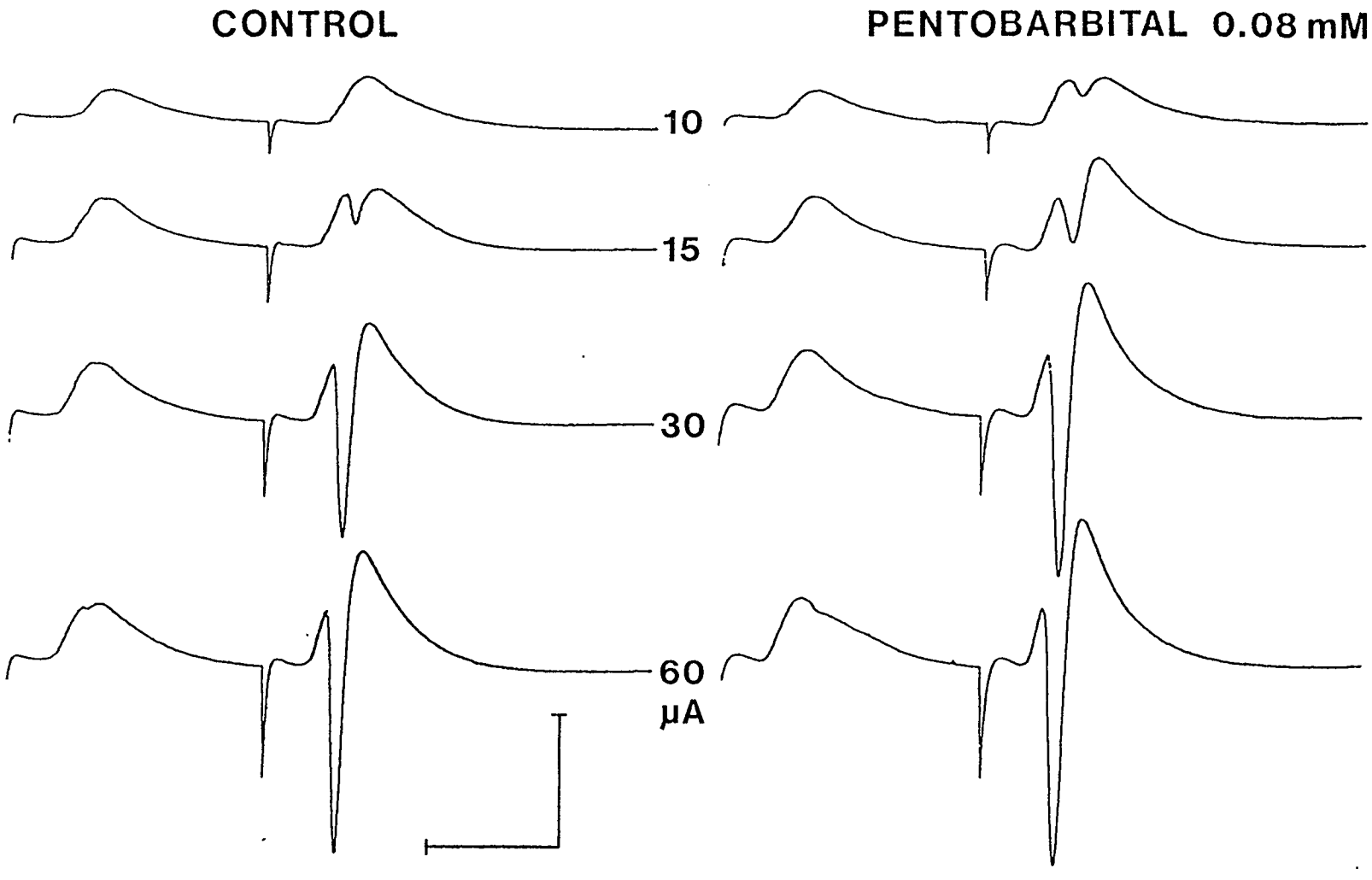
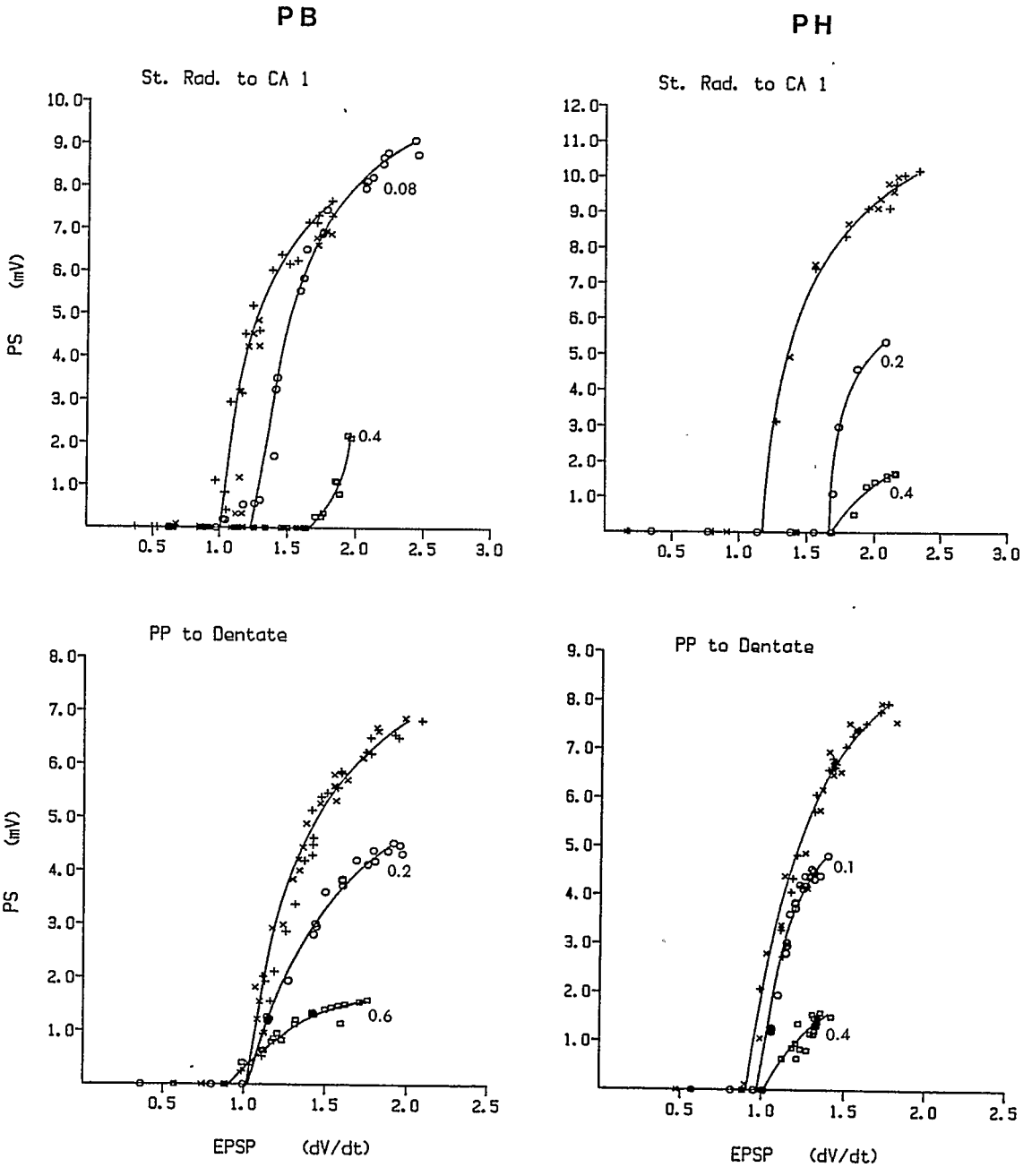

CONTROL
PENTOBARBITAL 0.08 mM
10
15
30
60
µA

Figure 13 - E-S curves showing the relationship between EPSP slopes (dV/dt in Volts/second) and population spike (PS) amplitudes (mV) at various stimulus intensities for stratum radiatum (St. Rad.) inputs to CA 1 and perforant path (PP) inputs to dentate neurons. Control responses before (+) and after washout (x) are compared with responses in the presence of low (○) and higher (□) concentrations of pentobarbital (PB) and phenobarbital (PH). Concentrations shown are in mM.


PB
St. Rad. to CA 1
PS (mV)
0.08
0.4
PH
St. Rad. to CA 1
0.2
0.4
PP to Dentate
PS (mV)
0.2
0.6
EPSP (dV/dt)
PP to Dentate
0.1
0.4
EPSP (dV/dt)

input accounted for the overall facilitation of field potential responses for radiatum inputs to CA 1 (Figure 13, upper left); EPSP dV/dt responses were increased from a maximum control value of 1.65 to over 2.3 V/s in the presence of 0.08 mM pentobarbital. Population spike responses, in contrast, were depressed at this low concentration; as evidenced by the smaller PS amplitudes produced by equivalent EPSP strengths. The depression of PS amplitudes appeared to result from an increase in discharge threshold, measured as a shift to the right of the EPSP axis intercept; stronger synaptic input was required to minimally discharge the CA 1 neurons. An increase in threshold (from 1.0 to 1.2 V/s) was observed in the presence of concentrations which increased the EPSP, but depressed PS amplitudes. The larger field potentials produced by pentobarbital (0.02 to 0.1 mM) must, therefore, be attributed to the increase in the radiatum evoked EPSP. Higher concentrations (0.1 to 0.4 mM) produced depression of field potentials associated with a further increase in threshold (> 1.6 V/s). Concentrations greater than 0.6 mM completely abolished the population spike and also depressed EPSP responses, at all levels of stimulation.

The depression of perforant path inputs to dentate granule neurons was not associated with an increase in threshold for discharge (Figure 13, lower left). Instead, the slope of the E-S curve was decreased, with the major effect on population spike amplitudes. At a concentration of 0.2 mM pentobarbital, the PS was reduced to 65 % of control, however, the EPSP was only reduced by approximately 8 %.

Higher concentrations (>0.4 mM) produced a further decrease in population spike amplitudes with little additional depression of EPSP responses. A decrease in the number of dentate granule neurons contributing to the PS or reduced depolarizing current flow in each neuron could account for the major action of pentobarbital on this pathway. The observed increases in EPSP amplitudes described above (Figure 13) were not reflected in increased dV/dt responses used for E-S analysis.

Only depression of population spike responses were observed over the effective concentration range for phenobarbital (0.02 to 0.4 mM) on radiatum inputs to CA 1. Depression of population spike amplitudes occurred with an increase in threshold of approximately 1.15 V/s (Figure 13, upper right). This increase in threshold, however, could not account for all of the observed depression of discharge, since comparable EPSP responses did not produce population spikes of equivalent amplitude for stimulus intensities above threshold.

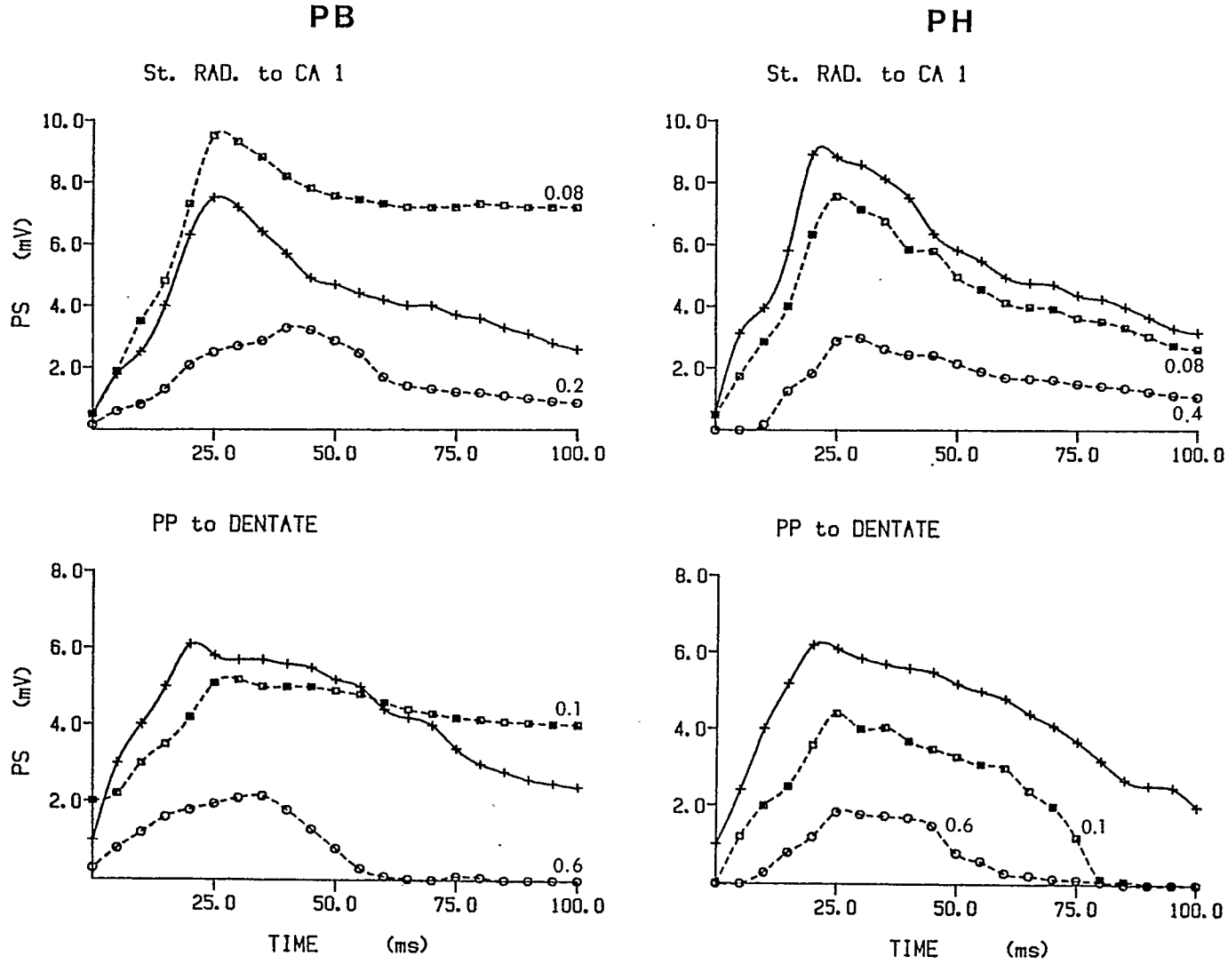
In the dentate area, low concentrations of phenobarbital (0.02 to 0.1 mM) produced a depression of the PS concomitant with a reduction of EPSP amplitudes (Figure 13, lower right). Higher concentrations (0.2 to 0.6 mM) produced marked reductions (50 to 90 %) of PS amplitudes without further depression of EPSP responses. Concentrations above 0.6 mM resulted in complete depression of PS responses and abolished the EPSP. Threshold for discharge did not appear to be altered at any effective concentration.

9.2.4 Effects on Paired Pulse Potentiation

It was apparent from the field potential records shown in Figures 10 and 12 that potentiation still occurred in the presence of the barbiturates. E-S analysis of conditioning and test stimuli, indicated that the amplitude of potentiation was not altered by the barbiturates (data not shown); however, the time course of potentiation was changed. Low concentrations of pentobarbital (0.02 to 0.08 mM) prolonged the time course of potentiation for radiatum inputs to CA 1 (Figure 14). The facilitation of transmission observed at these low concentrations accounts for some of the prolongation, since increasing stimulus intensity to produce comparable PS amplitudes also prolongs the period of late potentiation (see also Turner et al., 1984). In the presence of higher concentrations (> 0.1 mM) the earliest optimal period for potentiation was shifted from 25 ms to over 40 ms. This shift in optimal potentiation period may reflect an increase in feed-forward inhibition, as previously reported (Alger and Nicoll, 1982).

Pentobarbital increased both early and late periods of inhibition in the dentate area (Figure 14). This was evidenced by a shift to the right in the optimal time for peak potentiation (early) and as a steeper tailing of late responses. In contrast, phenobarbital did not produce major shifts in the latency to peak potentiation in either CA 1 or dentate.

Figure 14 - Latency profiles showing PS amplitudes at various inter-stimulus intervals for control (+), low concentrations (□), and higher concentrations (○) of pentobarbital (PB) or phenobarbital (PH) on stratum radiatum (St. RAD.) to CA 1 and perforant path (PP) to dentate responses. Concentrations are in mM.


PB
St. RAD. to CA 1
10.0
8.0
6.0
4.0
2.0
PS (mV)
0.08
0.2
25.0
50.0
75.0
100.0
PH
St. RAD. to CA 1
10.0
8.0
6.0
4.0
2.0
0.08
0.4
25.0
50.0
75.0
100.0
PP to DENTATE
8.0
6.0
4.0
2.0
PS (mV)
0.1
0.6
25.0
50.0
75.0
100.0
TIME (ms)
PP to DENTATE
8.0
6.0
4.0
2.0
0.6
0.1
25.0
50.0
75.0
100.0
TIME (ms)

9.3 Inhalation Anaesthetics

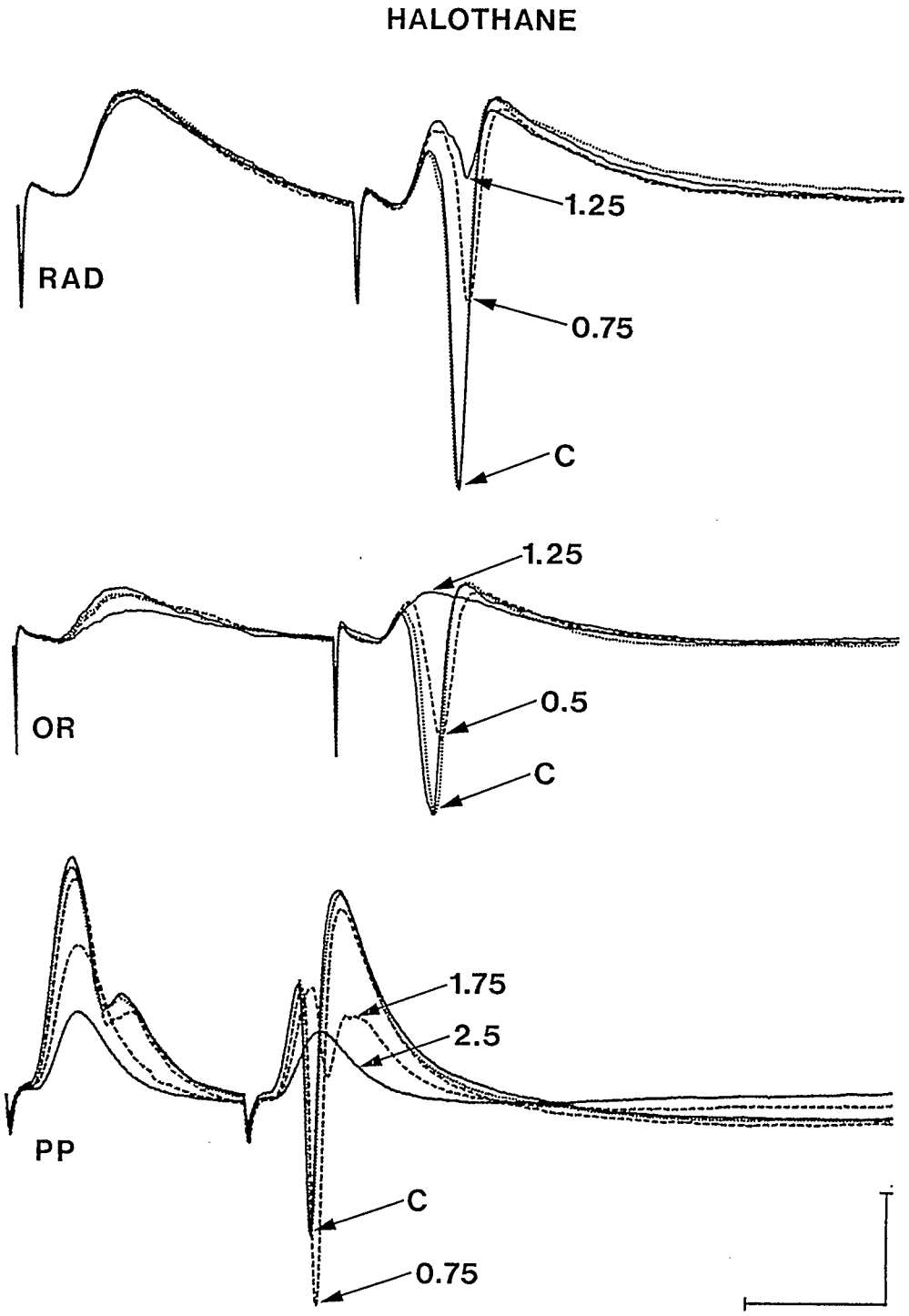
9.3.1 Halothane

Concentrations of halothane between 0.3 to 1.25 vol % produced depression of PS responses for RAD inputs to CA 1 neurons (upper recordings in Figure 15). PS depression occurred with little or no effect on field EPSP amplitudes (compare responses to the first vs. second stimuli). The depression of PS responses was not due to altered paired pulse potentiation, since facilitation of EPSP responses was not changed in the presence of halothane. The latency to onset and initial slope of the EPSP was not altered by concentrations up to 1.5 vol %; however, higher concentrations depressed EPSP responses and complete block of transmission occurred at 3.0 vol % (data not shown).

In contrast to the responses of RAD inputs, the EPSP evoked by OR inputs to CA 1 was depressed by lower concentrations of halothane (0.25 to 1.25 vol %; middle recordings of Figure 15). This depression of EPSP responses was accompanied by reduced PS amplitudes in response to the second stimulus pulse. Onset latency and paired pulse potentiation of the EPSP did not appear to be altered by concentrations that blocked PS responses (1.0 to 1.5 vol %).

