THE UNIVERSITY OF CALGARY

GENERAL ANAESTHETICS ALTER THE RHYTHMICAL ACTIVITY OF HIPPOCAMPAL NEURONS

by

Jagdeep Kohli

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "General Anaesthetics Alter the Rhythmical Activity of Hippocampal Neurons" submitted by Jagdeep Kohli in partial fulfillment of the requirements for the degree of Master of Science.

Supervisor, Dr. S.H. Roth
Department of Pharmacology and Therapeutics

Dr. B.H. Bland
Department of Psychology

Dr. S. Weiss
Department of Pathology

Dr. C. Triggle
Department of Pharmacology and Therapeutics

MAY 27, 1992
Date

ABSTRACT

Historically, general anaesthetics were thought to produce similar actions on the mammalian central nervous system (CNS). This was thought to be due to a common site and mechanism of action. Recently it has been demonstrated that anaesthetics can produce differential effects. The purpose of this thesis was to further document the differential effects of anaesthetics on the mammalian CNS using the carbachol-induced rhythmical population activity (RPA) of the *in vitro* hippocampal slice as a model system.

It was observed that ethanol and isoflurane depressed the power of RPA in CA1 neurons . In contrast, pentobarbital, halothane and enflurane produced biphasic effects. Ethanol and pentobarbital increased the length of a single burst of RPA and pentobarbital eventually abolished the pattern whereas the volatile anaesthetics shortened the length. Ethanol produced biphasic effects on the frequency of RPA while all the other anaesthetics decreased the frequency. These differential effects support the hypothesis that anaesthetics act via multiple sites and/or mechanisms of action.

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DEDICATION

This thesis is dedictated to my wife Dianne

and to my parents, Jatinder and Swaran

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INTRODUCTION

1.1 Overview

Historically, general anaesthetics were thought to have a common site and mechanism of action and thus would produce similar actions on the central nervous system. This 'unitary' theory of anaesthetic action was based upon Meyer and Overton's classic observation of the correlation between lipid solubility and anaesthetic potency which was the basis for the lipid solubility hypothesis. However, it has been recently demonstrated that anaesthetics have differential effects which suggest that multiple mechanisms and/or sites of action are involved. It is the goal of this thesis to provide further evidence that anaesthetics have differential effects on the mammalian central nervous system and thus support the multiple mechanism and/or site hypothesis. Differential actions have been demonstrated on single isolated neurons and synaptic transmission of neuronal preparations. Recently, it has been shown that the neuronal circuitry of the *in vitro* hippocampal preparation can generate rhythmic population activity which is similar to *in vivo* rhythmical slow wave (RSA or theta) activity. This network activity was used to demonstrate the differential actions of several general anaesthetic agents.

The characteristics of general anaesthetics, the mechanism and sites of action, the actions of anaesthetics on central nervous systems, the use of the *in vitro* hippocampal

formation as a model system, and finally, the hypothesis and specific objectives of this thesis will be presented in following sections of the INTRODUCTION.

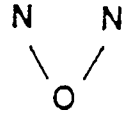
1.2 Characteristics of Anaesthetic Agents

General anaesthetics are an extremely diverse group of chemical compounds which are capable of producing analgesia, amnesia, loss of consciousness, inhibition of sensory and autonomic reflexes and skeletal muscle relaxation (Trevor and Miller, 1989). They range from simple molecules such as nitrous oxide to complex steroid molecules such as alfaxalone (Figure 1). Modern anaesthetics typically are, non-flammable, stable in oxygen and light and have low blood solubility (Terrell, 1984).

There is no apparent relationship between the chemical structure of anaesthetics and their pharmacological function (Halsey, 1984). This is in contrast to the typical interaction between drugs and biological systems which occur via associations with specific macromolecules known as receptors. Chemical structure determines what molecule will bind with what particular receptor among the vast array of chemically different binding sites and thus the ultimate pharmacological effect(s). Binding to a specific receptor is characterized by high affinity-low capacity profile, and a molecule binding tightly to its target tissue (i.e. binding energy >1000 kJ/Mol) in a structure-dependent manner with

Figure 1. The molecular structure of a few anaesthetic compounds including the ones used in this study: ethanol, pentobarbital, halothane, isoflurane and enflurane. Note that pentobarbital exists in two isomeric forms at the chiral carbon indicated by the arrow and that isoflurane and enflurane are structural isomers.

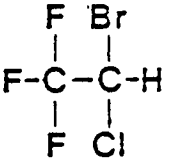
nitrous oxide:



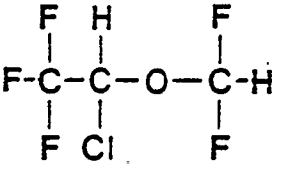
ethanol:

CH_3CH_2OH

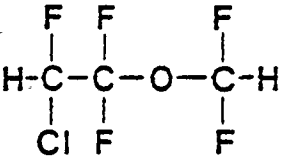
halothane:



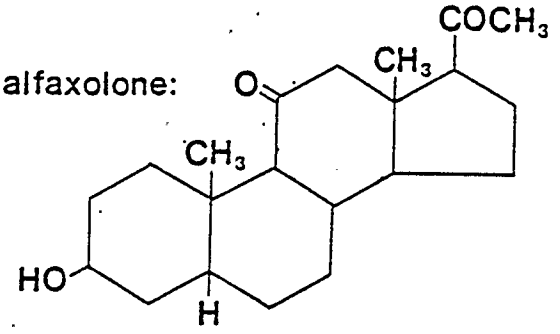
isoflurane:



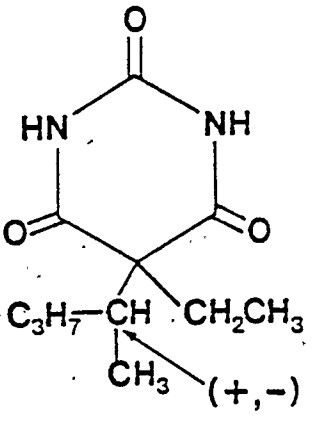
enflurane:



alfaxolone:



pentobarbital:



saturation of binding sites occuring at low concentrations (Terrell, 1984). Anaesthetic interactions with the nervous system has been demonstrated to be low affinity-high capacity with low binding energies and saturation of binding sites occurring only at concentrations well beyond clinically effective concentrations (Seeman, 1972; Roth, 1979; Miller, 1985). These observations have suggested that anaesthetics do not interact with a specific receptor but rather with multiple sites (Miller, 1985; Roth, 1988). Hence, the actions of anaesthetics are thought of as non-specific in nature.

Anaesthetics share the property of lipid solubity which was first reported, independently, by Meyer (1901) and Overton (1901). Specifically, they found that the relative potencies of anaesthetics was strongly correlated with the extent of their solubility in olive oil. The greater the solubility the more potent the anaesthetic. This correlation is now referred to as the Meyer-Overton rule of anaesthesia and is regarded primarily as a rule of efficacy (Roth, 1988). It did, however, lead to some early speculations on the site and mechanism of anaesthetic action which will be discussed in the following sections.

1.3 The Site of Anaesthetic Action

There have been two independent lines of research that have provided insight into the site of anaesthetic action. The first originated with the Meyer-Overton rule. The

observed correlation of lipid solubility and anaesthetic potency suggested to H.H. Meyer that the site of action was hydrophobic in nature. He specifically proposed that any substance that was soluble in the "vitally important lipoids of the cell" should produce an anaesthetic effect (Meyer, 1906). K.H. Meyer (1937) subsequently stated that the lipids of the cell membrane were the site of action. This hypothesis was vague since H.H. Meyer's suggestion predated the establishment of the structure of the cellular membrane (Miller, 1985).

Characterization of the lipid bilayer in the 1960's led to more rigorous testing of H.H. Meyer's original hypothesis. The first test of this hypothesis was carried out utilizing red blood cell ghosts and proved to very successful (Roth and Seeman, 1972a; 1972b). This led to more systematic investigations using lipid bilayers. Solubility studies using phosphatidylcholine bilayers showed that lipid bilayers demonstrated the Meyer-Overton correlation (Smith, Porter and Miller, 1981). Furthermore, it has long been known that homologous series of alkanes and alkanols of a certain carbon chain length (> 10 -12 carbons) lose their ability to cause anaesthesia. This is referred to as the "cut off phenomenon" and has been explained by membrane solubility (Miller, 1985). That is, these alcohols are unable to achieve a sufficient concentration in the membrane to cause anaesthesia. Thus, it appears that the cellular membrane is a site for anaesthetic

interaction. Whether this interaction leads to the state of anaesthesia will be discussed in section 1.4 which deals with mechanisms.

It is quite reasonable to propose that the hydrophobic chains of proteins might also be a site of anaesthetic action. In fact, general anaesthetics are capable of inhibiting the luminscence of luciferases and the extent of this inhibition correlates well with their solubility in lipid bilayers (Franks and Lieb, 1984). Moreover, inhibition of luciferases by anaesthetics also demonstrate a "cut off phenomenon" (Franks and Lieb, 1985). This observation succinctly demonstrates that anaesthetics are unable to distinguish a protein from a lipid bilayer (Miller, 1985). However, the only proteins examined to date which obey the Meyer-Overton rule are luciferases and bovine serum albumin (Miller, 1985). These proteins do not provide any direction into where in the central nervous system anaesthetic-protein interactions might occur. Therefore, it is necessary to pursue further investigations using excitable proteins.

A second line of research orginated in 1905 when Sowton and Sherrrington found that the concentration of chloroform required to block a reflex motor response was much smaller than that required to block a motor response elicited by electrical stimulation of the nerve itself. This suggested that synaptic transmission was more vulnerable to anaesthetic action than was the nerve impulse. This suggestion was later

confirmed by Larrabee and Posternak (1952) when they investigated the effects of anaesthetics on synaptic transmission in sympathetic ganglia. They found that the concentrations required to block a nerve impulse in the preganglionic fibre were 5-10 times greater than those required to block a nerve impulse in the postganglionic fibre. Although indirect, these experiments nonetheless demonstrated the selectivity of anaesthetics for synaptic transmission. It was not until Somjen and Gill (1963) and Somjen (1963) that the direct actions of anaesthetics on synaptic transmission were shown. They reported that excitatory postsynaptic potentials of motor neurons were depressed by ether and barbituates. These findings were extended to the hippocampal formation by Richards and White (1975) who found that a wide variety of anaesthetics were capable of depressing excitatory synaptic transmission.

It is now well accepted that synaptic transmission is the primary cellular site of anaesthetic action (Roth, 1988). In addition, the nature of the site appears to be hydrophobic with both protein and membrane being probable targets. Current research is focused on elucidating the mechanism(s) that account for the depression of excitatory synaptic transmission by anaesthetics.

1.4 The Mechanism of Anaesthetic Action

The Meyer-Overton rule af anaesthesia led to the

formulation of the first modern theory which attempted to explain the mechanism of anaesthetic action. K.H. Meyer (1937) proposed that anaesthesia would occur when any chemically indifferent substance attained a certain molar concentration in the cellular membrane. Mullins (1954) later modified this theory by suggesting that anaesthetic molecules fit into "free spaces" of the membrane thereby achieving a critical volume of occupation. This in turn, according to Mullins, would result in interference with the conductance of the membrane to certain ions. However, further investigations did not support this free-volume concept and Mullin's original hypothesis was modified to what is now known as the critical volume hypothesis (Miller, Paton, Smith and Smith, 1972). This hypothesis suggested that anaesthesia commenced when the volume of a hydrophobic region was forced to expand beyond a certain critical volume as a result of adsorption of the anaesthetic into this hydrophobic region (Roth, 1980). This hypothesis was the first truly mechanistic explanation of anaesthetic action.

Indeed, it was found that anaesthetics expanded biological membranes (Roth and Seeman, 1972). Quantitative measurements approximated this expansion, at clinical concentrations, in erythrocyte memebranes to be 0.4%. Further studies using spectroscopic methods reported that anaesthetics fluidized membranes (Roth, 1980). That is, the freedom of motion of lipids in the membrane increased.

Furthermore, high atmospheric pressures could reverse both the expansion and fluidization of the membrane. This latter observation provided important support for the membrane expansion/fluidization theories because it was well documented that pressure could reverse the effects of general anaesthesia (Kaufman, 1977). Thus, these theories were considered as tenable theories about the physical mechanism of anaesthetic action. The nonspecific nature of anaesthetics and the similar effects they produced on the cellular membrane led to a 'unitary theory' of anaesthesia. Simply stated, a 'unitary theory' implies that all anaesthetics act at a common site via a common mechanism (Roth, 1979; Roth, 1988).

Despite the apparent strength of a 'unitary theory' there are a number of serious criticisms. First of all, "a common physiochemical relationship may only explain the differences in relative potencies and the ability of the various compounds to reach the active site within the hydrophobic milieu of the cellular membrane" (Roth, 1979). It does not provide any data on the mechanism of action. Secondly, many lipid soluble substances have no anaesthetic effect and some can actually produce convulsions (Krantz, Esquibel, Truit, Ling, Kurland, 1958). Moreover, the fluidization of the membrane at clinical concentrations is relatively small compared to the same changes induced by temperature. It has been argued, therefore, that since temperature changes could not produce anaesthesia then membrane expansion or fluidity was not the

precise mechanism of action (Franks and Lieb, 1982). Lastly, a unitary theory is appealing because it explains the phenomenon of pressure reversal. However, pressure alone can produce central nervous stimulation, tremors and convulsions (Winter et al., 1976). Furthermore, dose-response profiles of anaesthetics are different at high pressures which suggests that the anatagonism is not direct (Roth, 1979). Therefore, it appears that although hydrophobicity is necessary for anaesthetic action, it is not in itself sufficient to cause anaesthesia.

The concept of a nonspecific unitary mode of action for general anaesthetics is an attractive one since it accounts for the diversity of anaesthetic compounds. However, it does not provide an explanation for the apparent clinical specificity of general anaesthetics. For example, hexafluoroisopropyl methyl ether and flurothyl are two structural isomers with virtually identical physiochemical properties; however, the former produces anaesthesia while the later induces convulsions (Seeman, 1972). Furthermore, it is well known that a variety of patterns of anaesthesia can be produced by anaesthetic agents that cannot be explained by differences in pharmacokinetic variables (Roth, 1979). Evidence against a 'unitary theory' has come from studies using *in vitro* preparations which have demonstrated differential actions.

1.5 The Differential Actions of Anaesthetic Agents

The concept of a single mechanism and site of action for all anaesthetics has been seriously challenged over the last decade. In order for a compound to produce anaesthesia it must possess the nonspecific requirement of lipid solubility. However, molecular structure may also be an important factor, which could give rise to selective effects. Selectivity could produce differential actions which is incompatibile with a 'unitary theory.' This suggests that a particular anaesthetic or group of anaesthetics might have a unique site or mechanism of action or both. Equally as likely is the possiblity that multiple sites or mechanisms are involved that are characteristic of an anaesthetic or group of anaesthetics. Investigations using various *in vitro* preparations have demonstrated that selectivity occurs at the levels of neuronal discharge activity, membrane responses, synaptic transmission, and synaptic plasticity. Each of these levels will be discussed in the context of the multiple mechanism and/or site hypothesis.

Firstly, it is worthwhile to recognize that Mullins (1954) was the first to propose selectivity in anaesthetic action. However, it was not until Roth's (1980) observations on the effects of anaesthetics on the discharge pattern of the crayfish stretch receptor neuron *in vitro* did the idea receive any direct experimental support. It was found that three different general anaesthetics (ethanol, halothane and

pentobarbital) each affected the discharge pattern in a unique manner. These characteristic burst patterns suggested that anaesthetics did not alter neuronal behavior in a similar fashion. Weston and Roth (1986) pursued these findings in more detail using several volatile anaesthetic agents. They reported that the discharge activity was selectively altered by halothane, isoflurane, enflurane and diethyl ether. This alteration could be dividied into four phases: enhancement (I), depression (II), partial recovery (III), and reversal upon washout (IV). Whereas halothane produced all four phases, isoflurane only produced phases II-IV. Enflurane and diethyl ether, however, produced biphasic effects. At concentrations below 3.5 vol% and 8.0 vol%, respectively, the profile was similar to isoflurane. At higher concentrations, enhancement and burst firing were observed to occur during phase I which was then proceeded by the remaining phases. Thus, it appears that a single isolated neuron is capable of discriminating the structural differences between anaesthetics.

Since anaesthetic agents presumably attain their effects by hydrophobic interactions with the cellular memebrane, it follows that selectivity should also be observed on membrane responses. Indeed, differential actions on neuronal membrane properties can be observed. MacIver and Roth (1987a) demonstrated that anaesthetics produced different, concentration-dependent membrane effects. They measured the

effects of ethanol, pentobarbital and halothane on membrane resting potential, resistance and spike threshold in the *in vitro* crayfish stretch receptor neuron. They observed that halothane and pentobarbital produced depolarization whereas ethanol hyperpolarized the resting potential. Biphasic effects were produced on membrane resistance by both halothane and pentobarbital. However, resistance returned to control values following an initial increase at lower concentrations with halothane in contrast to pentobarbital which decreased resistance at higher concentrations relative to control. Ethanol simply increased membrane resistance at all concentrations studied. Lastly, spike threshold increased for both halothane and ethanol, whereas for pentobarbital, spike threshold decreased at lower concentrations, and then increased at higher concentrations. It is clear, that although some common actions were observed, the profile of effects were quite dissimilar for the three compounds. These results support the hypothesis that anaesthetics act via selective mechanisms and/or sites at the membrane level.

Although it is evident that virually all general anaesthetics ultimately depress excitatory synaptic transmission, they may not all do so in a similar manner. A recent review by Pocock and Richards (1991) discussed the effects of anaesthetics on synaptic transmission. The spectrum of effects they described reinforces the concept that differential actions also occur at the synaptic level, since

not all agents produce the same effects. For example, anaesthetics can inhibit the secretion of neurotransmitter from nerve endings, enhance the secretion of the inhibitory amino acid GABA, desensitize the postsynaptic receptor, reduce the time of opening of postsynaptic ion channels as well as increase the open time of chloride channels and alter postsynaptic excitability. MacIver and Roth (1987; 1988) directly addressed the question of selectivity on synaptic transmission in both the crayfish stretch receptor neuron and the hippocampal formation *in vitro*. The stretch receptor neuron has only one type of synapse impinging upon it, a GABA inhibitory connection. The amplitude of GABA-mediated inhibitory postsynaptic potentials (IPSPs) was measured to determine the effects of various anaesthetics on the synaptic transmission between the inhibitory fiber and the stretch receptor neuron. Ethanol, pentobarbital and halothane all depressed the IPSP amplitude in a concentration-dependent manner. However, when GABA was applied exogenously to the stretch receptor, halothane and ethanol blocked GABA's postsynaptic responses (i.e. depolarization and a membrane conductance increase) in contrast to pentobarbital which augmented the effects of GABA. These results can best be explained by assuming that different sites and/or mechanisms were involved.

The actions of halothane, isoflurane and enflurane were then subsequently compared on neurotransmission in the

mammalian CNS. Specifically, the effects of these agents on the three principal pathways of the *in vitro* hippocampus were investigated. Halothane was found to depress postsynatpic excitability of CA1 pyramidal neurons (0.25-1.25 vol%) in response to stimulation of stratum radiatum synaptic inputs. Conversely, it also produced excitatory (0.25-1.25 vol%) and depressant (1.5-2.0 vol%) effects on dentate granule neuron excitability and perforant path evoked synaptic responses. In contrast, isoflurane increased CA1 postsynaptic excitability (0.25-0.75 vol%) and depressed dentate neurons (0.5-4.0 vol%). Enflurane also increased CA1 neuron excitability at equivalent concentrations but produced mixed excitatory (0.25-1.0 vol%) and depressant (1.0-4.0 vol%) effects on dentate synaptic responses. These varied and opposing effects extended the conclusions made in the invertebrate prepartion to the mammalian CNS that multiple mechanisms and/or sites appeared to be involved. Furthermore, the authors proposed that discrete recognition sites existed which could discriminate structural differences and which were heterogenously distributed on the different synaptic pathways. They found that a strong correlation existed between membrane/buffer coefficients and ED_{50} concentrations necessary to depress stratum radiatum activitated synaptic transmission. Thus, the results comply with the Meyer-Overton rule and suggest that these recognition sites are hydrophobic in nature.

There is a paucity of data investigating the effects of

anaesthetics on synaptic plasticity. Such studies are important when one considers the phenomenon of long-term potentiation (LTP) which is regarded as being involved in the cellular basis of memory (Teyler and Discenna, 1984). LTP occurs when a response is exposed to a train of stimuli, upon subsequent elicitation one finds the response has been enhanced. This enhancement to test stimuli can last for hours. Amnesia is a characteristic effect of most general anaesthetics and therefore such studies are directly relevant. It is no surprise that LTP does not respond in the same way to all anaesthetics. MacIver, Tauck and Kendig (1989) found that in the hippocampal slice preparation, halothane reduced the probability of LTP induction but methoxyflurane had no effect. This observation provides further evidence for the concept of selective actions.

It is presently unknown how anaesthetic molecules exhibit selectivity and perhaps differential actions. A discovery by Franks and Lieb (1988; 1991) offers an interesting example of how anaesthetic selectivity, and thus differential actions, might occur. They found a novel neuronal potassium current in the right parietal ganglion of the pond snail *Lymnea stagnalis*. Apparently, neurons in this ganglion exhibit an endogenous pattern of spontaneous firing and this steady-state firing activity was unaffected by surgical concentrations of halothane. However, one neuron in the cluster was extremely sensitive in that its cell membrane potential demonstrated a

large hyperpolarization in the presence of halothane. This hyperpolarization was enough to completely inhibit spontaneous firing activity of this particular neuron. Moreover, there was a direct correlation between the presence of an anaesthetic-induced potassium current and anaesthetic inhibition of spontaneous firing in a given neuron. This potassium current was unaffected by external applications of tetraethylammonium, 4-aminopyridine and cobalt ions, but, changes in the bath potassium concentration did affect it. Interestingly, dose-response curves of this current for halothane demonstrated saturability at low concentrations which suggested to the authors the possibility of anaesthetic binding to saturable sites. Furthermore, when the neuron was isolated and halothane solutions were microinjected onto different regions, anaesthetic sensitivity was greatest near the axon hillock region. This region contains many synaptic connections which suggested that the current may be normally activated by synaptic inputs. This latter observation is important because it is consistent with the "axiom" of synaptic transmission being the site of anaesthetic action. Thus, halothane selectively caused a cessation of neuronal firing activity as a result of an asymmetric distribution of a particular potassium channel, out of the vast array that exist, which was sensitive to halothane.

How does this example relate to the multiple mechanism and/or site hypothesis? In order to answer this question, we

must look to other actions of halothane in *Lymnea*. Girdlestone, McCrohan and Winlow (1989) also examined the effects of halothane on the CNS of *Lymnea*. They found that halothane decreased the mean spike frequency of spontaneously active cerebral giant cells (CGC) of the cerebral ganglia. However, this decrease in frequency appeared to be due to a sensitivity of the calcium-dependent components of the CGC action potential to halothane. This is in sharp contrast to the identified neuron in the right parietal ganglion which was inhibited due to a halothane activated potassium current. Therefore, it would seem that halothane differentialy affects neurons of the *Lymnea* CNS because it interacts with multiple sites.

Considering the body of evidence that has been presented, it would appear that a unitary concept of anaesthetic action is not an accurate picture. Although these data are certainly not conclusive, a particular anaesthetic appears to have, at the very least, multiple sites of action and possibly multiple mechanisms. Unfortunately, there is a formidable obstacle that must be overcome in order to resolve this controversy; the neurophysiological basis of consciousness. Understanding this concept would certainly determine whether a single mechanism and/or site of action is required for anaesthesia to occur or multiple mechanisms and/or sites of action are necessary.

1.6 The Spectrum of Effects of Anaesthetics on the CNS

It appears that anaesthetics affect virtually every aspect of the CNS imagineable. The task is to determine the key effects that lead to anaesthesia. As stated above, this is limited by our understanding of consciousness. However, one can critically evaluate which effects may be important by keeping in mind the concentrations that are necessary to produce anaesthesia in the whole animal. This section will attempt to present the most recent cellular and molecular effects that anaesthetics have been found to have on the CNS in the context of clinically effective concentrations.

Since Larrabee and Posternak's (1952) discovery of the vulnerability of synaptic transmission to anaesthetic action, there has been a wealth of research on the synaptic actions of anaesthetics. It is well documented that anaesthetics depress excitatory synaptic transmission (Roth, 1988; Popock and Richards, 1990; Miu and Puil, 1989; El-Beheiry and Puil, 1989). Presynaptically, there is strong evidence to suggest that anaesthetics inhibit neurotransmitter secretion. The first convincing demonstration came from Matthews and Quilliam (1964) when they showed that anaesthetics decreased the amount of acetylcholine released from postganglionic nerves upon stimulation of preganglionic nerves. Current research has continued to support this initial observation. For example, Bazil and Minnenan (1989) found that halothane reduced potassium-evoked release of norepinephrine from rat cortical

slices at concentrations of 1.25 vol%. Interestingly, halothane did not affect the release of acetylcholine at similar concentrations, nor did it affect potassium-evoked release of aspartate (Arai, Hatano and Mori, 1990). The norepinephrine and acetylcholine observations were subsequently replicated using an *in vivo* preparation (Bazil and Minneman, 1989). A number of mechanisms are possible for this type of action: (1) a decrease in the quantal content of transmitter substances in secretory vesicles; (2) a disruption of one or more of the processes leading to exocytosis; (3) an inhibition of voltage-gated calcium channels. Pocock and Richards (1987, 1988, 1990) reported that a variety of anaesthetics did not affect the leakage of catecholamines from bovine chromaffin cells. On the other hand, it has been shown that anaesthetics do suppress voltage-dependent calcium currents at clincically relevant concentrations (Krnjevic and Puil, 1988; Takenoshita and Steinbach, 1991; Tas, Kress and Koschel, 1989). Studies on the effects of anaesthetics on exocytotic process are presently quite scarce. Thus, from the available evidence, it would appear that depression of calcium currents may be a major candidate for the inhibition of neurotransmitter release.