The lower recordings in Figure 15 illustrate the different profile of effects produced by halothane on PP inputs to dentate granule neurons; compared with the CA 1 responses. Low concentrations (0.5 to 1.25 vol %) increased PS amplitudes, whereas EPSP responses were

Figure 15 - The effects produced by halothane on stratum radiatum (RAD), stratum oriens (OR) inputs to CA 1 neurons, and perforant path (PP) inputs to dentate granule neuron evoked field potentials. Each superimposed series of records shows control (C) responses before (solid line) and after washout (light dotted line) together with two or three concentrations of halothane (numbers refer to vol %). Calibration: 20 ms and 2.0 mV for Figures 3, 4 and 5.


HALOTHANE
RAD
1.25
0.75
C
OR
1.25
0.5
C
PP
1.75
2.5
C
0.75

either unchanged or slightly depressed. Latencies to onset of both the EPSP and PS were increased at concentrations which enhanced PS amplitudes. In the presence of concentrations greater than 1.5 vol %, both EPSP and PS responses were depressed, and paired pulse facilitation of the EPSP did not occur at concentrations above 2.0 vol % (compare responses to first and second stimuli at 2.5 vol % in Figure 15).

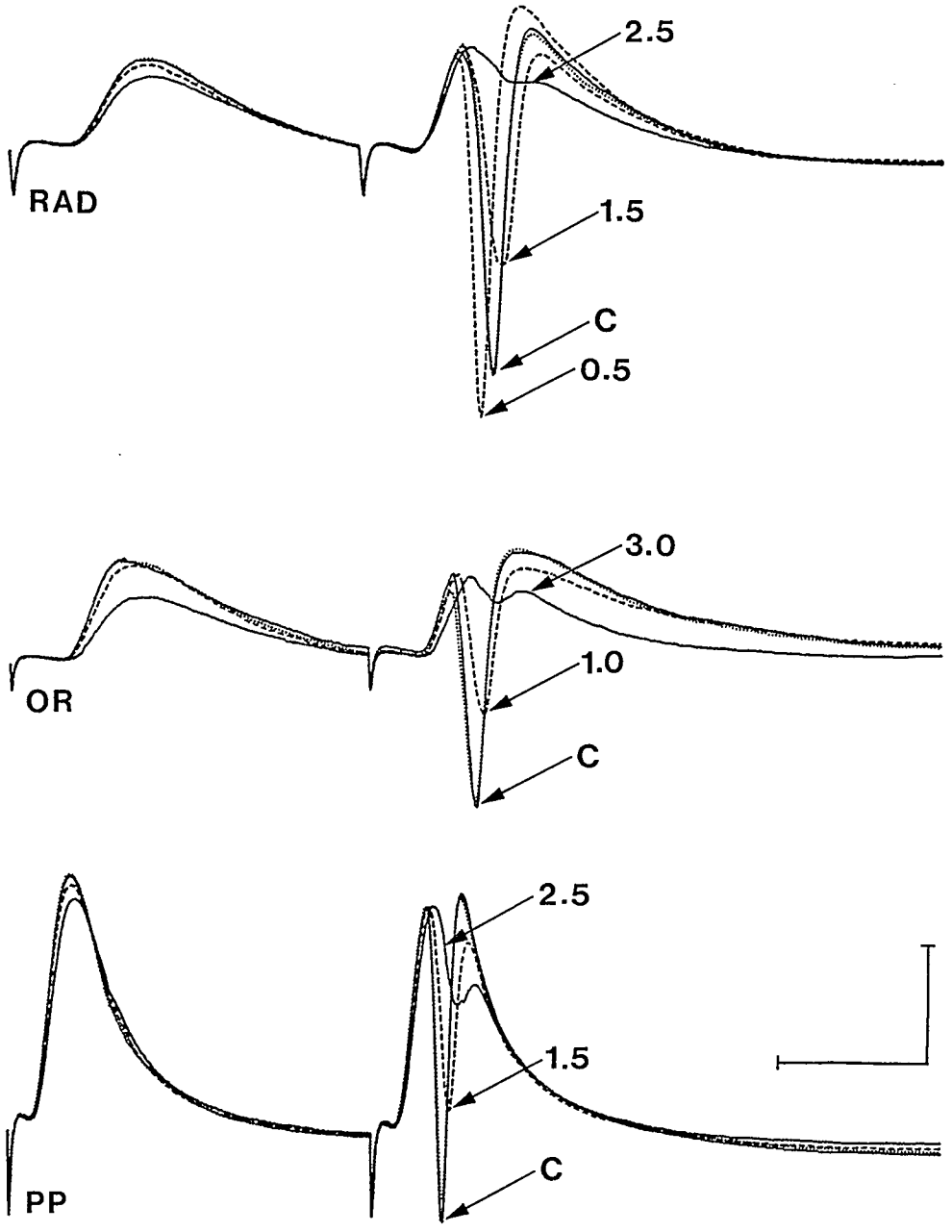
9.3.2 Isoflurane

The effects produced by isoflurane differed from the actions of halothane on both the RAD and PP synaptic pathways (compare Figures 15 and 16). Low concentrations of isoflurane (0.25 to 0.9 vol %) increased RAD to CA 1 PS amplitudes, whereas EPSP responses were unchanged or slightly depressed (upper recordings in Figure 16). Increased PS amplitudes were accompanied by decreased latencies of the spike; but onset latencies and rate of rise of the EPSP, and paired pulse potentiation were not markedly altered by these concentrations. Concentrations greater than 1.0 vol % produced depression of PS responses, accompanied by increased latencies and broadening of the negative spike (eg: 1.5 vol % in Figure 16). Concentrations above 2.0 vol % depressed synaptic (EPSP) responses and above 3.0 vol % completely blocked postsynaptic discharge, although paired pulse facilitation of EPSPs was still apparent.

In contrast to the biphasic actions on RAD inputs, isoflurane produced only depression of OR inputs to the same postsynaptic population (CA 1). Concentrations from 0.5 to 4.0 vol % depressed both the

Figure 16 - The effects produced by isoflurane on stratum radiatum (RAD), stratum oriens (OR), and perforant path (PP) evoked field potentials.

ISOFLURANE



EPSP and PS responses, accompanied by increased latencies (middle recordings in Figure 16). Paired pulse potentiation did not appear to be altered by concentrations up to 4.0 vol % for this pathway.

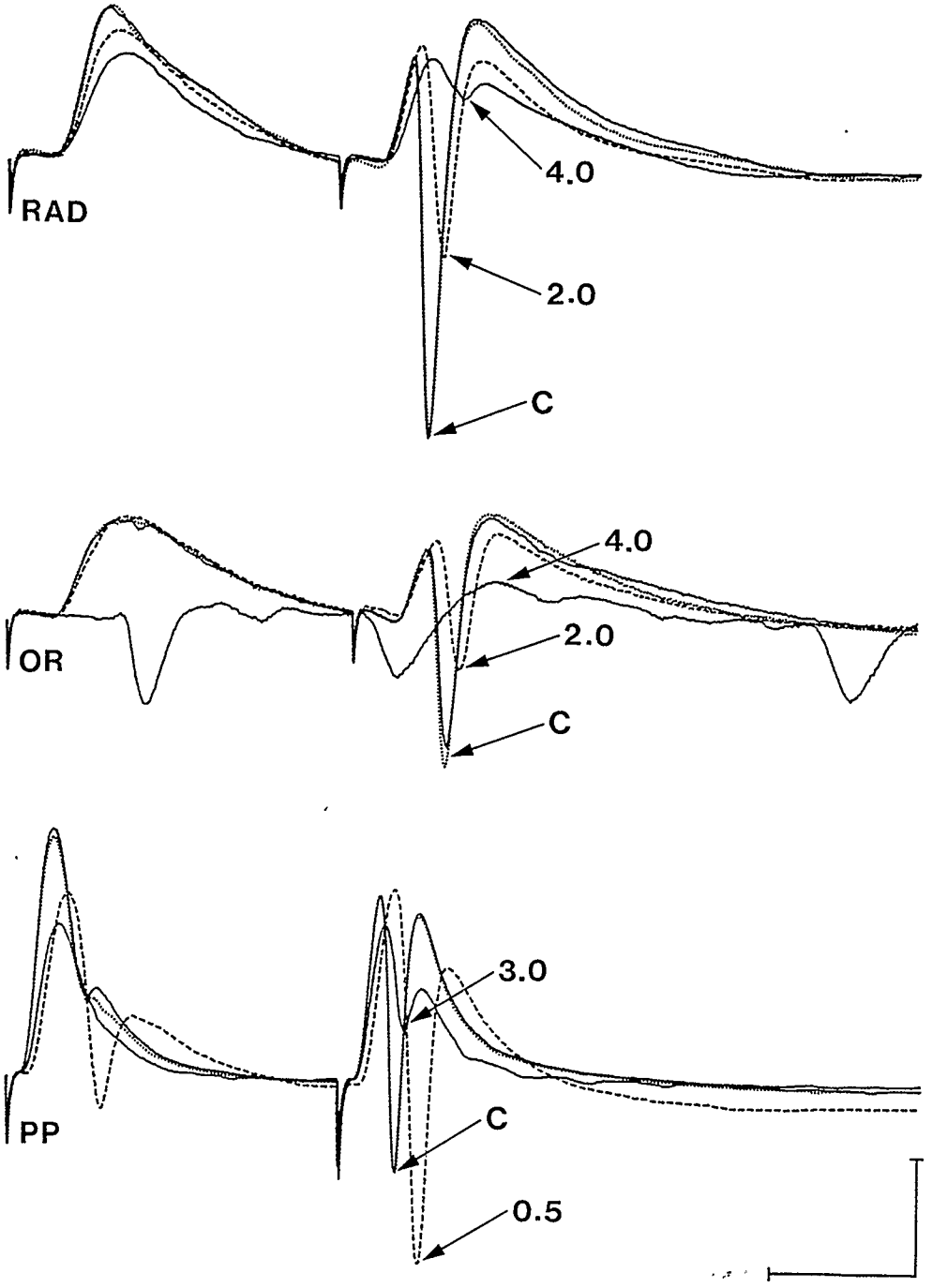
The actions of isoflurane on PP inputs to DG neurons are shown in the lower recordings of Figure 16. All effective concentrations depressed PS responses with only minimal effects on the EPSP. Even in the presence of concentrations which markedly depressed PS amplitudes (eg: 2.5 vol %), EPSP amplitudes, rise time, and onset latencies were only slightly altered (compare EPSP and PS responses to the first and second stimuli respectively). Concentrations above 3.0 vol % were required to depress EPSP responses in this pathway; well above the levels needed to alter EPSP amplitudes for either of the inputs to CA 1 neurons.

9.3.3 Enflurane

Field potential alterations produced by enflurane are illustrated in Figure 17; and differences in effects were observed compared with halothane and isoflurane. RAD inputs to CA 1 neurons were depressed by enflurane over the concentration range from 0.5 to 4.0 vol % (upper recordings in Figure 16). EPSP responses were depressed at the lowest concentrations, and reduced PS amplitudes appeared to result from depression of synaptic input. Paired pulse potentiation and onset latencies for EPSPs were not markedly altered by concentrations below 4.0 vol %, however, an increased latency for PS responses accompanied depression of spike amplitudes.

Figure 17 - Enflurane effects on evoked field potentials for stratum radiatum (RAD), stratum oriens (OR) and perforant path (PP) responses.

ENFLURANE



Low concentrations of enflurane (below 2.5 vol %) did not appreciably alter EPSP responses for OR inputs to CA 1 (middle recordings of Figure 17). PS amplitudes were depressed by concentrations as low as 0.5 vol % and complete depression occurred above 4.0 vol %. Concentrations above 2.0 vol % produced intermittant burst discharges from CA 1 neurons (see below). This siezure-like activity was observed spontaneously and in response to stimulation of both RAD and OR inputs to CA 1 neurons (eg: at 4.0 vol % in middle recordings of Figure 17). Burst firing did not occur in response to each stimulus, and non-burst responses were chosen for the other records shown in Figure 16, to permit comparisons with the non-burst responses of halothane and isoflurane.

Enflurane produced concentration-dependent biphasic actions on PP inputs to DG neurons (lower recordings in Figure 17), similar to the effects produced by halothane on this pathway. Concentrations from 0.25 to 1.0 vol % produced increased PS amplitudes accompanied by markedly prolonged spike latencies (eg: at 0.5 vol % in Figure 16) and increased EPSP onset latencies. Note, however, that the EPSP amplitude and rise time were depressed by concentrations which increased PS responses. Higher concentrations of enflurane (1.0 to 6.0 vol %) produced further reductions in EPSP responses, resulting in decreased PS amplitudes. Paired pulse potentiation was not altered by concentrations less than 4.0 vol %.

9.3.4 Concentration-Response Relationships

Log concentration vs. PS amplitude curves for halothane, isoflurane, and enflurane are shown in Figure 18. Note the dissimilar biphasic effects (enhancement/depression) on each pathway. Halothane was the most potent anaesthetic, producing half-maximal (ED_{50}) depression of PS amplitudes at 0.5, 0.8 and 1.5 vol % for oriens, radiatum, and perforant path inputs, respectively; isoflurane produced half-maximal effects at 1.25, 1.6 and 1.8 vol %, and enflurane was the least potent with ED_{50}'s of 1.65, 2.0, and 2.15 vol %. The ED_{50} values for depression of stratum radiatum inputs to CA 1 neurons corresponded to perfusate concentrations of 0.4, 0.6, and 0.9 mM for halothane, isoflurane, and enflurane, respectively; measured using gas chromatographic analysis (see METHODS). Depression of CA 1 antidromic responses required 5 to 10 times higher concentrations for minimal effects; half-maximal depression of antidromic PS amplitudes could only be achieved with isoflurane (4.3 vol %), limited by the maximum range of the vaporizers. Similar effects were produced by these agents on dentate granule neuron antidromic responses (data not shown), except in the presence of enflurane (0.5 to 3.0 vol %) which produced increased dentate antidromic PS amplitudes (see Figure 20).

9.3.5 Input/Output Relationships

9.3.5.1 Halothane

Population spike responses for RAD inputs to CA 1 appeared to be most sensitive to the depressant effects of halothane (upper left,

Figure 18 - Concentration-response curves for halothane (HAL), isoflurane (ISO), and enflurane (ENF) actions on stratum oriens (○), stratum radiatum (●), perforant path (■) and CA 1 antidromic (▲) responses. Population spike (PS) amplitudes are expressed as percent of control for various concentrations (vol %) of anaesthetic; each point is the mean +/- SEM from at least 3 experiments.

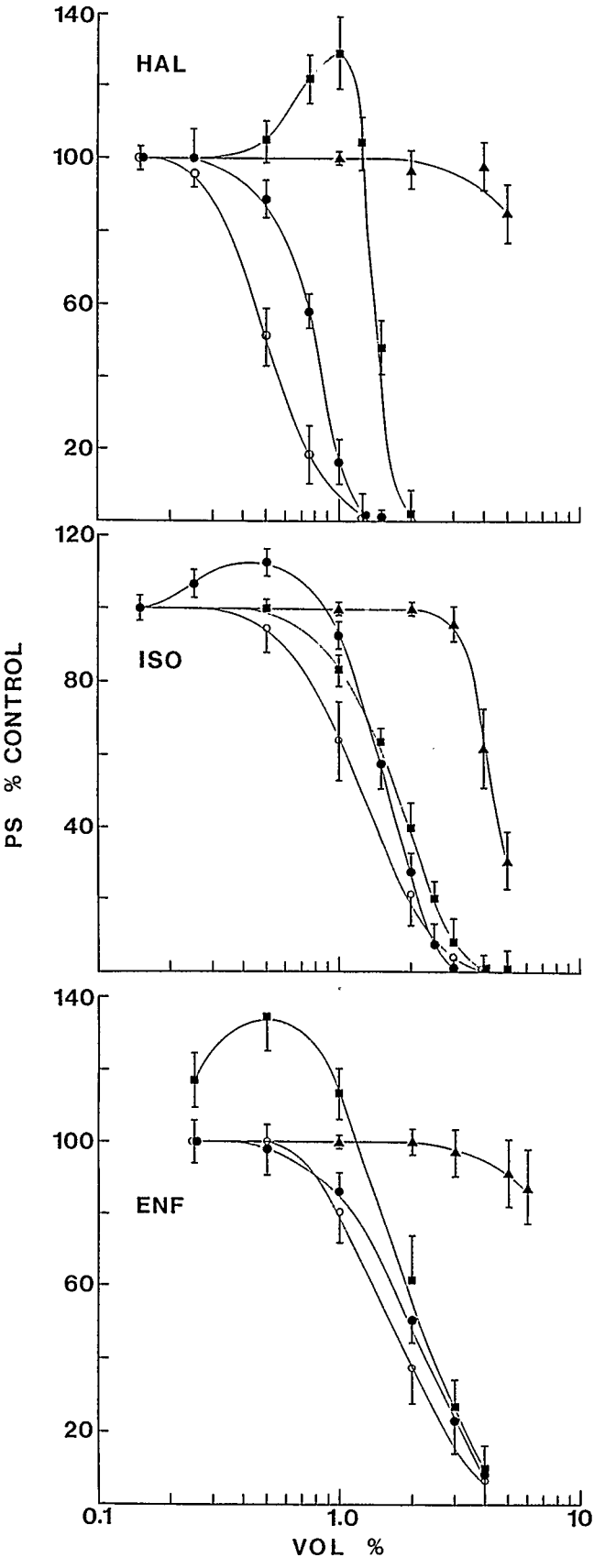

HAL
ISO
ENF
PS % CONTROL
VOL %
140
100
60
20
120
80
40
0.1
1.0
10

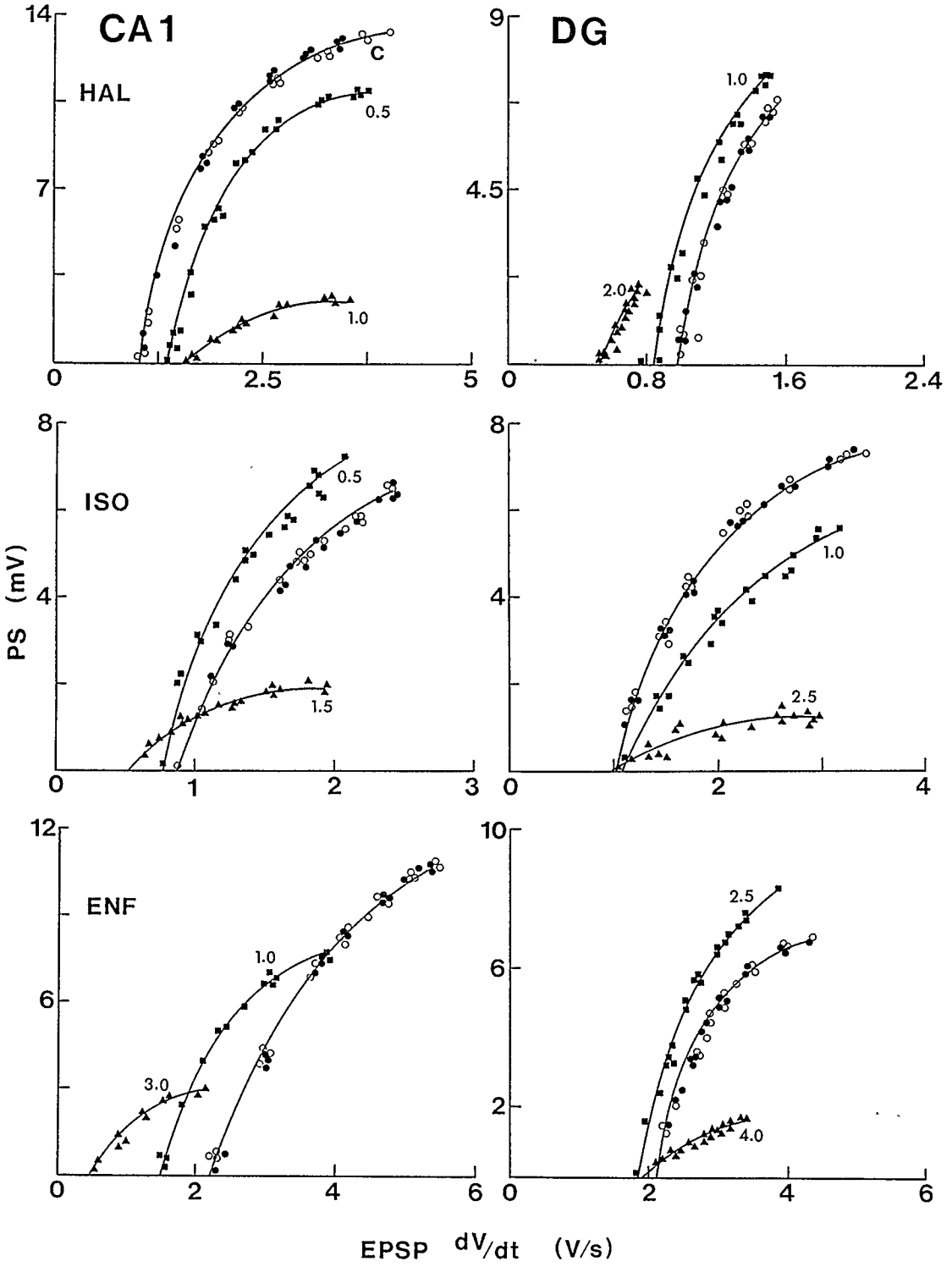
Figure 19). Stimuli which produced equivalent EPSP responses failed to elicite PS amplitudes comparable to control values; indicating that depression of postsynaptic excitability was the major action on this pathway. Depression of excitability was also apparent from the shift to the right of the EPSP axis intercept (threshold); such that larger EPSP responses were required to produce minimal discharge of the CA 1 population. This increase in threshold, however, could not account for all of the depression of discharge since stimuli above threshold resulted in relatively small PS amplitudes compared with control responses.