Post-synaptically, a variety of anaesthetic effects have also been reported. Firstly, pentobarbital, halothane, and methoxyflurane have been found to inhibit acetylcholine-evoked release of catecholamines from chromaffin cells in a

noncompetitive manner (Pocock and Richards, 1990). In addition, Aronstam, Anthony and Dennison (1986) reported that the affinity of carbachol for muscarinic receptors was not affected by halothane. These results suggest that direct interactions with the postsynaptic receptor do not occur. However, halothane has been found to disrupt receptor-G protein interactions (Baumgartner, Dennison, Narayanan and Aronstam, 1990). These effects may be somewhat agent specific since the adrenergic receptor complex was affected by a halothane concentration of 1.0 vol% whereas disruption of muscarinic receptor-G protein coupling was reported only at high halothane concentrations (i.e. 2 vol%) (Anthony, Dennison, Aronstam, 1989).

Secondly, anaesthetics can alter postsynaptic ionic currents that are the result of a neurotransmitter binding to its postsynaptic receptor. Weight, Lovinger, White and Peoples (1991) observed that ethanol and diethyl ether inhibited a N-methyl-D-aspartate (NMDA) activated current whereas pentobarbital had no effect on this current. Analysis of single ion channels activated by acetylcholine has revealed that halothane and isoflurane reduced the amplitude of channel currents, shorten the duration of individual channel openings and decrease the time constant of the decay phase of the current (Wachtel and Wegrzynowicz, 1991; Sokoll, Davies, Bhattacharyya and Zwagerman, 1989).

One interesting controversy that is presently being

debated in experimental studies is the effect of anaesthetics on inhibitory synaptic transmission. It is well documented that barbituates enhance GABA-activiated responses, primarily through prolonging the mean open time of chloride channels (Ho and Harris, 1981; Gage, McKinnon, Robertson, 1986; Gage and Robertson, 1985). However, evidence for both sides of the argument have been presented for volatile anaesthetics. Gage and Robertson (1985) observed that halothane increased IPSPs in CA1 pyramidal cells of the rat hippocampus. Nakahiro, Yeh, Brunner and Narahashi (1989) found that halothane, isoflurane, and enflurane increased ionic currents induced by exogenously applied GABA to cultured rat dorsal root ganglion neurons. These latter results must be viewed cautiously as high concentrations were used (i.e. 2 times the minimum alveolar concentration or MAC), but since the effects of these anaesthetics were so large at these concentrations, significant effects may have been possible at lower concentrations. Further support for anaesthetics enhancing GABA-mediated responses has come from whole cell patch clamp recordings which have demonstrated that pentobarbital and halothane increase the decay time constants of GABA-medited IPSCs (MacIver, Tanelian and Mody, 1990). These authors also found that halothane required an increase in intracellular calcium levels to prolong chloride currents whereas pentobarbital did not. In contrast to these studies, a few investigators have reported that anaesthetics do not

differentiate between inhibitory and excitatory synaptic transmission; that is, volatile anaesthetics can also depress inhibitory synaptic transmission (El-Beheiry and Puil, 1989; Fujiwara, Higashi, Nishi, Shimoji, Sugita and Yoshimura, 1988; Yoshimura, Higashi, Fujita, Shimoji, 1985). Based on these observations one can conclude that volatile anaesthetics may enhance inhibition whereas it appears to the major action for the barbituates.

In addition to synaptic effects, anaesthetics also exert nonsynaptic actions. At the membrane level, hyperpolarization of the resting potential is observed when anaesthetics are applied to rat motor and hippocampal neurons, as well as human neocortical neurons (Nicoll and Madison, 1982; Berg-Johnsen and Langmoen, 1987; MacIver and Kendig, 1991). This effect appears to be associated with an increase in conductance. Nicoll and Madison (1982) suggested that this could be due to an increase in a potassium conductance as a result of a rise in intracellular calcium levels. Although current research continues to support the former observation, it is clearly in contradiction to the latter suggestion (Franks and Lieb, 1988; Puil, El-Beheiry and Bambridge, 1990). The conductance effect may not be the same for all anaesthetics; for example, enflurane appears to decrease conductance whereas isoflurane has no consistent effects (MacIver and Kendig, 1991). Lastly, spontaneous firing patterns of hippocampal neurons are also affected by

anaesthetics. Halothane, isoflurane and enflurane have been shown to decrease the spontaneous firing of CA1 pyramidal neurons of the rat hippocampus (Fujiwara, Higashi, Shimoji, Sugita and Yoshimura, 1988). Interestingly, this depression was not accompanied by changes in resting membrane properties. This observation was reported for concentrations less than approximately 0.4 vol% for all three anaesthetics. Fujiwara et al. provided evidence for an anaesthetic-induced increase in the threshold for spike generation to account for the change in firing activity. This has also been seen in myelinated peripheral nerves of frogs (Butterworth, Raymond, and Roscoe, 1989).

In summary, it is quite evident that at even clinically relevant concentrations there is a broad range of effects produced by anaesthetic agents. Not only are there multiple actions, but it appears that the spectrum of effects are different depending upon the agent. Which of these effects are responsible for the state of anaesthesia cannot be determined at this time; however, based on the published reports presented, one is inclined to conclude that multiple sites and possibly mechanisms may be involved.

1.7 The Hippocampus as a Model System

In order to gain an understanding of how anaesthetics affect the mammalian CNS, various *in vitro* preparations have been employed. This type of analysis represents a

reductionist's approach to the problem which rests upon the fundamental assumption that by understanding an isolated neuron or a single synaptic connection or a simple neural network, one can extrapolate to the entire CNS. To this end, the *in vitro* hippocampal slice prepartion can be a useful model because it is well defined anatomically, much is known about its physiology and pharmacology and it affords a number of practical advantages. Since a comprehensive review of these areas is beyond the scope of this thesis, only those aspects that are relevant to this thesis will be discussed. The reader is directed to a number of excellent reviews if a more in depth and detailed understanding of one or more of these areas is desired (Lopes da Silva, Witter, Boeijing and Lohman, 1990; Amaral and Witter, 1989; Schwartzkroin and Mueller, 1987; Dingledine, 1984).

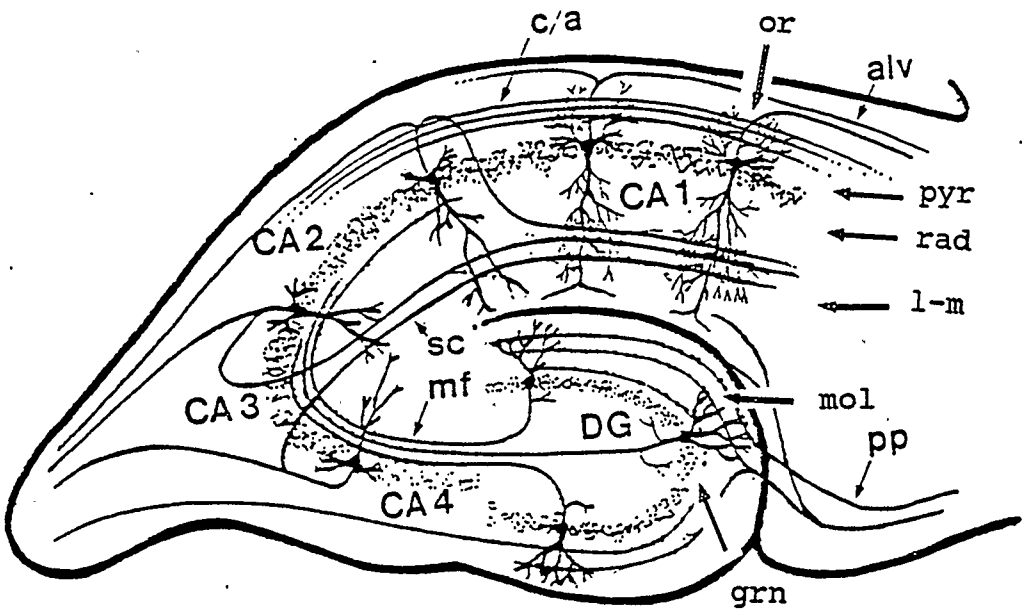
1.7.1 Microanatomy of the Hippocampal Formation

The cortex of the mammalian telencephalon is divided into two subdivisions based on phylogenetic and cytoarchitectonic data. These are the pentalayered neocortex or the uni or bilayered allocortex. The allocortex is further divided into the paleocortex, or the pyriform cortex, and the archicortex, or the hippocampal formation (Lopes da Silva et al., 1990). The hippocampal formation appears as two C-shaped interlocking cell layers and is specifically comprised of the dentate gyrus, the hippocampus proper or Ammon's horn, the subicular

complex and the entorhinal cortex. A schematic of the *in vitro* hippcampal formation is shown in Figure 2. The two cell layers that are present are the granular cell layer of the dentate gyrus and the pyramidal cell layer of Ammon's horn and the subiculum. Traditionally, the dentate gyrus is considered to have a trilayered structure: the molecular or dendritic layer, the granule cell layer and the hilar region which consists of widely dispersed polymorphic neurons. Ammon's horn is also considered to have a layered structure: stratum moleculare, which contains fibers and dendritic terminals; stratum-lacunosum, which is comprised of collaterals of the pyramidal cells; stratum radiatum has several fiber systems the most important being the Schaffer collaterals; stratum pyramidale is the cell body layer of the pyramidal cells, and lastly, stratum oriens, which contains the basal dendritic arborizations of the pyramidal cells, different cell types and more axon collaterals. Ramon y Cajal further subdivided the pyramidal cells into three fields based on morphology. These are the small pyramids of CA1 and CA2 (15-25 μm) and the giant pyramids of CA3 (20-35 μm). It is important to note that interspersed throughout the cell layers are numerous interneurons, the predominant type being the inhibitory basket cell.

The intrinsic circuitry of the hippocampal formation can be thought of as having a lamellar organization. The concept was orginally proposed by Andersen, Bliss and Skrede (1971)

Figure 2. A schematic of the *in vitro* hippocampal slice preparation showing the location of the pyramidal neurons (CA1 to CA4) and dentate granule (DG) neurons. Included are also some of the fiber pathways: sc - Schaffer collaterals, mf - mossy fibers, c/a - commissural/associational, alv - alveus, pp - perforant path, or - stratum oriens, pyr - pyramdial cell layer, rad - stratum radiatum, l-m - straum lacanoum-moleculare, mol - straum moleculare, grn - granule cell layer


c/a
or
alv
CA1
pyr
rad
l-m
CA2
sc
mf
CA3
DG
mol
pp
CA4
grn

based on anatomic and electrophysiological evidence. A lamella occurs transverse to the longitudinal or septotemporal axis of the hippocampus and contains a defined three-membered pathway in its entirety. This pathway consists of mossy fibers (long thin axons) from the dentate granule cells, Schaffer collaterals of the CA3 pyramidal cells and CA1 axons in the alveus. Corticocortical association fibers converge onto the entorhinal area which in turn projects to the molecular layer of the dentate gyrus, the Ammon's horn, and the subiculum. The most dense projection is to the dentate gyrus and occurs via the perforant pathway (Nauta, Fiertag, and Freeman, 1986). In the dentate gyrus, entorhinal synapses constitute at least 85% of the total number of synapses (reviewed by Lopes da Silva et al., 1990). The transmitter associated with these synapses is most likely glutamate or aspartate, although somatostatin, enkephalin, and cholecystokinin may also be present (reviewed by Lopes da Silva et al., 1990). This pathway does not follow the lamellar organization of the intrinsic hippocampal circuitry since it projects along the septotemporal axis (Amaral and Witter, 1989). The granule cells, in, turn, send mossy fibers to the pyramidal cells of the CA3 region. Here, they form en passsant synapses on the proximal dendrites of the pyramidal cells. The mossy fiber synaptic transmitter also appears to be glutamate or aspartate (reviewed by Lopes da Silva et al., 1990). CA3 pyramidal cells send their axons to form an

intrinsic fiber tract, the alveus, which becomes the fornix. They also give rise to the collateralized axons, the Schaffer collaterals, which provide the major input to the CA1 field (Nauta et al., 1986), where they form synapses on the apical dendrites of these pyramidal cells. Glutamate or aspartate is also the most likely neurotransmitter at these synapses (reviewed by Lopes da Silva et al., 1990). The Schaffer collaterals also distribute to the subiculum and, in turn, both the CA1 and the subiculum contribute to form the fornix. The fornix carries the output of the hippocampus to various structures. In particular, fibers from the CA fields project only to the septum and the contralateral CA fields (Nauta et al., 1986). The rest of the fornix, arising from the subiculum, project to the nucleus accumbens, mammillary body, and the anterior nucleus of the thalamus (Nauta et al., 1986).

One important connection that must be further discussed are the projections to and from the septal area. As already mentioned, axons from the CA fields project to the septum, however, it must be emphasized that almost all pyramidal cells of Ammon's horn and subiculum participate in the hippocamposeptal projection (Lopes da Silva et al., 1990). Axons from the septal cells terminate, within Ammon's horn, in the stratum radiatum and the stratum oriens of CA3 and in the stratum oriens of CA1 (Lopes da Silva et al., 1990). They also terminate in the hilar region of the dentate gyrus just below the granule cell layer. It has been demonstrated that

these projections contain acetylcholine as their neurotransmitter (reviewed by Lopes da Silva et al., 1990). However, a significant portion of the septohippocampal fibers also arise from GABAergic neurons (reviewed by Lopes da Silva et al., 1990).

1.7.2 The Cholinergic Neurotransmitter System

Although there are numerous neurotransmitter systems in the hippocampus, this discussion will be limited to the cholinergic system. Autoradiographic methods have revealed the distribution of binding sites for cholinergic ligands in the hippocampus. Of the two types of cholinergic receptors, muscarinic binding sites have been found in the stratum radiatum and stratum oriens of the CA fields, and the molecular layer of the dentate gyrus (Lopes da Silva et al., 1990). This distribution coincides closely with the termination of septohippocampal fibers within Ammon's horn. It has been suggested that muscarinic binding sites may be located presynaptically on the terminals of the Schaffer collaterals since the bindings sites correlate, to a large extent, with the distribution of these collaterals (Aguilar, Jerusalinsky, Stockert, Medina and Roberts, 1982). Based on the relative sensitivity of muscarinic responses to the tricyclic compound pirenzepine (a muscarinic antagonist), muscarinic receptors have been divided into M1 and M2 subtypes; the M1 subtype being 100 times more sensitive to the

antagonist (Hammer, Berrie, Birdsall, Burgen and Hulme, 1980). Recently, it has been demonstrated that the antagonist gallamine has a high affinity to the M2 subtype in the rat brain (Burke, 1986). Unfortunately, pharmacological methods have been less successful in identifying muscarinic receptor subtypes than molecular cloning methods, which have identified four functional muscarinic receptor clones (Bonner, Buckley, Young and Brann, 1987). Moreover, the pyramidal cells of the rat hippocampus express the mRNAs of all four clones and, cholinergic respones, elicited by carbachol, can be selectively antagonized by both pirenzepine and gallamine (Palocios, Mengod, Vilaro, Wiederhold, Boddeke, Alvarez, Chinaglia and Probst, 1990; Dutar and Nicoll, 1988). Thus, at the very least, cholinergic responses within the hippocampus appear to be mediated by M1 and M2 receptors, with the possibility of the involvement of two more cholinergic receptor subtypes.

1.7.3 Cholinergic Responses of Hippocampal Neurons

There are a number of characteristic electrophysiological responses elicited from *in vitro* hippocampal neurons when acetylcholine or its analogues are admininstered. Dodd, Dingledine and Kelly (1981) reported that 10 μM of muscarine produces depolarization, an increase in firing rate and membrane resistance when applied to CA1 pyramidal cells. More detailed examinations have revealed the ionic basis of the

depolarization and resistance increase. Specifically, a voltage-dependent potassium conductance, called the M-current, is blocked by the cholinergic agonist carbachol (Halliwell, 1990; Halliwell and Adams, 1982; Brown and Adams, 1980). In addition, a rapidly inactiviating outward potassium current called the A-current, a calcium activated potassium current called the afterhyperpolarization or I_{AHP}, and a voltage-insensitive "leak" potassium conductance are all suppressed by cholinergic agonists (Halliwell, 1990; Nakajima, Nakajima, Leonard,Yamaguuchi, 1986; Madison, Lancaster and Nicoll , 1987). Both the M-current and "leak" current are persistent outward currents that, when inhibited, lead to a depolarization of the membrane and a general increase in neuronal excitability. These currents "place a hippocampal neuron in a state of readiness to give a brisker response when an excitatory input arrives" (Halliwell, 1990). However, inhibition of the A-current and I_{AHP} lead to a modulation of ongoing neuronal activity (Halliwell, 1990). Whereas suppression of I_{AHP} changes the firing pattern of hippocampal neurons from phasic to tonic (i.e. a decrease in accomodation), inhibition of the A-current results in a decrease in the threshold required to initiate action potentials and a change in the spacing of action potentials. Interestingly, amongst the the cholinergic actions in hippocampal neurons, I_{AHP} and the "leak" conductance are the most sensitive (Halliwell, 1990; Lopes da Silva et al., 1990).

Moreover, the muscarinic-induced depolarization and the attenuation of I_{AHP} appear to be mediated by the M1 receptor, since they are blocked by pirenzepine, and inhibition of the M-current is antagonized by gallamine which suggests an involvement of the M2 receptor (Dutar and Nicoll, 1988).

1.7.4 Practical Advantages of the *In Vitro* Hippocampal Slice

Generally speaking, the hippocampal slice serves as a convenient model for telencephalic function because it displays the variability in neuron structure and aspects of the complexity in interneuronal circuitry that is found throughout the cortex (Turner and Schwartzkroin, 1984). Furthermore, because of its relative simplicity, investigations of the hippocampus may lead to a more in depth understanding of the CNS and, thus, how anaesthetics might alter CNS funtion. In addition, the hippocampal slice preparation offers some practical advantages. Firstly, improved visualization of the tissue allows one to precisely record from the desired sites. Optical techniques allows this precision to achieve the level of axons, dendrites and the soma (Schwartzkroin, 1981). Secondly, there is direct access to the extracellular space thus one can equilibrate the ionic and drug concentrations of the bath with the space. This allows one to accurately control and easily change the ionic and/or drug composition and concentrations the cells are exposed to in the surrounding environment. Unfortunately,

there is a serious drawback to the preparation: the tissue is not in its normal state because it is separated from its normal afferents and efferents. In addition, it is difficult to know what the relationship is between neuronal activity in the slice and behaviour. These limitations may be at least partially overcome by using the carbachol-induced rhythmical population activity (RPA) that can be generated in the hippocampal slice. This will be discussed in section 1.11 (rationale).

1.8 Hippocampal Theta Activity

Since Saul and Davis (1933) first reported the existence of "biological currents" within the hippocampus *in vivo*, there has been a tremendous effort into explaining their anatomy, physiology, pharmacology and behavioral relations. Theta rhythms, as they are now referred to, can be described as rhythmical sinusoidal-like slow waves. They are considered to be the largest most prominent rhythmical waveforms produced by the mammalian brain (Andersen, Bland, Myhrer and Schwartzkroin, 1979). In the last few years, it has been demonstrated that *in vitro* hippocampal slices are capable of generating rhythmical field potential activity that is very similar to theta rhythms (MacIver, Harris, Konopacki, Roth and Bland, 1986). The following discussion will focus on these *in vitro* "theta" rhythms, however, a brief background to *in vivo* theta will be provided.

1.8.1 Characteristics of Theta

From the early investigations into theta there emerged two classic papers that paved the road for all subsequent research. The first, by Green and Arduini in 1954, set the tone for theories about the functional significance of hippocampal theta activity. In their paper, they proposed that theta represented a "specialized paleocortical arousal reaction." Several years later, Vanderwolf (1969) carried out the first systematic studies that linked theta to overt behavior. He stated that the electroencephalographic (EEG) activity in the 6-12 Hz range produced by the hippocampus "may be a reflection of the activity of some of the complex-circuitry which appears necessary for voluntary movement." Since these early beginnings much has been learned about theta rhythms. So much so that inclusion of that information within this thesis would leave the reader searching for the relevance to the main objectives of this thesis. However, there are a few facts that are directly relevant. Firstly, two types of theta have been identified, type 1 and type 2. Type 1 is associated with a certain class of movements observed in rats, guinea pigs and rabbits that Vanderwolf (1969) originally termed voluntary. These are walking, running, swimming, rearing, jumping, digging, manipulation of objects with the forelimbs, isolated movements of the head or one limb and shifts of posture (reviewed by Bland, 1986). Type 1 has a frequency range of approximately 7-12 Hz and is resistant to

muscarinic antagonism by atropine sulfate (Bland, 1986). Lesions to the medial septum and diagonal band of Broca also abolish this type of theta. Although the neurotransmitter systems involved in the generation of type 1 theta is not known, some evidence in support of a serotonergic pathway has been presented (Vanderwolf and Baker, 1986). Type 2 theta, on the other hand, has a slightly lower frequency range that type 1, that being approximately 4-9 Hz. This type of theta occurs in the abscence of movement in response to sensory stimuli of any modality. Based on this observation, it has been concluded that type 2 theta is an electrical sign of sensory processing. Pharmacologically, this type of theta is quite distinct in that it is abolished by atropine sulfate. Furthermore, evidence for a muscarinic cholinergic mediation of type 2 theta is quite strong and, thus, a different neurotransmitter system appears to be involved in the generation of each type of theta (Vanderwolf, 1975; Robinson and Green, 1980; Rowntree and Bland, 1986). However, lesions of the medial septum and diagonal band also abolish type 2 theta which suggests that there is anatomical overlap of these systems, at least at the level of the medial septum-diagonal complex (Andersen et al., 1979; Sainsbury and Bland, 1981).

1.8.2 Cellular Mechanisms of Theta Generation

Despite the difficulties of recording intracellularly from *in vivo* preparations, several investigators have reported

some interesting observations during theta activity. Firstly, it is well documented that during theta rhythm, both CA1 pyramidal and dentate granule neurons show rhythmic membrane potential oscillations that are phase-locked to the extracellularly recorded theta activity in those regions (Munoz, Nunez and Garcia-Austt, 1990; Nunez, Garcia-Austt, Buno, 1990; Leung and Yim, 1986; Fox, 1989). Impedance changes observed during this "intracellular" theta imply conductance changes (Fox, 1989). However, the precise nature of these conductance changes has not been investigated. Investigations into the synaptic contributions to theta genesis is an ongoing debate. Fujita and Soto (1964) suggested that excitatory postsynaptic potentials (EPSPs) contributed to intracellular theta recorded from CA1 pyramidal cells and that inhibitory postsynaptic potentials were not involved. Contrary to these findings, Leung et.al. (1986) concluded that somatic IPSPs were predominantly important to the genesis of intracellular theta in CA1 neurons, however they could not rule out the possibility of an involvement of dendritic EPSPs. They based their conclusions on the observation that current injection and acetate ion diffusion, which reversed IPSPs evoked by stimulation of the alveus, altered the phase relationship between intracellularly and extracellularly recorded theta. Recently, Nunez et al. (1990) and Munoz et.al. (1990) have provided more evidence to support Fujita and Soto's (1964) original conclusion. They found that

chloride or cesium ion diffusion had no effect on intracellular theta whereas hyperpolarizing current injections increased the amplitude; the expected result if EPSPs were involved. Thus, presently, it appears that the weight of evidence favors the involvement of EPSPs.

1.8.3 *In Vitro* Theta

Given the cholinergic mediation of type 2 theta, Rowntree and Bland (1986) hypothesized that microinfusions of cholinergic agonists directly onto cholinoceptive neurons within the hippocampal formation should produce theta activity. Indeed, eserine and carbachol both induced theta, the frequency of which was related to the concentration used. Moreover, this activity occured in areas containing high levels of cholinergic markers, that is, the stratum oriens of and radiatum of CA1 and CA3, the stratum moleculare and granulosum of the dentate gyrus. This was in agreement with previous *in vivo* findings which demonstrated the existence of two theta generators, one in the stratum oriens of CA1 and the other in the stratum moleculare of the dentate gyrus (Winson, 1974; Bland, Andersen and Ganes, 1975). This observation lead to the first demonstration that the cholinergic agonist carbachol could produce rhythmical sinusoidal-like field potential oscillations when perfused onto *in vitro* hippocampal slices (Konopacki, MacIver, Bland and Roth, 1987; MacIver et al., 1986). This activity exhibited a similar frequency (4-9

Hz), amplitude (0.2-1.0 mV) and sensitivity to atropine sulfate as type 2 theta. Subsequently, Konopacki, Bland and Roth (1988a) demonstrated that muscarinic receptors were involved in the generation of these *in vitro* carbachol-induced RPA. Further investigations revealed that there were two generators of RPA identical to *in vivo* findings about type 2 theta. This conclusion was based on the observations that CA1 and dentate neurons were pharmacologically distinct in terms of drug sensitivity (or selectivity or kinetics), that in transected slices the CA1 and dentate areas could independently produce theta and that theta rhythms recorded in these areas could be as much as 180 degrees out of phase (Roth, Konopacki and Bland, 1987; Konopacki, Bland, MacIver and Roth, 1987b; Konopacki et al., 1987). This later finding is also a well documented finding in *in vivo* theta research (Bland and Whishaw, 1976). Given the many similarities between RPA and type 2 theta, it appears that RPA is a realistic simulation of type 2 theta.

With the advent of an *in vitro* model of hippocampal theta rhythms there came the ability to pursue more detailed studies. MacVicar and Tse (1989) provided important insights into the characteristics and possible mechansim of carbachol-induced RPA using intracellular recordings of CA3 neurons. They found that RPA consisted of oscillatory depolarizations which had a rise time much slower than EPSPs recorded with the same neuron. When these depolarizations were studied under

current-clamp conditions, the temporal pattern was not altered when the membrane potential was significantly hyperpolarized or depolarized. However, the amplitude was increased or decreased, respectively, like conventional EPSPs. Since intrinsic ionic conductances are voltage-gated (Llinas, 1988), they suggested that such ionic conductances were not involved but rather the depolarizations were generated synaptically. In accordance with this hypothesis, it was found that a broad-spectrum excitatory amino acid antagonist, and not selective N-methyl-D-aspartate (NMDA) antagonists, blocked RPA. This meant that non-NMDA excitatory amino acid synapses were involved. Moreover, atropine also blocked the activity but selective $GABA_A$ and $GABA_B$ antagonists appeared to have no effect. The former finding is in agreement with previous work while the later finding lends support to the suggestion that IPSPs are not involved in the cellular mechanism of theta generation (Nunez et al., 1990; Munoz et al., 1990). Furthermore, evidence was presented which showed that action potential propagation through this synaptic network was necessary since RPA were blocked by sodium and calcium channel blockers. Lastly, simultaneous recordings between neurons revealed that RPA was synchronous in a large population of CA3 neurons.