In the upper right of Figure 19, effects of halothane on PP inputs to dentate granule neurons are shown. Low concentrations (eg: 0.5 vol %) produced a shift to the left in the EPSP axis intercept and increased PS amplitudes were observed for comparable control EPSP responses; indicating a decreased threshold for discharge of granule neurons could account for the enhancement of field potentials at concentrations of 0.25 to 1.25 vol %. In the presence of higher concentrations (eg: 1.5 vol %), depression of synaptic input resulted in decreased PS amplitudes, even though discharge threshold was further reduced.

9.3.5.2 Isoflurane

Isoflurane produced opposite effects, compared to halothane, on input/output profiles for the RAD and PP pathways. The effects produced by isoflurane on RAD inputs to CA 1 neurons are shown in the

Figure 19 - Input/output curves for stratum radiatum inputs to CA 1 pyramidal neurons and perforant path inputs to dentate granule (DG) neurons in the presence of halothane (HAL), isoflurane (ISO) and enflurane (ENF); numbers refer to concentrations of anaesthetic (vol %). Control (C) responses are also shown; pre-anaesthetic (○) and post-recovery (●).


CA1
DG
HAL
ISO
ENF
C
0.5
1.0
2.0
1.5
2.5
3.0
4.0
PS (mV)
EPSP dV/dt (V/s)

middle left graph of Figure 19. Low concentrations (eg: 0.5 vol %) enhanced PS amplitudes for equivalent control EPSP responses and also appeared to decrease the discharge threshold of the CA 1 population. Higher concentrations (eg: 2.0 vol %) produced a further reduction of discharge threshold, however, at higher stimulus intensities, PS amplitudes were reduced. Thus, isoflurane produced two opposing effects on postsynaptic excitability; smaller EPSP responses were required to minimally discharge the CA 1 population (enhanced excitability) but the level of discharge was depressed for stimuli above threshold.

The major action of isoflurane on PP inputs to dentate neurons was a depression of PS amplitudes (middle right of Figure 19). Even in the presence of high concentrations (eg: 2.5 vol %) synaptic responses were only slightly depressed, while PS amplitudes were reduced to approximately 20 % of control values. This is opposite to the depression of EPSP responses and enhanced excitability produced by halothane on this pathway. Isoflurane did not alter the EPSP axis intercept, suggesting that the depression of granule neuron discharge did not result from an increase in threshold.

9.3.5.3 Enflurane

All effective concentrations of enflurane produced a dramatic reduction of discharge threshold for CA 1 neurons (lower left of Figure 19), which could account for the spontaneous and stimulus evoked seizure-like burst firing produced by this anaesthetic (see section

9.3.6). The dominant effect on RAD inputs to CA 1, however, was depression of synaptic responses, resulting in an overall attenuation of field potential amplitudes. Thus, enflurane appeared to act on at least two independent sites to produce opposing actions on synaptic transmission and CA 1 neuron excitability.

PP to dentate neuron EPSP responses were also depressed by enflurane (lower right of Figure 19) but the major effects occurred on postsynaptic excitability. In the presence of low concentrations (eg: 0.25 vol %) depressed synaptic responses were accompanied by increased PS amplitudes, which appeared to involve a reduction in discharge threshold (left shift of the EPSP axis intercept). Higher concentrations (eg: 3.0 vol %) produced a further reduction of EPSP responses and marked depression (approx. 25 % of control) of PS amplitudes. Enflurane did not produce dramatic reductions in discharge threshold of granule neurons and burst firing of dentate cells was never observed.

9.3.6 Enflurane-Induced Burst Activity

Figure 20 shows example recordings from CA 1 and DG regions during enflurane-induced field bursts. Burst discharges were concentration-dependent, with low concentrations (0.5 to 1.0 vol %) producing only small amplitude discharges, and higher levels (2.0 to 4.0 vol %) producing large amplitude field bursts which increased in frequency (Figure 20). Both spontaneous and stimulus-evoked burst activities were observed in the CA 1 area; however, the burst

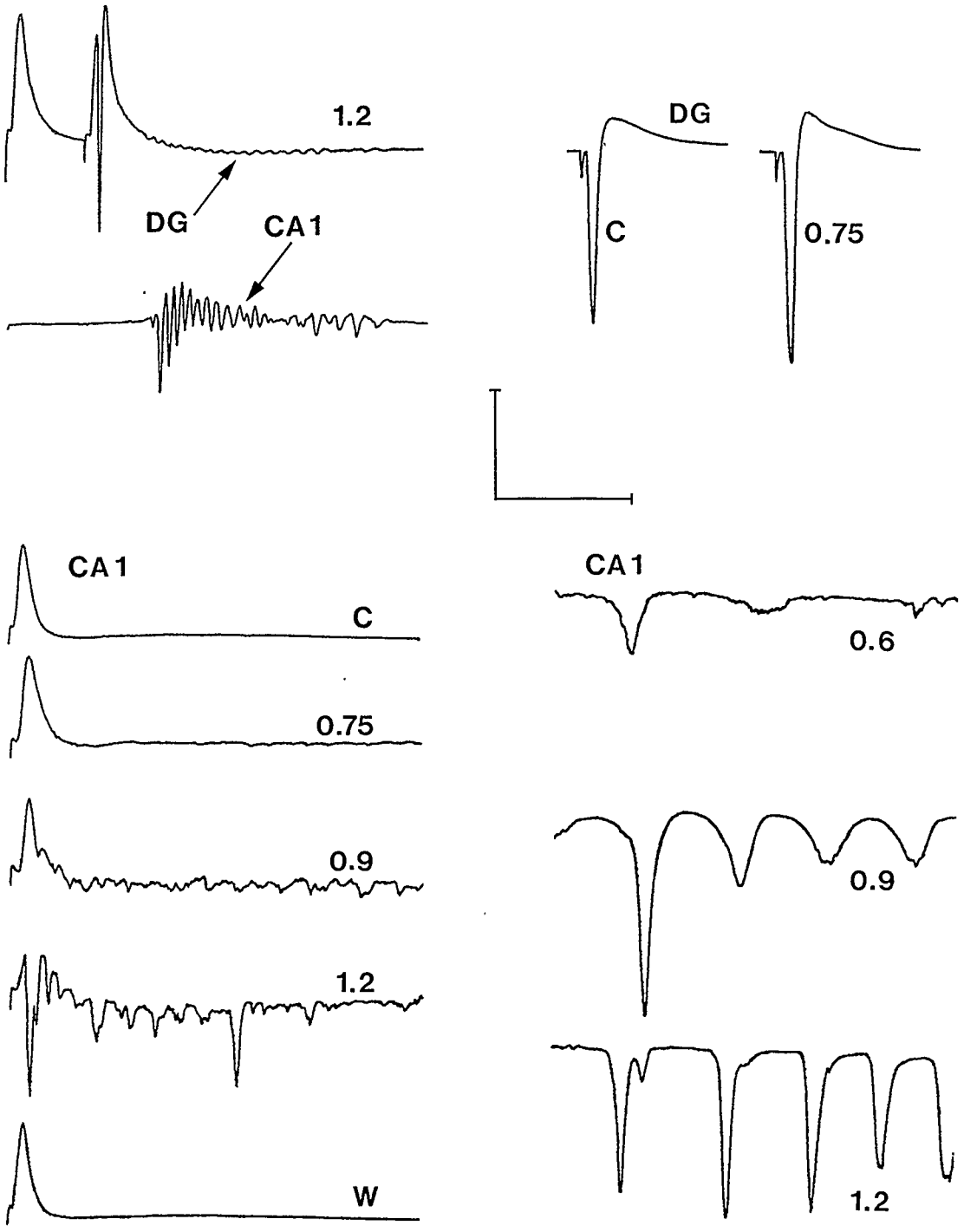
Figure 20 - Enflurane-induced burst discharges recorded in CA 1 and DG areas:

Upper left - perforant path evoked field potential responses recorded from DG and CA 1 neurons from the same slice are shown in the presence of 1.2 mM enflurane. The late 'ringing' of DG responses (arrow) corresponds with burst firing in CA 1 (arrow).

Lower left - field EPSP responses evoked by low intensity stimulation of st. radiatum are shown for control (C), and in the presence of enflurane. Depression of EPSP amplitude and field bursting were concentration dependent and reversible on washout (W).

Upper right - antidromic field potentials from DG neurons are shown for control (C) and in the presence of enflurane.

Lower right - spontaneous field bursts recorded from the CA 1 region were also concentration dependent. Concentrations shown are in mM; calibration: 5 mV, 40 ms (left), 20 ms (right).


1.2
DG
CA1
DG
C
0.75
CA1
C
0.75
0.9
1.2
W
CA1
0.6
0.9
1.2

discharges were periodic and did not occur in response to each stimulus. Spontaneous burst discharges were not observed in DG neurons, and perforant path evoked responses appeared relatively normal at concentrations of enflurane which produced marked seizure-like activity in the CA 1 area. Enflurane-induced burst firing of CA 1 neurons could occasionally be recorded in the dentate area (volume conduction), and resulted in a late 'ringing' of the perforant path to DG neuron evoked responses which occurred 15 to 20 ms after the DG population spike (Figure 20). These late responses appeared to be CA 1 neuron bursts which were synchronized with the perforant path stimulus; perhaps triggered via DG neuron activation of CA 3 neurons which subsequently synapse onto CA 1 cells (Andersen, 1975), or via direct perforant path activation of CA 1 neurons (Andersen, 1981). CA 3 neuron firing patterns were not altered in the presence of enflurane (data not shown; 3 experiments), and multiple fiber-volleys were not recorded in stratum radiatum at concentrations which produced spontaneous or evoked seizure discharges in CA 1 neurons. Multiple firing of DG neurons was never observed even in the presence of vapour concentrations which produced increased field potential responses (eg: 0.25 to 1.0 vol %). Thus, enflurane-induced seizure activity appeared to be localized to the CA 1 region; however, a more detailed study of CA 1 and CA 3 responses is required to determine how perforant path activation may result in CA 1 neuron burst firing.

9.3.7 Enflurane Effects on Antidromic Field Potentials

Concentrations which produced enhanced perforant path to DG responses also increased dentate antidromic responses (Figure 20). Depression of antidromic spike amplitudes required concentrations above 5.0 vol % which reduced synaptic transmission by greater then 90 %. Multiple antidromic discharges were produced in the CA 1 area when spontaneous and synaptically evoked seizure activity was present, however, antidromic bursts were not observed in the DG area.

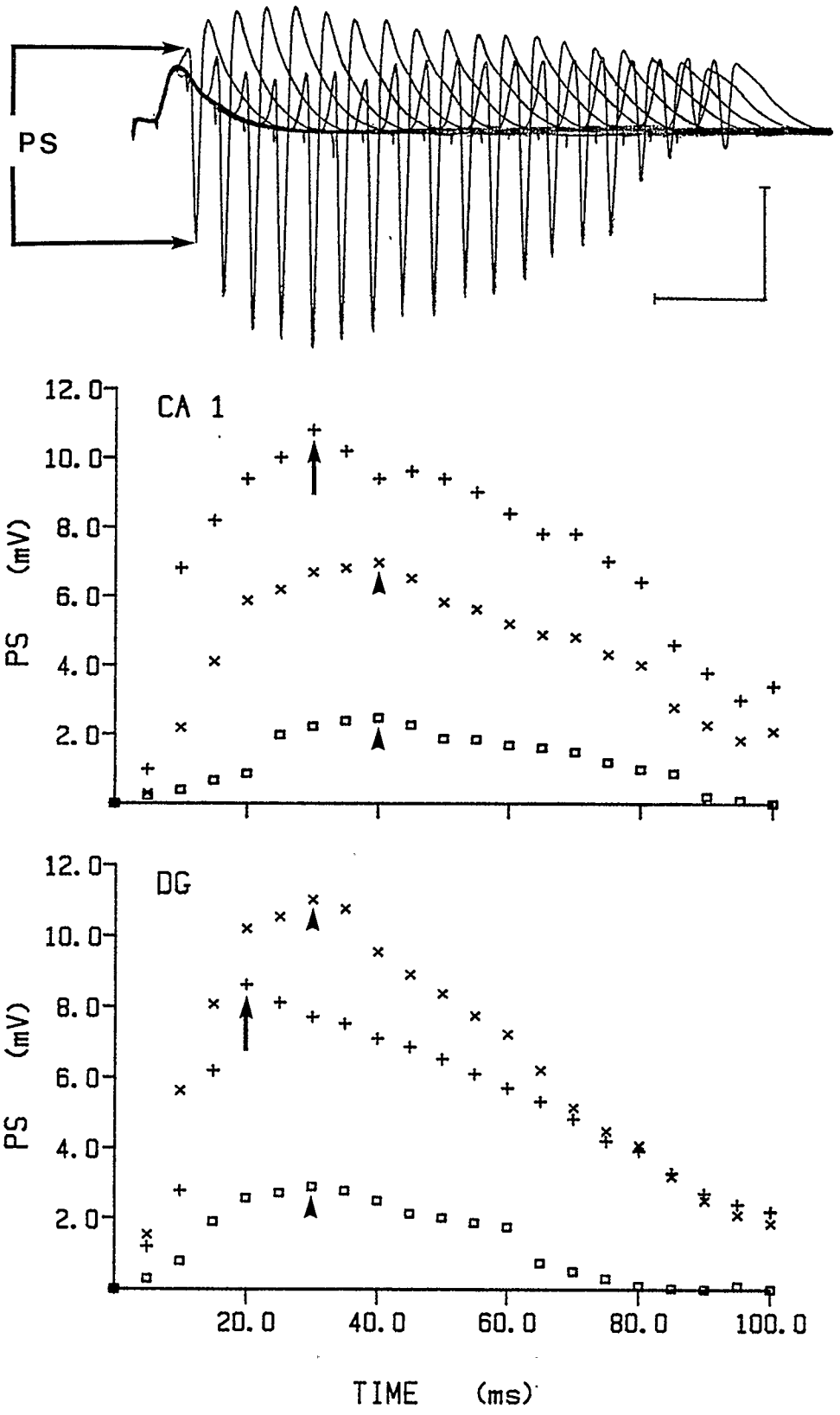
9.3.8 Enflurane Effects on Paired Pulse Potentiation

In the presence of enflurane, paired pulse potentiation still occurred and enhanced potentiation was associated with increased response amplitudes produced by low concentrations in the DG area; reduced potentiation accompanied depression of responses in both pathways. Low concentrations also appeared to enhance inhibition in both pathways and higher concentrations resulted in further enhancement of late inhibition in DG neurons with no additional effect in the CA 1 area. Increased inhibition was observed as a prolongation of the early component (optimal interstimulus intervals were shifted from 30 to > 40 ms) and steeper tailing in late responses (Figure 21). This interpretation of latency responses is supported by earlier observations which demonstrated similar effects produced by pentobarbital (section 9.2; Gribkoff and Ashe, 1985; Ashton and Wauquier, 1985). Pentobarbital has been shown to enhance local inhibition in the hippocampal formation (Nicoll et al., 1975).

Figure 21 - Enflurane effects on paired pulse latency profiles:

Upper - twenty superimposed recordings of St. Radiatum to CA 1 evoked responses are shown at various interstimulus intervals. PS amplitude measurements (indicated by arrows) were used for subsequent paired pulse latency plots.

Lower - enflurane decreased CA 1 PS amplitudes and shifted the control (+) optimal latencies of 30 ms (arrow) to 40 ms in the presence of the anaesthetic (arrowheads; x - 0.75 mM; □ - 1.2 mM). A similar shift in optimal latencies were observed for DG neuron responses at concentrations which enhanced (x; 0.75 mM) or depressed (□; 1.2 mM) PS amplitudes. Calibration: 4 mV and 20 ms.


PS
CA 1
DG
PS (mV)
12.0
10.0
8.0
6.0
4.0
2.0
20.0
40.0
60.0
80.0
100.0
TIME (ms)

10.0 DISCUSSION

10.1 The Hippocampal Slice as a Model System

The advantages and limitations of brain slice preparations have been discussed elsewhere (Kerkut and Wheal, 1982; Dingledine, 1984), and, in general, *in vitro* preparations have been shown to offer several important advantages in some types of experiments. The major advantage in the present study was the ability to accurately control and measure anaesthetic concentrations in the perfusate. The *in vitro* preparation also facilitated placement of stimulating and recording electrodes on visually identified pathways. Several studies have shown that synaptic transmission and electrical activity in the hippocampal formation is altered during anaesthesia (Leung, 1982; Winters, 1982; Kramis et al., 1975); however, this could involve anaesthetic actions on ascending inputs (eg: septo-hippocampal inputs; Dutar et al., 1986). The best evidence supporting an involvement of the hippocampus in anaesthesia is from *in vitro* studies which demonstrate pronounced anaesthetic effects at relatively low (clinical) concentrations (Carlen et al., 1982; Nicoll and Madison, 1982; Yoshimura et al., 1985). Furthermore, the hippocampal formation has been shown to be more sensitive to anaesthetics than some other cortical areas (eg: olfactory, *in vitro*, Richards and White, 1975; and neocortex, *in vivo*, Kott and Winters, 1979; Winters, 1982). The results of the present study also demonstrate that hippocampal physiology can be altered by anaesthetic concentrations equivalent to therapeutic ranges.

10.2 Paired Pulse Potentiation

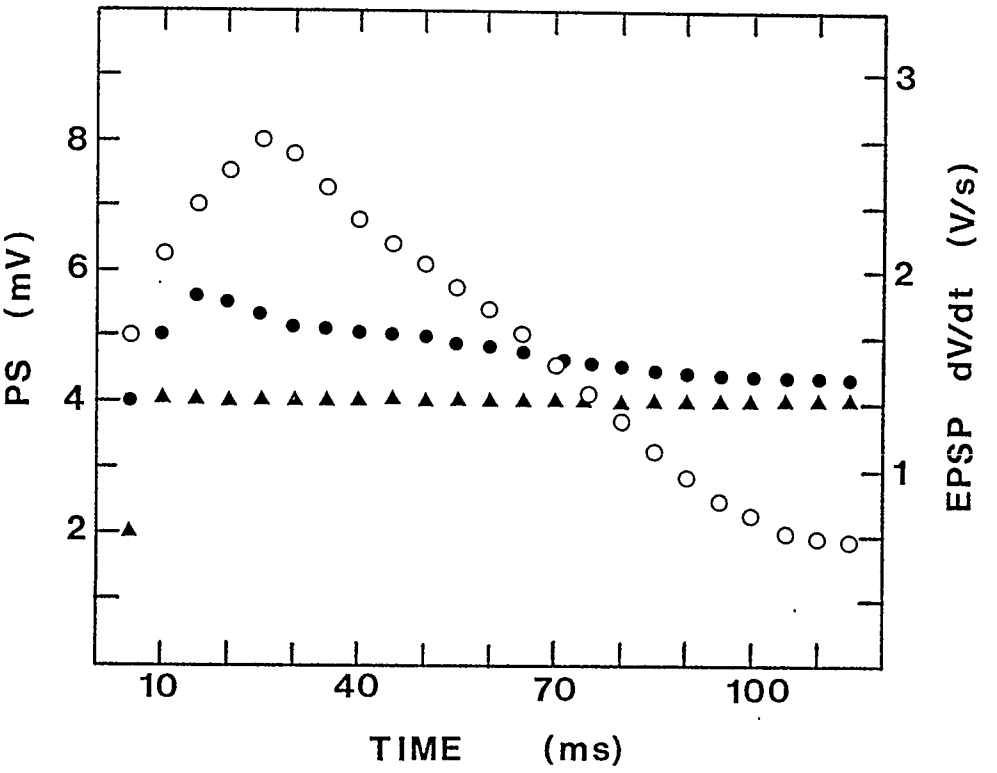
Although mechanisms underlying paired pulse potentiation are unknown, both an increase in synaptic strength and postsynaptic excitability were apparent from the E-S curves in Figure 9. These observations are consistent with other reports (eg. Bliss and Gardner-Medwin, 1973; Assaf and Miller, 1978, 1981; Creager et al., 1980; Abraham et al., 1985). Paired pulse potentiation may also be associated with the resetting of random discharge by the conditioning stimulus, resulting in increased synchronous firing in response to the test stimulus (Assaf and Miller, 1981). Support for this is provided in Figure 9; EPSP and PS responses to test stimuli were consistently less variable than conditioning stimuli responses.

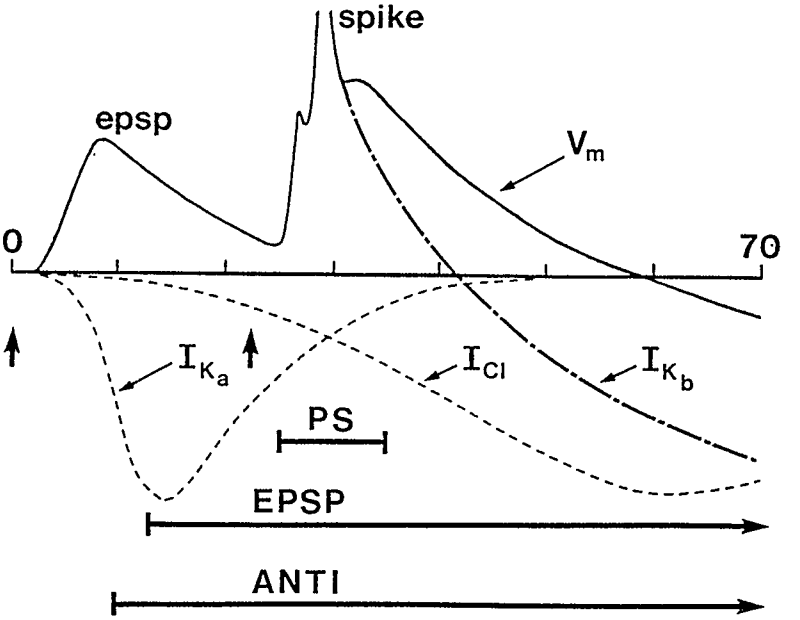
Figure 22 shows the observed latencies for orthodromic and antidromic peak amplitude responses and a relationship to the time course of known inhibitory membrane currents in CA 1 neurons. For interpulse intervals less than 10 ms, the reduced level of potentiation is most likely related to the relative refractory period of the CA 1 neurons. During this time both antidromic and EPSP response amplitudes are depressed relative to conditioning stimulus responses. EPSP response amplitudes become potentiated after 15 ms, although the population spike does not reach peak amplitudes until 25 to 30 ms have elapsed between conditioning and test stimuli. It is postulated that the difference between maximal EPSP potentiation and the optimal interstimulus intervals for orthodromic population spike potentiation is due to the activation of the early outward potassium current,

Figure 22 - Upper - Graph showing amplitudes of antidromic (▲) and orthodromic (○) population spikes (PS), together with dendritic EPSP (●) responses at increasing interstimulus intervals recorded from the cell body layer of CA 1. Orthodromic EPSP and PS responses are plotted as the increase in amplitude relative to control; antidromic PS responses are actual amplitudes.