Tse and MacVicar (1989) further investigated the pharmacological mechanisms involved in RPA generation. It was found that pre-incubation of hippocampal slices in pertussis

toxin or in lithium prevented induction of activity by carbachol. This suggested that a GTP-binding protein linked to the phophoinositide second-messenger system was involved. These findings are consistent with the observations that the muscarinic responses of *in vitro* hippocampal neurons involve G-proteins and the phosphoinositide system (Ghodsi-Hovespian, Messer and Hoss, 1990).

Recently, a novel finding was presented by Heynen and Bilkey (1990). They found that RPA could not be obtained in hippocampal slices obtained from adult rats but could be consistently obtained from juvenile rats. This finding replicated the observation made by MacVicar and Tse (1989) that 21- to 28-day-old rats were the most consistent in generating RPA. Furthermore, Heynen et al. demonstrated that concurrent excitation (by glutamate) and disinhibition (by picrotoxin) could elicit rhythmical field potential oscillations in slices from adult rats. They suggested that a lack of development of inhibitory circuitry in the hippocampus of juvenile rats was the reason why only excitation by carbachol was necessary to produce activity. Thus, in the adult, this inhibition, being fully developed, would have to be blocked in order for activity to occur. This would explain why GABA antagonists had apparently no effect in the experiments conducted by MacVicar and Tse (1989). However, the picture is not as clear as it may seem. Firstly, *in vivo* experiments have demonstrated that microinfusions of

carbachol in the hippocampi of adult rats elicited theta activity (Rowntree and Bland, 1986). Secondly, if in fact there is a lack of inhibition in juvenile rats and only excitation is necessary for activity, then simply perfusing, for example, glutamate onto juvenile slices should produce theta-like activity. Konopacki et al. (1998a) found that perfusing glutamate onto juvenile slices did not produce RPA. Clearly simple excitation is not sufficient, theta-like activity requires the distinct pharmacological actions that occurs with the activation of muscarinic receptors. The difference between adult and juvenile hippocampal slices remains to be resolved.

1.9 Hypothesis

It is hypothesized that general anaesthetics will differentially alter the carbachol-induced rhythmical population activity observed in CA1 pyramidal and dentate granule neurons of the *in vitro* hippocampal formation.

1.10 Objectives

The objectives of this thesis were:

(1) to generate a concentration-response curve for carbachol-induced RPA recorded from dentate granule neurons

(2) to determine the threshold of effect for ethanol, pentobarbital, halothane, isoflurane and enflurane on RPA recorded from CA1 pyramidal and dentate granule neurons

(3) to generate a concentration-response curve for the above listed anaesthetics on RPA recorded from CA1 pyramidal and dentate granule neurons

(4) to determine the reversibility/irreversibility of the anaesthetic effects on RPA recorded from CA1 pyramidal and dentate granule neurons

(5) to establish a rank order of *in vitro* potencies

(6) to compare the effective *in vitro* concentrations (i.e. EC_{50}) to those reported in the whole animal

(7) to evaluate the usefulness of carbachol-induced RPA as a model system for studying the actions of anaesthetics on the CNS

1.11 Rationale

Studies have shown that anaesthetics are capable of producing multiple actions in the CNS which could be due to multiple sites and/or mechanisms of action. However, these studies use neuronal model systems in which activity has no functional/behavioral consequence. Behavior and other functions of the CNS are dependent upon both the properties of individual neurons and neural networks (Keeler, Pichler, and Ross, 1989). Although the mechanism by which the functions of individual neurons are translated into behavior is not understood, it has been proposed that network oscillations are involved in functional states (for example, attention) and motor coordination (Llinas, 1988). Electroencephalographic

(EEG) activity, in part, is the electrophysiological manifestation of these network oscillations (Traub, Miles, and Wong, 1989). It also reflects the "instantaneous changes in the integrated synaptic activity of the CNS, which most probably represents.....all ongoing processes under higher nervous control "(Fox, Lawndes and Bierkamper, 1982). Changes in the characteristics of the EEG are thought to be indicative of defined aspects of behavior, therefore, changes in EEG should be reflected by alterations in behavior and vice versa (Fox et al., 1982).

There are very few studies that have investigated the effects of anaesthetics on network oscillations. Carbachol-induced RPA appears to an *in vitro* simulation of type 2 theta EEG activity observed in the hippocampi of whole animals. This activity reflects some aspect of sensory processing (Bland, 1986) and memory (Winson, 1978), features that are relevant to the state of anaesthesia. The study of the effects of anaesthetics on RPA should provide information on how anaesthetics affect the network activity of neurons and if a particular agent affects the network activity in a unique manner.

METHODS

2.1 Dissection

Male Sprague Dawley rats (21 days old) from the University of Calgary Biosciences Vivarium were anaesthetized with diethyl ether and, upon cessation of breathing, were decapitated. A longitudinal incision was made in the scalp, the skin pulled back and the cranium was fractured along the midline by inserting one blade of a pair scissors through the foramen magnum. A small pair of Rongeurs were subsequently used to remove the two halves of the cranium and the dura was carefully removed with a scalpel. The cerebellum was then separated from the striate cortex and a sharpened stainless steel spatula, with a slight upward curve, was utilized to cut the olfactory nerves and gently lift the brain out of the cranium. The brain was rapidly placed in a petri dish containing cooled (5°C) and oxygenated artificial cerebrospinal fluid (ACSF). ACSF was cooled to slow cellular metabolism and increase oxygen solubility. The brain was then transected along the longitudinal fissure and the right hemisphere was placed with the neocortex side down onto a filter paper moistened with ACSF. The hippocampal formation was dissected out by placing a small stainless steel spatula into the overlying lateral ventricle and pushing back on the neocortex to reveal the entire dorsal side of the hippocampus. A similar spatula was then placed beneath the hippocampus which was then cautiously pulled upwards and outward.

Simultaneously, the other spatula was used to sever the fornix and the connections to the entorhinal cortex and basal ganglia. Extreme care was taken during this entire procedure. Furthermore, the spatulas were covered with a thin layer of ACSF so that there was minimal contact between the tissue and the metal surface. The hippocampus was then placed on another piece of moistened filter paper which was located on the teflon stage of a tissue chopper (Stoelting). Care was taken to align the fibers on the dorsal surface with the blade of the chopper so that slices preserved the alignment of synaptic pathways. Transverse slices with a thickness of 350 microns were made and the slices were gently transferred with a fine brush to another petri dish containing ACSF. Four or five slices were placed in a tissue chamber using a wide mouth glass pipette to minimize mechanical disturbance of the tissue. The entire dissection period, including placement into the chamber, took no longer than 6 minutes.

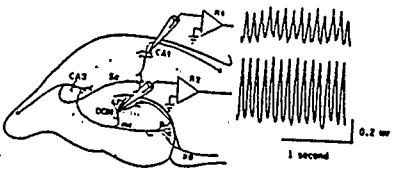
Hippocampal slices were placed on a nylon mesh screen of a gas/liquid interface McIlwain style tissue chamber. The upper sufaces of the slices were exposed to an atmosphere of pre-humidified and warmed 95% O_2/5% CO_2. Slices were maintained at a temperature of 35°C and the flow rate of the ACSF was approximately 1 ml/min. The internal electronic heating system was a circulating water bath (Haake) controlled by a Yellow Springs, Inc. thermistor feedback system.

2.2 Experimental Protocol

A schematic of the experimental protocol is shown in Figure 3. Slices were allowed to equilibrate for 90 minutes prior to start of the experiment. Following this time period, the viability of the slices were evaluated by placing a stimulating electrode on the Schaffer collaterals and measuring the corresponding population spike in stratum pyramdale of the CA1 neurons (see MacIver and Roth (1988) for stimulation parameters). Slices that had a population spike of at least 8 mV were considered to be healthy and were used to generate RPA (Konopacki et al., 1988). At this point, 75 μM of carbachol was perfused through the chamber to induce RPA. The activity was recorded with glass recording electrodes (3-5 Megohms; 2 M NaCl) made from Kwik-Fil capillaries 1 mm in diameter (W-P Instruments, CT, USA) and pulled on a Narishige electode puller. Electrodes were positioned by a Burleigh Inchworm Controller (PZ-550) set to move in 2 micrometer steps. Recorded signals were filtered (1-30 Hz, band pass) and amplified (x1000) using a Grass Instruments P-15 preamplifier and displayed on a digital oscilloscope (Gould 0S4020). Activity was recorded in either the stratum radiatum of CA1 or the stratum moleculare of the dentate gyrus (Figure 3). Following a 20 - 30 minute equilibration period with carbachol, a 5 - 10 minute control recording was made of the field potential activity. All signals were recorded onto VCR tape via a Neurodata Neuro-

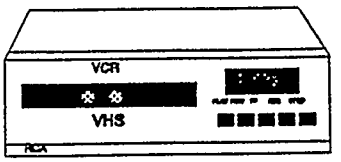
Figure 3. A schematic of the experimental protocol showing the hippocampal slice, extracellular recording sites, viewing, storage and subsequent analysis of the data.

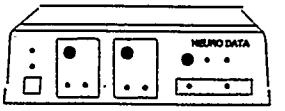
SPRAGUE DAWLEY RAT (21 DAYS)



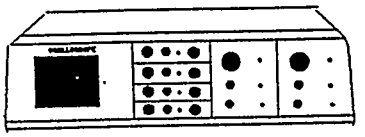
75 μM CARBACHOL

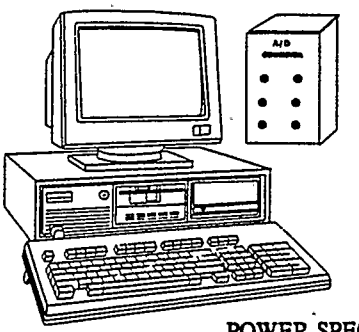
LOW PASS FILTER (30 Hz)





NEURO-CORDER



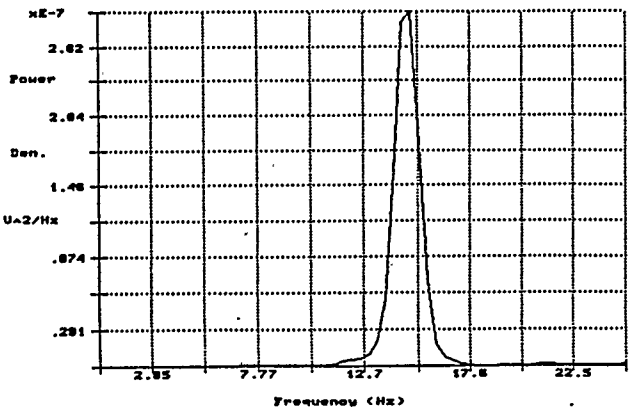


A/D CONVERSION

12 BITS @ 102.4 Hz

FFT ANALYSIS

POWER SPECTRAL ANALYSIS



10s POWER SPECTRUM

Corder DR 390 for later analysis. Following the control recording anaesthetics were administered. Sodium pentobarbital and ethanol were administered as solutions whereas the volatile anaesthetics were delivered by commercial vaporizers via the O_2/CO_2 flow. Appropriate amounts of pentobarbital powder were weighed accurately and dissolved in artificial cerebrospinal fluid (ACSF), which contained carbachol at a concentration of 75 μM. Ethanol was appropriately diluted in ACSF, which also contained carbachol at a concentration of 75 μM. Selected concentrations of a volatile anaesthetic were allowed to equilibrate for 15 minutes before RPA was recorded again. Anaesthetic solutions were allowed to equilibrate for 20 minutes. At this point, another 5 - 10 minute recording of the activity was made. A maximum of 3 different concentrations were used per experiment and a cumulative dose-response protocol was followed.

2.3 Power Spectral Analysis: Theory

Power spectral analysis is a mathematical means of describing a waveform. It was first developed for signal analysis in communication systems but has since been used extensively in clinical electroencephalography. This method of analysis was chosen to study the effects of anaesthetics on RPA because: (1) this activity is a continuous, periodic, sinusoidal-like waveform (2) this activity consists of features which cannot be measured by visual methods (3) power

spectral analysis is a sensitive method in which one can examine those features in detail. The following discussion will present the priniciples of power spectral analysis.

In communication systems there are two fundamental types of signals, deterministic and random signals (Stanley, 1981). Deterministic waveforms are those whose instantaneous values as a function of time can be completely predicted. Conversely, a random signal is one whose instantaneous values cannot be predicted at any given time. However, random signals can behave in certain well defined patterns, that is, they have a probability distribution hence they can be described in statistical or probabilistic terms. These types of signals can also be periodic, that is, the pattern is predictable, or nonperiodic. A nonperiodic signal can be thought of as having a period which approaches infinity.

Any signal can be transformed into a sum of a series of sine and cosine functions with different frequencies (periods) and amplitudes. In the simplest case, if we consider a deterministic periodic signal, it can be described in the frequency domain by a Fourier series spectral representation (Cooper, J.W. Osselton and Shaw, 1980; Stanely, 1981; Dumermuth and Molinari, 1987). To determine the frequencies that are present in the original signal, the signal is multiplied by a complex sum of sine and cosine functions. This operation is equivalent to multiplying each of the signal's frequency components separately and adding the

results together. The mathematical outcome is that only a component with the same frequency as the sine or cosine function will have a product with some nonzero finite value. On the other hand, components of the signal with different frequencies will have a product of zero. Suppose that a 10 second segment of a signal that repeats itself every 10 seconds is analyzed. The lowest frequency sine or cosine wave in this signal is one that has a period of 10 seconds or a frequency of 0.1 Hz. Thus, the lowest frequency that can be detected is 0.1 Hz. If the signal is multiplied by a sine or cosine function of this frequency, only if the signal has this frequency will the product be a nonzero value. This frequency is called the **fundamental frequency** and the component frequencies of the signal that can be determined are **harmonics** (integer multiples) of the fundamental frequency (Cooper et al., 1980). These product values are the coefficients of the Fourier series of the signal. The sum of these values is referred to as the Discrete Fourier Transform. Conversion to the original signal is simply attained by summing the spectral components.

In the case of nonperiodic signals, the Fourier series does not exist. Therefore, power spectral density (psd) functions are used to describe such signals in the frequency domain. In such cases, the Fourier transform of the autocorrelation function is its psd function (Dumermuth and Molinari, 1987). The Fourier transform is a limiting case of

the Fourier series calculation to nonperiodic signals which is achieved by letting the period approach infinity. This results in a continuous rather than a discrete spectrum. A crosscorrelation function is a measure of the degree of similarity or linearity between two waveforms. An autocorrelation, then, is a crosscorrelation of a waveform with a time-shifted version of itself. It is used to detect rhythmical or periodical components in the signal. Therefore, a Fourier transform of the signal's autocorrelation function will reveal peaks in the psd function where periodicities (i.e. rhythmical components) in the signal exist.

EEG activity, or any similar biological activity, is considered a random nonperiodic signal, however, power spectral analysis can be extended to random signals if it meets the assumption of stationarity (Cooper et al., 1980; Stanely, 1981; Dumermuth and Molinari, 1987). That is, the statistics of a signal cannot be affected if the signal is time shifted within the segment that is being analyzed. Another way of conceptualizing stationarity, using a 10 second segment of some signal, is by determining if the autocorrelation function changes with a time shift within this segment. If it does not, then the signal is stationary. Power spectral analysis does not apply to nonstationary signals but will apply to approximately stationary signals provided the autocorrelation condition is met for a given segment of data to be analyzed.

There is another way to calculate the psd function for a random nonperiodic signal. This is accomplished by taking the Fourier transform of the signal and then squaring the results. Mathematically, it can be shown that this function is an estimate of the psd function calculated from the signal's autocorrelation function (Cooper et al., 1980; Dumermuth and Molinari, 1987). In the present study, this procedure was employed to calculate the psd functions for carbachol-induced RPA. The advantage of this procedure is a practical one; it decreases the computational overhead which is extremely large using the above method. However, it increases the variability or scatter of the estimate around the 'true' psd function calculated from the autocorrelation function. Thus, one must employ mathematical means to smooth out the spectrum, which was done using appropriate software for data analysis.

One important feature that can be extracted from power spectral analysis is the electrical energy of the original waveform or of selected bandwidths. A common assumption made in signal analysis is to use a 1 ohm resistor as a reference. Power is the rate of change of electrical energy as a function of time, and the power dissipated in a resistor is equal to V^2/R. Thus, with a resistance of 1 ohm, power is simply V^2, and since a signal is represented by voltage values sampled over time, the power is simply the square of the signal. Note that the power is directly proportional the energy of the signal. However, the power spectral density function is not

the Fourier transformation of the squared signal, but rather, the transform of the signal's autocorrelation function. Therefore, in the frequency domain, the power of a signal is represented by its power density spectrum and the total energy of the signal is proportional to the area under the power spectrum function over the segment length being analyzed. In addition, the amplitude and the area under the peaks in the power spectral density curve are directly related to the energy content of the periodicity (Findeiss, Kien, Huse and Linde, 1969). It must be emphasized that since a 1 ohm reference is used, the energy of the signal is normalized and thus is not the absolute value.

2.4 Power Spectral Analysis of RPA

Data on the VCR tape was inputted into a Sperry PC/IT personal computer via an A/D board (Data Translation 2801). These data were digitized at a sampling rate of 102.4 Hz (Nyquist criterion) and divided into 10 second segments. Data were stored on 1.2 Megabyte diskettes. For each 10 second segment, the power spectral density function was computed using computer software developed in this laboratory which was written in Turbo Pascal 5.0 using the Asyst 3.0 Fast Fourier Transform alogrithms. "Leakage" due to time limiting the signal was minimized using the Blackman window.

For each segment the total power, the peak frequency and the duration of an individual burst of RPA were determined

(burst length). Total power represented the area under the curve from 0 - 25 Hz. Peak frequency was the frequency which had the greatest amount of power in that segment. The duration of a burst of RPA was visually determined by measuring from the time of onset to the time the burst ceased. Since the baseline field potential, before perfusion with carbachol and between bursts of RPA after perfusion with carbachol, was flat (i.e. no spontaneous spiking or spiking between bursts), the onset of the burst was defined as any observable activity above the baseline. Thus, the end of a burst was defined as a return to the baseline. For calculating the averages for each measure, all 10 second segments were pooled across all slices, corresponding to CA1 pyramidal or dentate granule neurons, for a single experimental condition. In this thesis, the 'n' values refer to the number of slices (obtained from different animals), however the number of 10 second segments for a given experimental condition were used in the statistical analysis. ANOVA and Student's t-test were used to determine statistical signficance of the data. All data are expressed as mean ± S.E.M..

2.5 Analysis of Volatile Anaesthetic Concentrations

Concentrations of volatile anaesthetics in the perfusate were determined using a gas chromatograph (HP 5890A fitted with a flame ionizer detector). Temperatures for the

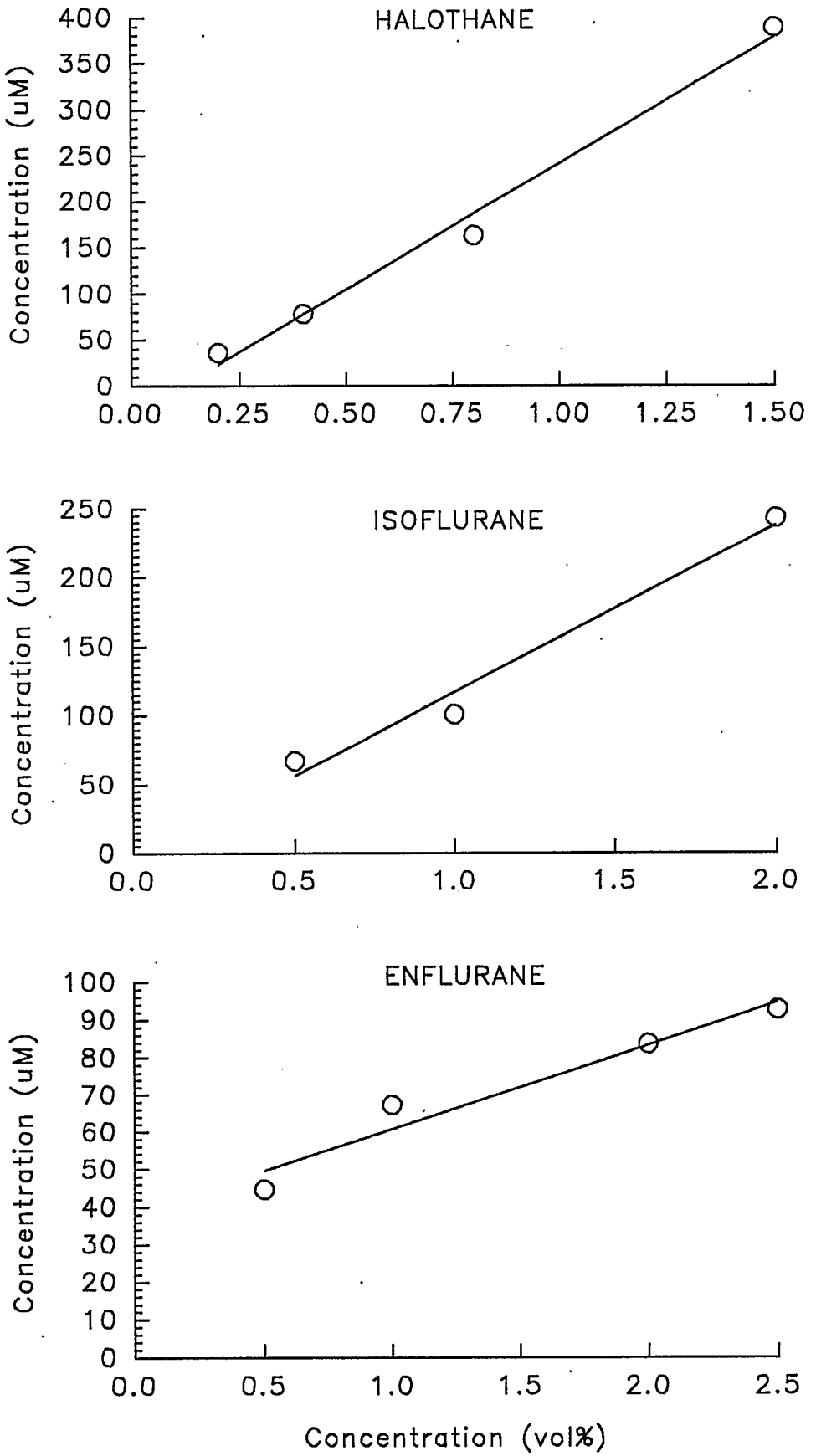
injector, oven and detector were 200, 125 and 250 °C, respectively. A flame-ionization detector was used and the carrier gas was helium at a flow rate of approximately 45 cm/sec.

Aliquots of aqueous test samples (100 μl) were taken from the tissue chamber at different vaporizer settings with a gas tight Hamilton syringe and injected directly onto the column. Concentrations of test samples were determined by comparing their peak areas with areas determinined from known standards. Column retention times were approximately 3 to 5 minutes for the volatile anaesthetics. Figure 3 shows the relationship between vol% settings on the vaporizer and the calculated molar concentrations in the tissue chamber. The scales on each calibrated vaporizer appeared to be ideal for the effective concentration range to produce effects on RPA in this study. The lowest concentration that could be detected, and thus studied, was limited by the settings on the vaporizer.

2.6 Materials

Rats were obtained from the University of Calgary Biosciences Vivarium. Drugs used in this study were obtained from the following suppliers: sodium pentobarbital (BDH chemicals, Ont.); halothane, isoflurane, and enflurane (Anaquest); carbachol and ethanol (Sigma). Halothane was

Figure 4. Results of the GC analysis showing the relationship between the vaporizer settings (vol%) and the measured concentrations (μM) of the volatile anaesthetics in the tissue chamber.


HALOTHANE
Concentration (uM)
400
350
300
250
200
150
100
50
0
0.00
0.25
0.50
0.75
1.00
1.25
1.50
ISOFLURANE
Concentration (uM)
250
200
150
100
50
0
0.0
0.5
1.0
1.5
2.0
ENFLURANE
Concentration (uM)
100
90
80
70
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
2.0
2.5
Concentration (vol%)

99.99% pure (containing 0.01% thymol as a preservative) and isoflurane, enflurane and ethanol were 100% pure. The vaporizers used to administer the volatile anaesthetics were obtained from Foothills Hospital Department of Anaesthesia; manufactured by Ohio Medical Products (Madison, Wis.) and Benson Medical Industries (Markham, Ont).

The ACSF physiological solution had the following composition (mM): NaCl 124, KCl 5, $CaCl_2$ 2, NaH_2PO_4 1.25, $MgSO_4$ 2, $NaHCO_3$ 26, glucose 10 (Schwartzkroin, 1977). All chemicals were reagent grade or better and made with water that was prefiltered to 18 Megohm by a RO pure LP low pressure reverse osmosis system (Barnstead). ACSF had an osmolarity of 286 +/- 1.55 mmol/kg (n=7) (Wescor 5500 Vapor Pressure Osmometer) and a pH of 7.38 +/- 0.08 (Fisher, model 620) following saturation with 95% O_2/ 5% CO_2 for approximately one hour. ACSF and drug solutions were made fresh prior to each experiment and saturated with 95% O_2/ 5% CO_2. Carbogen and the specialty gases for GC analysis were obtained from Medigas Ltd. (Calgary, Alberta) and were manufactured by Union Carbide Canada Ltd. (Edmonton, Alberta).

RESULTS

3.1 Control - Carbachol Concentration-Response

Carbachol-induced activity was observed within 5 minutes of perfusion with the peak effect occurring by approximately 10 minutes. As reported by Konopacki et al. (1988), over the next 10-15 minutes there was a subsequent decrease in amplitude which was then followed by a stable period lasting several hours. Population spiking could be observed with carbachol concentrations as low as 12.5 μM, however, at this low concentration the activity occurred without any distinct temporal pattern (Figure 5). At a concentration of 25 μM there was a dramatic change in the pattern of the activity; a short burst followed by a quiescent period (interburst interval) which was then proceeded again by a short burst (Figure 5). This behavior was repetitive and stable for several hours. Concentrations up to 100 μM also demonstrated this pattern but with a subtle change; there was an increase in burst length with increasing carbachol concentrations (Figure 5). Figure 6 summarizes the linear relationship between the carbachol concentration and burst length and Table 1 gives the range of values that were determined for each concentration. Using linear regression analysis, the equation that best fit the data was $y = 0.0634x + 2.52$ ($x \geq 25$ μM) ($R = 0.998$). For a given preparation the burst length and interburst interval were extremely consistent. Both parameters had a predictable range given a certain

Figure 5. Example recordings of RPA from dentate granule neurons at different carbachol concentrations. Note the change in pattern of the activity at 25 μM as compared to 12.5 μM and that the length and frequency of the activity increases with increasing concentrations. At 125 μM the pattern starts to become disrupted.

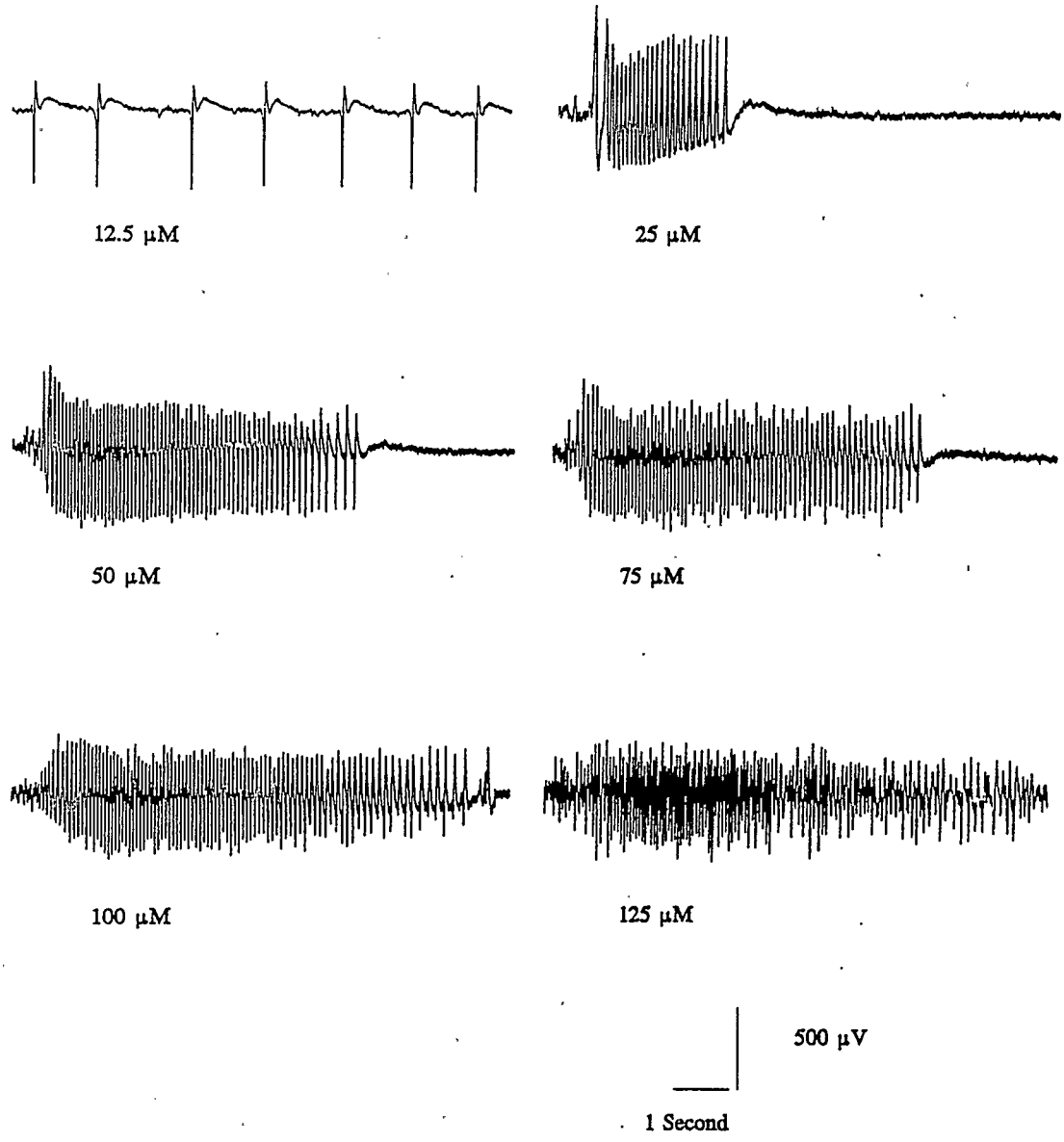

12.5 µM
25 µM
50 µM
75 µM
100 µM
125 µM
500 µV
1 Second

Figure 6. A graph showing the linear relationship between the carbachol concentration and burst length of RPA recorded from dentate granule neurons. Each point represents the mean (+/- S.E.M.) of 4 - 5 experiments.

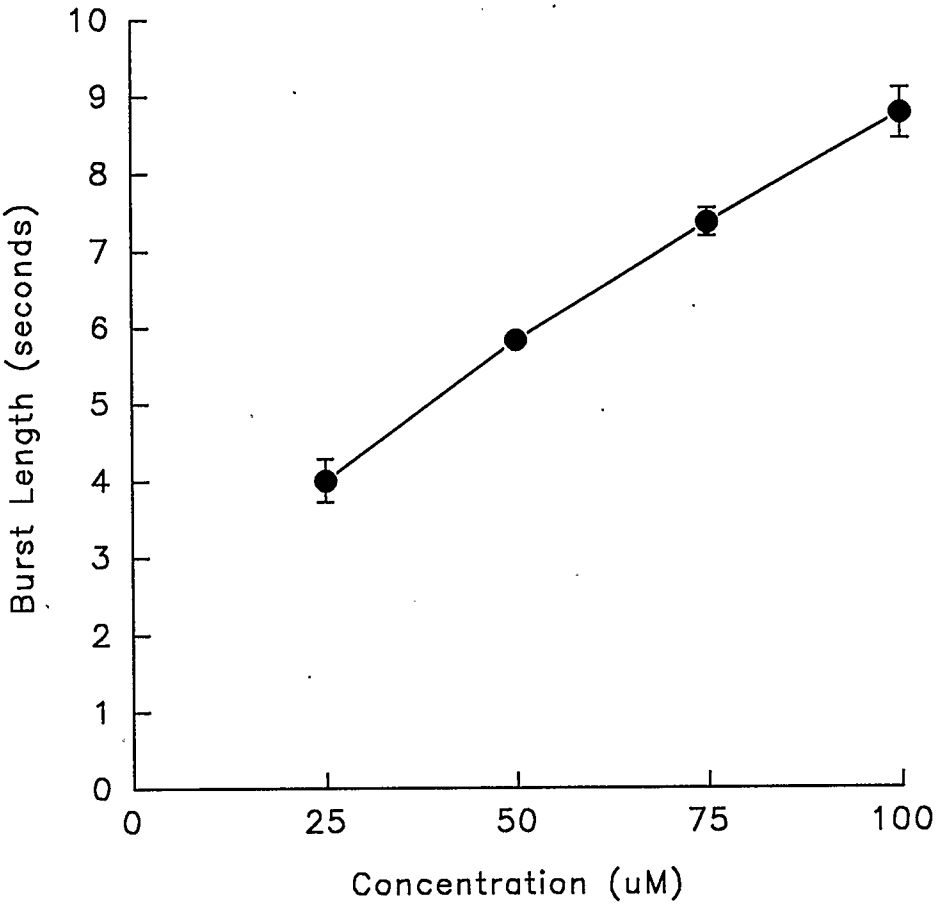

Burst Length (seconds)
10
9
8
7
6
5
4
3
2
1
0
0
25
50
75
100
Concentration (uM)

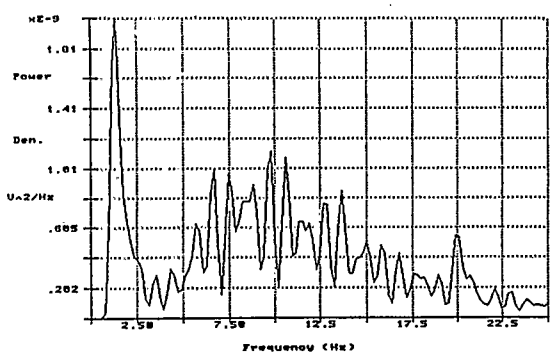
Table 1. The range of values determined for peak frequency, burst length and interburst interval in dentate granule neurons at different concentrations of carbachol. Determinations were made from at least 4 different preparations. The values in parenthesis are data from CA1 pyramidal neurons.

Concentration (μM)	**Peak Frequency (Hz)**	**Burst Length (sec)**	**Interburst Interval (sec)**
12.5	0.7 - 1.1	N/A	N/A
25	2.9 - 14.9	1.1 - 6.5	11.7 - 49.3
50	6.5 - 15.1	4.7 - 7.2	15.9 - 22.5
75	9.3 - 16.8 (8.9 - 17.2)	5.2 - 10.4 (5.1 - 11.0)	14.5 - 24.6 (11.6 - 32.6)
100	9.3 - 17.9	6.9 - 14.4	22.7 - 25.9

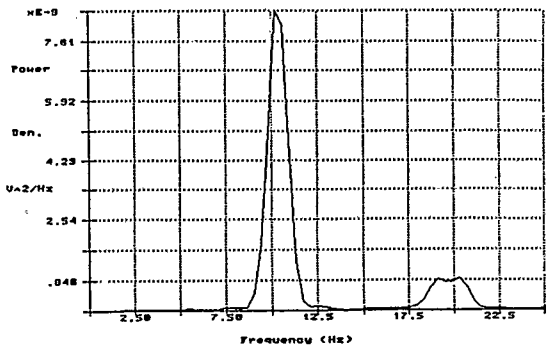
concentration for the sample of animals that were used in the control studies (Table 1). Concentrations higher than 100 μM appeared to disrupt the temporal pattern such that the interburst interval contained population spiking in addition to the regular bursting pattern (Figure 5). Thus the threshold appeared to be 25 μM with an effective concentration range of 25 to 100 μM. The length of a single burst of RPA and the interburst interval recorded from CA1 pyramidal neurons were not significantly different from those of dentate granule neurons. For dentate neurons, exposed to 75 μM carbachol, the mean burst length and interburst interval were 7.4 ± 0.2 sec and 19.9 ± 0.4 sec, respectively. For CA1 neurons, they were 7.3 ± 0.1 sec and 17.9 ± 2.3 sec, respectively. The range of values for the burst length and interburst interval of CA1 neurons are shown in Table 1. Note that for dentate neurons the standard deviation burst length at a carbachol concentration of 75 μM (i.e. control) was 1.3 sec.

Frequency was also dependent upon the carbachol concentration. Figure 7 demonstrates this effect and average results are summarized in Figure 8. There was a sharp significant increase ($n=4$; $p<0.0005$) in frequency from 12.5 (0.8 ± 0.01 Hz) to 25 μM (10.1 ± 0.6 Hz) with a further increase up to 75 μM at which the maximum frequency appeared to be reached (12.4 ± 0.5 Hz). The mean peak frequency at a concentration of 50 μM was significantly different from the

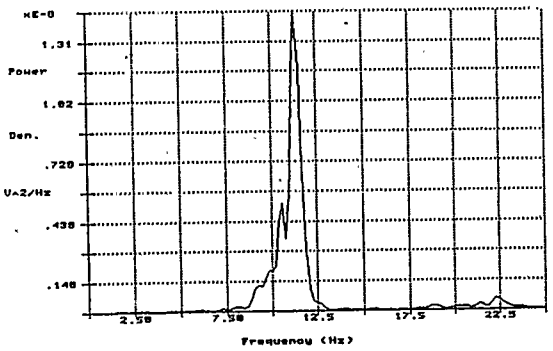
Figure 7. Example power spectra of RPA recorded from dentate granule neurons at different carbachol concentrations. Note the shift in frequency of the peak with increasing concentrations and the 'scatter' of the spectum at 12.5 μM and 125 μM. Also note that the values of the ordinates are not similar.



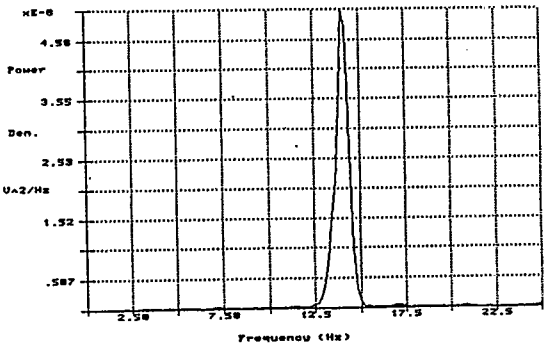
12.5 μM



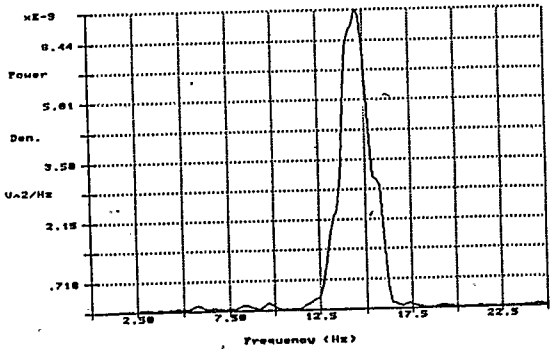
25 μM



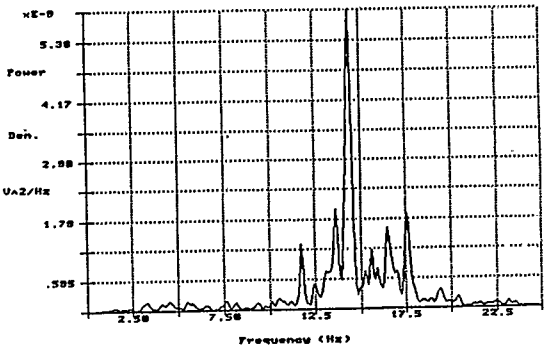
50 μM



75 μM

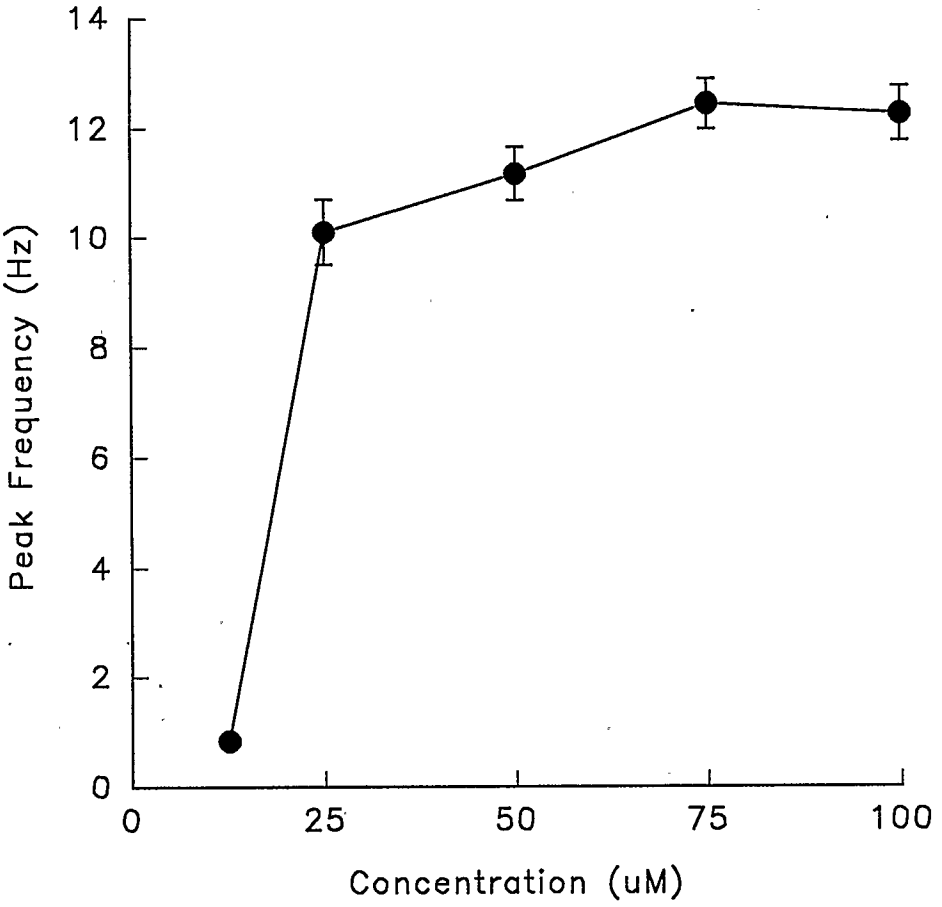


100 μM



125 μM

Figure 8. The relationship between carbachol concentration and the mean peak frequency of RPA recorded from dentate granule neurons. Each point represents the mean (+/- S.E.M.) of 4 - 5 experiments.


14
12
10
8
6
4
2
0
Peak Frequency (Hz)
0
25
50
75
100
Concentration (uM)

mean peak frequency at a concentration of 75 μM ($n=5$; $p<0.05$). However, the mean peak frequencies at concentrations of 25 and 50 μM were not significantly different ($n=4$; $p>0.05$). The peak frequency of CA1 pyramidal neurons was not different from dentate granule neurons. For example, at a concentration of 75 μM the mean peak frequency for dentate granule neurons was 12.4 ± 0.5 Hz whereas for CA1 pyramidal neurons it was 11.8 ± 0.3 Hz. The range of peak frequencies at carbachol concentrations of 12.5 to 100 μM are shown in Table 1. The peak frequency also appeared to be extremely consistent for a given preparation and was confined to a predictable range in the sample of animals used in this study, given a certain carbachol concentration (Table 1).

There was no apparent relationship between RPA power and the carbachol concentration. Although power values were consistent within a given preparation at a certain recording site, there was variation depending on the placement of the recording electrode. In addition, there was also substantial variation from preparation to preparation.

For the anaesthetic experiments, a concentration of 75 μM was selected to induce RPA. This concentration of carbachol was selected for the following reasons: (1) Konopacki, MacIver, Bland and Roth (1988a) reported that 85% of slices demonstrated this activity in response to carbachol at concentrations of 50 μM and higher whereas only 25% did so below this concentration. Thus, using concentrations above 50

μM allows a greater experimental success rate (2) 75 μM elicited the maximal frequency response which suggests that this concentration recruited the maximum number of neurons and that the synaptic network was maximally functional (3) there was less variation in the peak frequency as compared to any other concentration (Table 1) which would allow more confidence in the anaesthetic effect and comparisons between preparations (4) at 100 μM there was more variability in the burst length which would make it more difficult to compare data across animals and assess anaesthetic effects (Table 1) (5) 100 μM is close to the concentration at which the effects of overstimulation were observed (i.e. 125 μM) which could mean that the activity is reaching a pathological state (i.e. epileptic-like).

3.2 Effects of Ethanol on RPA

3.2.1 Total Power

Ethanol depressed the power of carbachol-induced activity of both CA1 pyramidal and dentate granule neurons. Figure 9 demonstrates this observation and Figure 10 summarizes these effects at concentrations of 20-80 mM. In the CA1, 20 mM decreased the power to 68.2 ± 16% (n=3) of control values, which was followed by further decreases at concentrations of 30-50 mM. In the dentate, 20 mM produced a greater depression as compared to the CA1 since power levels were decreased to 45.0 ± 4.5% (n=3) of control values. This suggests that the

Figure 9. Example recordings of RPA from CA1 and dentate neurons at different concentrations of ethanol. The control recordings demonstrate the difference in amplitude of RPA between the two neuronal populations. Note the decrease in amplitude and frequency with increasing ethanol concentrations and the lack of activity in the CA1 at 80 mM relative to the dentate.

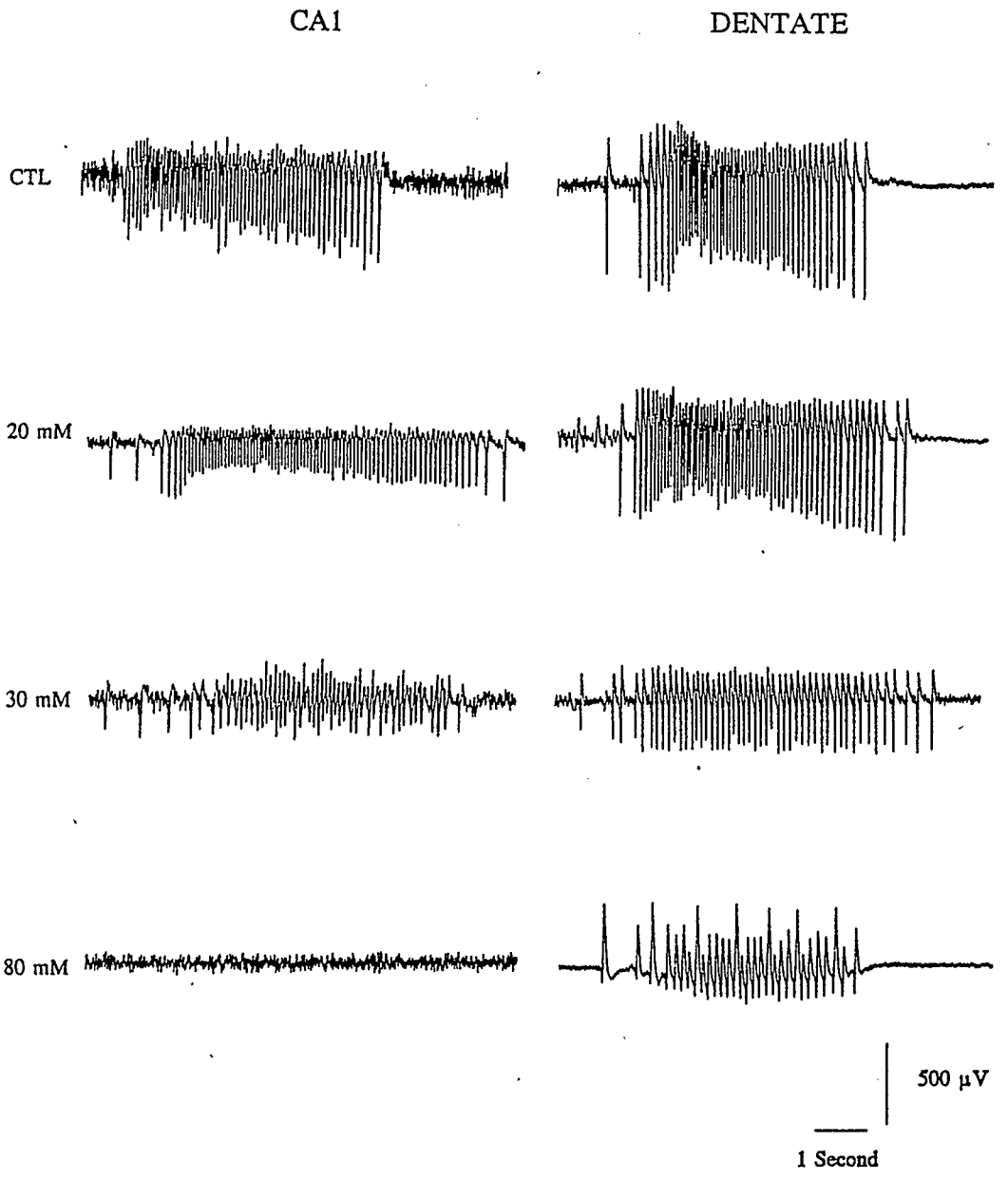
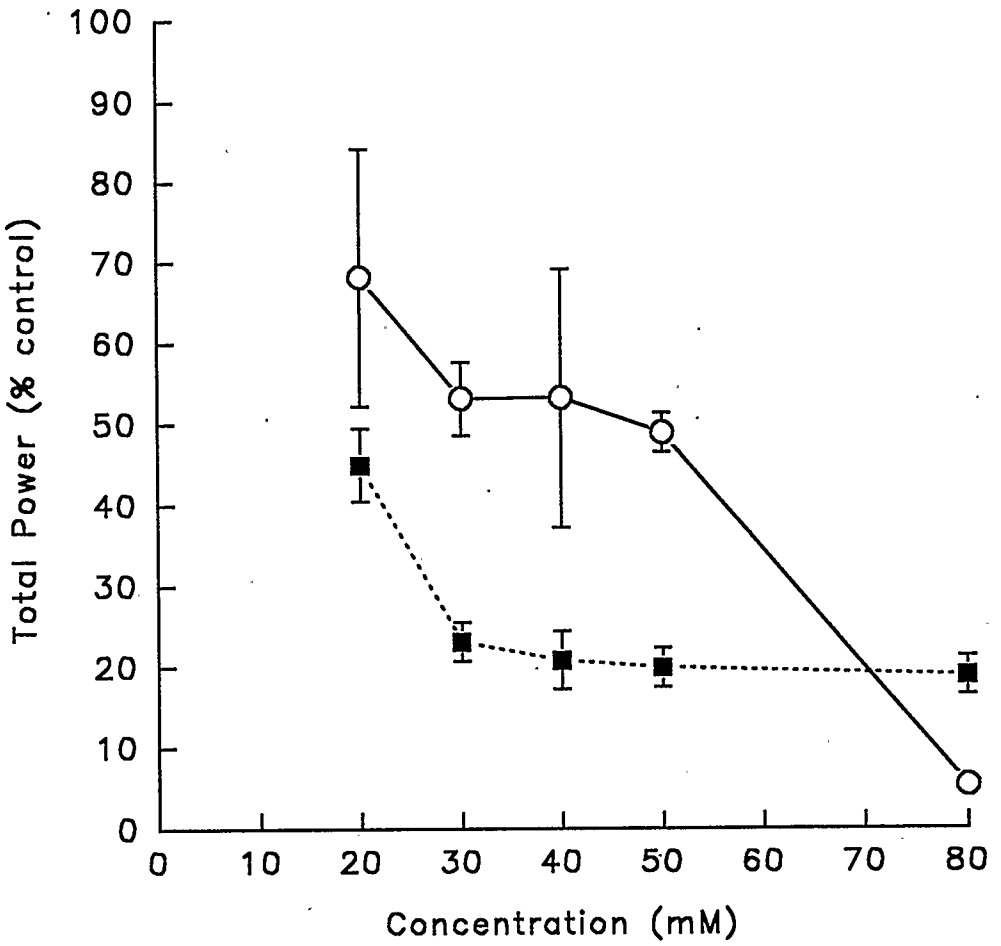

CA1
DENTATE
CTL
20 mM
30 mM
80 mM
500 µV
1 Second

Figure 10. A summary of the concentration-dependent effects of ethanol on the total power of RPA recorded from CA1 (○) and dentate (■) neurons. Each point represents the mean (+/- S.E.M.) of 3 experiments.