Lower -

Diagram showing the relationship between the time course of paired pulse potentiation and membrane currents which may underly early and late periods of inhibition in CA 1 pyramidal neurons (see text). Membrane potential (Vm) following paired pulse stimulation (arrows) shows the time course of voltage change observed during potentiation (adapted from: Turner, Richardson, and Miller, 1985). A subthreshold stimulus produces an EPSP (epsp) in response to the first pulse and a larger EPSP, which results in an action potential (spike), following the second stimulus. The optimal time for population spike potentiation occurs between the early potassium (Ka), and late chloride (Cl) and potassium (Kb) inhibitory currents. The time course for onset of potentiation of population spike (PS), EPSP, and antidromic (ANTI) responses are shown relative to the 70 ms time scale for membrane potential and current diagrams.


PS (mV)
EPSP dV/dt (V/s)
TIME (ms)
8
6
4
2
3
2
1
10
40
70
100


spike
epsp
V_m
0
70
I_{K_a}
I_{Cl}
I_{K_b}
PS
EPSP
ANTI

described by other investigators (Brown and Johnston, 1983; Segal and Barker, 1984). This potassium current demonstrates the exact activation kinetics and half-life required to clamp the neurons for the 5 to 10 ms needed to account for the difference between EPSP and population spike potentiation (Figure 22).

EPSP responses remain potentiated for a much longer time period (> 1200 ms) than PS responses (25 to 40 ms; Figure 22). The most likely explanation for the faster decrease in PS amplitudes is the activation of late inhibitory currents which depress discharge. The Cl conductance activated by GABA mediated synaptic inhibition may account for late depression (Schwartzkroin, 1983). This current activates at the onset of the synaptically evoked EPSP (probably because the basket cell inhibitory neurons receive the same afferent drive from stratum radiatum fibers; Schwartzkroin and Knowles, 1983), but it requires 60 to 80 ms to fully develop and could, therefore, account for much of the suppression of discharge (see Figure 22). The Ca-dependent K conductance associated with the prolonged after hyperpolarization and/or voltage dependent K currents could also contribute to suppression of population spike potentiation with interstimulus intervals over 40 or 50 ms (Carlen et al., 1983).

10.3 Barbiturates

The present study demonstrates that pentobarbital and phenobarbital produce concentration-dependent and pathway-specific effects on synaptic transmission in the isolated hippocampal formation. The con-

centration range utilized is associated with the state of anaesthesia (0.06 to 0.1 mM; Ho and Harris, 1981; Richards, 1980; MacDonald and Barker, 1978; MacDonald et al., 1986), and the different effects observed on the three synaptic pathways suggest multiple and selective membrane sites (Richards, 1983). Pentobarbital, for example, must act at two or more independent sites on the radiatum to CA 1 pathway, to account for synaptic enhancement at the same time that depression of population spike responses were observed (0.08 mM, Figure 13).

10.3.1 Pathway-Specific Barbiturate Effects

Stratum oriens inputs to CA 1 were consistently more sensitive to the depressant actions of both barbiturates and did not exhibit biphasic (enhancement/depression) responses, whereas, radiatum and perforant path EPSPs demonstrated biphasic responses. Although increased EPSP responses were observed for radiatum inputs to CA 1 and perforant path inputs to dentate neurons, only the CA 1 population spike was enhanced; whereas, dentate spike responses were depressed. It is unlikely that similar actions at common sites could be compatible with these differential effects on the three synaptic pathways.

The radiatum pathway is one of only two CNS systems described to date which consistently shows barbiturate-induced facilitation of excitatory transmission (Roth et al., 1983; Roth et al., 1986; Morris, 1978). A previous report described only depressant actions of anaesthetics (including pentobarbital and phenobarbital) on hippocampal neurons, observed as a membrane hyperpolarization (Nicoll and

Madison, 1982). It is possible that membrane hyperpolarization could account for the increased EPSP responses (by shifting the membrane potential away from the EPSP reversal potential) and also produce the postsynaptic depression described in the present study. While results are compatible with direct hyperpolarizing actions of the barbiturates, additional actions on synaptic and membrane functions are required to account for the differential effects produced on the three pathways. Furthermore, membrane hyperpolarization cannot account for the opposite effects produced by low concentrations (< 0.1 mM) of pentobarbital and phenobarbital on radiatum inputs to CA 1 neurons.

10.3.2 Stereo-Selective Actions of Pentobarbital

The stereoisomers of pentobarbital produced qualitatively similar, but quantitatively different effects. Previous reports have suggested that the (-) isomer may have a more depressant effect than the (+) isomer (Huang and Barker, 1980; Barker and Mathers, 1981). In view of the concentration-dependent biphasic actions and differences in potency, it is likely that the previous studies examined concentrations on either side of the 'turnover point' for enhancement and depression (ie. 0.1 mM). Alternatively, the stereoisomers may be capable of producing quite different effects on cultured spinal cord neurons (Huang and Barker, 1980); opposite effects were produced by pentobarbital on different neuronal populations in the present study.

10.3.3 Excitatory and Depressant Effects

Mixed excitatory and depressant actions suggest independent effects occurring at different membrane sites, and could account for the selectivity described for the stereoisomers of pentobarbital (Huang and Barker, 1980) and structural analogs of other barbiturates (Ho and Harris, 1981). Selective actions are also consistent with the differential effects of convulsant, anaesthetic, and anticonvulsant barbiturates on synaptic transmission (Nicoll, 1979; MacDonald and Barker, 1979).

Excitatory and depressant barbiturate effects have been observed in animal studies as: 1) increased EEG fast activity, 2) hypersynchronization of thalamic relays, and 3) increased unit activity in midbrain reticular centers (Rosner and Clark, 1973; Winters and Kott, 1979); and depression of cortical evoked potentials. While it is possible that concentration-dependent biphasic effects might be explained by pharmacokinetics; evidence from invertebrate studies demonstrate that excitation and depression occur at the cellular level (MacIver et al., 1979; Johnston, 1978; Roth et al., 1986). The results of the present study are consistent with the hypothesis that barbiturates produce concentration-dependent excitatory and depressant effects on synaptic and cellular function (Winters, 1982).

10.3.4 Effects on Paired Pulse Potentiation

The effects produced by pentobarbital on the CA 1 latency profile are consistent with the above interpretation of inhibitory currents

limiting discharge. Thus, the early shift in the optimal period for population spike potentiation (from 25 to 40 ms; Figure 14) could represent a pentobarbital-induced prolongation of an early K current (Figure 22). Pentobarbital can enhance and/or prolong potassium currents in Retzius cells of leech (Kleinhaus and Prichard, 1979) and mammalian CNS neurons (Barker, 1975; Nicoll and Madison, 1982). Similarly, the observed increase in late inhibition could correspond to enhancement of GABA mediated Cl currents consistent with numerous other reports (eg. Nicoll et al., 1975; Barker and Ransom, 1978; Jackson et al., 1982) (see Figure 22). Barbiturate effects on the time course of short-term potentiation could contribute to the alteration of neuronal information processing which may be involved in anaesthesia (Winters, 1982).

10.3.5 Multiple Actions of Barbiturates

The present results suggest that several modes of action may underly barbiturate effects on excitatory transmission in hippocampal pathways. First, the observed synaptic facilitation and perhaps some postsynaptic depression could be attributed to increased release of intracellular calcium (Krnjevic, 1972; Carlen et al., 1983). Elevated [Ca]i would be expected to increase transmitter release from synaptic terminals, accounting for radiatum to CA 1 and perforant path to dentate EPSP facilitation in the presence of anaesthetic concentrations of pentobarbital, and would also activate calcium-dependent potassium conductances which would stabilize postsynaptic excitability. Second, paired-pulse results confirm earlier observations that pentobarbital

also prolongs inhibitory conductances (Jackson et al., 1983; Judge, 1983) which would further stabilize postsynaptic discharge. Finally, other actions on transmitter sensitivity, threshold, and/or membrane electrical properties may contribute to depression in the presence of higher concentrations (Judge, 1983; Richards, 1983). The major common effect of each barbiturate appeared to be postsynaptic depression. Pentobarbital may produce depression via enhancement of inhibitory conductances, but it is likely that phenobarbital acts via depression of excitatory currents, because little or no effect on paired pulse latencies was observed and a direct depression of threshold was apparent from E-S analysis (Figure 13). It is important to note that differential effects were apparent on EPSP and PS responses; dependent on the barbiturate, the concentration, and synaptic pathway studied. Thus, pentobarbital and phenobarbital appear to interact at selective recognition sites which are unevenly distributed between the CA 1 and dentate granule neuron populations.

10.4 Inhalation Anaesthetics

Halothane, isoflurane and enflurane depressed field potential responses in the three synaptic pathways studied; however, the concentration-dependent effects were different for each anaesthetic. This is apparent when comparing the biphasic (facilitation/depression), versus monophasic actions on population spike amplitudes (Figure 18). Furthermore, depression of field potential amplitudes could result from opposite actions on synaptic responses and postsynaptic excitability of CA 1 pyramidal and dentate

granule neurons (Figure 19). These varied and opposite effects on neuronal transmission suggest that inhalation anaesthetics act via different mechanisms at selective cellular (or membrane) sites. Alternatively, the anaesthetics may be acting at different sites to produce the varied effects observed.

Differences between the actions of volatile anaesthetics on membrane excitability have been reported previously. Landau et al. (1979a) have shown that ether and methoxyflurane produce biphasic actions on miniature end plate currents at the frog neuromuscular junction, which also appear to involve 'selective' interactions. They conclude that "the effect of such structurally non-specific drugs comprises a combination of discrete and distinguishable events which are specific and dose dependent for each of the agents". In a more detailed study of volatile agents on the postsynaptic sensitivity to glutamate and GABA at crab neuromuscular junctions these authors described further selective and differential actions which led them to conclude that the transmitter-coupled 'gating molecules are housed in specific subregions of membrane' (Landau, Richter, and Cohen 1979b), which demonstrate different susceptibilities for perturbation by volatile agents. Results of the present study support these ideas, and extend the earlier observations, on which they were based, to include selective and differential actions on mammalian CNS neurons. The question remains whether the inhalation anaesthetics act at different sites. To address this question, the effects of the anaesthetics were compared on three synaptic pathways, which differ in the composition

of membrane components; and, perhaps, in the distribution of anaesthetic 'recognition' sites.

10.4.1 Pathway-Specific Actions of Inhalation Anaesthetics

Aside from the obvious morphological differences between CA 1 pyramidal neurons and dentate granule cells, several important differences in membrane physiology are also apparent (Turner and Schwartzkroin, 1984). Granule neurons demonstrate a relatively high resistance to convulsant-induced burst firing, which has been attributed to a lack of inward calcium currents in these cells (Fricke and Prince, 1984). CA 1 pyramidal neurons, in contrast, exibit a low threshold for convulsant-induced siezure activity, and large inward calcium currents have been recorded (Benardo, Masukawa and Prince, 1982). Thus, the distribution of membrane calcium channels appears to differ between pyramidal and granule neurons. The distribution of glutamate receptor sub-types also differs for granule and pyramidal neurons (Kelly, 1982; Collinridge, 1985), which may have direct bearing on the differential actions of anaesthetics observed in the present study, since glutamate is thought to be the major transmitter in both perforant path and Schaffer-collateral (RAD) systems. Other differences in membrane protein distribution between neuron types also exist (see review by Poo, 1985). If anaesthetic recognition sites are differentially distributed in membranes, then inhalation anaesthetics should exhibit pathway-specific effects.

Marked differences in anaesthetic effects on synaptic responses and postsynaptic excitability were observed between the different pathways. Halothane blocked stratum radiatum transmission by depressing CA 1 neuron excitability, but perforant path inputs to dentate were blocked via a reduction in synaptic strength; sufficient to overcome a halothane-induced increase in granule neuron excitability (Figure 19). Enflurane, in contrast, enhanced the discharge of CA 1 neurons but depressed the stratum radiatum evoked EPSP; the major effect on dentate granule neurons was a depression of discharge. EPSP responses for radiatum and oriens inputs to CA 1 neurons appeared to be more sensitive to isoflurane than EPSP responses for perforant path inputs to dentate neurons (Figure 17) and depression of discharge was observed on both populations of neurons (Figure 19).

These anaesthetic effects involve actions on mixed excitatory and inhibitory fiber systems projecting to each neuronal population. Stimulation of stratum radiatum results in activation of numerous fibers, from several brain areas, which contribute to the recorded field potentials; although Schaffer-collateral fibers contribute most to the response. A similar situation exists for stimulus evoked potentials from the other two pathways. It is likely that some of the differences in effects between pathways represents a non-homogenous distribution of fiber (and transmitter) systems; which could partially explain differential anaesthetic actions between pathways. Anaesthetic effects on interneurons could also contribute to apparent differences in effects on the principal neurons (Gage, McKinnon and

Robertson, 1986).

Results with the inhalation agents suggest that some of the anaesthetic 'recognition' sites may be different on the same postsynaptic neurons. Isoflurane, for example, enhanced the discharge of CA 1 neurons by reducing threshold (Figure 19), and this resulted in larger population spike amplitudes following stimulation of radiatum inputs. This reduction in threshold for radiatum inputs was not generalized to inputs from oriens fibers, since only a depression of oriens evoked population spikes was observed (Figure 18). Similarly, enflurane also reduced the discharge threshold of CA 1 neurons when radiatum inputs were tested, but an increase in oriens evoked population spikes was not observed, even though EPSP amplitudes were not depressed and spontaneous burst discharges were generated by the CA 1 neuron population. Thus, the anaesthetics produced quite different effects on the postsynaptic discharge of CA 1 neurons, dependent on which synaptic input brought the membrane to threshold. Anaesthetic recognition sites in the basilar dendrites must be different from those in the apical dendrites of CA 1 neurons to account for the different effects on postsynaptic excitability.

10.4.2 Concentration-Dependent Biphasic Responses

General anaesthetics produce more than a simple depression of CNS excitability; instead, a concentration-dependent continuum of excitatory and depressant actions have been described, based on measurements of cortical EEG, brain stem, and thalamic neuron activities, and

evoked potentials recorded from cats with chronically implanted electrodes (Winters, 1982; Stevens et al., 1984). Furthermore, different anaesthetics produce unique patterns of excitatory and depressant effects, *in vivo*, and specific brain regions are differentially affected by the same anaesthetic (Winters and Kott, 1979; Winters, 1982; Stevens et al., 1984). Similar patterns of concentration-dependent biphasic actions have been reported for anaesthetics on invertebrate neuronal preparations (Johnston, 1978; Kleinhaus and Prichard, 1979; Landau, Richter and Cohen, 1979b; Judge, 1983) and include differential, biphasic actions, on single isolated neurons (MacIver and Roth, 1987c; Roth, Tan and MacIver, 1986; Weston and Roth, 1987).

Previous studies of general anaesthetic effects on isolated mammalian CNS preparations did not describe biphasic excitatory and depressant actions. Depression of excitatory synaptic responses (Richards, 1983), enhancement of synaptic inhibition (Owen et al., 1986; Gage, McKinnon and Robertson, 1986), or decreased postsynaptic excitability (Morris, 1986; Macdonald, Skerritt and Werz, 1986) appeared to be the dominant actions on isolated mammalian neurons. These effects would produce only depression of CNS electrical activity. Recent studies using the rat hippocampal slice preparation (Nicoll and Madison, 1982; Carlen, Gurevich and O'Beirne, 1984) implicate an enhanced potassium conductance as the major action leading to depression of CNS excitability (see also: Krnjevic, 1972; 1986).

A common or 'unitary' effect (eg: enhanced potassium conductance) cannot account for the selective and pathway-specific effects observed in this study. The data are more compatible with results from intact mammalian preparations (Winters, 1982) which clearly demonstrate biphasic and multiple mechanisms of anaesthetic action. A 'unitary' mechanism of action for general anaesthetics, leading to a concentration-dependent depression of the CNS (eg: Nicoll and Madison, 1982), is incompatible with the different profiles of effects observed clinically and on CNS electrical activity (Winters, 1982).

10.5 Enflurane Induced Seizure-Like Activity

The seizure-like burst discharges produced by enflurane are consistent with recent (Stevens et al., 1984) and earlier (Darimont and Jenkins, 1977; Black, 1979) observations from mammalian *in vivo*, preparations. These studies reported both excitatory and depressant actions of enflurane on CNS electrical activity. The present results extend these observations to include a cortical site of action for biphasic and convulsive effects of enflurane, since ascending afferent systems are not operative in the *in vitro* brain slice preparation. Drug effects on ascending inputs to cortical centers may exacerbate seizure-like actions in hippocampal and neocortical areas, *in vivo*. The present results demonstrate the intrinsic seizure susceptibility of the hippocampal formation and relate these anaesthetic-induced effects to actions at a neuronal level.

Enflurane did not appear to depress synaptic inhibition in either DG or CA 1 pathways; instead, enhanced inhibition was apparent from the paired pulse latency analysis. Increases in background firing activity of inhibitory interneurons also suggests an enhancement of inhibition. Stevens and co-workers (1984) noted that multiple unit activity in brainstem regions could be increased by enflurane, which may reflect a generalized stimulatory action on neuronal excitability in the CNS. Enflurane appeared to increase postsynaptic excitability in both CA 1 and DG regions, since increased PS amplitudes were observed in response to equivalent EPSP input (see Figure 19). Similar excitatory effects have been observed in the presence of other volatile anaesthetics, in vivo (Winters, 1982).

10.5.1 Mechanism of Burst Firing.

The seizure-like actions produced by enflurane were correlated with decreases in the threshold for discharge of CA 1 neurons. This was particularly evident from input/output analysis of recordings in the CA 1 region (Figure 19). All effective concentrations produced a shift to the left in the input/output relationship indicating that less synaptic input was required to produce postsynaptic discharge. This shift in the stratum radiatum EPSP axis intercept is consistent with a decrease in threshold of the CA 1 neurons, and would be expected to accompany enflurane-induced spontaneous burst firing. A direct excitation of pyramidal neurons appears to be the most likely mechanism of enflurane-induced seizure activity in the present study, and has been previously postulated to underly the epileptogenic

effects of kainate and folate (Olney, Fuller and deGubareff, 1981; Clifford and Ferrendelli, 1983) By comparison, DG neuron excitability did not appear to be altered by enflurane, since a shift in the perforant path EPSP axis intercept was not observed (see Figure 19). This is consistent with a lack of enflurane-induced seizure activity in the dentate area. Enflurane-induced increases in DG field potential responses appear to be produced via a postsynaptic effect, since equivalent EPSP input resulted in larger PS amplitudes, however, this enhanced response was not associated with a lower threshold for discharge.