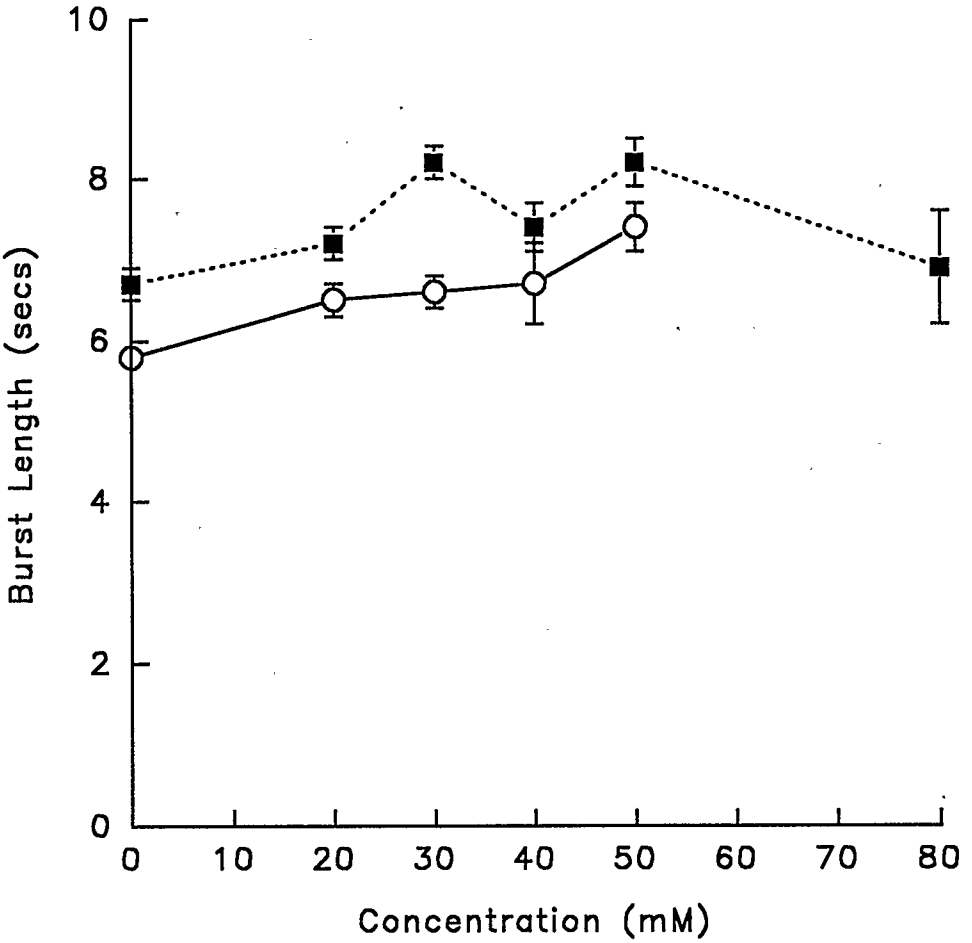

Total Power (% control)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
Concentration (mM)

dentate was more sensitive to ethanol, however, the difference was not statistically significant ($p>0.05$). A concentration of 30 mM further depressed the activity in the dentate to 23.1 ± 2.4% of control. An ethanol concentration of 80 mM appeared to abolish RPA recorded from CA1 pyramidal but not dentate granule neurons. In one experiment, concentrations up to 150 mM did not appear to have any further effects on RPA of dentate granule neurons.

3.2.2 Burst Length

Ethanol prolonged RPA burst length of CA1 pyramidal and dentate granule neurons. This effect is demonstrated in Figure 8 and summarized in Figure 11. In the CA1, 20 mM significantly increased the length from 5.8 ± 0.1 sec to 6.5 ± 0.9 sec ($n=3$; $p<0.0005$). Although this difference appears to be within the variability of the burst length of control (and thus not significant) (see Table 1), the variation of the burst length of control for the ethanol series of experiments was very small; i.e. a standard deviation of 0.8 sec. In addition, the variation at 20 mM was equally as small (i.e. 0.9 sec). The effect on the burst length was concentration-dependent since higher concentrations produced further increases. In the dentate, an increase was also observed at 20 mM, however, a significant effect was not observed until 30 mM ($n=3$; $p<0.0005$). At this concentration, ethanol increased the length from 6.7 ± 0.2 to 8.2 ± 0.2 sec and concentrations

Figure 11. A summary of the concentration-dependent effects of ethanol on the burst length of RPA recorded from CA1 (○) and dentate (■) neurons. Each point represents the mean (+/- S.E.M.) of 3 experiments.


10
8
6
4
2
0
Burst Length (secs)
0
10
20
30
40
50
60
70
80
Concentration (mM)

up to 50 mM produced further increases. However, a clear concentration-dependency was not observed in the dentate. At 80 mM, the effect appeared to be reversing since the length returned to control values. It was interesting to note that ethanol did not affect the burst/interburst pattern of the activity itself.

3.2.3 Peak Frequency

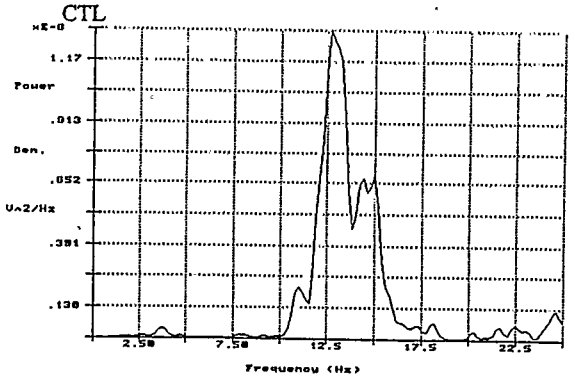
For both the CA1 and dentate ethanol had a biphasic effect on the mean peak frequency of carbachol-induced RPA (Figures 12 and 13). In the CA1, ethanol increased the frequency from 13.5 ± 0.2 to 14.9 ± 0.3 Hz ($n=3$; $p<0.0005$) at a concentration of 20 mM. Similarily in the dentate, this concentration increased the peak frequency of the activity from 13.7 ± 0.3 to 15.6 ± 0.2 Hz ($n=3$; $p<0.0005$). However, in both neuronal populations, an abrupt decrease was observed at 30 mM to 7.8 ± 0.4 Hz ($n=3$; $p<0.0005$) and 8.9 ± 0.3 Hz ($n=3$; $p<0.0005$) for dentate and CA1 neurons, respectively. Concentrations up to 50 mM ($n=3$) did not significantly further reduce the frequency but at 80 mM rhythmical activity appeared to be abolished in the CA1 ($n=4$) whereas it was still present in the dentate ($n=4$). Simultaneous recordings made in the CA1 and dentate at 80 mM provided further confirmation of this differential sensitivity.

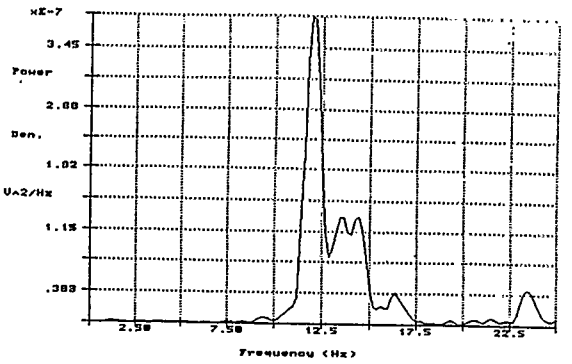
Figure 12. Example power spectra of RPA recorded from CA1 and dentate neurons at different concentrations of ethanol. Note the shift to the right (i.e. increase in frequency) in the peak of the spectra at 20 mM followed by a shift to the left (i.e. decrease in frequency) at 30 mM relative to control. Also at 20 mM the spectral power density of the peak frequency is decreased relative to control. At 80 mM the peak of the CA1 spectrum is 'noise' whereas the peak of the dentate spectrum is RPA. In addition, also note that the values of the ordinaates are not similar.

CA1

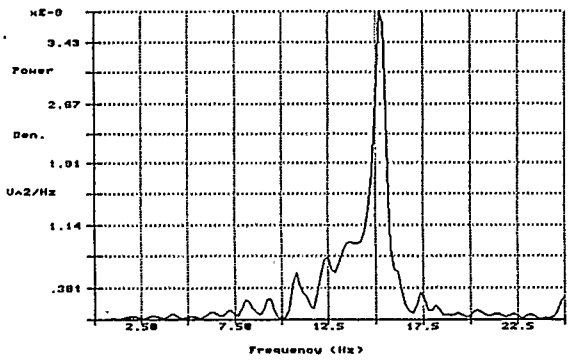
DENTATE

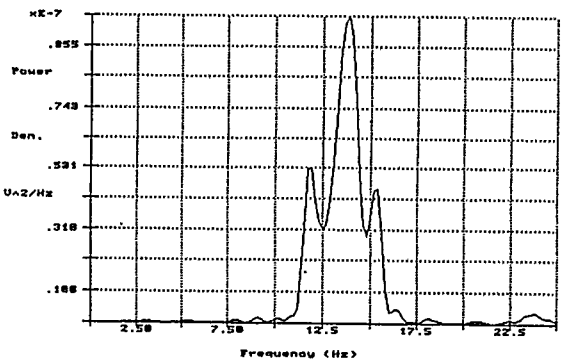
CTL



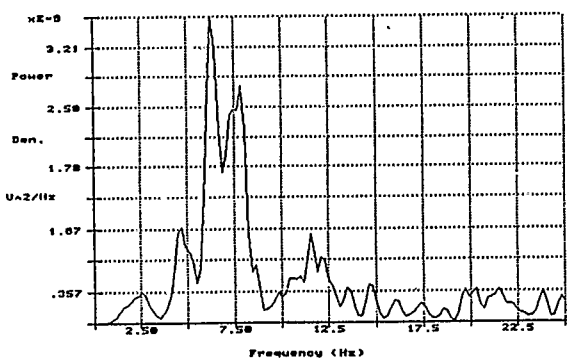


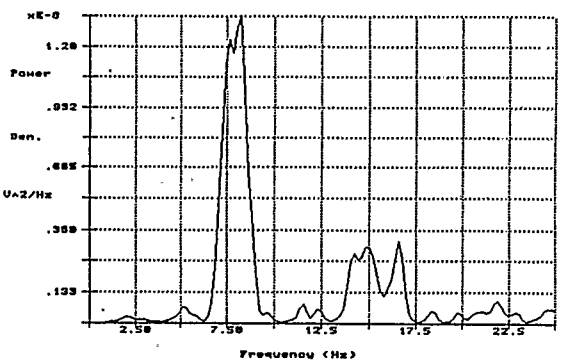
20 mM



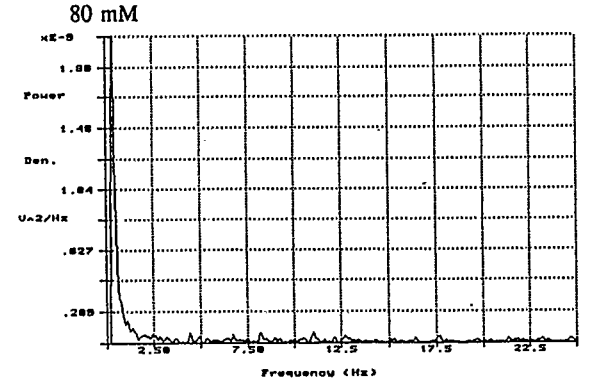


30 mM





80 mM



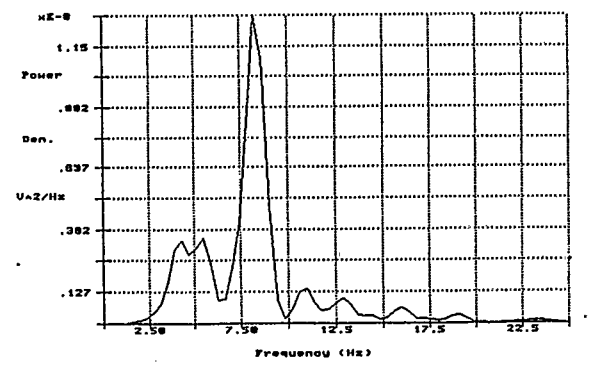
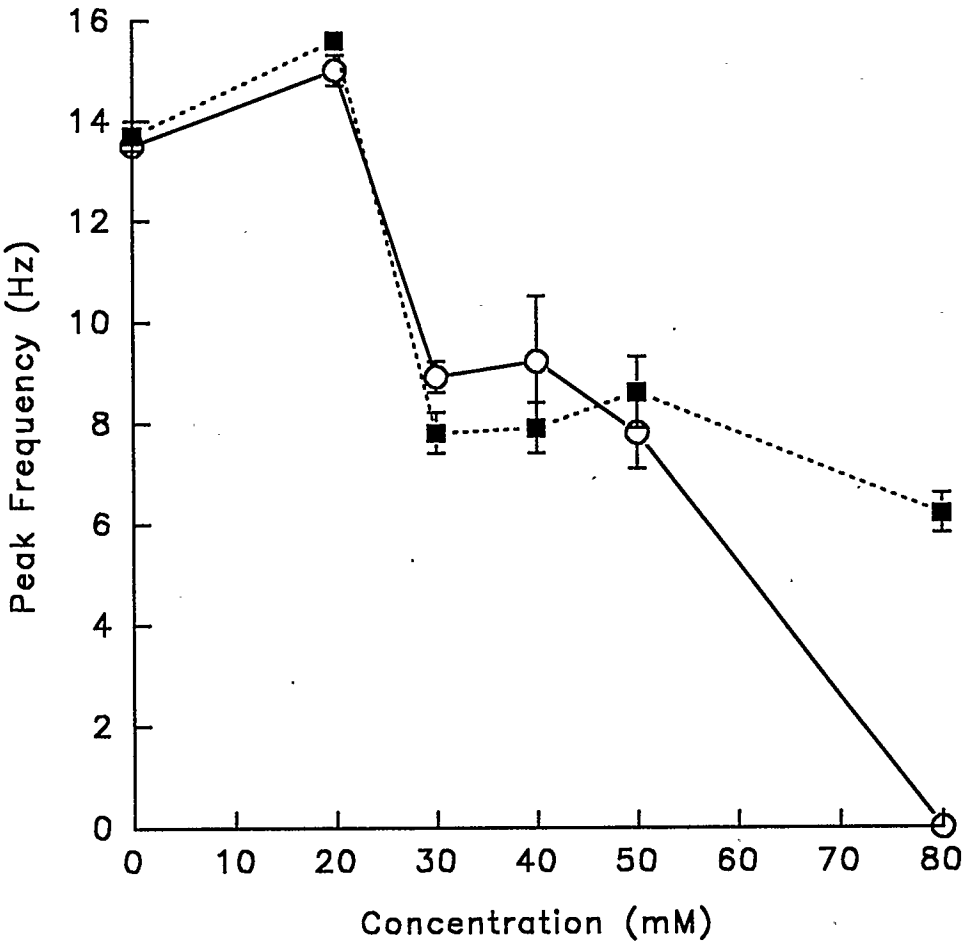


Figure 13. A summary of the concentration-dependent effects of ethanol on the peak frequency of RPA recorded from CA1 (○) and dentate (■) neurons. Each point represents the mean (+/- S.E.M.) of 3 experiments.


16
14
12
10
8
6
4
2
0
Peak Frequency (Hz)
0
10
20
30
40
50
60
70
80
Concentration (mM)

3.2.4 Reversibility/Irreversibility

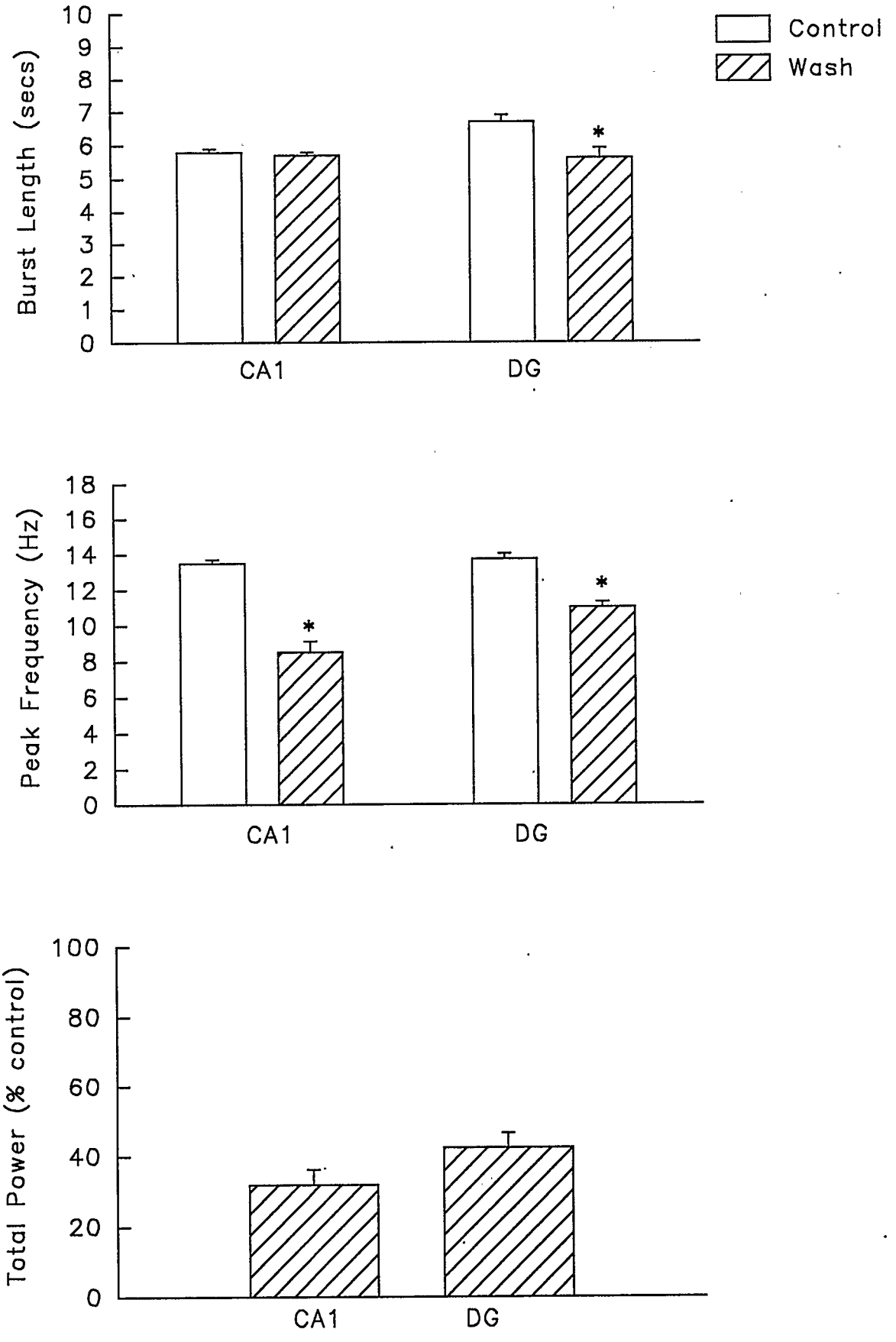
Figure 14 summarizes the reversal of the ethanol effects on burst length, peak frequency and total power after 60-90 minutes of washing with control solution. In general, the return of activity after washout of 80 mM ethanol was poor. The burst length of CA1 RPA returned to control values, however, dentate RPA had a length that was signficantly shorter than control (6.7 ± 0.2 sec and 5.6 ± 0.3 sec, for the control and wash, respectively; n=3; $p<0.05$). The peak frequency of the activity did not return to control values for CA1 neurons but approached control for dentate neurons. CA1 RPA had a mean control peak frequency of 13.5 ± 0.2 Hz whereas upon washout it only returned to 8.5 ± 0.6 Hz (n=3; $p<0.0005$). Dentate RPA had a mean peak control frequency of 13.7 ± 0.3 Hz whereas upon washout of ethanol it was 11.0 ± 0.3 Hz. Although the return approached control values this difference was significant (n=3; $p<0.0005$). For both neuronal populations total power did not return to control levels. The power of CA1 RPA returned to 31.9 ± 4.3% of control (n=3) and to 42.4 ± 4.2% (n=3) for dentate RPA.

3.3 Effects of Pentobarbital on RPA

3.3.1 Total Power

Pentobarbital had a different effect on RPA of CA1 neurons as compared to dentate neurons. For CA1 neurons, a biphasic response was observed; pentobarbital enhanced the

Figure 14. The average values for burst length, peak frequency and power in comparison to their respective controls after washout of ethanol. Each bar represents the mean (+/- S.E.M.) of 3 - 4 experiments and * refers to a significant difference ($p<0.05$) between the means of the wash and control.


Control
Wash
Burst Length (secs)
10
9
8
7
6
5
4
3
2
1
0
*
CA1
DG
Peak Frequency (Hz)
18
16
14
12
10
8
6
4
2
0
*
*
CA1
DG
Total Power (% control)
100
80
60
40
20
0
CA1
DG

power of the activity at low concentrations (20-40 μM) up to 179.4 ± 28.4% of control (n=3) and produced depression at higher concentrations (50-80 μM) (Figures 15 and 16). In contrast, only depression was observed when recording from dentate neurons (n=3) (Figures 15 and 16). Initially, pentobarbital reduced the power to 86.4 ± 1.4% of control at 20 μM and then substantially decreased power at 60 μM to 18.6 ± 0.2%. At 80 μM all activity was abolished (n=3), but this was observed for the dentate neurons only.

3.3.2 Burst Length

Pentobarbital also appeared to different effects on the RPA burst length of CA1 and dentate neurons. No consistent effects were found on the burst length of dentate RPA (Figures 15 and 17) whereas in the CA1 pentobarbital increased the length from 6.4 ± 0.2 sec to 7.8 ± 0.6 sec (n=3; $p<0.01$) up to a concentration of 40 μM (Figures 15 and 17). In both neuronal populations, higher concentrations changed the activity from the characteristic burst/interburst pattern to a continuous spiking pattern (Figure 15). Unlike dentate neurons, the continuous spiking of the CA1 pyramidal neurons was unaffected by concentrations as high as 80 μM whereas it appeared to abolish RPA of dentate granule neurons.

Figure 15. Example recordings of RPA from CA1 and dentate neurons at different concentrations of pentobarbital. Notice that 20 μM increased the amplitude of the CA1 activity but slightly depressed the amplitude of the dentate activity. At 60 μM the burst/interburst pattern is disrupted in both neuronal populations and at 80 μM all activity is abolished in the dentate whereas it is still present in the CA1.

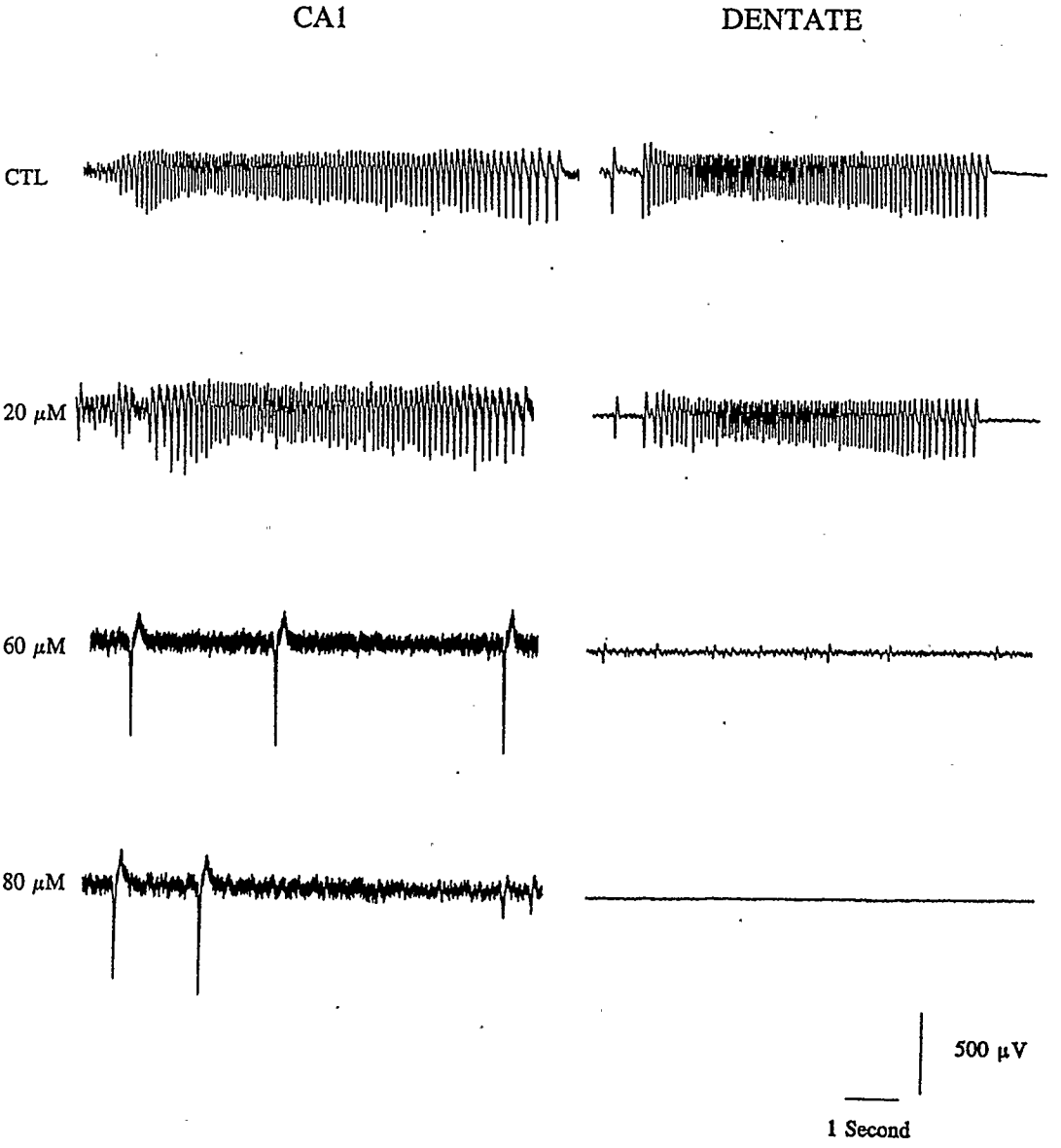

CA1
DENTATE
CTL
20 μM
60 μM
80 μM
500 μV
1 Second

Figure 16. A summary of the concentration-dependent effects of pentobarbital on the power of RPA recorded from CA1 (O) and dentate (■) neurons. Each point represents the mean (+/- S.E.M.) of 3 - 4 experiments.

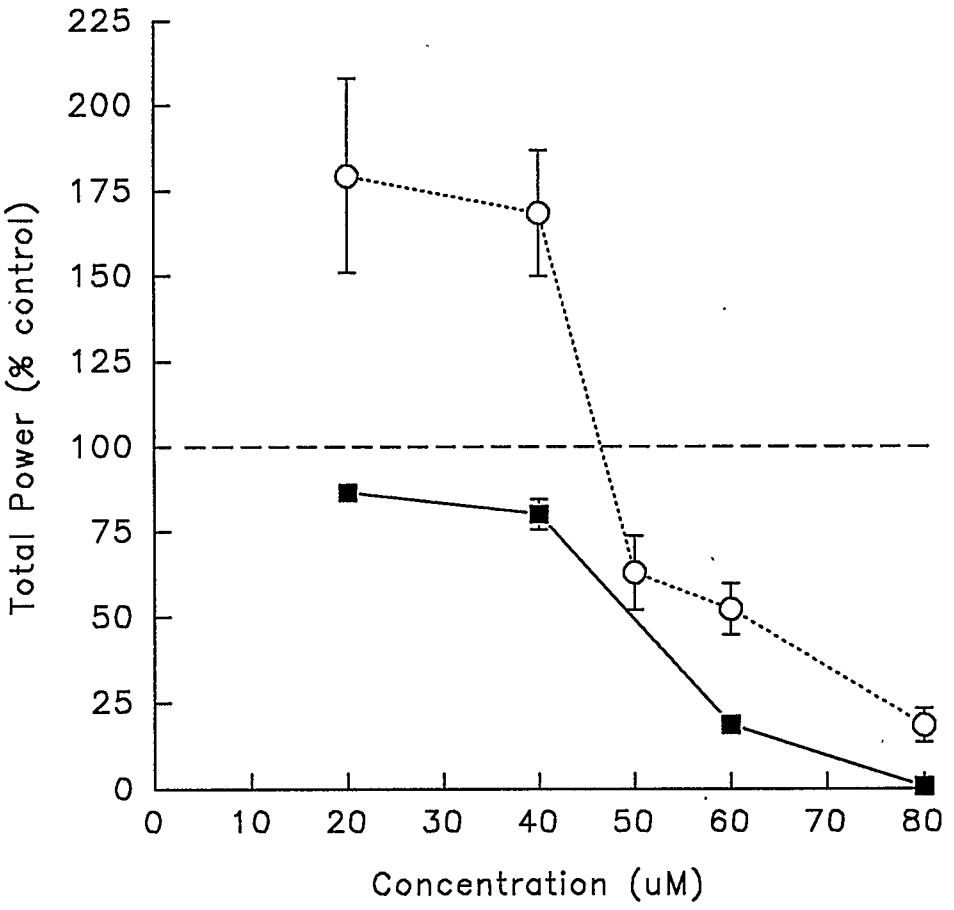
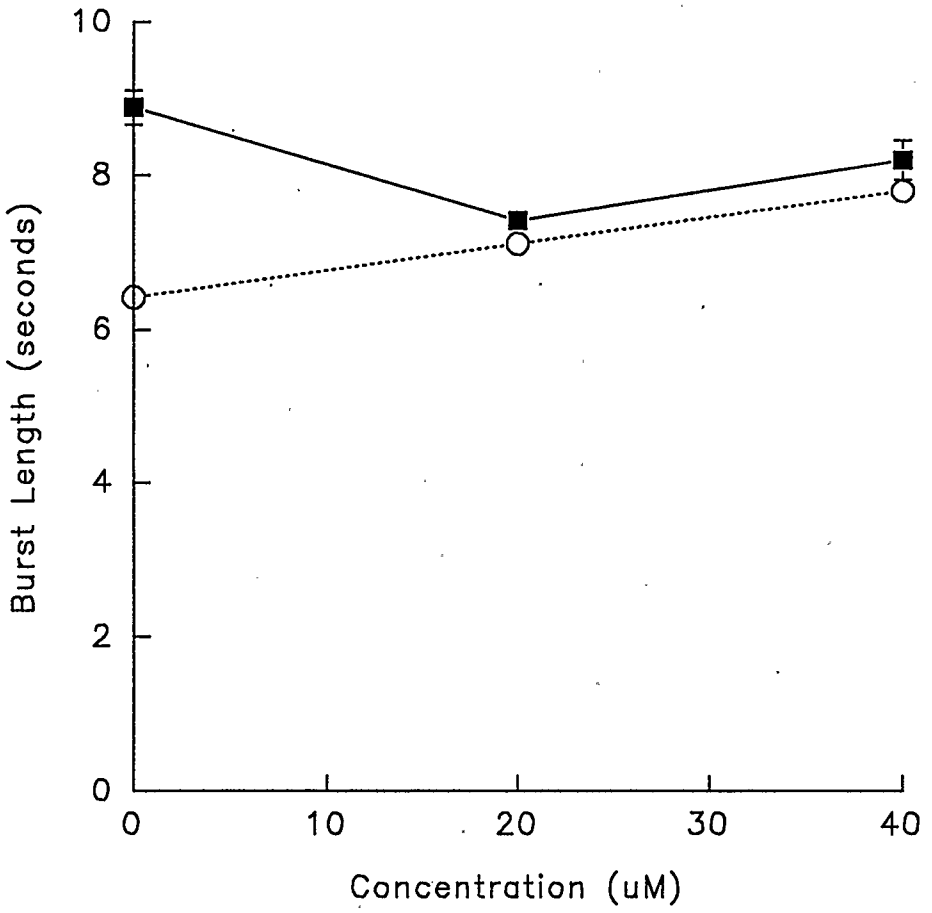

225
200
175
150
125
100
75
50
25
0
Total Power (% control)
0
10
20
30
40
50
60
70
80
Concentration (uM)

Figure 17. A summary of the effects of pentobarbital on the burst length of RPA recorded from CA1 and dentate neurons. Each experiment represents the mean (+/- S.E.M.) of 3 - 4 experiments.


10
8
6
4
2
0
Burst Length (seconds)
0
10
20
30
40
Concentration (uM)

3.3.3 Peak Frequency

Pentobarbital decreased the peak frequency of carbachol-induced RPA recorded from CA1 pyramidal and dentate granule neurons. This effect was concentration-dependent with a significant decrease starting at 40 μM for both neuronal populations (Figures 18 and 19). At this concentration pentobarbital reduced the peak frequency of CA1 RPA from 16.0 ± 0.9 Hz to 7.4 ± 0.4 Hz ($n=3$; $p<0.0005$) and dentate RPA from 12.2 ± 0.4 Hz to 8.9 ± 0.3 Hz ($n=3$; $p<0.0005$). In one experiment 20 μM caused a slight increase in the peak frequency of CA1 RPA. The maximum depression in peak frequency appeared to be reached at 80 μM for CA1 RPA (4.1 ± 0.3 Hz) although the depression in peak frequency appeared to plateau at 50 μM. Dentate RPA appeared to be abolished at 80 μM and therefore had a relative peak frequency of zero.

3.3.4 Reversibility/Irreversibility

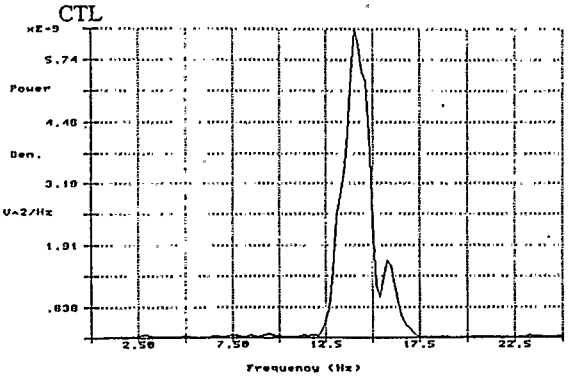
RPA returned following exposure to 80 μM pentobarbital approximately 40 minutes after washout with control solution. The burst length and the peak frequency returned to near control values for both CA1 pyramidal and dentate granule neurons (Figure 20). However, power levels did not return to control values and there were differences between CA1 and dentate neurons. For CA1 neurons, a rebound effect was observed, total power (% control) returned to 142.8 ± 11.9% ($n=3$) whereas for dentate neurons the return was only to

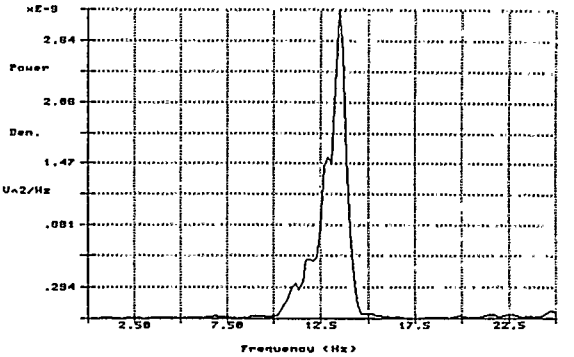
Figure 18. Example power spectra of RPA recorded from CA1 and dentate neurons at different concentrations of pentobarbital. Note the increase spectral power density at 20 μM relative to control for CA1 neurons in contrast to the decrease for dentate neurons. Higher concentrations shifted the peak of the spectra to the left (i.e. to a lower frequency). At 80 μM the peak of the CA1 spectrum is the peak frequency of RPA whereas the peak of the dentate spectrum is 'noise'. Also, that the values of the ordinates are not similar.

CA1

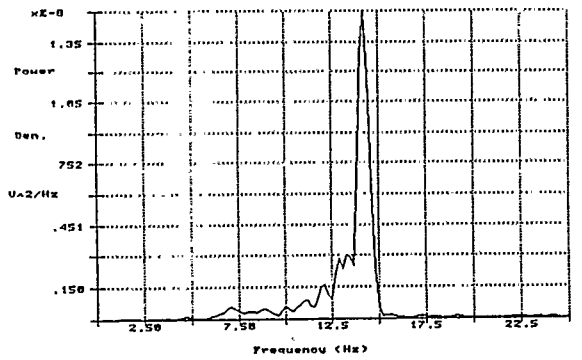
DENTATE

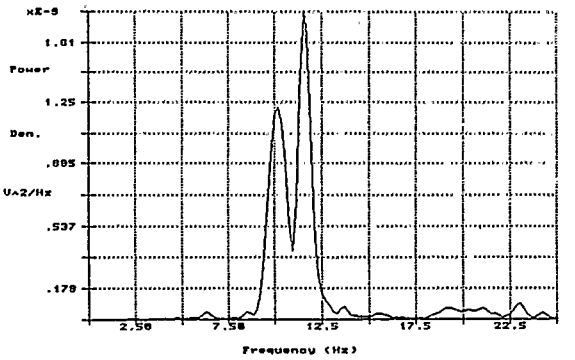
CTL



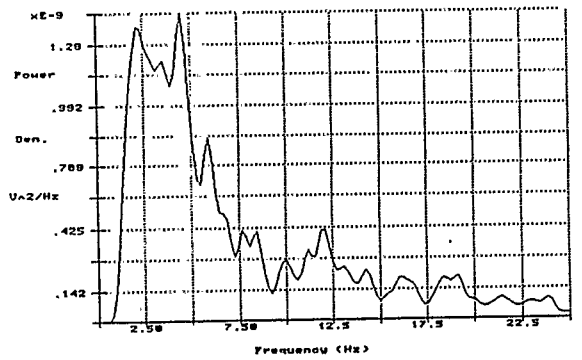


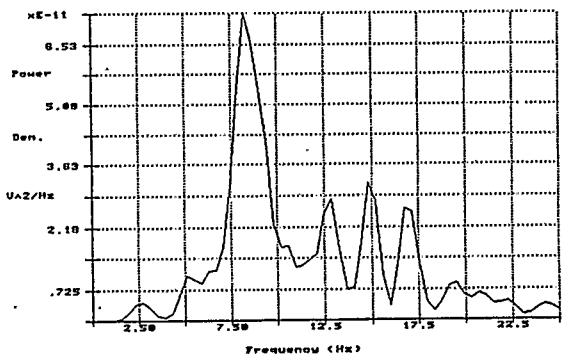
20 μM



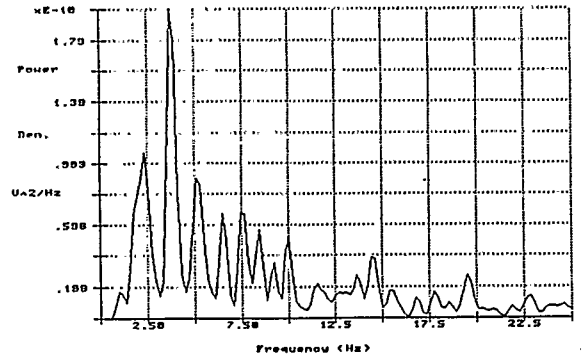


60 μM





80 μM



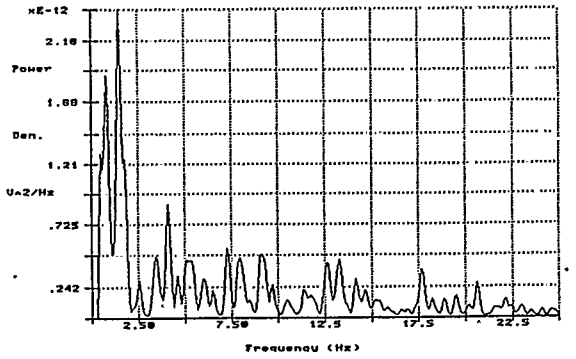


Figure 19. A graph summarizing the concentration-dependent effects of pentobarbital on the peak frequency of RPA recorded from CA1 (○) and dentate granule (■) neurons. Each point represents the mean (+/- S.E.M.) of 3 - 4 experiments.

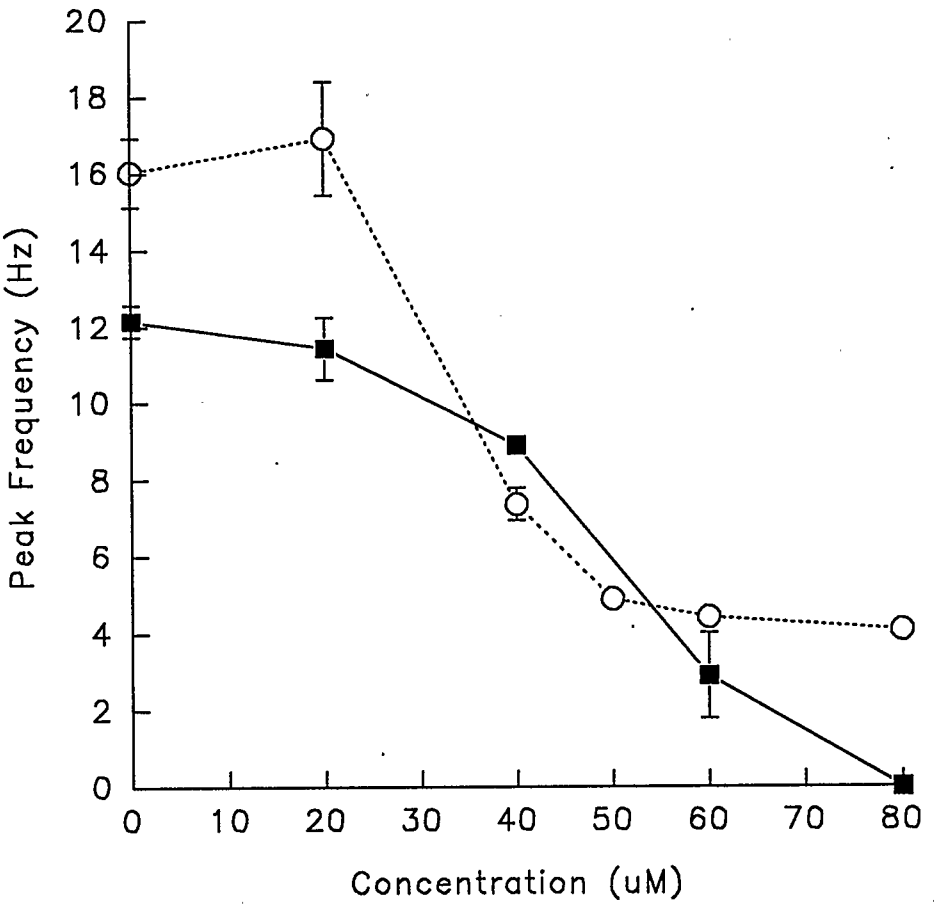
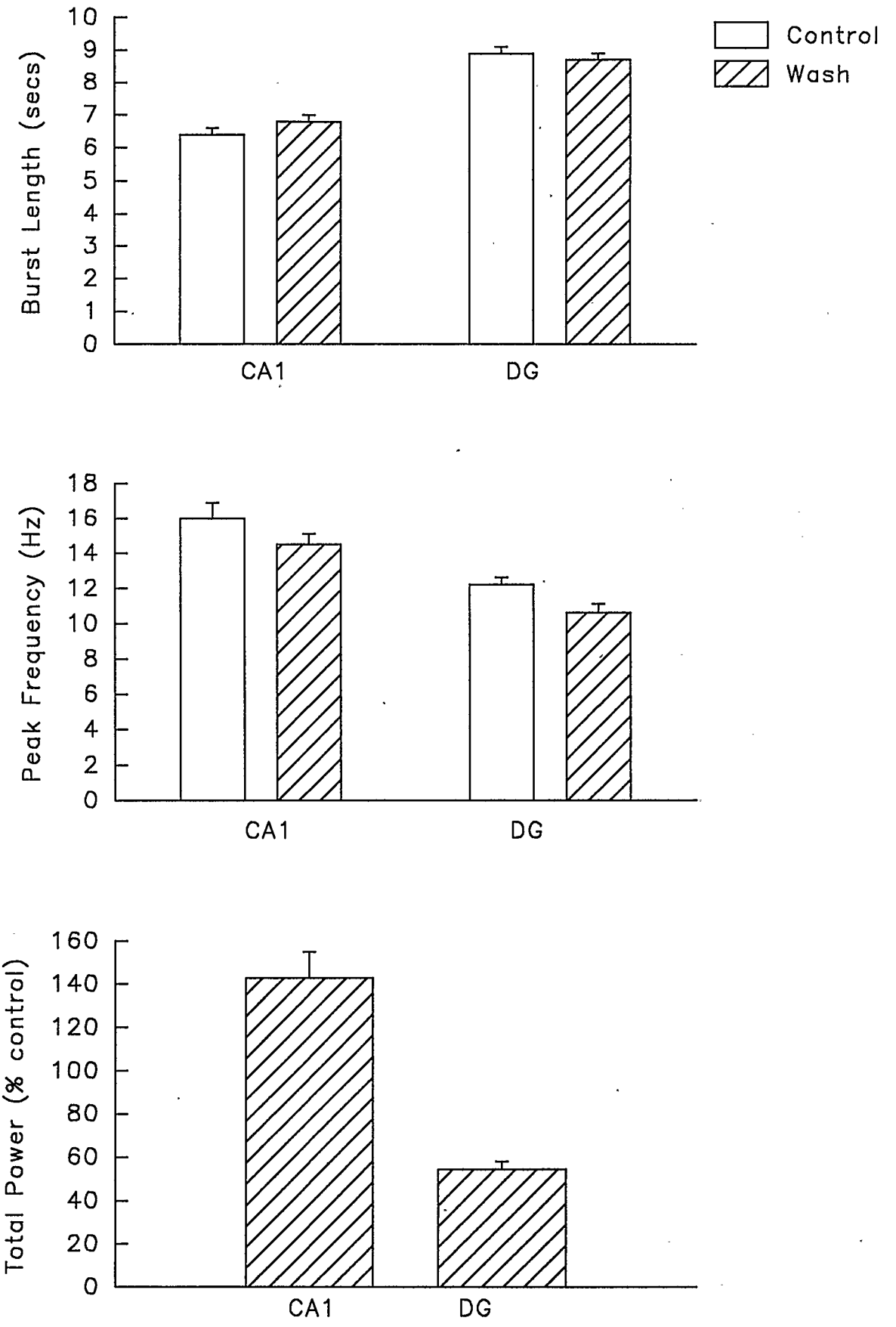

20
18
16
14
12
10
8
6
4
2
0
Peak Frequency (Hz)
0
10
20
30
40
50
60
70
80
Concentration (uM)

Figure 20. The average values of burst length, peak frequency and power in comparison to their respective controls after washout of pentobarbital for the CA1 and dentate neuronal populations. Each bar represents the mean (+/- S.E.M.) of at least 3 experiments. The differences between the controls and the values for the wash burst length and peak frequency are not significant.


Control
Wash
Burst Length (secs)
0
1
2
3
4
5
6
7
8
9
10
CA1
DG
Peak Frequency (Hz)
0
2
4
6
8
10
12
14
16
18
CA1
DG
Total Power (% control)
0
20
40
60
80
100
120
140
160
CA1
DG

54.4 ± 3.5% (n=3) (Figure 20).

3.4 Effects of Halothane on RPA

3.4.1 Total Power

A biphasic effect was observed on carbachol-induced RPA recorded from CA1 pyramidal neurons. At concentrations up to 0.4 vol% halothane increased the power of RPA to 154.3 ± 24.4% of control values. Concentrations higher than 0.4 vol% decreased power levels to below control values (Figure 21 and 22). In contrast, a dramatic concentration-dependent depression was observed on dentate RPA at concentrations as low as 0.2 vol%. At this concentration, halothane depressed RPA power to 42.0 ± 5% of control. Although at 1.0 vol% RPA power levels for both CA1 and dentate neurons were extremely low halothane did not abolish all activity. A concentration of 1.5 vol% (the next setting on the vaporizer), however, did abolish all activity.

3.4.2 Burst Length

Significant effects (i.e. $p<0.05$) on burst length were not observed until higher concentrations (0.8-1.0 vol%) were reached. At these concentrations halothane shortened RPA burst length of both CA1 and dentate neurons (Figures 21 and 23). A significant effect occurred at a concentration of 0.8 vol% for both CA1 ($n=4$; $p<0.0005$) and dentate neurons ($n=4$; $p<0.0005$). Although not statistically significant, a greater reduction appeared to occur in the dentate (4.4 ± 1.9 secs) as

Figure 21. Example recordings of RPA from CA1 and dentate neurons at different concentrations of halothane. Notice that the amplitude of CA1 RPA increased at a concentration of 0.4 vol% in contrast to the dentate which decreased in amplitude. In addition, there was a noticeable decrease in frequency of the activity for both neuronal populations. At 1.0 vol% the amplitude of RPA for both neuronal populations was depressed but the burst pattern remained intact.

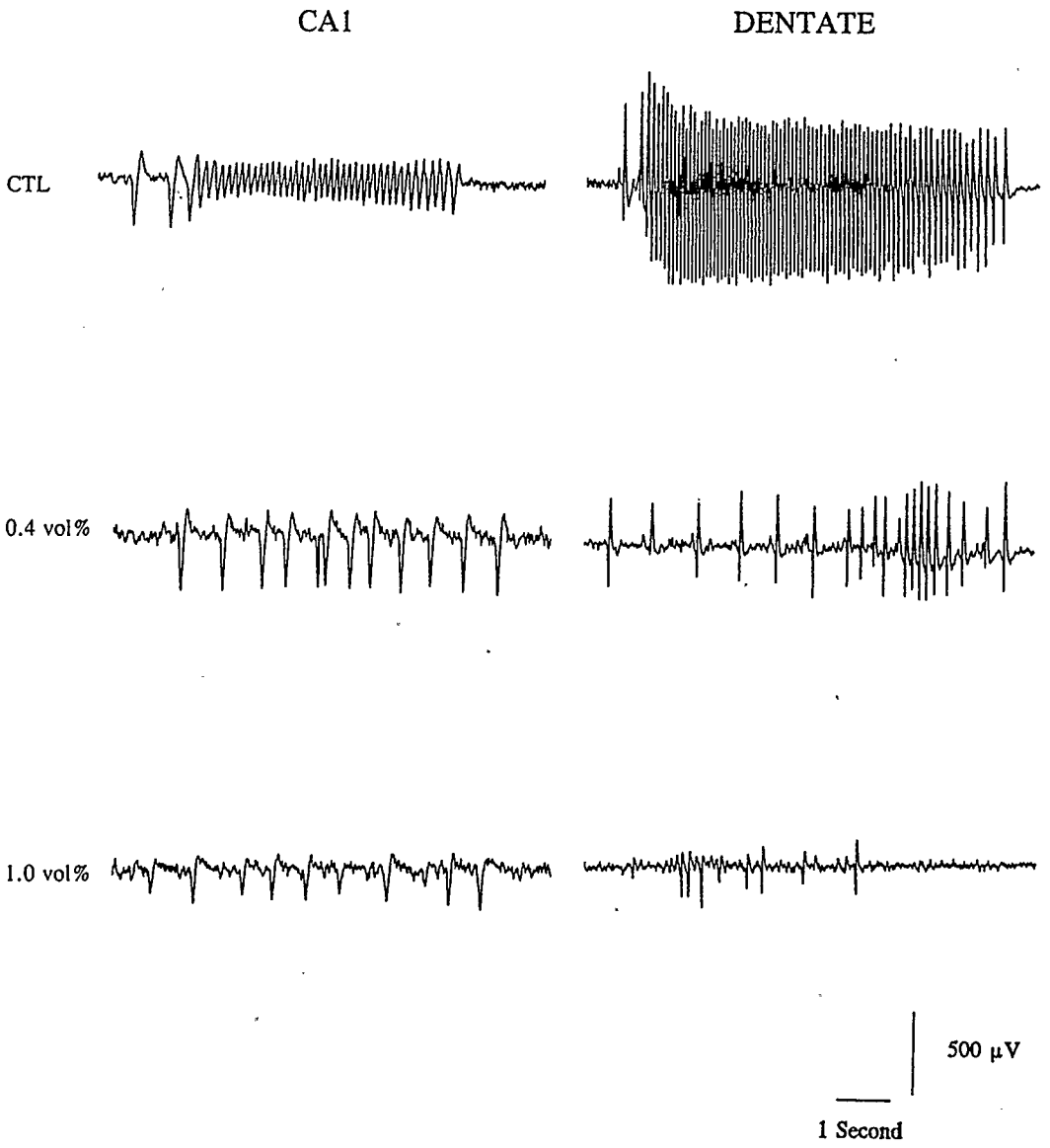

CA1
DENTATE
CTL
0.4 vol%
1.0 vol%
500 µV
1 Second

Figure 22. A graph summarizing the concentration-dependent effects of halothane on the power of RPA recorded from the CA1 (○) and dentate (■) neuronal populations. Each point represents the mean (+/- S.E.M.) of 5 - 7 experiments.