10.5.2 Differential Burst Sensitivity of Hippocampal Regions.

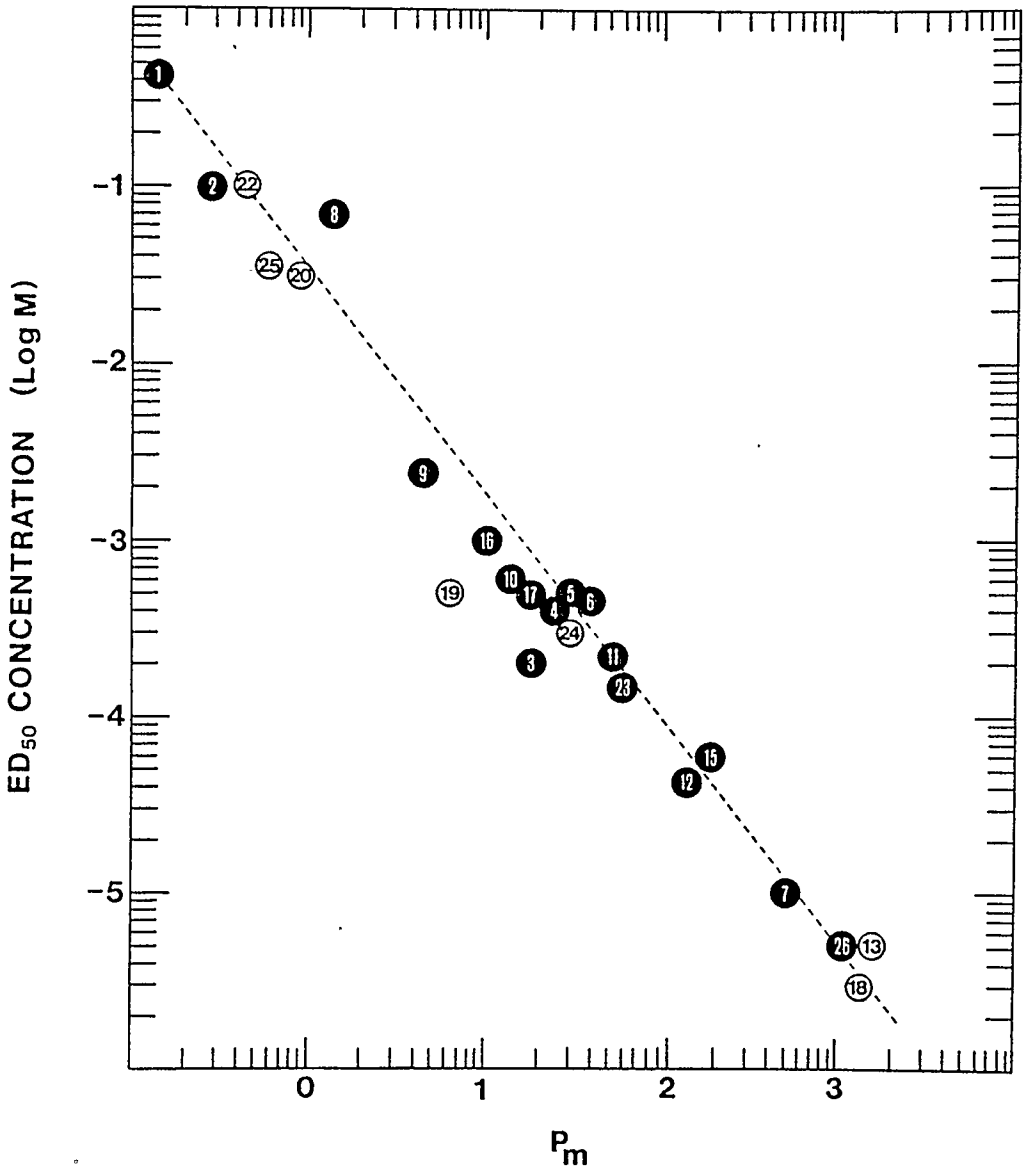
Inputs to CA 1 neurons were altered by the lowest vapour concentrations of enflurane; significant depression occurred in this pathway although DG responses remained virtually unchanged or enhanced. This differential sensitivity cannot be explained on the basis of drug distribution or kinetics, since the concentration at these sites should be similar in thin (400 μ) slices of tissue. Furthermore, onset times of drug effects for the two pathways were identical and rapid (< 60 s). Differences in drug efficacy could be due to a differential localization and/or composition of sites of action in the pathways studied. It is well known, for example, that DG neurons are less sensitive to the actions of other convulsants than the pyramidal regions of hippocampus, and this may reflect dominant inhibitory mechanisms in the DG area (Schwartzkroin, 1983). Recent studies of pencillin and GABA antagonist actions on DG neurons also demonstrated a relatively

high resistance to convulsant-induced burst firing (Fricke and Prince, 1984), which was attributed to the apparent lack of inward calcium currents in these cells. CA 1 and CA 3 neurons are also more sensitive than DG cells to the non-specific effects produced by anoxia and mechanical damage (Kelly, 1982; Lipton and Whittingham, 1979). These latter two forms of perturbation produce seizure activity which is similar to that produced by enflurane; however, important differences are apparent. Anoxia and mechanical damage, for example, produced irreversible or only partially reversible alterations (Whittingham, Lust and Passonneau, 1984), while enflurane effects were readily reversible. Furthermore, enflurane-induced seizure activity tended to be intermittent, and burst responses did not correlate directly with stimulus intensity. Anoxic burst responses, in contrast, increased at higher stimulus intensities and occurred in response to each stimulus (MacIver and Roth, unpublished observation; see also Schwartzkroin, 1983).

10.6 Correlation Between Synaptic Actions and Lipid Solubility

Major support for a 'unitary' theory of anaesthetic action comes from the correlation between anaesthetic potencies and partition coefficients for membrane/buffer (Pm) or solvent/water systems (Roth 1979; Roth and Miller, 1986). The potency of an anaesthetic varies exponentially with respect to it's lipid solubility, ie: Log [anaesthetic] = a (Log Pm) + b, which yields a linear relationship when anaesthetic concentrations are plotted against lipid solubility (eg: Pm) on a Log - Log axis. Figure 23 illustrates that this correlation exists for

Figure 23 - Graph showing the relationship between ED_{50} concentrations for alteration of stretch receptor discharge activity and membrane/buffer partition coefficients for a number of anaesthetics (data from MacIver and Roth, 1987). Open circles are for anaesthetics which increased the discharge rate, closed circles are for agents which produced depression. Enflurane - 4, halothane - 6, pentobarbital - 17, and phenobarbital - 19, isoflurane was not examined in this study; number 1 is ethanol and 18 is the steriod anaesthetic, alphaxalone.


ED50 CONCENTRATION (Log M)
-1
-2
-3
-4
-5
0
1
2
3
Pm
1
22
8
25
20
9
16
10
17
5
4
6
24
11
3
23
15
12
7
26
13
18
19

anaesthetic effects on electrically active membranes; in this case for the discharge activity of isolated crayfish stretch receptor neurons, regardless of whether an anaesthetic produces an excitatory (increased discharge rate) or depressant action (data from MacIver and Roth 1987). Table 1 lists the partition coefficients for membrane/buffer (Pm) and octanol/water (Pow) for the anaesthetics used in the present study, together with ED 50 values for depression of hippocampal transmission and predicted potencies for producing anaesthesia. Figure 24 demonstrates that the anaesthetic potency for depression of RAD population spike amplitudes varied exponentially with respect to membrane/buffer partition coefficients; as predicted by the unitary theory. A correlation ($r = 0.983$) exists between Log membrane/buffer partition coefficients and Log ED 50 concentrations required to depress stratum radiatum synaptic transmission in the present study (Figure 25), also in keeping with a unitary theory. Depression of stratum radiatum transmission was better correlated with predicted *in vivo* potencies than were the minimum effective concentrations for producing hyperpolarization reported by Nicoll and Madison (1982). This correlation was improved ($r = 0.977$) by omitting the data for phenobarbital (Figure 26), which is not a particularly good anaesthetic (Olsen et al., 1986; Roth and Miller, 1986).

A rather poor correlation ($r = 0.634$) was found between Log ED 50 values and Log octanol/water partition coefficients for the anaesthetics (Figure 27), although the correlation was improved ($r = 0.931$) when data for pentobarbital was excluded. This anomally for pentobar-

TABLE 1: HALF-MAXIMAL CONCENTRATIONS FOR DEPRESSION

OF HIPPOCAMPAL SYNAPTIC RESPONSES AND SOLUBILITY OF ANAESTHETICS

ANAESTHETIC	SYNAPTIC PATHWAY RAD	OR	PP	IN VIVO POTENCY	Pm	Pow
PENTOBARBITAL	0.22	0.10	0.25	0.14	46.0 (1)	135
PHENOBARBITAL	0.16	0.10	0.16	0.60	59.0 (2)	295
HALOTHANE	0.40	0.20	0.60	0.30	32.4 (1)	199
ISOFLURANE	0.60	0.45	0.65	0.45	27.8 (1)	139
ENFLURANE	0.90	0.85	0.92	0.60	25.2 (1)	126

Footnotes:

(a) RAD - stratum radiatum inputs to CA 1, OR - stratum oriens inputs to CA 1 pyramidal neurons, PP - perforant path inputs to dentate granule neurons; half-maximal (ED_{50}) concentration for depression of population spike amplitudes, POTENCY data are from reference 18 of Nicoll and Madison (1982), all concentrations are in mM.

(b) References for membrane/buffer partition coefficients (Pm) are 1 - Hansch et al (1975), 2 - Roth and Miller (1986), and octanol/water (Pow) data are from Roth and Miller (1986), except for isoflurane and phenobarbital which calculated (5 x Pm) as per Seeman (1972).

Figure 24 - Graph showing the relationship between ED for depression of stratum radiatum (RAD) inputs to CA 1 and membrane/buffer partition coefficients (Pm from Table 1). Abbreviations for this and subsequent figures: ENF - enflurane, ISO - isoflurane, HAL - halothane, PB - pentobarbital, PH - phenobarbital, ED_{50} - half-maximal concentration.

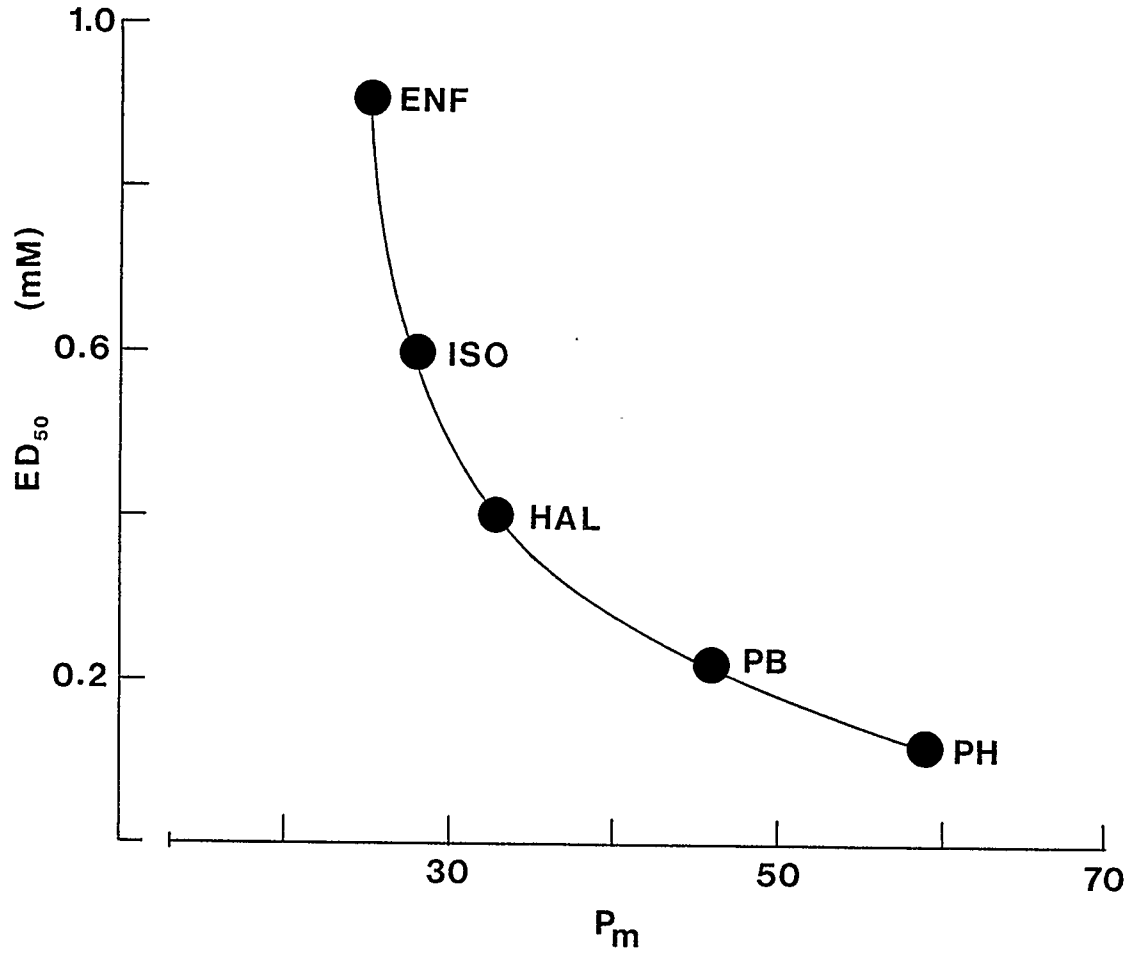

1.0
0.6
0.2
(mM)
ED_{50}
ENF
ISO
HAL
PB
PH
30
50
70
P_m

Figure 25 - Graph showing the relationship between ED_{50} for depression of RAD inputs to CA 1 and membrane/buffer partition coefficients (Pm from Table 1), plotted on Log - Log axis. Dashed line is the linear regression analysis.

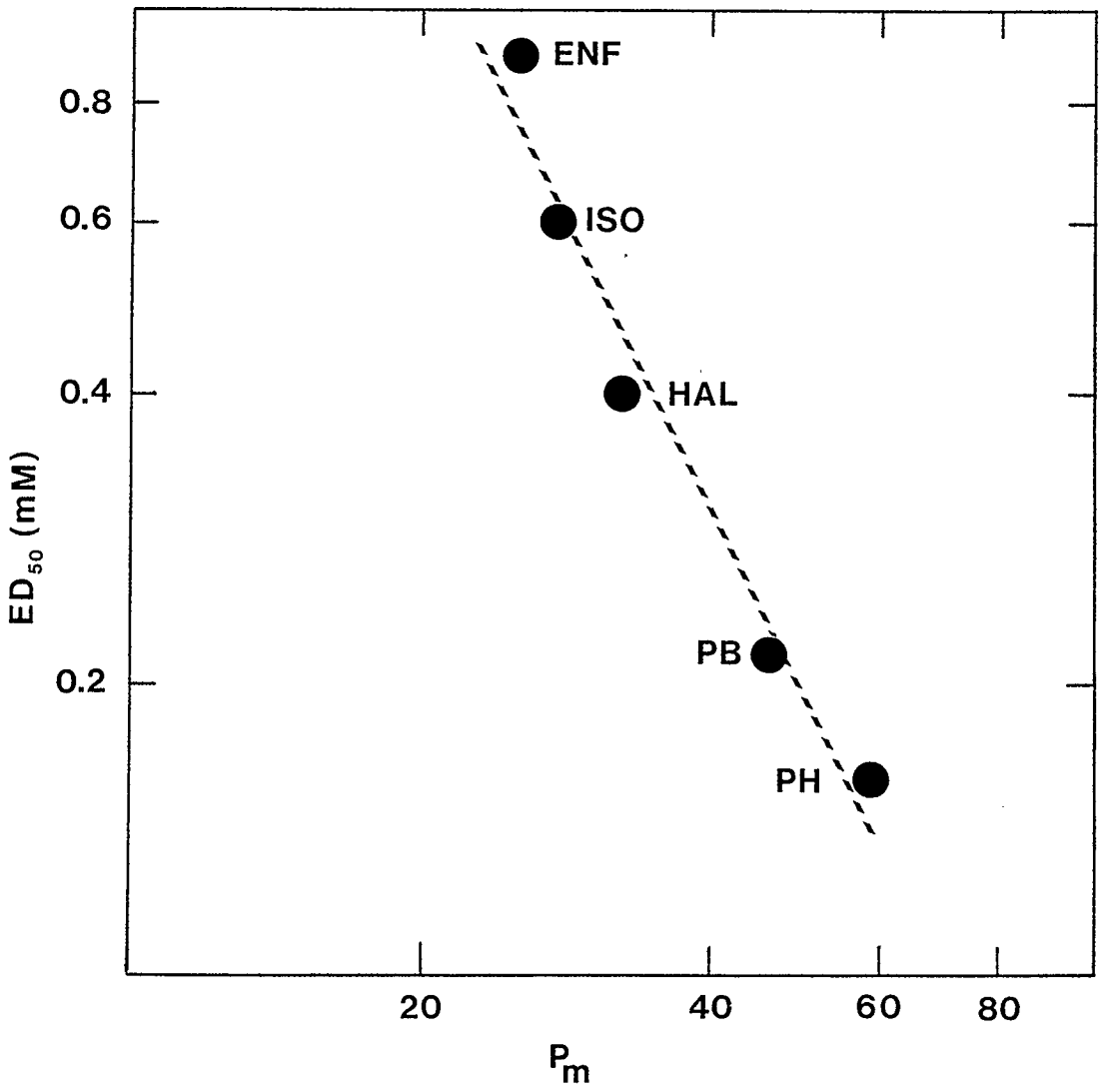

ENF
ISO
HAL
PB
PH
0.8
0.6
0.4
0.2
ED50 (mM)
20
40
60
80
Pm

Figure 26 - Graph showing the relationship between ED_{50} for depression of RAD inputs to CA 1 (circles) or minimum effective concentrations for hyperpolarization (squares; data from Nicoll and Madison, 1982) and predicted in vivo potencies for the anaesthetics (data from reference 18 of Nicoll and Madison, 1982). Dashed line is the linear regression analysis, excluding data for phenobarbital (PH), for the RAD ED_{50} data only. Note that this is a linear - linear plot.

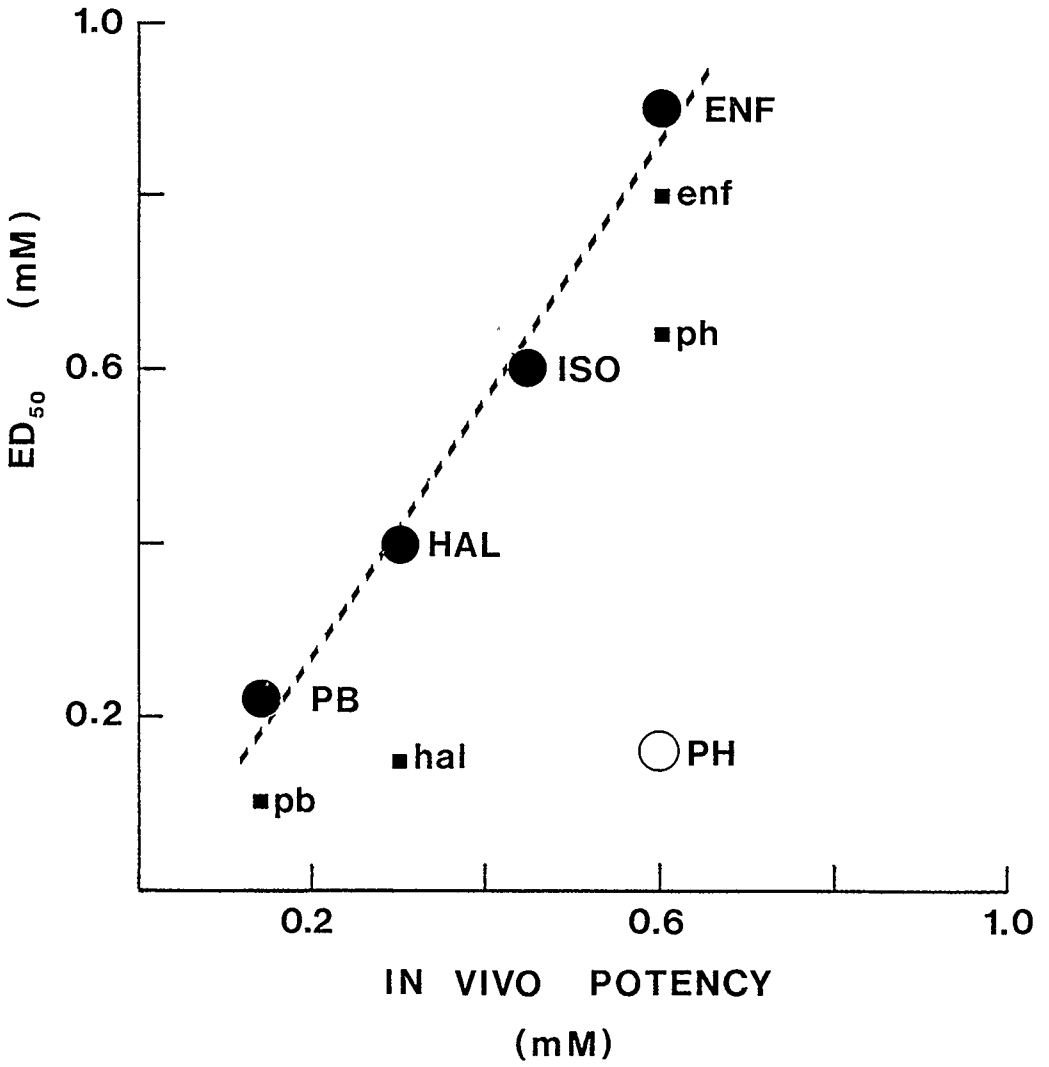
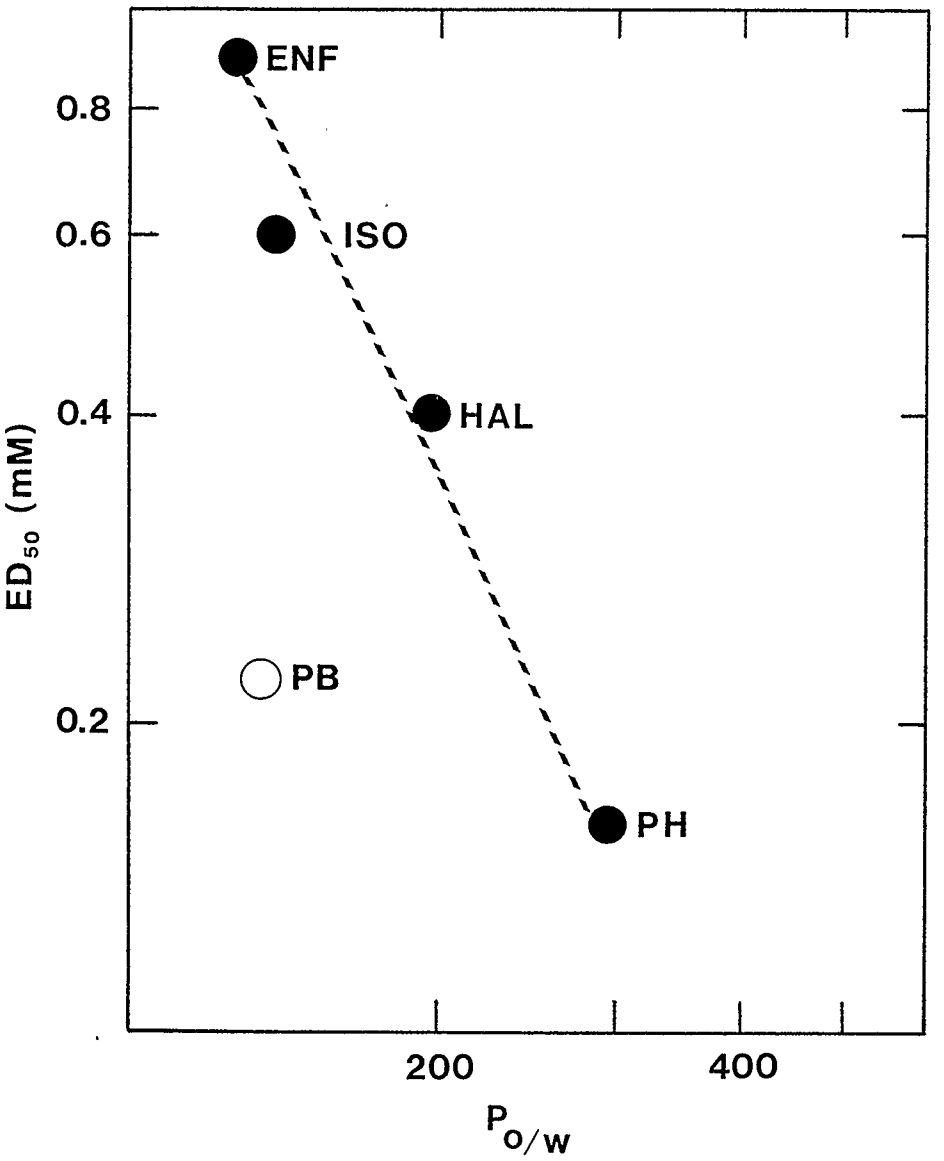

1.0
0.6
0.2
ED50 (mM)
ENF
enf
ph
ISO
HAL
PB
hal
PH
pb
0.2
0.6
1.0
IN VIVO POTENCY
(mM)

Figure 27 - Graph showing the relationship between ED_{50} for depression of RAD inputs to CA 1 and octanol/water partition coefficients (Pow from Table 1). Dashed line is the linear regression analysis, excluding the data for PB.