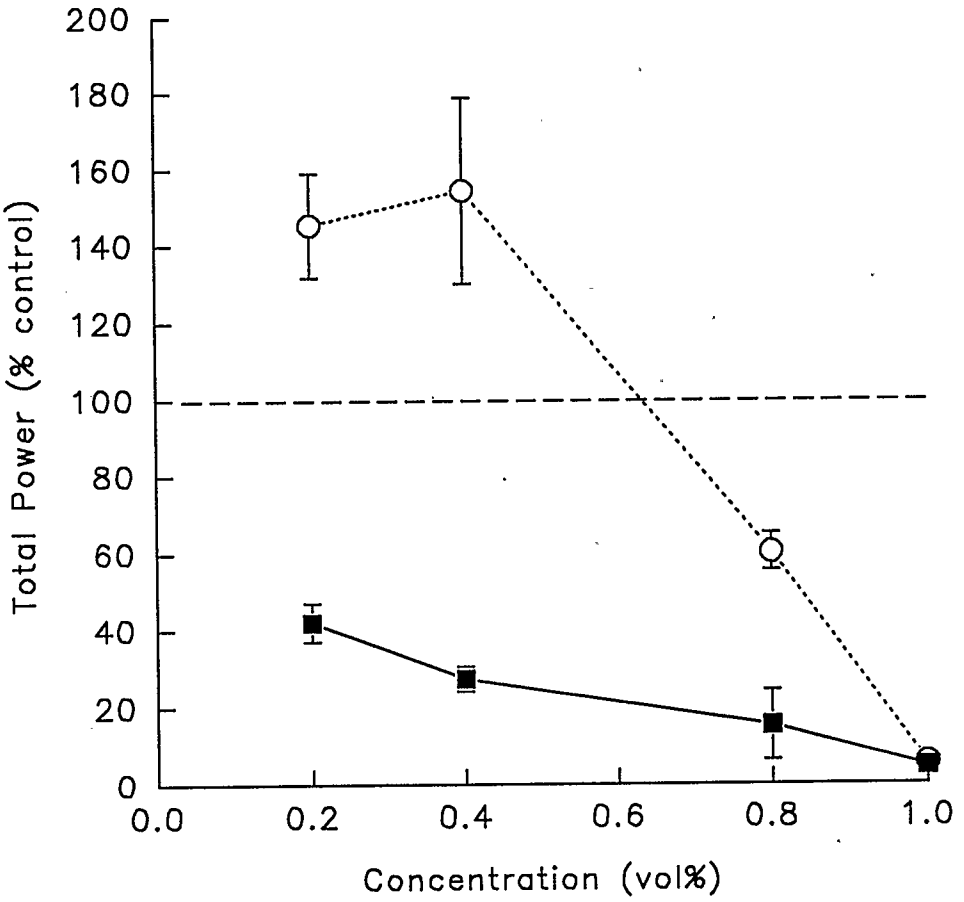
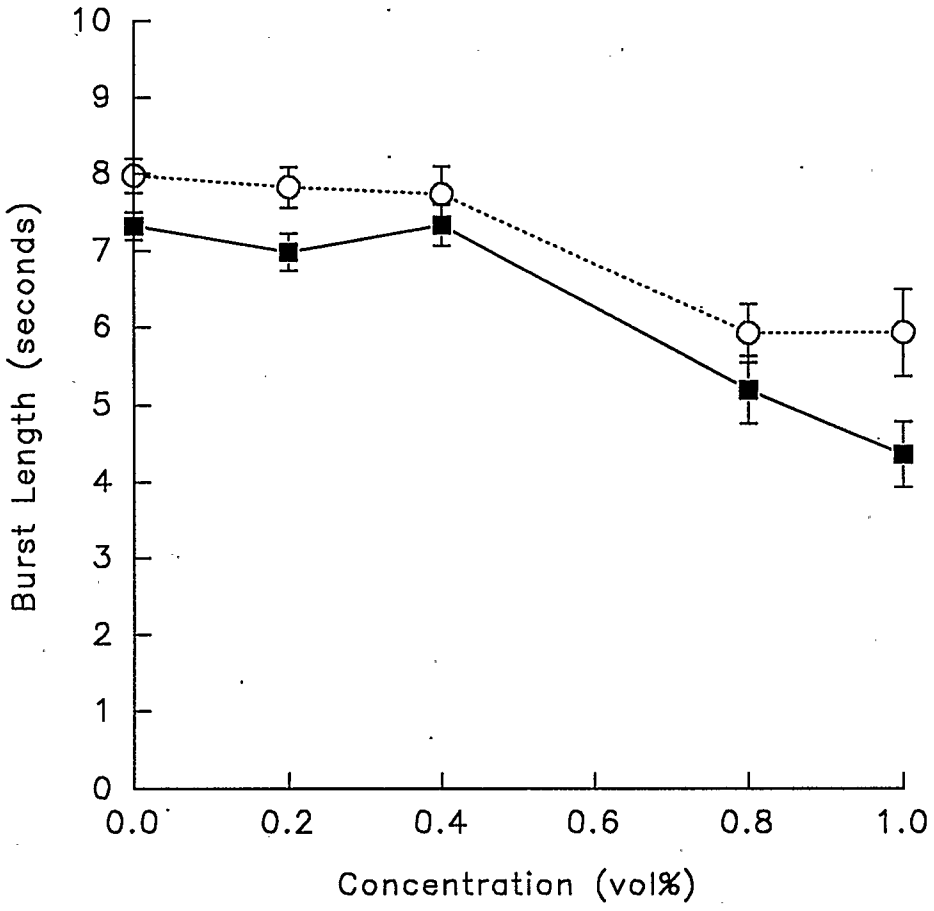

200
180
160
140
120
100
80
60
40
20
0
Total Power (% control)
0.0
0.2
0.4
0.6
0.8
1.0
Concentration (vol%)

Figure 23. A summary of the effects of halothane on the burst length of RPA recorded from the CA1 (○) and dentate (■) neuronal populations. Each point represents the mean (+/- S.E.M.) of 5 - 7 experiments.


Burst Length (seconds)
10
9
8
7
6
5
4
3
2
1
0
0.0
0.2
0.4
0.6
0.8
1.0
Concentration (vol%)

compared to the CA1 (5.9 ± 1.6 sec). Moreover, the greatest effect was achieved at a slightly lower concentration (0.8 vol%) in CA1 neurons than in dentate neurons (1.0 vol%). It was interesting to note that even at 1.0 vol% the burst/interburst pattern of the activity in both neuronal populations was not disrupted.

3.4.3 Peak Frequency

Halothane produced a concentration-dependent decrease of RPA mean peak frequency recorded from both CA1 and dentate neurons (Figures 24 and 25). This effect could be observed at concentrations as low as 0.2 vol% for both the CA1 ($n=5$; $p<0.025$) and the dentate ($n=6$; $p<0.0005$). Remarkably, this effect was virtually identical for CA1 and dentate neurons. It appears there was a minimum to which halothane could lower the peak frequency after which there was no further reduction. This occurred at a concentration of 0.8 vol% for both the CA1 and dentate with the minimum frequency being 5.0 ± 1.4 and 5.0 ± 0.6 Hz, respectively.

3.4.4 Reversibility/Irreversibility

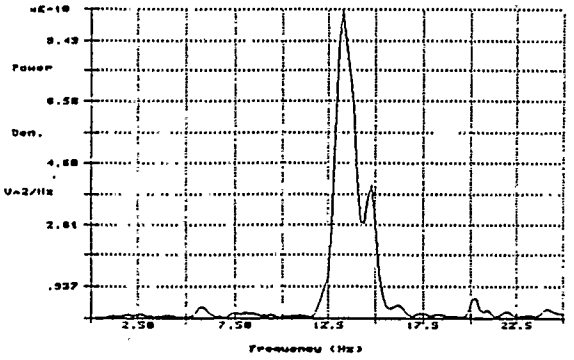
Anaesthetic effects could be reversed within 5 minutes of cessation of administration. The return of RPA burst length for dentate neurons was closer to control values than it was for CA1 neurons (Figure 26). For dentate RPA, there was a slight increase (7.3 ± 0.2 sec for the control; 7.7 ± 0.2 sec

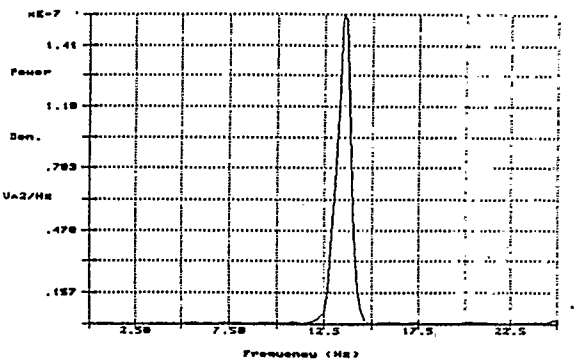
Figure 24. Example power spectra of RPA recorded from the CA1 and dentate at different concentrations of halothane. Notice that at 0.4 vol% the peak of the spectra shifts to the left (i.e. decrease) for both neuronal populations. In addition, the spectral power density of the peak frequency increases for the CA1, relative to control, whereas it decreases for the dentate (compare ordinates to control). At 1.0 vol%, there is a further shift to the left of the peak frequency and the spectral power density is depressed, relative to control, for both neuronal populations.

CA1

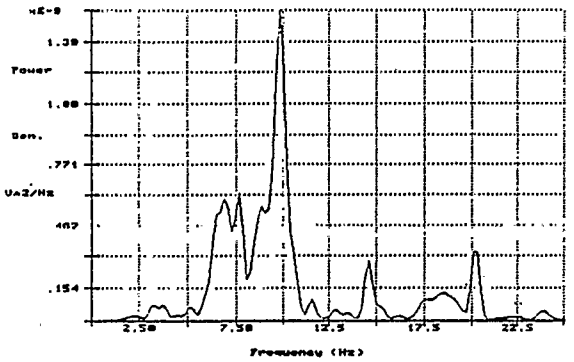
DENTATE

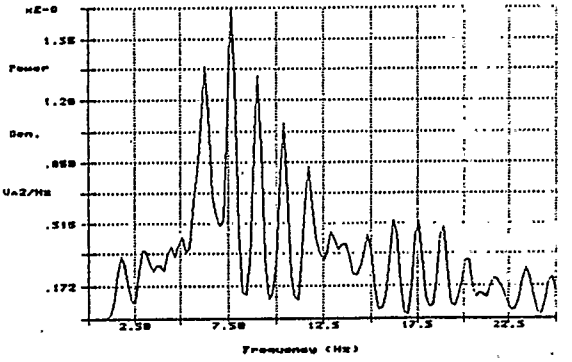
CTL



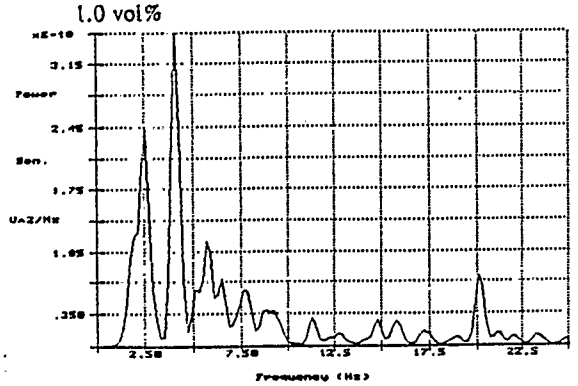


0.4 vol%





1.0 vol%



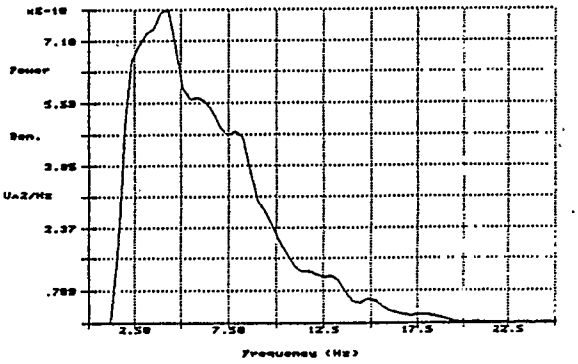


Figure 25. A graph summarizing the concentration-dependent effects of halothane on the peak frequency of RPA recorded from CA1 (○) and dentate (■) neurons. Each point represents the mean (+/- S.E.M.) of 5 - 7 experiments.

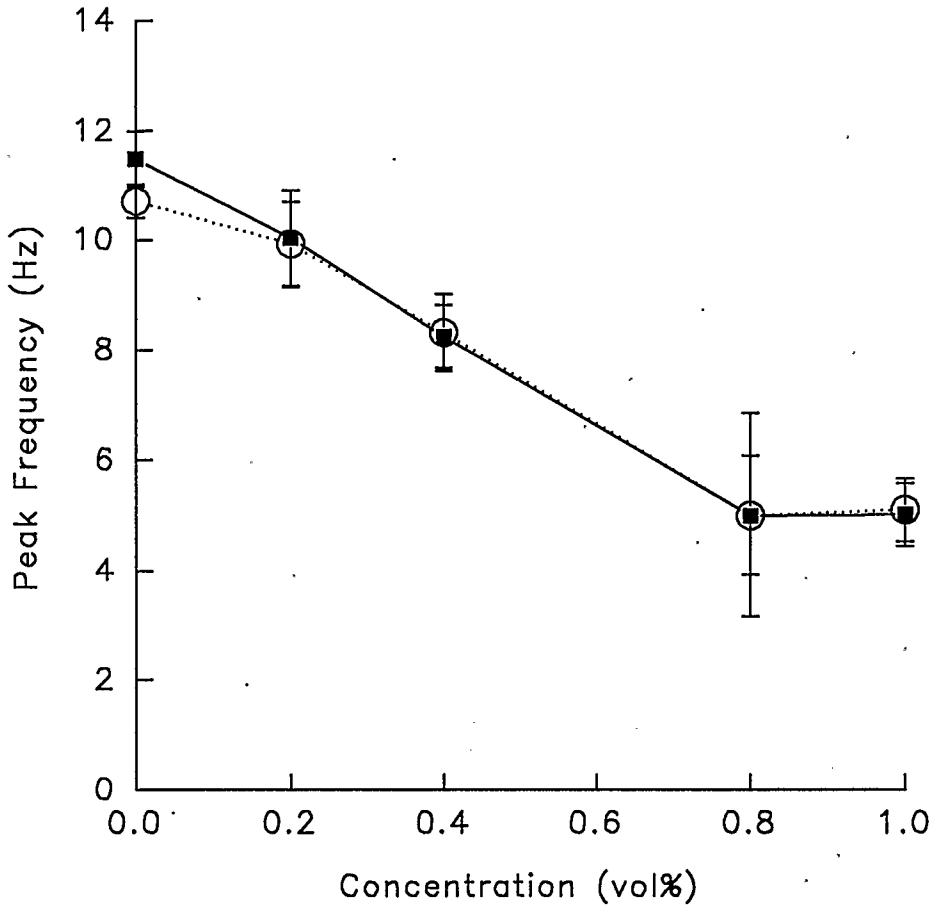
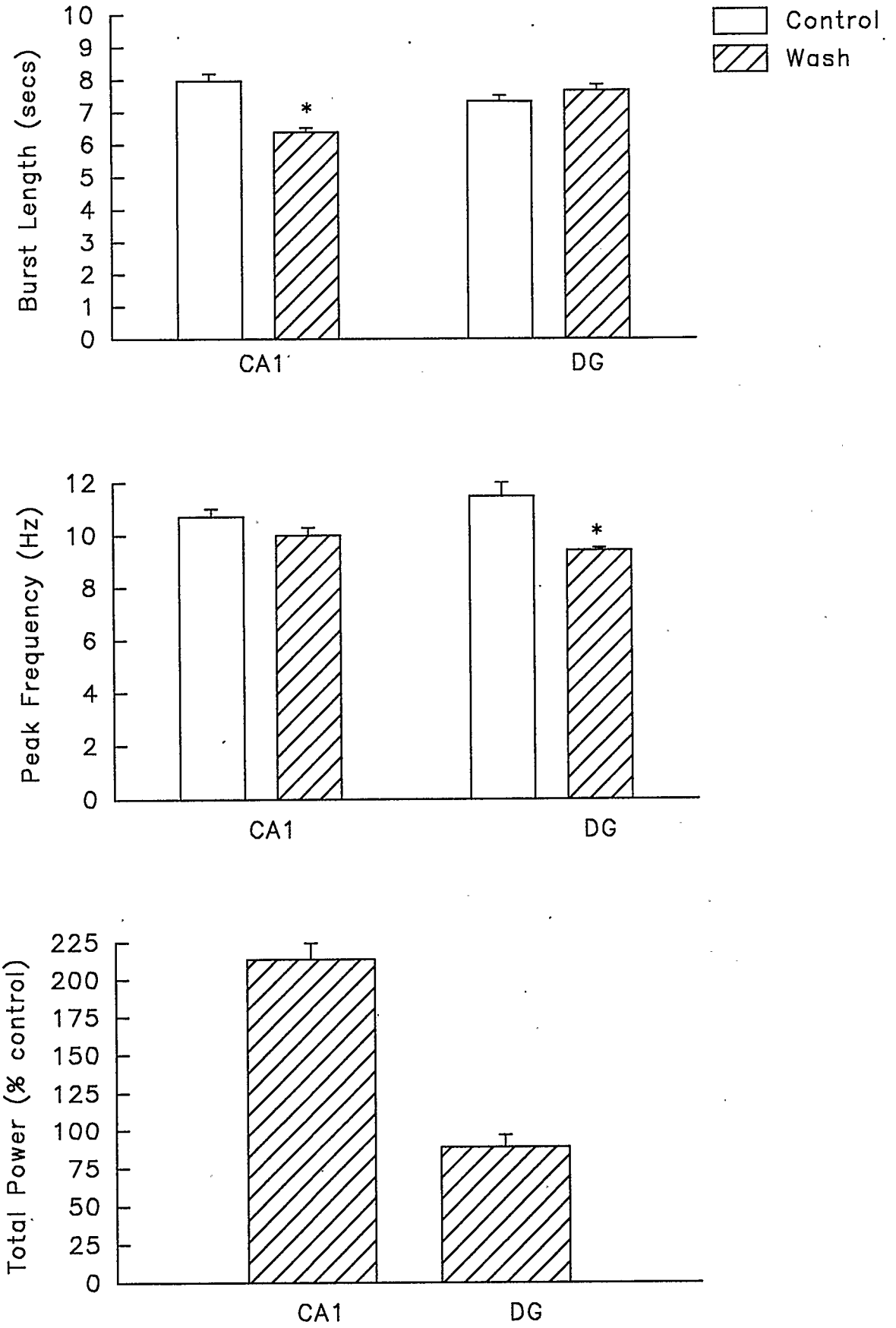

14
12
10
8
6
4
2
0
Peak Frequency (Hz)
0.0
0.2
0.4
0.6
0.8
1.0
Concentration (vol%)

Figure 26. The average values for burst length, peak frequency and power in comparison to their respective controls after washout of halothane. Each bar represents the mean (+/- S.E.M.) of 5 - 7 experiments and * refers to a significant difference ($p<0.05$) between the means of the control and wash.


Control
Wash
Burst Length (secs)
0
1
2
3
4
5
6
7
8
9
10
*
CA1
DG
Peak Frequency (Hz)
0
2
4
6
8
10
12
*
CA1
DG
Total Power (% control)
0
25
50
75
100
125
150
175
200
225
CA1
DG

for the wash) whereas the burst length of CA1 RPA (6.4 +/- 0.1 secs.) was shorter compared to the control (6.4 ± 0.1 sec and 8.0 ± 0.2 sec, respectively). Although the increase for dentate neurons was not significant (n=6; $p>0.05$), the difference between the wash and and the control for CA1 neurons was statistically significant (n=4; $p<0.0005$). The opposite was found with respect to the peak frequency (Figure 26). The RPA mean peak frequency for CA1 neurons returned to near control values (10.7 ± 0.3 Hz for the control; 10.0 ± 0.3 Hz for the wash) but the peak frequency of the wash for dentate neurons (9.4 ± 0.1 Hz) was significantly depressed relative to the control (11.5 ± 0.5 Hz; n=6; $p<0.05$). A large rebound effect was observed for CA1 neurons (n=4; 213.5 ± 10.7%) with respect to total power levels (Figure 26). For dentate neurons, power levels returned to near control values (n=6; 88.8 ± 8.2%).

3.5 Effects of Isoflurane on RPA

3.5.1 Total Power

Isoflurane depressed the total power of carbachol-induced RPA in both CA1 pyramidal and dentate granule neurons in a concentration-dependent manner (Figures 27 and 28). This effect could be observed at the lowest concentration studied (0.5 vol%). However, dentate granule neurons were more sensitive to this depression since total power values were reduced to a greater degree (n=6; $p<0.0005$). For example, for

Figure 27. Example recordings of RPA from CA1 and dentate neurons at different concentrations of isoflurane. A decrease in amplitude and frequency of the activity, relative to control, can be observed for both neuronal populations at 1.0 and 2.0 vol%. The burst pattern remained unaltered even at a concentration of 2.0 vol%.

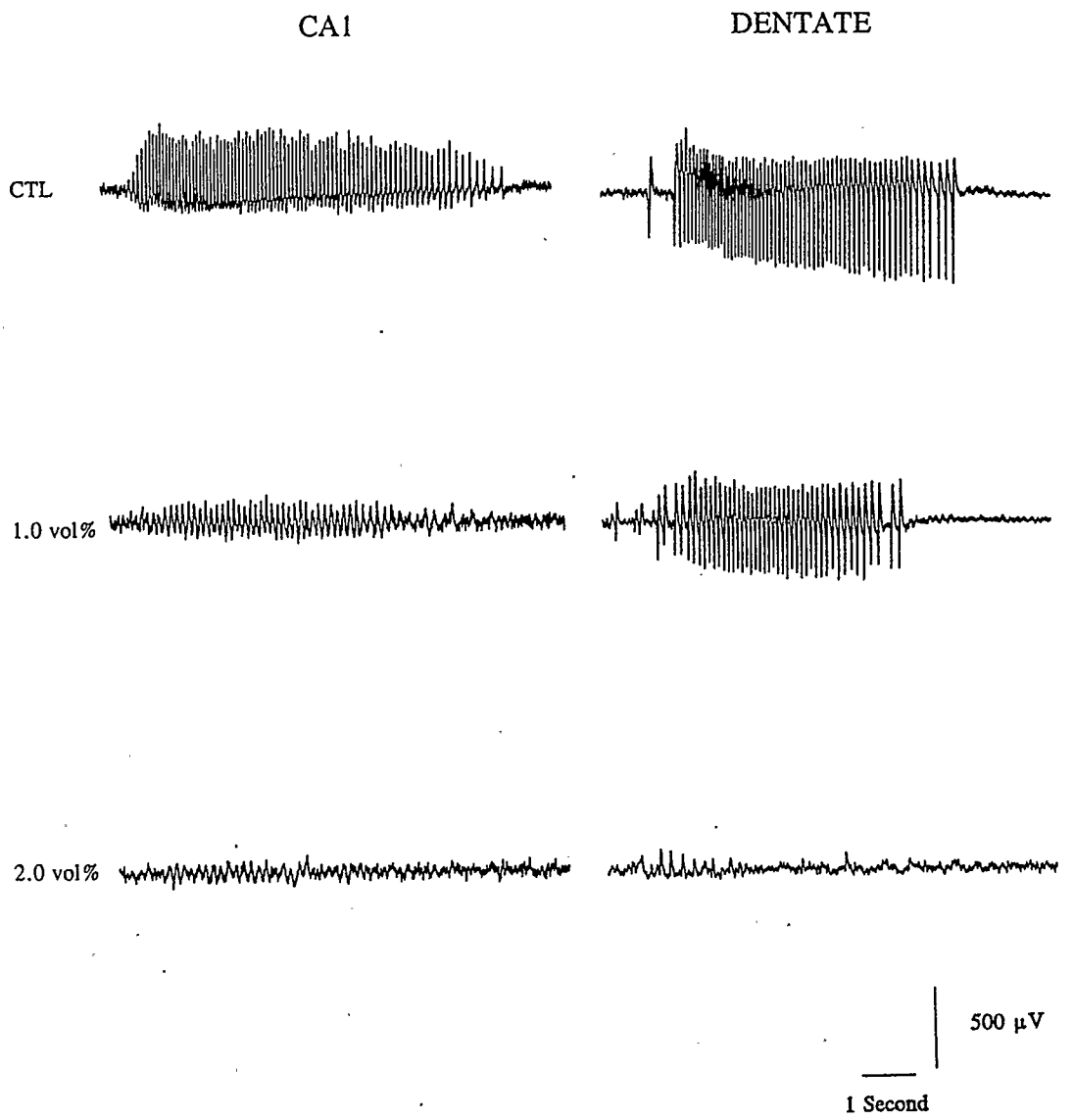
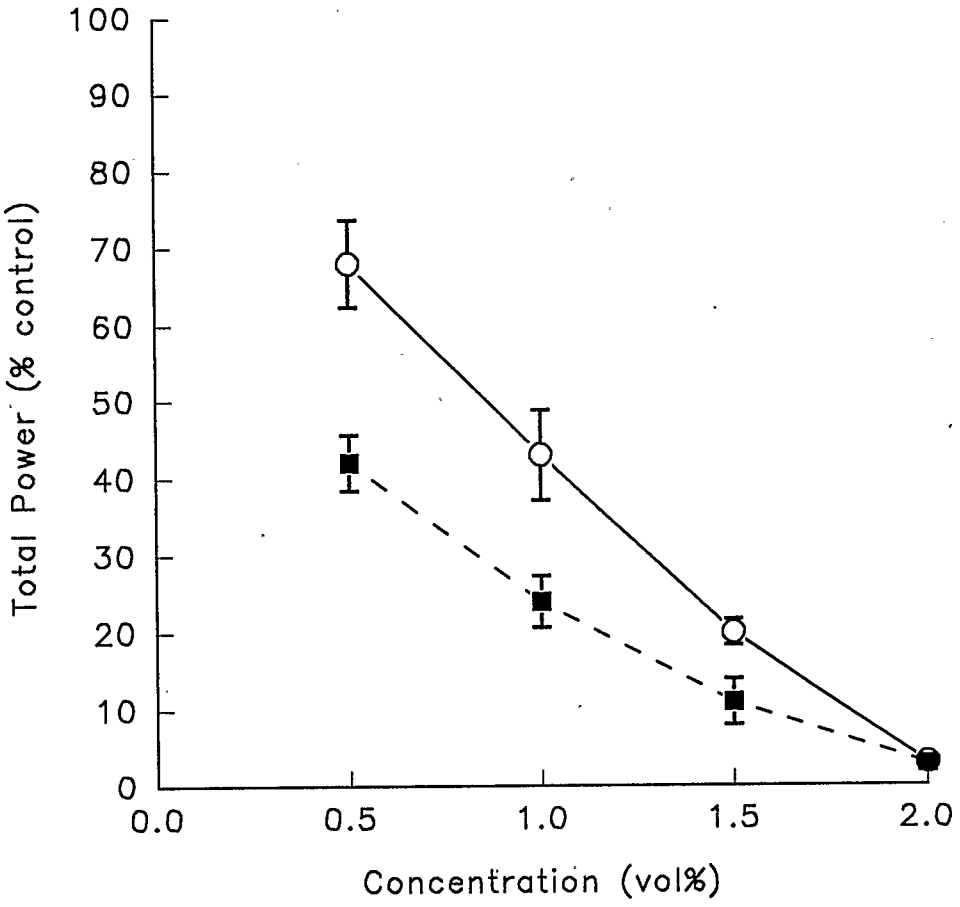

CA1
DENTATE
CTL
1.0 vol%
2.0 vol%
500 µV
1 Second

Figure 28. A graph summarizing the concentration-dependent effects of isoflurane on the power of RPA recorded from CA1 (O) and dentate (■) neurons. Each point represents the mean (+/- S.E.M.) of 5 - 7 experiments.