ENF
ISO
HAL
PB
PH
0.8
0.6
0.4
0.2
ED50 (mM)
200
400
Po/w

bital (and other negatively charged anaesthetics) has been noted previously (Roth and Seeman, 1972; Seeman, 1972), and may be related to the degree of ionization at physiological pH (pKa for pentobarbital = 8.1; phenobarbital = 7.4). An alternative explanation for the anomally is that the potency for pentobarbital is enhanced via actions on selective membrane sites (receptors), which are not present in simple solvent/water systems (eg: for octanol/water; Pow). A receptor site for pentobarbital has been described in rat cortical neuron membranes and this site discriminates between pentobarbital (IC 50 = 100 uM) and phenobarbital (IC 50 = 4000 uM), which could account for the difference in potency vs. Pow for the two barbiturates (Olsen et al., 1986). Furthermore, this receptor site also discriminates between (+) and (-) pentobarbital, with an IC 50 ratio of 1.4:1 comparable to the potency ratio (1.6:1) found in the present study for these stereoisomers (Olsen et al., 1986).

An additional explanation can be offered to account for the rather poor correlation observed between ED 50 concentrations and Pow (Figure 27). The 'traditional' correlations between anaesthetic potencies and partition coefficients (or lipid solubility) work best when comparing anaesthetics which differ over several orders of magnitude in potency (eg: Seeman, 1972; Seeman and Roth, 1972; Franks and Lieb, 1986; MacIver and Roth, 1987; see Figure 23). The anaesthetics used in the present thesis differ by less than 1 order of magnitude in potency. A good correlation does not always exist over a narrow potency range, as pointed out by Elliott and Haydon (1986), and this

may reflect an ability of biological membranes to discriminate between lipid-soluble agents on the basis of slight structural differences (Landau, Richter and Cohen, 1979a, 1979b).

10.7 Summary of Differential Effects on Synaptic Transmission

Hippocampal synaptic pathways are capable of discriminating amongst the five anaesthetics studied, evidenced by the differential effects produced on radiatum, oriens and perforant path field potential responses. Furthermore, the CA 1 pyramidal and dentate granule neuron populations also responded differently to the five anaesthetics studied. A summary of the differential effects observed is provided in Table 2. The only agent which produced a common action on all three pathways and two neuron populations was phenobarbital (row two of Table 2), evidenced by a 'universal' depression of EPSP, PS and antidromic field potentials. Pentobarbital, halothane, isoflurane and enflurane produced pathway-specific actions (compare biphasic and monophasic actions on EPSP and PS of RAD, OR and PP synaptic responses; rows 1, and 3 to 5 of Table 2). The anaesthetics also altered the postsynaptic excitability of CA 1 and DG neuron populations in a differential manner, eg: halothane, which increased the excitability of DG but depressed CA 1 neurons (row 3, columns 8 and 10). The other agents studied did not alter DG neuron excitability, but produced variable effects on CA 1 discharge threshold. As pointed out earlier, the marked decrease in CA 1 discharge threshold produced by enflurane may account for its seizure-inducing properties.

TABLE 2: DIFFERENTIAL EFFECTS OF ANAESTHETICS ON HIPPOCAMPAL SYNAPTIC TRANSMISSION

ANAESTHETIC		EFFECTS ON FIELD POTENTIAL RESPONSES									
		RAD		OR		PP		CA 1		DG	
		EPSP	PS	EPSP	PS	EPSP	PS	ANTI	EXCIT	ANTI	EXCIT
		1	2	3	4	5	6	7	8	9	10
PENTOBARBITAL	1	↑↓	↑↓	↓	↓	↑↓	↓	↓	↓	↓	–
PHENOBARBITAL	2	↓	↓	↓	↓	↓	↓	↓	↓	↓	–
HALOTHANE	3	↓	↓	↓	↓	↓	↑↓	↓	↓	↓	↑
ISOFLURANE	4	↓	↑↓	↓	↓	↓	↓	↓	↑	↓	–
ENFLURANE	5	↓	↓	↓	↓	↓	↑↓	↓	↑	↑↓	–

Footnotes:

(a) RAD - stratum radiatum inputs to CA 1, OR - stratum oriens inputs to CA 1 pyramidal neurons, PP - perforant path inputs to dentate granule (DG) neurons, ANTI - antidromic field potential amplitude, EXCIT - excitability, from the EPSP axis intercept of Input/Output curves, increased excitability means a smaller EPSP dV/dt was able to produce discharge of the postsynaptic neurons.

(b) Dark arrow - major effect, light arrow - minor effect, and direction of arrow indicates an increase (up) or decrease (down) in response relative to pre-anaesthetic control responses. Concentration-dependent biphasic effects are indicated (↑↓), with the arrow on the left for the lowest effective concentration. Lack of effect indicated by –.

Several of the recorded responses were 'universally' depressed by all five anaesthetics (columns 3,4 and 7 of Table 2), although the degree of depression produced varied between agents; eg: the major action of pentobarbital on OR evoked responses was depression of PS amplitudes, in contrast to phenobarbital which produced depression of synaptic input (EPSP) to a greater extent than CA 1 discharge (PS). All other responses (columns 1,2,5,6,8,9 and 10) were differentially affected by the anaesthetics; eg: pentobarbital and isoflurane produced biphasic effects on RAD evoked PS responses, phenobarbital and halothane produced marked depression, and enflurane only marginally depressed this response (column 2). The barbiturates and halothane depressed CA 1 discharge, but enflurane and isoflurane increased the excitability of CA 1 neurons (column 8). Thus, the anaesthetics studied exhibited some common actions, but also a number of distinctly different effects. The particular profile of effects observed was dependent on the anaesthetic, concentration, synaptic pathway, neuron population and/or the response (EPSP or PS) measured.

These results allow two conclusions to be offered:

1) anaesthetics produce more than a simple depression of synaptic responses and excitability,
2) different anaesthetics do not produce the same profile of effects on an <u>in vitro</u> preparation of mammalian CNS neurons.

Why have previous studies of anaesthetic actions on hippocampal slices reported common effects produced by all agents? The methods

used in the present study could provide an explanation. Previous studies (Nicoll and Madison, 1982; Carlen et al., 1984; Yoshimura et al., 1985) used intracellular recording techniques, while extracellular field potentials were measured for this thesis. Intracellular recording from hippocampal neurons is difficult, and stable responses last for relatively brief periods (0.5 to 4 hr). Constraints are imposed which limit the experimental protocol; eg: a concentration-dependency of recorded responses was not provided in previous reports. If only a single (higher) concentration was considered for each anaesthetic in the present study, then only a depression of field potentials would have been apparent (except for enflurane-induced burst activity). In addition, interpretation of intracellular data is complicated by the variability in observed anaesthetic effects. For example, Nicoll and Madison (1982) reported that halothane hyperpolarized CA 1 pyramidal neurons; Yoshimura et al. (1985) reported that halothane depolarized these neurons.

Incomplete concentration-response analysis could account for some differences, but can be excused, given the inherent difficulties of intracellular studies; but there is a further problem with the intracellular technique which is harder to reconcile. The increased 'resolution' gained with intracellular recording is offset by a decrease in 'scope'; ie: only a small portion of a system can be measured at a given time. This problem is well illustrated by comparing the effects produced by phenobarbital and pentobarbital on isolated crayfish stretch receptor neurons (Roth, Tan and MacIver, 1986). Using intra-

cellular recording techniques, both barbiturates were shown to depolarize the membrane potential, increase membrane resistance, decrease discharge threshold, depress synaptic potentials (GABA-mediated IPSP), and (at higher concentrations) both barbiturates reduced action potential peak amplitudes, spike dV/dt, and slowed conduction velocities (Tan, MacIver and Roth, 1981). Equimolar concentrations (eg: 0.1 to 0.5 mM) of phenobarbital and pentobarbital produced similar effects. Taken together, these actions on membrane electrical properties should result in increased stretch-induced discharge; that is, the 'system' consisting of physiological input (stretch), membrane integration, and action potential output should be affected in the same way by both barbiturates. The intact system response (measured with extracellular techniques) is not affected in the same way; phenobarbital increases discharge activity (as expected), but pentobarbital produces depression of stretch-induced discharge (Roth, Tan and Tooth, 1983). Thus, drug effects on an integrated system may not be reflected at the level of individual membrane effects (Roth, Tan and MacIver, 1986), even when the 'system' consists of only a single isolated neuron. Measurement of effects at the cellular level are prerequisite to an understanding of differential anaesthetic actions on membrane electrical properties (MacIver and Roth, 1987b). For example, the different effects produced by barbiturates on discharge activity can only be understood when the entire stretch receptor system (including GABA inhibitory modulation) is considered (Roth, Tan and MacIver, 1986).

Anaesthetic effects on hippocampal synaptic transmission and neuronal excitability also involve multiple and selective actions on a complicated 'system(s)'. It is not surprising that effects on field potential responses should reflect such complexity (see Table 2). Differential actions, together with the excitatory and depressant effects observed, were predicted from studies of anaesthetic responses in the intact central nervous system (Clark and Rosner, 1973; Stockard and Bickford, 1975; Winters, 1982; Stevens et al., 1984). A detailed understanding of the membrane mechanisms of anaesthetic action will include an explanation for effects at higher levels of organization (synaptic and cellular).

10.8 Membrane Recognition Sites and Mechanisms of Anaesthetic Action

Neuronal membranes exhibit a high degree of molecular structure resulting from the nonrandom distribution of membrane associated proteins (reviewed by Poo, 1985). Membrane proteins are often organized into functional macromolecular complexes (eg: receptors and ion channels) which occur in abundance at cellular specializations (eg: dendritic spines, spike trigger zones). In addition, phospholipid and cholesterol molecules forming the bilayer also appear to be heterogenously distributed in microdomains of membrane (Severs and Robenek, 1983; Berdan and Shivers, 1985). As a consequence, neuronal membranes provide numerous distinct hydrophobic zones, differing in protein/lipid composition (hence solubility), which provide distinctly different sites for anaesthetic interaction (Figure 28). The growing literature demonstrating selective anaesthetic interactions have led

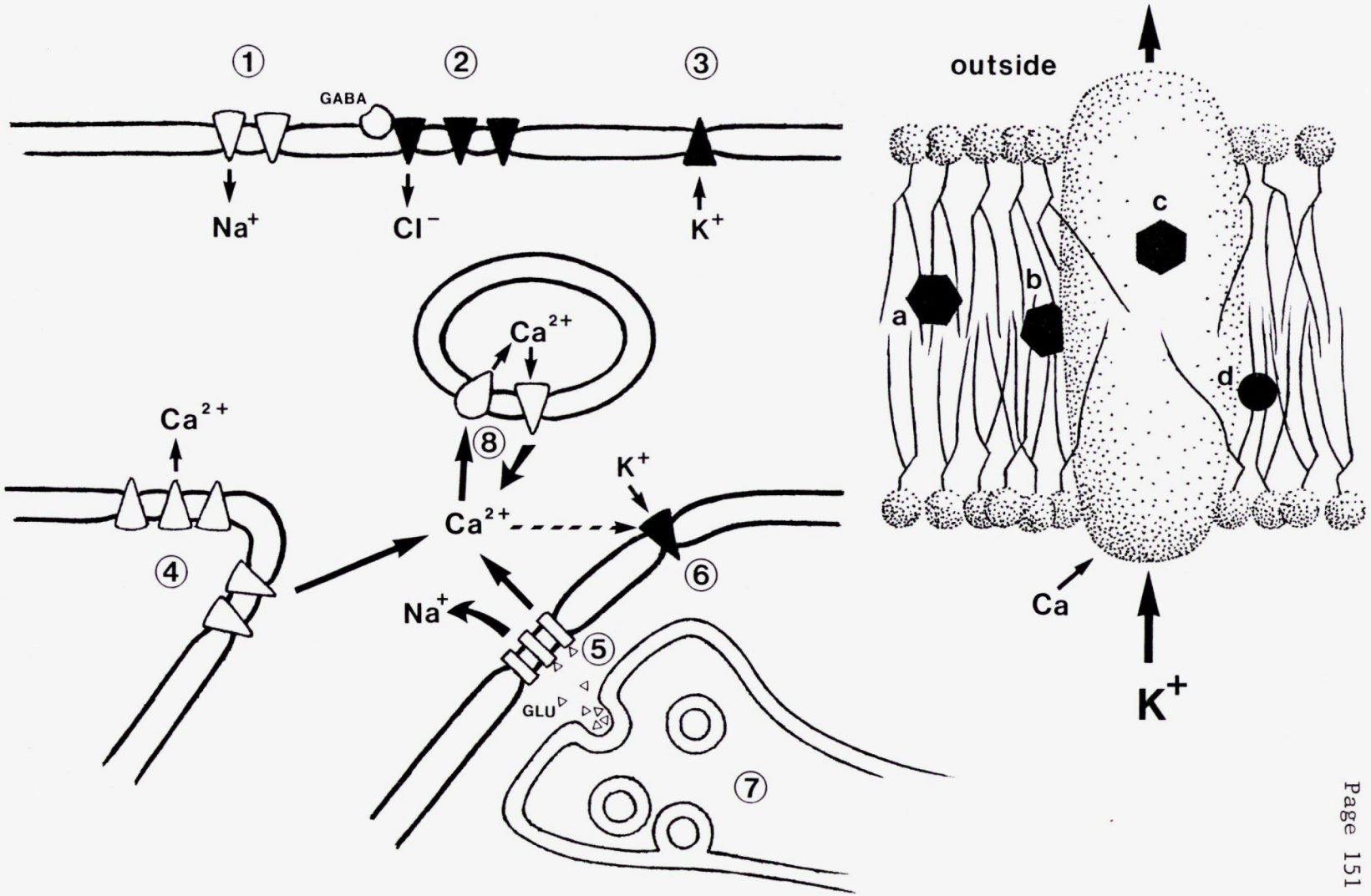
several independent investigators to propose 'recognition sites' for anaesthetics (Landau, Richter and Cohen, 1979; Richards et al., 1978; Roth, 1980). Results of the present thesis are best explained by anaesthetic interactions with hydrophobic microdomains in neuronal membranes; accounting for the correlation between potency and membrane/buffer partition coefficients (Figure 25). A difference in the lipid/protein composition of some microdomain(s) results in differential susceptibilities to anaesthetic perturbation; providing anaesthetic recognition sites which discriminate between agents on the basis of molecular structure (Figure 28). A high degree of selectivity is exhibited by anaesthetic recognition sites, evidenced by the different potencies for stereoisomers of pentobarbital, and the differential (eg: biphasic vs monophasic and burst-inducing) effects of structural isomers (enflurane and isoflurane). The existence of recognition sites may permit further, detailed, studies of anaesthetic Structure-Activity Relationships, which could provide a powerful new approach for probing selective actions on excitable membranes and synaptic transmission.

Figure 28 - Diagram of some probable sites of action of general anaesthetics. On the right, three possible sites of anaesthetic action are shown with respect to a transmembrane ion (potassium) channel; a - lipid site, b - a site at the interface between lipid and protein, c - protein site (see reviews by Roth, 1979; Miller, 1985). The results of the present study do not offer the resolution to discriminate between a, b, or c; however, it was apparent that structural differences between anaesthetics resulted in differential responses which may come about via interactions at different sites (eg: compare b and d). It is argued that the protein/lipid composition in different microdomains of membrane determines the solubility and structural requirements of a given anaesthetic 'recognition' site (see text). Different microdomains of membrane, in turn, result from the non-random (heterogenous) distribution of proteins which can be differentially localized to subsynaptic membranes and afferent fiber terminals (see review by Poo, 1985).

(Continued...)

Figure 28 (Cont.)

On the left, a hypothetical region of dendrite is shown receiving an excitatory synapse (lower right). A heterogenous distribution of membrane proteins in CA 1 neuron dentrites may exist for: 1) sodium channels coupled to glutamate receptors (giving rise to 'hot spots' for responses to iontophoretically applied glutamate), 2) chloride channels coupled to GABA-A receptors on somal and GABA-B receptors on dendritic membrane, 3) voltage dependent potassium channels, modulated by muscarinic cholinergic agents, 4) calcium channels at dendritic spike initiation zones (branch points?), 5) calcium channels coupled to glutamate (NMDA) receptors at subsynaptic zones, 6) calcium-dependent potassium channels, 7) proteins in or on pre-synaptic terminal membranes, 8) proteins regulating intracellular calcium concentrations. Anaesthetic actions at each of these sites have been described (see reviews by Judge, 1983; Collingridge, 1985; Krnjevic, 1986; Morris, 1986; Richards, 1983), and this is only a partial catalogue of probable sites of anaesthetic action. It is postulated that the multiple effects produced by anaesthetics are related to the large number of membrane microdomains with which they can interact, and differential effects occur via selective interactions at some of these sites (anaesthetic recognition sites).


①
②
③
GABA
Na+
Cl−
K+
Ca2+
Ca2+
⑧
K+
Ca2+
④
⑥
Na+
⑤
GLU
⑦
outside
a
b
c
d
Ca
K+

11.0 REFERENCES

1. Abraham, W. C., Bliss, T. V. P., and Goddard, G. V. (1985) Heterosynaptic changes accompany long-term but not short-term potentiation of the perforant path in the anaesthetized rat. J. Physiol., 363 pp. 335-349.
2. Adams, D. J., Gage, P. W., and Hamill, O. P. (1982) Inhibitory postsynaptic currents at Aplysia cholinergic synapses: effects of permeant anions and depressant drugs. Proc. Royal Soc. Lond. B, 214 pp. 335-337.
3. Adams, P. R. (1976) Drug blockade of open end-plate channels. J. Physiol., 260 pp. 531-552.
4. Adams, P. R. (1974) The mechanism by which amylobarbitone and thiopentone block the end-plate response to nicotinic antagonists. J. Physiol., 241 pp. 41P.
5. Alger, B. E. and Nicoll, R. A. (1982) Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied in vitro. J. Physiol., 328 pp. 125-141.
6. Andersen, P., Bliss, T. V. P., and Skrede, K. K. (1971) Unit analysis of hippocampal population spikes. Exp. Brain Res., 13 pp. 208-221.
7. Andersen, P., Blackstad, T. W., and Lomo, T. (1966) Location and identification of excitatory synapses on hippocampal pyramidal cells. Exp. Brain Res., 1 pp. 236-248.
8. Andersen, P., Bliss, T. V. P., and Skrede, K. K. (1971) Lamellar

organization of hippocampal excitatory pathways. Exp. Brain Res., 13 pp. 222-238.

9. Andersen, P., Sundberg, S. H., Sveen, O., Swann, J. W., and Wigstrom, H. (1980) Possible mechanisms for long-lasting potentiation of synaptic transmission in hippocampal slices from guinea pigs. J. Physiol., 302 pp. 463-482.
10. Andersen, P., Bland, B. H., and Dudar, J. D. (1973) Organization of hippocampal output. Exp. Brain Res., 17 pp. 152-168.
11. Andersen, P. (1975) Organization of hippocampal neuurons and their connections, in The Hippocampus (Vol. 1), edited by R. L. Isaacson and K. H. Pribram (Plenum Press, New York) pp. 155-175.
12. Andersen, P. (1981) Properties of glutamate excitation of hippocampal pyramidal cells, in Glutamate: Transmitter in the Central Nervous System, edited by P. J. Roberts, J. Storm-Mathisen and G. A. R. Johnston (Wiley, New York)
13. Andrews, P. R., Jones, G. P., and Lodge, D. (1979) Convulsant, anitconvulsant and anaesthetic barbiturates. Eur. J. Pharmacol., 55 pp. 115-120.

15. Assaf, S. Y. and Miller, J. J. (1981) Commissural potentiation of perforant path evoked responses in the dentate gyrus of the rat. Can. J. Physiol. Pharmacol., 59 pp. 1117-1121.
16. Assaf, S. Y. and Miller, J. J. (1978) Neuronal transmission in the dentate gyrus: role of inhibitory mechanisms. Brain Res., 151 pp. 587-592.
17. Ault, B. and Nadler, J. V. (1983) Effects of baclofen on

synaptically-induced cell firing in the rat hippocampal slice. Br. J. Pharmacol., 80 pp. 211-219.

18. Balestrino, M., Aitken, P. G., and Somjen, G. G. (1986) The effects of moderate changes of extracellular K and Ca on synaptic and neural function in the CA 1 region of the hippocampal slice. Brain Res., 377 pp. 229-239.
19. Bangham, A. D. and Mason, W. (1979) The effect of some general anaesthetics on the surface potential of lipid monolayers. Br. J. Pharmacol., 6 pp. 259-265.
20. Bangham, A. D. and Mason, W. T. (1980) Anesthetics may act by collapsing pH gradients. Anesthesiol., 53 pp. 135-141.
21. Barker, J. L. and Mathers, D. A. (1981) GABA receptors and the depressant action of pentobarbital. TINS, January pp. 10-13.
22. Barker, J. L. (1975) CNS depressants: effect on postsynaptic pharmacology. Brian Res., 92 pp. 35-42.
23. Barker, J. L. and Gainer, H. (1973) Pentobarbital: selective depression of excitatory postsynaptic potentials. Science, 182 pp. 720-722.
24. Barker, J. L. and Ransom, B. R. (1978) Amino acid pharmacology of mammalian central neurones grown in tissue culture. J. Physiol. (Lond.), 280 pp. 331-354.
25. Benardo, L. S., Masukawa, L. M., and Prince, D. A. (1982) Electrophysiology of isolated hippocampal pyramidal dendrites. J. Neurosci., 2 pp. 1614-1622.
26. Berdan, R. C. and Shivers, R. R. (1985) Filipin-cholesterol complexes in plasma membranes and cell junctions of Tenebrio molitor

epidermis. Tissue & Cell, 17 pp. 177-187.

27. Black, G. W. (1979) Enflurane. Br. J. Anaesthes., 51 pp. 627.

28. Bliss, T. V. P. and Gardner-Medwin, A. T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the unanaesthetized rabbit following stimulation of the perforant path. J. Physiol., 232 pp. 357-374.

29. Bliss, T. V. P. and Richards, C. D. (1971) Some experiments with in vitro hippocampal slices. J. Physiol., 214 pp. 7-9P.

30. Bowyer, J. F. and Winters, W. D. (1981) The effects of various anesthetics on amygdaliod kindled seizures. Neuropharmacol., 20 pp. 199-209.

31. Bradley, D. J. and Richards, C. D. (1984) Temperature-dependence of the action of nerve blocking agents and its relationship to membrane-buffer partition coefficients: thermodynamic implications for the site of action of local anaesthetics. Br. J. Pharmacol., 81 pp. 161-167.

32. Braswell, L. M., Sauter, J-F., and Miller, K. W. (1984) Interactions between the effects of pressure and general anesthetics on the binding of acetylcholine to receptors from Torpedo. Br. J. Pharmacol., 83 pp. 305-311.

33. Brown, T. H. and Johnston, D. (1983) Voltage-clamp analysis of mossy fiber synaptic input to hippocampal neurons.. J. Neurophysiol., 50 pp. 487-507.

34. Bruce, D. and Christiansen, R. (1965) Morphological changes in the giant amoeba (Chaos chaos) induced by halothane and ether. Exp. Cell Res., 40 pp. 544-553.

35. Bull, M. H., Brailsford, J. D., and Bull, B. S. (1982) Erythrocyte membrane expansion due to the volatile anesthetics, the 1-alkanols, and benzyl alcohol. Anesthesiol, 57 pp. 399-403.

36. Butler, T. C. (1950) Theories of general anesthesia. Pharmacol. Rev., 2 pp. 121-160.

37. Caldwell, K. K. and Harris, R. A. (1985) Effects of anesthetic and anticonvulsant drugs on calcium-dependent efflux of potassium from human erythrocytes. Eur. J. Pharmacol., 107 pp. 119-125.

38. Carlen, P. L. (1985) Enhanced neuronal K conductance: possible common mechanisms for sedative-hypnotic drug action. Can. J. Physiol. Pharmacol., 63 pp. 831-837.

39. Carlen, P. L., Gurevich, N., and O'Beirne, M. (1984) Electrophysiological evidence for increased calcium-mediated potassium conductance by low-dose sedative hypnotic drugs., in Calcium in Biological Systems, edited by E.P. Rubin D. Weiss and J.W. Putney (Plenum, New York) pp. 193-200.

40. Carlen, P. L., Gurevich, N., and Durand, D. (1982) Ethanol in low doses augments calcium-mediated mechanisms measured intracellularly in hippocampal neurons. Science, 215 pp. 306-308.

41. Catterall, W. (1984) The molecular basis of neuronal excitability. Science, 223 pp. 653-661.

42. Catterall, W. A. (1975) Activation of the action potential Na ionophore by neurotoxins: An allosteric model. J. Biol. Chem., 252 pp. 8669-8676.

43. Chalazonitis, N. (1967) Selective actions of volatile anesthetics on synaptic transmission and autorhythmicity in single

identifiable neurons. Anesthesiol., 28 pp. 111-122.

44. Chaykowski, F. T. (1979) Interaction of small molecules with phospholipid bilayer membranes: A spin label study. Chem. Phys. Lipids, 23 pp. 111-123.

45. Clark, D. L. and Rosner, B. S. (1973) Neurophysiologic effects of general anesthetics I. The electroencephalogram and sensory evoked response in man. Anesthesiol., 38 pp. 564-582.

46. Clarke, R. F., Daniels, S., Harrison, C. B., Jordan, M. J., Paton, W. D. M., Smith, E. B., and Smith, R. A. (1978) Potency of mixtures of general anaesthetic agents. Br. J. Anaesthes., 50 pp. 979-987.

47. Clifford, D. B. and Ferrendelli, J. A. (1983) Neurophysiologic effects of folate compounds in hippocampus, in vitro. Brain Res., 266 pp. 209-216.

48. Coleman, P. and Roth, S. H. (1986) Combinations of volatile anaesthetics do not produce simple additive effects on a single isolated neuron. Can. Anaesthetists Soc. J., 33 pp. S108-S109.

49. Collingridge, G. L. (1985) Long term potentiation in the hippocampus: Mechanisms of initiation and modulation by neurotransmitters. TIPS, 6 pp. 407-411.

50. Collins, G. G. S. (1980) Release of endogenous amino acid neurotransmitter candidates from rat olfactory cortex: possible regulatory mechanisms and the effects of pentobarbitone.. Brain Res., 190 pp. 517-525.

51. Cote, I. L., Zbicz, K. L., and Wilson, W. A. (1978) Barbiturate-induced outward currents in Aplysia neurones. Nature,

274 pp. 594-596.

52. Cote, I. L. and Wilson, W. A. (1980) Effects of barbiturates on excitatory and inhibitory responses to applied neurotransmitters in Aplysia. Nature (Lond.), 274 pp. 594.

53. Crawford, J. M. (1970) Anaesthetic agents and the chemical sensitivity of cortical neurons. Neuropharmacol., 9 pp. 31-46.

54. Creager, R., Dunwiddie, T., and Lynch, G. (1980) Paired-pulse and frequency facilitation in the CA 1 region of the in vitro rat hippocampus. J. Physiol, 299 pp. 409-424.

55. Crossland, J. and Merrick, A. J. (1954) The effect of anaesthetics on the acetylcholine content of brain. J. Physiol. (Lond.), 186 pp. 121-136.

56. Crunelli, V., Forda, S., and Kelly, J. S. (1983) Blockade of amino acid-induced depolarizations and inhibition of excitatory post-synaptic potentials in rat dentate gyrus. J. Physiol., 341 pp. 627-640.

57. Cutler, R. W. P., Markowitz, D., and Dudzinski, D. S. (1974) The effect of barbiturates on (H3)GABA transport in rat cerebral cortex slices. Brain Res., 81 pp. 189-197.

58. Darimont, P. C. and Jenkins, L. C. (1977) The influence of i.v. anaesthetics on enflurane-induced central nervous system seizure activity. Can. Anaesth. Soc. J., 24 pp. 42.

59. Diamond, B. I. (1975) Differential membrane effects of general and local anesthetics. Anesthesiol., 43 pp. 651-660.

60. Dingledine, R. and Gjerstad, L. (1980) Reduced inhibition during epileptiform activity in the in vitro hippocampal slice. J.

Physiol., 305 pp. 297-313.

61. Dingledine, R. (1984) in Brain Slices, (Plenum Press, New York) pp. 1-442.

62. Dutar, P., Lamour, Y., and Jobert, A. (1986) Septo-hippocampal neurons in the rat: Further study of their physiological and pharmacological properties. Brain Res., 365 pp. 325-334.

63. Elliott, J. R. and Haydon, D. A. (1986) Mapping of general anaesthetic target sites. Nature, 319 pp. 77-78.

64. Esplin, B., Theoret, Y., Seward, E., and Capek, R. (1985) Epileptogenic action of penicillin derivatives: structure activity relationship. Neuropharmacol., 24 pp. 571-575.

65. Eyring, H., Woodbury, J. W., and D'Arrigo, J. S. (1973) A molecular mechanism of general anesthesia. Anesthesiol., 38 pp. 415-424.

66. Fink, B. R. and Cairnes, A. M. (1984) Differential slowing and block of conduction by lidocaine in individual afferent myelinated and unmyelinated axons. Anesthesiol., 60 pp. 111-120.

67. Firestone, L. L., Sauter, J., Braswell, L. M., and Miller, K. W. (1986) Actions of general anesthetics on acetylcholine receptor-rich membranes from Torpedo californica. Anesthesiol., 64 pp. 694-702.

68. Frank, G. B. and Sanders, H. D. (1965) A proposed common mechanism of action for general and local anaesthetics in the CNS. Br. J. Pharmacol. Chemother., 21 pp. 1-9.

69. Franks, N. and Lieb, W. (1982) The molecular mechanism of general anaesthesia: A review. Nature, 300 pp. 487-493.

70. Franks, N. P. and Lieb, W. R. (1979) The structure of lipid bilayers and the effects of general anaesthetics. J. Mol. Biol., 133 pp. 469-500.

71. Franks, N. P. and Lieb, W. R. (1986) Do direct protein/anesthetic interactions underlie the mechanism of general anesthesia, in Molecular and Cellular Mechanisms of Anesthetics, edited by S. H. Roth and K. W. Miller (Plenum, New York) pp. 319-332.

72. Fricke, R. A. and Prince, D. A. (1984) Electrophysiology of dentate gyrus granule cells. J. Neurophysiol., 51 pp. 195-209.

73. Fung, S-C and Fillenz, M. (1984) The actions of barbiturates on release of noradrenaline from rat hippocampal synaptosomes. Neuropharmcol., 23 pp. 1113-1116.

74. Gage, P. W. and Sah, P. (1982) Postsynaptic effects of some central stimulants at the neuromuscular junction. Br. J. Pharmacol., 75 pp. 493-499.

75. Gage, P. W., McBurney, R. M., and Schneider, G. T. (1975) Effects of some aliphatic alcohols on the conductance change caused by a quantum of acetylcholine at the toad neuromuscular junction. J. Physiol., 244 pp. 409-429.

76. Gage, P. W., Hamill, O. P., and Helden, D. Van (1979) Daul effects of ether on end-plate currents. J. Physiol., 287 pp. 353-361.

77. Gage, P. W., McKinnon, D., and Robertson, B. (1986) The influence of anesthetics on postsynaptic ion channels, in Molecular and Cellular Mechanisms of Anesthetics, edited by S. H. Roth and K. W.

Miller (Plenum, New York) pp. 139-153.

78. Gage, P. W. and Hamill, O. (1981) Effects of anaesthetics on ion channels in synapses, in Neurophysiology IV. Int. Rev. Physiol., 25, edited by R. Porter Baltimore) pp. 1-14.

79. Gage, P. W. and Hamill, O. (1975) General anaesthetics: Synaptic depression consistent with increased membrane fluidity. Neurosci. Lett., 1 pp. 61-65.

80. Galindo, A. (1969) Effects of procaine, pentobarbital and halothane on synaptic transmission in the central nervous system. J. Pharmacol. Exp. Ther., 169 pp. 185-195.

81. Gierasimov, V. and Janiszewski, L. (1967) The effect of urethane on some electrical properties of molluscan giant neurones. Experientia, 23 pp. 643-649.

82. Gissen, A. J., Covino, B. G., and Gregus, J. (1980) Differential sensitivities of mammalian nerve fibers to local anesthetic agents. Anesthesiol., 53 pp. 467-474.

83. Gjerstad, L., Andersen, P., Langmoen, I. A., Lundervold, A., and Hablitz, J. (1981) Synaptic triggering of epileptiform discharges in CA 1 pyramidal cells in vitro. Acta Physiol. Scand., 113 pp. 245-252.

84. Gribkoff, V. K. and Ashe, J. H. (1985) Responses of opossum and rat hippocampal CA 1 cells to paired stimulus volleys. Brain Res. Bull., 15 pp. 273-279.

85. Halsey, M. J. (1984) A reassessment of the molecular structure-functional relationships of the inhaled general anaesthetics. Br. J. Anaesthesia, 56 pp. 9S-25S.

86. Halsey, M. J., Brown, F. F., and Richards, R. E. (1978) Perturbations of model protein systems as a basis for the central and peripheral mechanisms of general anaesthesia. Proc. Ciba Foundation Symp., 60 pp. 123-128.

87. Halsey, M. J. and Eger, E. I. (1971) The effect of pressure on the anesthetic potency of nitrous oxide. Fed. Proc., 30 pp. 442.

88. Harris, R. A. (1984) Differential effects of membrane perturbants on voltage-activated sodium and calcium channels and calcium-dependent potassium channels. Biophys. J., 45 pp. 132-144.

89. Harrison, N. L. and Simmonds, M. A. (1983) Two distinct interactions of barbiturates and chlormethiazole with GABA receptor complex in rat cuneate nucleus in vitro. Br. J. Pharmacol., 80 pp. 387-394.

90. Haydon, D. A. and Urban, B. W. (1986) The actions of some general anaesthetics on the potassium current of the squid giant axon. J. Physiol. (Lond.), 373 pp. 311-327.

91. Hille, B. (1977) Local anesthetics: hydrophilic and hudrophobic pathways for the drug-receptor reaction. J. Gen. Physiol., 69 pp. 497-515.

92. Ho, I. K. and Harris, R. A. (1981) Mechanism of action of barbiturates. Ann. Rev. Pharmacol. Toxicol., 21 pp. 83-111.

93. Huang, L-Y. M. and Barker, J. L. (1980) Pentobarbital: Stereospecific actions of (+) and (-) isomers revealed on cultured mammalian neurons. Science, 207 pp. 195-197.

94. Iadarola, M. J., Fanelli, R. J., McNamara, J. O., and Wilson, W.

A. (1985) Comparsion of the effects of diphenylbarbituric acid, phenobarbital, pentobarbital and secobarbital on GABA-mediated inhibition and benzodiazepine binding. J. Pharmacol. Exp. Ther., 232 pp. 127-133.

95. Jackson, M. B., Lecar, H., Mathers, D. A., and Barker, J. L. (1982) Single channel currents activated by gamma-aminobutyric acid, muscimol, and (-)-pentobarbital in cultured mouse spinal neurons. J. Neurosci., 7 pp. 889-894.

96. Janoff, A. S. and Miller, K. W. (1982) A critical assessment of the lipid theories of general anesthetic action, in Biological Membranes, edited by D. Chapman (Academic Press, New York) pp. 417-476.

97. Janoff, A. S., Pringle, M. J., and Miller, K. W. (1981) Correlation of general anesthetic potency with solubility in membranes. Biochim. Biophys. Acta, 649 pp. 125-128.

98. Johnson, F. H., Brown, D., and Marsland, D. (1942) A basic mechanism in the biological effects of temperature pressure and narcotics. Science, 95 pp. 200-203.

99. Johnson, F. H. and Flagler, E. A. (1951) Activity of narcotized amphibian larvae under hydrostatic pressure. J. Cell Physiol., 37 pp. 15-25.

100. Johnston, D. (1978) Phenobarbital: concentration-dependent biphasic effects on aplysia burst-firing neurons. Neurosci. Lett., 10 pp. 175-180.

101. Judge, S. E. (1983) Effect of general anaesthetics on Synaptic ion channels. Br. J. Anaesth., 55 pp. 191-200.

102. Kamaya, H. (1979) Antagonism between high pressure and anesthetics in the thermal phase-transition of dipalmitoyl phosphatidycholine bilayer. Biochim. Biophys. Acta, 550 pp. 131-137.

103. Katz, Y. and Simon, S. A. (1977) Physical parameters of the anesthetic site. Biochim. Biophys. Acta, 471 pp. 1-15.

104. Kaufman, R. D. (1977) Biophysical mechanisms of anesthetic action: Historical perspective and review of current concepts. Anesthesiology, 46 pp. 49-62.

105. Kelly, J. S. (1982) Intracellular recording from neurons in brain slices in vitro, in Handbook of Psychopharmacology, edited by Snyder (Plenum, New York) pp. 95-183.

106. Kendall, T. J. G. and Minchin, M. C. W. (1982) The effects of anaesthetics on the uptake and release of amino-acid neurotransmitters in thalamic slices. Br. J. Pharmacol., 75 pp. 219-224.

107. Kendig, J. J. and Cohen, E. N. (1976) Neuromuscular function at hyperbaric pressures: Pressure anesthetic interactions. Am. J. Physiol., 230 pp. 1244-1249.

108. Kendig, J. J. and Grossman, Y. (1986) Homogenous and branching axons: Differing responses to anesthetics and pressure, in Molecular and Cellular Mechanisms of Anesthetics, edited by S. H. Roth and K. W. Miller (Plenum, New York) pp. 333-340.

109. Kendig, J. J., Courtney, K. R., and Cohen, E. N. (1979) Anesthetics: Molecular correlates of voltage and frequency dependent sodium channel block in nerve. J. Pharmacol. Exp. Ther., 210 pp. 446-452.

110. Kennedy, R. D. and Galindo, A. D. (1975) Comparative site of action of various anaesthetic agents at the mammalian myoneural junction. Br. J. Anaesth., 47 pp. 533-540.

111. Kerkut, G. A. and Wheal, H. V. (1981) in Electrophysiology of Isolated Mammalian CNS Preparations, (Academic Press, London)

112. Kleinhaus, A. L. and Pritchard, J. W. (1977) Pentobarbital actions on a leech neuron. Comp. Biochem. Physiol., 58C pp. 61-66.

113. Kleinhaus, A. L. and Prichard, J. W. (1979) Interaction of divalent cations and barbiturates on four identified leech neurons. Comp. Biochem. Physiol., 63C pp. 351-357.

114. Koblin, D. D. and Lester, H. A. (1979) Voltage-dependent and voltage-independent blockade of acetylcholine receptors by local anaesthetics in Electrophorus electroplaques. Mol. Pharmacol., 15 pp. 559-565.

115. Koblin, D. D. and Eger, E. I. (1980) An update on the theories of narcosis. New England J. Med., 37 pp. 63-69.

116. Kramis, R., Vanderwolf, C. H., and Bland, B. H. (1975) Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: relations to behaviour and effects of atropine, diethyl ether, urethane and pentobarbital. Exp. Neurol., 49 pp. 58-85.

117. Krnjevic, K. (1974) Central actions of general anaesthetics, in Molecular Mechanisms in General Anaesthesia, edited by M.J. Halsey, R. Millar, J.A. Sutton (Churchill-Livingston, New York) pp. 65-89.

118. Krnjevic, K. (1986) Cellular and synaptic effects of general anesthetics, in Molecular and Cellular Mechanisms of Anesthetics, edited by S. H. Roth and K. W. Miller (Plenum, New York) pp. 3-16.

119. Krnjevic, K. (1972) Excitable membranes and anesthetics, in Cellular Biology and Toxicity of Anesthetics, edited by B. R. Fink (Williams and Wilkins, Baltimore) pp. 3-9.

120. Krnjevic, K., Morris, M. E., and Reiffenstein, R. J. (1982) Stimulation-evoked changes in extracellular K and Ca in pyramidal layers of the rat's hippocampus. Can. J. Physiol. Pharmacol., 60 pp. 1643-1657.

121. Landau, E. M., Richter, J., and Cohen, S. (1979) The mean conductance and open-time of the acetylcholine receptor channels can be independently modified by some anesthetic and convulsant ethers. Mol. Pharmacol., 16 pp. 1075-1083.

122. Landau, E. M., Richter, J., and Cohen, S. (1979) Differential solubilities in subregions of the membrane: A nonsteric mechanism of drug specificity. J. Med. Chem., 22 pp. 325-327.

123. Langmoen, I. A. and Andersen, P. (1983) Summation of excitatory postsynaptic potentials in hippocampal pyramidal cells. J. Neurophysiol., 50 pp. 1320-1329.

124. Larrabee, M. G. and Posternak, J. M. (1952) Selective action of anesthetics on synapses and axons in mammalian sympathetic ganglia. J. Neurophysiol., 15 pp. 91-114.

125. Lawrence, D. K. and Gill, E. W. (1975) Structurally specific effects of some steroid anesthetics on spin-labeled liposomes. Mol. Pharmacol., 11 pp. 280-286.

126. Lee, A. G. (1976) Model for action of local anaesthetics. Nature, 262 pp. 545-548.

127. Lenaz, G., Curatola, G., Mazzanti, L., Bertoli, E., and Pastuszko, A. (1979) Spin label studies on the effect of anaesthetics in synaptic membranes. J. Neurochem., 32 pp. 1689-1694.

128. Lenaz, G. (1978) Spin label studies on the effect of anaesthetics in synaptic membranes. J. Neurochem., 32 pp. 1689-1695.

129. Leung, L-W S. (1981) Differential effects of pentobarbital and ether on the synaptic transmission of the hippocampal CA 1 region in the rat. Electroenceph. Clin. Neurophysiol., 51 pp. 291-305.

130. Lever, M. K., Miller, K. W., Paton, W. D. M., and Smith, E. B. (1971) Pressure reversal of anaesthesia. Nature, 231 pp. 368-371.

131. Lipton, P. and Whittingham, T. W. (1979) The effect of hypoxia on evoked potentials in the in vitro hippocampus. J. Physiol., 287 pp. 427-438.

132. Little, H. J. and Thomas, D. L. (1986) The effects of anaesthetics and high pressure on the responses of the rat superior cervical ganglion in vitro. J. Physiol. (Lond.), 374 pp. 387-399.

133. Loh, H. H. and Law, P. Y. (1980) The role of membrane lipids in receptor mechanisms. Ann. Rev. Pharmacol. Toxicol., 20 pp. 201-234.

134. Lomo, T. (1971) Patterns of activation in a monosynaptic cortical pathway: the perforant path input to the dentate area of the hippocampal formation. Exp. Brain Res., 12 pp. 18-45.

135. MacIver, M. B., Hanna, T., and Roth, S. H. (1979) Biphasic

effects of anaesthetic agents on an isolated neuron. Proc. Can. Fed. Biol. Soc., 22 pp. 55.

136. MacIver, M. B. and Roth, S. H. (1980) The effects of halothane on the neruonal output, membrane properties and synaptic transmission of an isolated neuron. Proc. West. Pharmacol. Soc., 23 pp. 405-411.

137. MacIver, M. B. and Roth, S. H. (1987b) Anesthetics produce differential actions on membrane responses of the crayfish stretch receptor neuron. Eur. J. Pharmacol., (submitted)

138. MacIver, M. B. and Roth, S. H. (1987) Anesthetics produce differential actions on the discharge activity of a single neuron. Eur. J. Pharmacol., (submitted)

139. Macdonald, R. L. and Barker, J. L. (1978) Different actions of anticonvulsant and anaesthetic barbiturates revealed by use of cultured mammalian neurons. Science, 200 pp. 775-777.

140. Macdonald, R. L. and Barker, J. L. (1979) Anticonvulsant and anaesthetic barbiturates: Different postsynaptic actions in cultured mammalian neurons. Neurology, 29 pp. 432-447.

141. Macdonald, R. L., Skerritt, J. H., and Werz, M. A. (1986) Barbiturate and benzodiazepine actions on mouse neurons in cell culture, in Molecular and Cellular Mechanisms of Anesthetics., edited by S.H. Roth and K.W. Miller (Plenum, Boston) pp. 17-25.

142. Maleque, M. A., Warnick, J. E., and Albuquerque, E. X. (1981) The mechanism and site of action of ketamine on skeletal muscle. J. Pharmacol. Exp. Ther., 219 pp. 638-643.

143. Mastrangelo, C. J. and Trudell, T. R. (1978) Effect of clinical

concentrations of halothane on phospholipid-cholesterol membrane fluidity. Mol. Pharmacol., 14 pp. 463-467.

144. Mastrangelo, C. J. (1979) Nerve membrane lipid fluidity: Opposing effects of high pressure and ethanol. Undersea Biomed. Res., 6 pp. 47-53.

145. Matthews, E. K. and Quilliam, J. P. (1964) Effects of central depressant drugs on acetylcholine release. Br. J. Pharmacol. Chemother., 22 pp. 415-424.

146. Metcalfe, J. C., Seeman, P., and Burgen, A. S. V. (1968) The proton relaxation of benzyl alcohol in eyrthocyte membranes. Mol. Pharmacol., 4 pp. 87-95.

147. Meyer, H. (1899) Welche Eigenschaft der Anasthetica bedingt ihre narkitische Wirkung?. Arch. Exp. Pathol. Pharmacol., 42 pp. 109-118.

148. Meyer, H. (1901) Zur Theorie der Alkolnarkose. Der Einfluss wechselnder Temperatur auf Wirkungsstarke und Theilungscoefficient der Narcotica. Arch. Exp. Pathol. Pharmakol., 46 pp. 338-346.

149. Meyer, K. H. (1937) Contributions to the theory of narcosis. Trans. Faraday Soc., 33 pp. 1062-1068.

150. Miller, J. C. and Miller, K. W. (1975) Approaches to the mechanism of action of general anesthetics, in Physiological and Pharmacological Biochemistry, Biochemistry Series One, edited by H. F. K. Blaschko (Butterworth, London) pp. 33-76.

151. Miller, K. W. (1977) The opposing physiological effects of high pressures and inert gases. Fed. Proc., 36 pp. 1663-1667.

152. Miller, K. W. (1985) The nature of the site of general

anesthesia. Int. Rev. Neurobiol., 27 pp. 1-61.

153. Miller, K. W. (1974) Intermolecular forces. Br. J. Anaesthesia, 46 pp. 190-201.

154. Miller, K. W., Paton, W. D. M., Smith, R. A., and Smith, E. B. (1973) The pressure reversal of anesthesia and the critical volume hypothesis. Mol. Pharmacol., 9 pp. 131-143.

155. Miller, K. W. and Wilson, M. W. (1978) The pressure reversal of a variety of anesthetic agents in mice. Anesthesiology, 48 pp. 104-110.

156. Minchin, M. C. W. (1981) The effect of anaesthetics on the uptake and release of gamma-aminobutyrate and D-aspartate in rat brain slices. Br. J. Pharmacol., 73 pp. 681-693.

157. Moore, R. Y. and Halaris, A. E. (1975) Hippocampal innervation by serotonin neurons of the midbrain raphe in the rat. J. Comp. Neurol., 152 pp. 163-174.

158. Mori, K., Kawamata, M., Miyajima, S., and Fujita, M. (1972) The effects of several anesthetic agents on the neuronal reactive properties of thalamic relay nuclei in the cat. Anesthesiol., 36 pp. 550-557.

159. Mori, K. (1973) Excitation and depression of CNS electrical activities induced by general anesthetics, in Proceedings of the 5th World Congress of Anaesthesiology, edited by M. Fujita (Excerpta Medica, Amsterdam) pp. 40.

160. Morris, M. E. (1986) General anesthetics and intracellular free calcium ions, in Molecular and Cellular Mechanisms of Anesthetics, edited by S. H. Roth and K. W. Miller (Plenum, New York) pp. 65-

74.

161. Morris, M. E. (1978) Facilitation of synaptic transmission by general anaesthetics. J. Physiol., 284 pp. 307-325.

162. Mullins, L. J. (1954) Some physical mechanisms in narcosis. Chem. Rev., 54 pp. 289-323.

163. Nathon, P. W. and Sears, T. A. (1961) Some factors concerned in differential nerve block by local anesthetics. J. Physiol., 157 pp. 565-580.

164. Nicoll, R. A., Eccles, J. C., Oshima, T., and Rubia, F. (1975) Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. Nature, 258 pp. 625.

165. Nicoll, R. A. (1979) Differential postsynaptic effects of barbiturates on chemical transmission, in Neurobiology of Chemical Transmission, edited by M. Otsuka and Z. Hall (Wiley, New York) pp. 267-278.

166. Nicoll, R. A. and Madison, D. V. (1982) General anesthetics hyperpolarize neurons in the vertebrate central nervous system. Science, 217 pp. 1055-1057.

167. Olney, J. W., Fuller, T. A., and deGubareff, T. (1981) Kainate-like neurotoxicity of folates. Nature, 292 pp. 165.

168. Olsen, R. W., Fischer, J. B., and Dunwiddie, T. V. (1986) Barbiturate enhancement of gamma-aminobutyric acid receptor binding and function as a mechanism of anesthesia, in Molecular and Cellular Mechanisms of Anesthetics., edited by S.H. Roth and K.W. Miller (Plenum, Boston) pp. 165-177.

170. Olsen, R. W. (1981) GABA-Benzodiazepine-Barbiturate receptor interactions. J. Neurochem., 37 pp. 1-13.

171. Olsen, R. W. (1982) Drug interactions at the GABA receptor-ionophore complex. Ann. Rev. Pharmacol. Toxicol., 22 pp. 245-277.

172. Overton, E. (1901) Studien uber die Narkose zugleich ein Beitrag zur allgemeinen Pharmakologie. Jena, Verlag von Gustav Fischer

173. Owen, D. G., Barker, J. L., Segal, M., and Study, R. E. (1986) Postsynaptic actions of pentobarbital in cultured mouse spinal neurons and rat hippocampal neurons, in Molecular and Cellular Mechanisms of Anesthetics., edited by S.H. Roth and K.W. Miller (Plenum, Boston) pp. 27-41.

174. Persson, A., Peterson, E., and Wahlin, A. (1978) EEG changes during general anaesthesia with enflurane (Ethrane) in comparison with ether. Acta Anaesthesiol. Scand., 22 pp. 339.

175. Poo, M. (1985) Mobility and localization of proteins in excitable membranes. Ann. Rev. Neurosci., 8 pp. 369-406.

176. Potashner, S. J., Lake, N., Langlois, E. A., Ploffe, L., and Lecavalier, D. (1980) Pentobarbital: differential effects on amino acid transmitter release, in Molecular Mechanisms of Anesthesia (Progress in Anesthesiology, Vol. 2), edited by B. R. Fink (Raven Press, New York) pp. 469-482.

177. Pritchard, J. W. (1972) Action of phenobarbital on a leech neuron. Neuropharmacol., 11 pp. 585-593.

178. Quastel, D. M. J. and Linder, T. M. (1975) Pre- and postsynaptic actions of central depressants at the mammalian neuromuscular

junction. Prog. Anesthesiology, 1 pp. 157-168.

179. Quastel, D. M. J., Hackett, J. T., and Okamoto, K. (1972) Presynaptic action of central depressant drugs: inhibition of depolarization-secretion coupling. Can. J. Physiol. Pharmacol., 50 pp. 279-287.

180. Quastel, J. H. (1956) Action of drugs on enzyme systems, in Enzymes: Units of Biological Structure and Function, edited by O. H. Gaebler (Academic Press, New York) pp. 523-535.

181. Ransom, B. R. and Barker, J. L. (1975) Pentobarbital modulates transmitter effects on mouse spinal neurones grown in tissue culture. Nature, 254 pp. 703-705.

182. Richards, C. D. (1983) Actions of general anaesthetics on synaptic transmission in the CNS. Br. J. Anaesth., 55 pp. 201-207.

183. Richards, C. D. and Smaje, J. C. (1976) Anaesthetics depress the sensitivity of cortical neurones to L-glutamate. Br. J. Pharmacol., 58 pp. 347-357.

184. Richards, C. D. (1980) In search of the mechanisms of anaesthesia. Trends in Neuroscience, 3 pp. 9-13.

185. Richards, C. D., Russell, W. J., and Smaje, J. C. (1975) The action of ether and methoxyflurane on synaptic transmission in isolated preparations of mammalian cortex. J. Physiol., 248 pp. 121-142.

186. Richards, C. D., Martin, K., Gregory, S., Keightley, C. A., Hesketh, T. R., Smith, G. A., Warren, G. B., and Metcalfe, J. C. (1978) Degenerate perturbations of protein structure as the

mechanism of anaesthetic action. Nature, 276 pp. 775-779.

187. Richards, C. D. (1973) On the mechanism of halothane anaesthesia. J. Physiol., 233 pp. 439-456.

188. Richards, C. D. (1978) The actions of anaesthetics on synaptic transmission. Gen. Pharmacol., 9 pp. 287-293.

189. Richards, C. D. and Smaje, J. C. (1974) The actions of halothane and pentobarbitone on the sensitivity of neurones in the guinea-pig prepiriform cortex to iontophoretically applied L-glutamate.. J. Physiol., 239 pp. 103-105.

190. Richards, C. D. and White, A. E. (1975) The actions of volatile anaesthetics on synaptic transmission in the dentate gyrus. J. Physiol. (Lond.), 252 pp. 241-263.

191. Richards, C. D. (1972) On the mechanism of barbiturate anaesthesia. J. Physiol. (Lond.), 227 pp. 749-756.

192. Richards, C. D. and Tegg, W. J. G. (1977) A superfusion chamber suitable for maintaining mammalian brain tissue slices for electrical recording. Br. J. Pharmacol., 59 pp. 526P.

193. Richards, C. D. and White, A. E. (1981) Additive and non-additive effects of mixtures of short-acting intravenous anaesthetic agents and their significance for theories of anaesthesia. Br. J. Pharmacol., 74 pp. 161-168.

194. Rosner, R. S. and Clark, D. L. (1973) Neurophysiological effects of general anesthetics. Anesthesiology, 39 pp. 59-81.

195. Roth, S. H., Tan, K-S., and MacIver, M. B. (1986) Selective and differential effects of barbiturates on neuronal activity, in Molecular and Cellular Mechanisms of Anesthetics, edited by S.H.

Roth and K.W. Miller (Plenum, Boston) pp. 43-56.

196. Roth, S. H. (1979) Physical mechanisms of Anaesthesia. Ann. Rev. Pharmacol. Toxicol., 19 pp. 159-178.

197. Roth, S. H. (1980) Membrane and cellular actions of anesthetic agents. Fed. Proc., 39 pp. 1595-1599.

198. Roth, S. H. and Jay, A. W. L. (1977) Permeation of erythocyte membrane by chlorpromazine and its relation to cell shape. Proc. Can. Fed. Biol. Soc., 20 pp. 41.

199. Roth, S. H., Jay, A. W. L., and Beck, J. S. (1974) Expansion of red blood cell membrane by butanol. Fed. Proc., 33 pp. 1591.

200. Roth, S. H., Smith, R. A., and Paton, W. D. (1976) Pressure antagonism of anesthetic induced conduction failure in frog peripheral nerve. Br. J. Anaesthesia, 48 pp. 621-628.

201. Roth, S. H. (1980) Mechanisms of Anaesthesia: A Review. Can. Anaesth. Soc. J., 27 pp. 433-439.

202. Roth, S. H. (1980) Membrane and cellular actions of anesthetic agents. Fed. Proc., 39 pp. 1595-1599.

203. Roth, S. H. and Seeman, P. (1972) The membrane concentrations of neutral and positive anesthetics (alcohols, chlorpromazine, morphine) fit the Meyer-Overton rule of anesthesia; negative narcotics do not. Biochim. Biophys. Acta, 255 pp. 207-219.

204. Roth, S. H. and Omand, W. D. (1982) Pregnane steriod anesthetics exhibit selective action on the firing activity of an isolated neuron. Proc. West. Pharmacol. Soc., 25 pp. 421-425.

205. Sato, M., Austin, G. M., and Yai, H. (1967) Increase in permeability of the postsynaptic membrane to potassium produced by

'Nembutal'. Nature, 215 pp. 1506-1508.

206. Schultz, D. and Macdonald, R. L. (1981) Barbiturate enhancement of GABA-mediated inhibition and activation of chloride ion conductance: Correlation with anticonvulsant and anaesthetic actions. Brain Res., 209 pp. 177-188.

208. Schwartzkroin, P. A. and Knowles, W. D. (1983) Local interactions in the hippocampus. Trends in Neurosciences, 6 pp. 88-92.

209. Schwartzkroin, P. A. (1983) Local circuit considerations and intrinsic neuronal properties involved in hyperexcitability and cell synchronization, in Basic Mechanisms of Neuronal Hyperexcitability, (Alan R. Liss, New York) pp. 75-108.

210. Seeman, P. (1972) The membrane actions of anesthetics and tranquilizers. Pharmacological Reviews, 24 pp. 583-655.

211. Segal, M. and Barker, J. L. (1984) Rat hippocampal neurons in culture: voltage-clamp analysis of inhibitory synaptic connections. J. Neurophysiol., 52 pp. 469-487.

212. Severs, N. J. (1983) Detection of microdomains in biomembranes: an appraisal of recent developments in freeze-fracture cytochemistry. Biochim. Biophys. Acta, 737 pp. 373-408.

213. Smaje, J. C. (1976) General anaesthetics and the acetylcholine-sensitivity of cortical neurones. Br. J. Pharmacol., 58 pp. 359-366.

214. Somjen, G. G. (1967) Effects of anesthetics on spinal cord of mammals. Anesthesiol., 28 pp. 135-143.

215. Somjen, G. G. (1963) Effects of ether and thiopental on spinal presynaptic terminals. J. Pharmacol. Exp. Ther., 140 pp. 396-402.

216. Staiman, A. and Seeman, P. (1977) Conduction-blocking concentrations of anesthetics increase with nerve axon diameter: studies with alcohol, lidocaine and tetrodotoxin on single myelinated fibers. J. Pharmacol. Exp. Ther., 201 pp. 340-349.

217. Steen, P. A. and Michenfelder, J. D. (1979) Neurotoxicity of anesthetics. Anesthesiology, 50 pp. 437.

218. Stevens, J. E., Fuginaga, M., Oshima, E., and Mori, K. (1984) The biphasic pattern of the convulsive property of enflurane in cats. Br. J. Anaesth., 56 pp. 395-403.

219. Stockard, J. and Bickford, R. (1975) The neurophysiology of anesthesia, in A Basis and Practice of Neuroanesthesia, edited by E. Gordon (Excerpta Med., Amsterdam) pp. 3-46.

220. Storm-Mathisen, J. (1977) Localization of transmitter candidates in the brain: The hippocampal formation as a model. Prog. in Neurobiology, 8 pp. 119-181.

221. Tan, K-S., MacIver, M. B., and Roth, S. H. (1981) Phenobarbital and pentobarbital produce opposite effects on a single isolated neuron. Proc. Can. Fed. Biol. Soc., 24 pp. 171.

222. Terrell, R. C. (1984) Physical and chemical properties of anaesthetic agents. Br. J. Anaesthesia, 56 pp. 3S-7S.

223. Teyler, T. J. (1980) Brain slice preparation: Hippocampus. Brain Res. Bull., 5 pp. 391-403.

224. Teyler, T. J. and DiScenna, P. (1984) Long-term potentiation as a candidate mnemonic device. Brain Res. Reviews, 7 pp. 15-28.

225. Trudell, J. R. (1977) A unitary theory of anesthesia based on lateral phase separations in nerve membranes. Anesthesiol., 46

pp. 5-10.

226. Turner, D. A. and Schwartzkroin, P. A. (1984) Passive electronic structure and dendritic properties of hippocampal neurons, in Brain Slices, edited by R. Dingledine (Plenum Press, New York) pp. 25-50.

227. Turner, R. W., Richardson, T. L., and Miller, J. J. (1984) Extracellular fields influence transmembrane potentials and synchronization of hippocampal neuronal activity. Brain Res., 294 pp. 255-262.

228. Turner, R. W., Richardson, T. L., and Miller, J. J. (1984) Ephaptic interactions contribute to paired pulse and frequency potentiation of hippocampal field potentials. Exp. Br. Res., 54 pp. 567-572.

229. Ueda, I., Hirakawa, M., Arakawa, K., and Kamaya, H. (1986) Do anesthetics fluidize membranes. Anesthesiology, 64 pp. 67-71.

230. Ueda, I., Kamaya, H., and Eyring, H. (1977) Depression of phase transition temperature in a model cell membrane by local anesthetics. Anesthesiology, 46 pp. 327-332.

231. Ueda, I. and Kamaya, H. (1973) Kinetic and thermodynamic aspects of the mechanism of general anesthesia in a model system of firefly luminescence in vitro. Anesthesiol., 38 pp. 425-436.

232. Ueda, I. and Kamaya, H. (1984) Molecular mechanisms of anesthesia. Anesthes. Analg., 63 pp. 929-945.

233. Vanderkooi, G., Shaw, J., Storms, C., Vennerstrom, R., and Chignell, D., On the mechanism of action of anesthetics. 1981.

234. Vanderkooi, J. M., Landesberg, R., Selick, H., and MacDonald, G.

G. (1977) Interaction of general anesthetics with phospholipid vesicles and biological membranes. Biochim. Biophys. Acta, 464 pp. 1-16.

235. Wachtel, R. E. (1984) Effects of some depressant drugs on synaptic responses to glutamate at the crayfish neuromuscular junction. Br. J. Pharmacol., 83 pp. 387-391.

236. Wardley-Smith, B. and Halsey, M. J. (1984) Mechanisms of general anaesthesia, in Anaesthesia Review, edited by L. Kaufman (Churchill-Livingston, Edinburgh) pp. 63-72.

237. Wardley-Smith, B. and Halsey, M. J. (1985) Mixtures of inhalation and i.v. anaesthetics at high pressure. Br. J. Anaesthesia, 57 pp. 1248-1256.

238. Weakly, J. N. (1969) Effect of barbiturates on quantal synaptic transmission in spinal motoneurones. J. Physiol. (Lond.), 204 pp. 63-71.

239. Weston, G. A. and Roth, S. H. (1985) The volatile anaesthetics halothane, enflurane and isoflurane produce selective effects on the firing pattern of a single isolated neuron. Can. Anaesthetists Soc. J., 32 pp. S96-S97.

240. Whittingham, T. M., Lust, W. D., and Passonneau, J. V. (1984) An in vitro model of ischemia: metabolic and electrical alterations in the hippocampal slice. J. Neuroscience, 4 pp. 793-802.

241. Winter, P. M. and Miller, J. N. (1984) Anesthesiology. Scientific American, April pp. 124-131.

242. Winters, W. D. (1982) A review of the continuum of drug-induced states of excitation and depression. Prog. in Drug Res., 26 pp.

225-263.

243. Winters, W. D. and Kott, K. S. (1979) Continuum of sedation, activation and hypnosis or hallucinosis: a comparison of low dose effects of pentobarbital, diazepam, or gamma-hydroxybutyrate in the cat. Neuropharmacology, 18 pp. 877-884.

244. Winters, W. D., Mori, K., Spooner, C. E., and Kado, R. T. (1967) Correlation of reticular and cochlear multiple unit activity with auditory evoked responses during wakefulness and sleep. Electroenceph. Clin. Neurophysiol., 23 pp. 539-548.

245. Winters, W. D. (1969) Neuropharmacological studies of drug induced states of CNS excitation and depression, in Drugs and the Brain, edited by P. Black (John Hopkins Univ. Press, Baltimore) pp. 93-114.

246. Wong, E. H. F., Leeb-Lundberg, L. M. F., Teichberg, V. I., and Olsen, R. W. (1984) Gamma-Aminobytyric acid activation of Cl-flux in rat hippocampal slices and its potentiation by barbiturates. Brain Res., 303 pp. 267-275.

247. Wong, R. K. S. and Prince, D. A. (1978) Participation of calcium spikes during intrinsic burst firing in hippocampal neurons. Brain Res., 159 pp. 385-390.

248. Yoshimura, M., Higashi, H., Fujita, S., and Shimoji, K. (1985) Selective depression of hippocampal inhibitory potentials and spontaneous firing by volatile anesthetics. Brain Res., 340 pp. 363-368.