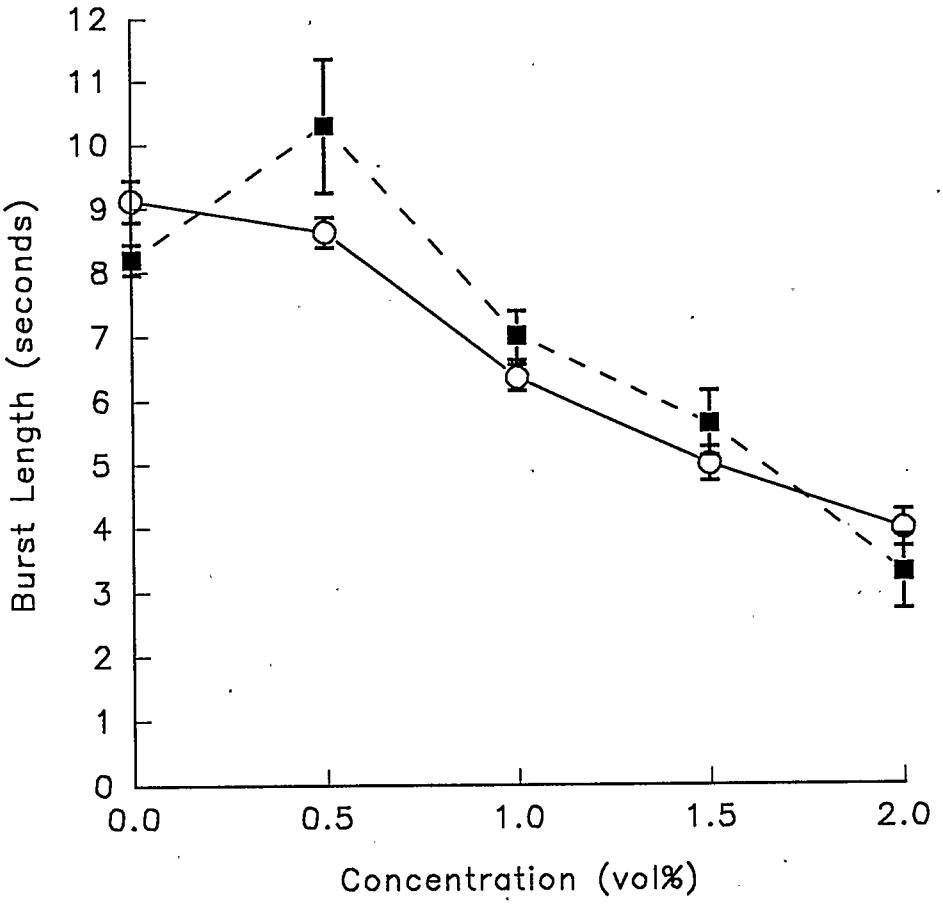

100
90
80
70
60
50
40
30
20
10
0
Total Power (% control)
0.0
0.5
1.0
1.5
2.0
Concentration (vol%)

CA1 neurons at 0.5 vol%, total power levels were 68 ± 5.6% of control whereas they were 42 ± 3.6% for dentate neurons. At 2.0 vol% activity was still present but at very low power levels. Higher concentrations (i.e. 2.5 vol%) abolished all activity.

3.5.2 Burst Length

RPA burst length was also altered by isoflurane. A biphasic effect was observed when recording from dentate neurons (Figures 27 and 29). At 0.5 vol% the length increased from 8.2 ± 0.2 sec to 10.3 ± 1.1 sec, however, this effect was not statistically significant ($p>0.05$) although the trend was evident in 3/6 experiments. In other experiments, 0.5 vol% had no effect. At concentrations of 1.0 vol% and higher isoflurane shortened the length of the activity in a concentration-dependent manner. In contrast, a prolonging of RPA burst length of CA1 neurons was not observed. Rather, isoflurane shortened the activity in a concentration-dependent manner at all concentrations studied (Figures 27 and 29). This effect became statistically significant ($p<0.0005$) at 1.0 vol%. For both neuronal populations, the maximum effect appeared to be reached at 2.0 vol%. It was interesting to note that, unlike pentobarbital, the burst/interburst pattern of the activity was not disrupted even at the highest concentration studied for both neuronal populations.

Figure 29. A summary of the concentration-dependent effects of isoflurane on the burst length of RPA recorded from CA1 (O) and dentate (■) neurons. Each point represents the mean (+/- S.E.M.) of 5 - 7 experiments.


Burst Length (seconds)
12
11
10
9
8
7
6
5
4
3
2
1
0
0.0
0.5
1.0
1.5
2.0
Concentration (vol%)

3.5.3 Peak Frequency

Both CA1 and dentate neurons responded similarily to isoflurane. As with halothane, there was a concentration-dependent decrease in the mean peak frequency (Figures 30 and 31). Although this effect could be observed at the lowest concentration studied it did not achieve statistical significance for dentate neurons until 1.0 vol% ($n=6$; $p<0.0005$) in comparison to 0.5 vol% ($n=6$; $p<0.0005$) for CA1 neurons. Maximal effects were attained at 2.0 vol% for both neuronal populations.

3.5.4 Reversibility/Irreversibility

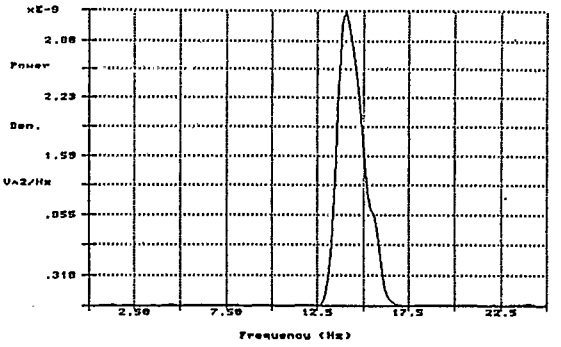
The burst length of carbachol-induced activity returned to near control values for CA1 neurons within 5 minutes following cessation of anaesthetic administration (9.1 ± 0.3 sec for control compared to 8.5 ± 0.3 sec upon washout) (Figure 32). For dentate neurons, a rebound effect was observed, the length was longer upon washout (10.6 ± 0.5 sec) than the control value (8.2 ± 0.2 sec). In addition, a slight rebound effect was also observed for dentate neurons with respect to the peak frequency, although this was not significant (13.0 ± 0.5 Hz for the control; 14.3 ± 1.5 Hz for the wash; $n=3$; $p>0.05$). The peak frequency of CA1 RPA also returned to near control values although the frequency of the wash (14.0 ± 0.8 Hz) was 1.6 Hz lower than the control (15.6 ± 0.5 Hz) (Figure 32). Unlike halothane, RPA power for both

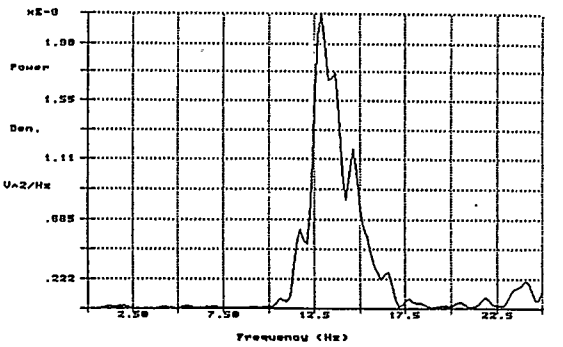
Figure 30. Example power spectra of RPA recorded from the CA1 and dentate neuronal populations at different concentrations of isoflurane. Note that the peak of the spectra shifts to the left (i.e. decrease) for both neuronal populations, relative to control, at both 1.0 and 2.0 vol%. In addition, the spectral power density of the peak is depressed relative to the control for both neuronal populations at both concentrations shown (compare ordinates to control).

CA1

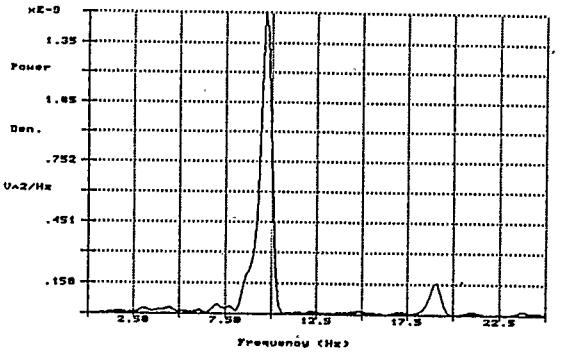
DENTATE

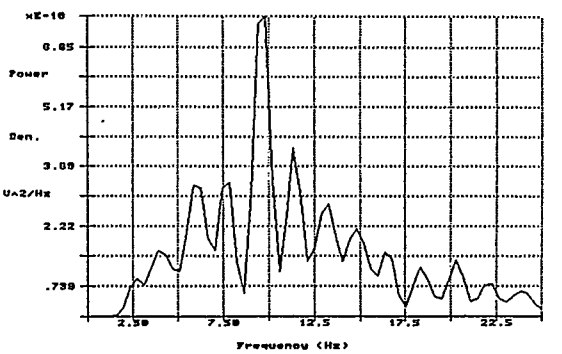
CTL



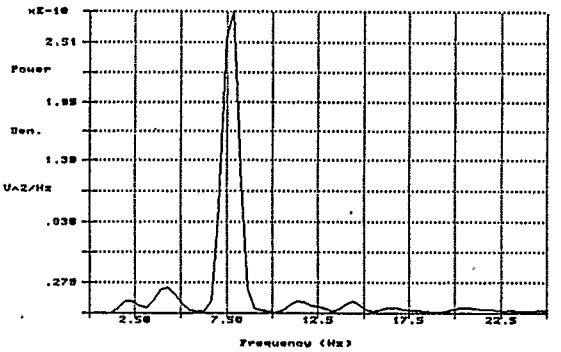


1.0 vol%





2.0 vol%



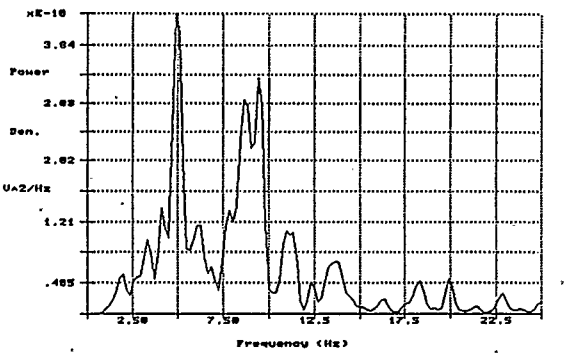


Figure 31. A graph summarizing the concentration-dependent effects of isoflurane on the peak frequency of RPA recorded from the CA1 (O) and dentate (■) neuronal populations. Each point represents the mean (+/- S.E.M.) of 5 - 7 experiments.

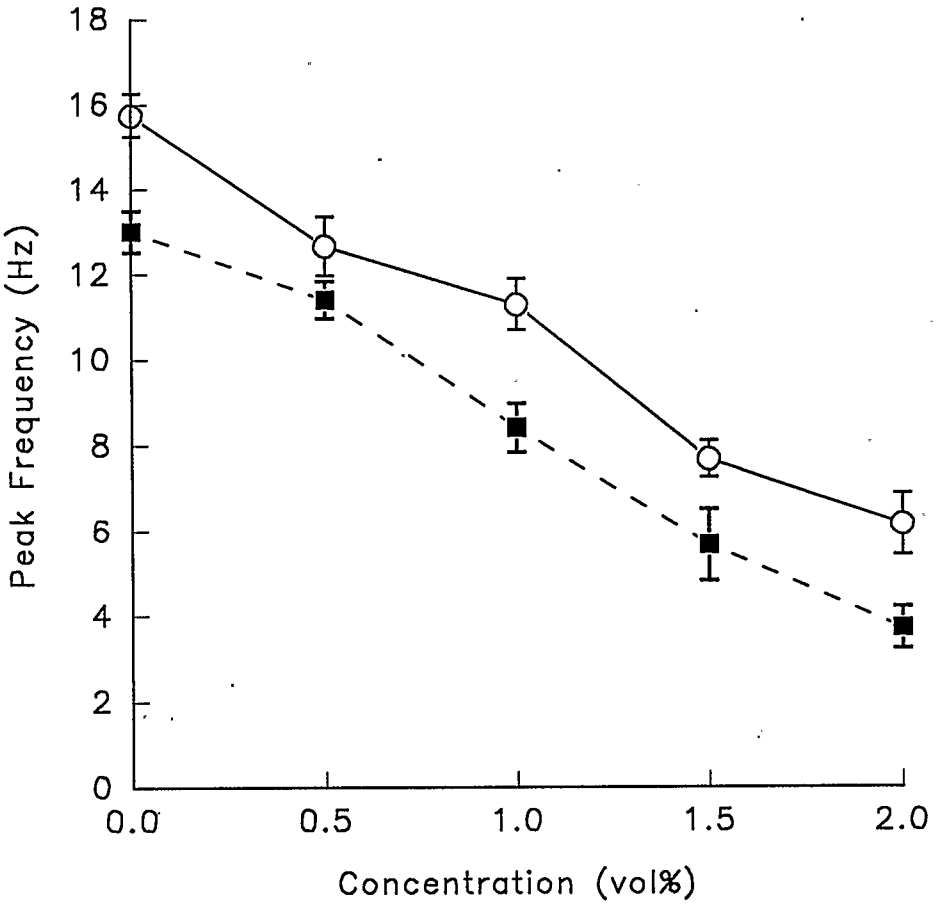
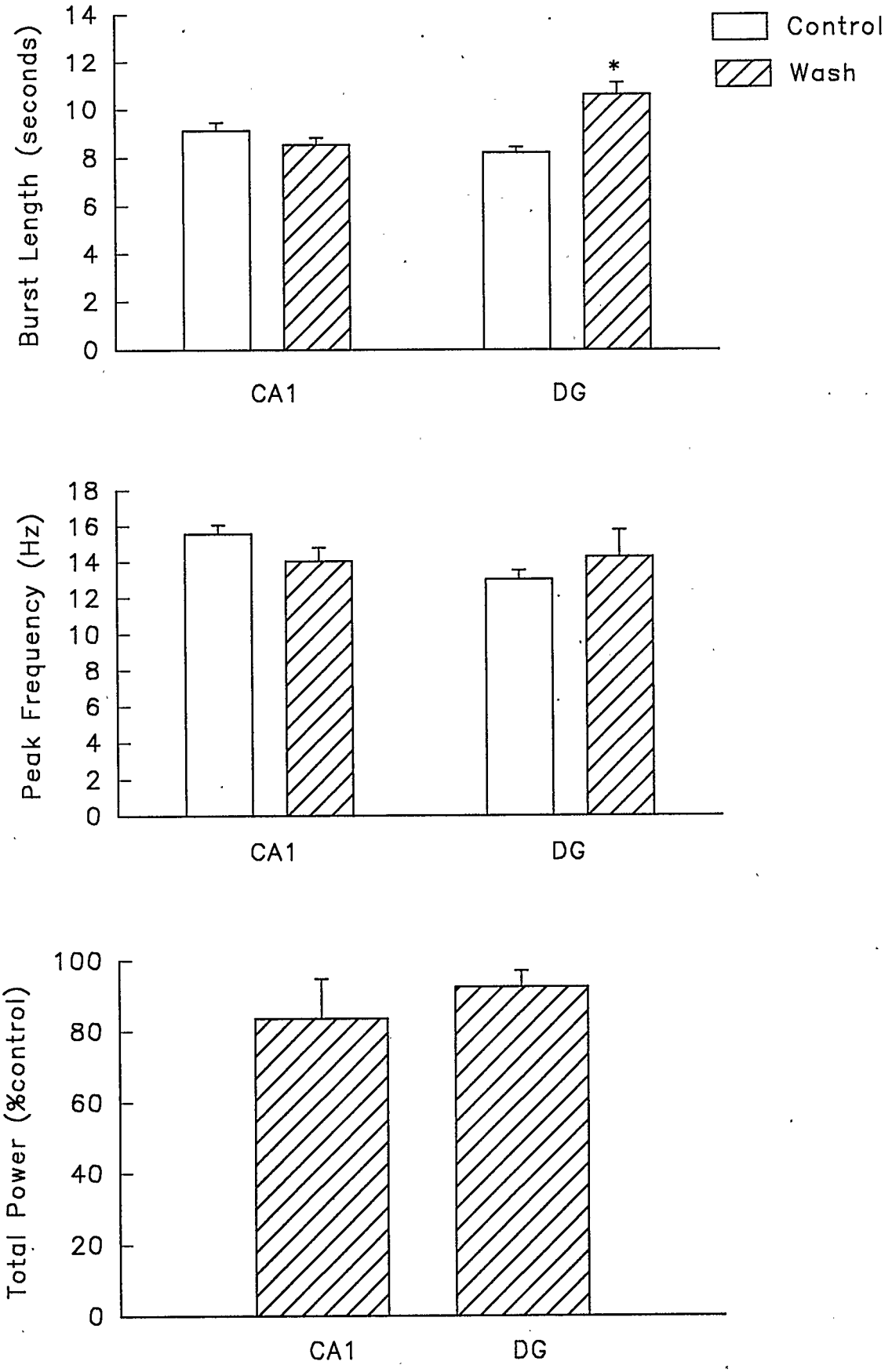

18
16
14
12
10
8
6
4
2
0
Peak Frequency (Hz)
0.0
0.5
1.0
1.5
2.0
Concentration (vol%)

Figure 32. The average values for burst length, peak frequency and power relative to their respective controls after washout of isoflurane. Each bar represents the mean (+/- S.E.M.) of 5 - 7 experiments and * refers to a significant difference ($p<0.05$) between the means of the wash and control.


Control
Wash
Burst Length (seconds)
14
12
10
8
6
4
2
0
*
CA1
DG
Peak Frequency (Hz)
18
16
14
12
10
8
6
4
2
0
CA1
DG
Total Power (%control)
100
80
60
40
20
0
CA1
DG

CA1 and dentate neurons returned to approximately control levels (83.6 ± 11.2% and 92.5 ± 4.5%, respectively) (Figure 32).

3.6 Effects of Enflurane on RPA

3.6.1 Total Power

As with halothane and pentobarbital, enflurane produced two distinct and opposing actions on CA1 as compared to dentate neurons. Low concentrations (0.5-1.0 vol%) increased RPA power of CA1 neurons to as high as 167.4 ± 23.4% of control (Figures 33 and 34). Higher concentrations (1.5-2.0 vol%) depressed the activity. This biphasic effect was not observed on dentate neurons, rather, the total power was depressed at all concentrations studied. This depressant effect was quite pronounced even at concentrations as low as 0.5 vol% (31.1 ± 4.6% of control). The difference between CA1 and dentate RPA was statistically significant ($n=5$; $p<0.0005$). Maximal depressant effects were reached at 1.5 vol% for dentate neurons (2.4 ± 0.4% of control) and 2.0 vol% for CA1 neurons (25.6 ± 3.3% of control). Despite the extremely low power levels in the dentate at these higher concentrations, activity was still present. Concentrations higher than this (i.e. 2.5 vol%) appeared to abolish all activity in both neuronal populations.

Figure 33. Example recordings of RPA recorded from the CA1 and dentate neuronal populations at different concentrations of enflurane. Notice that the amplitude of the activity increased in the CA1, relative to control, at a concentration of 0.5 vol% while it decreased in the dentate. In addition, frequency changes are also evident at this concentration for both neuronal populations. At 2.0 vol%, the amplitude and frequency of the activity is depressed in both neuronal populations. At this concentration enflurane occassionally disrupted the burst pattern of RPA. The CA1 record demonstrates this effect while the dentate recording represents those experiments in which the burst pattern was intact. Disruption of the burst pattern was not confined to the CA1.

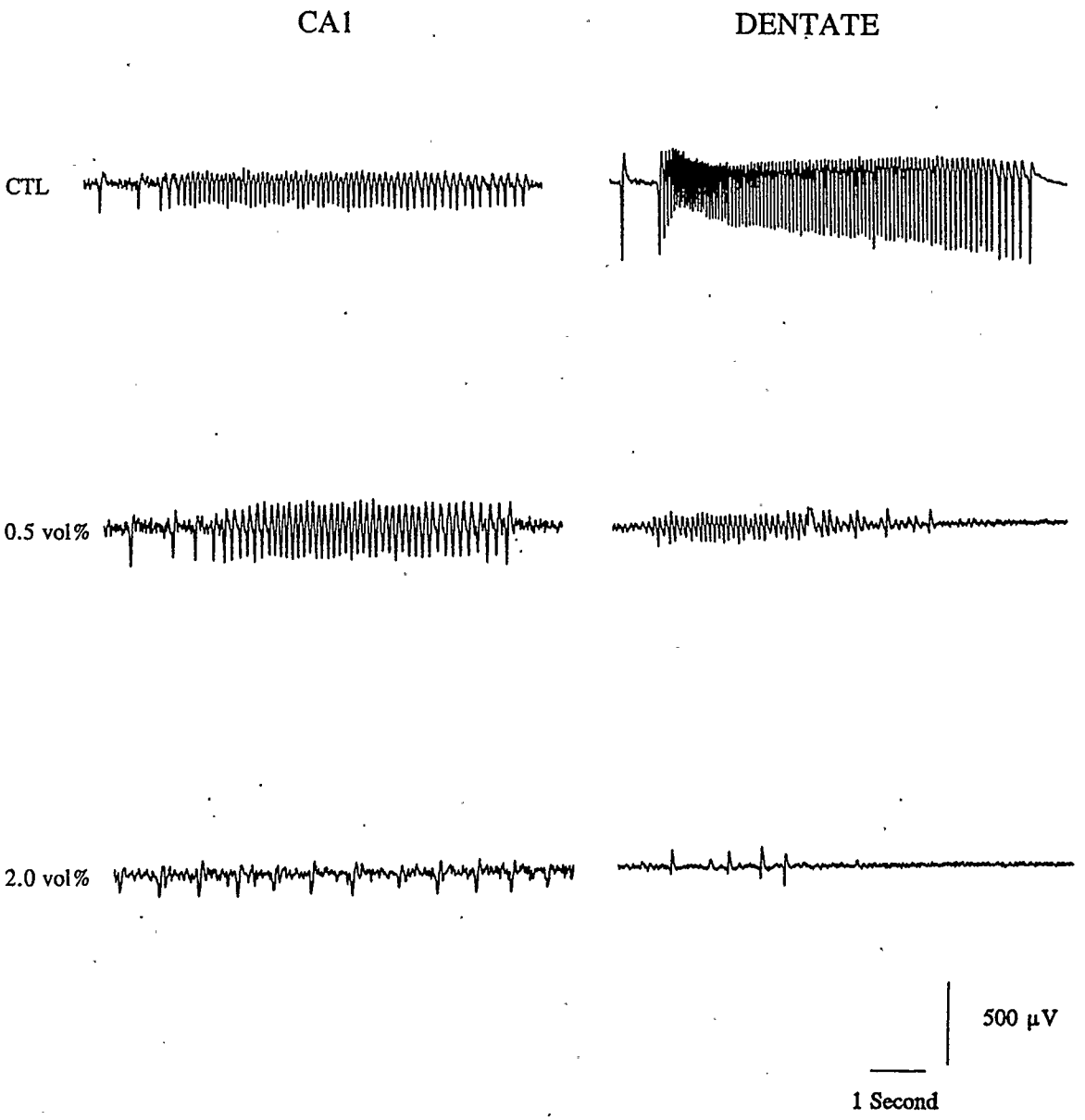
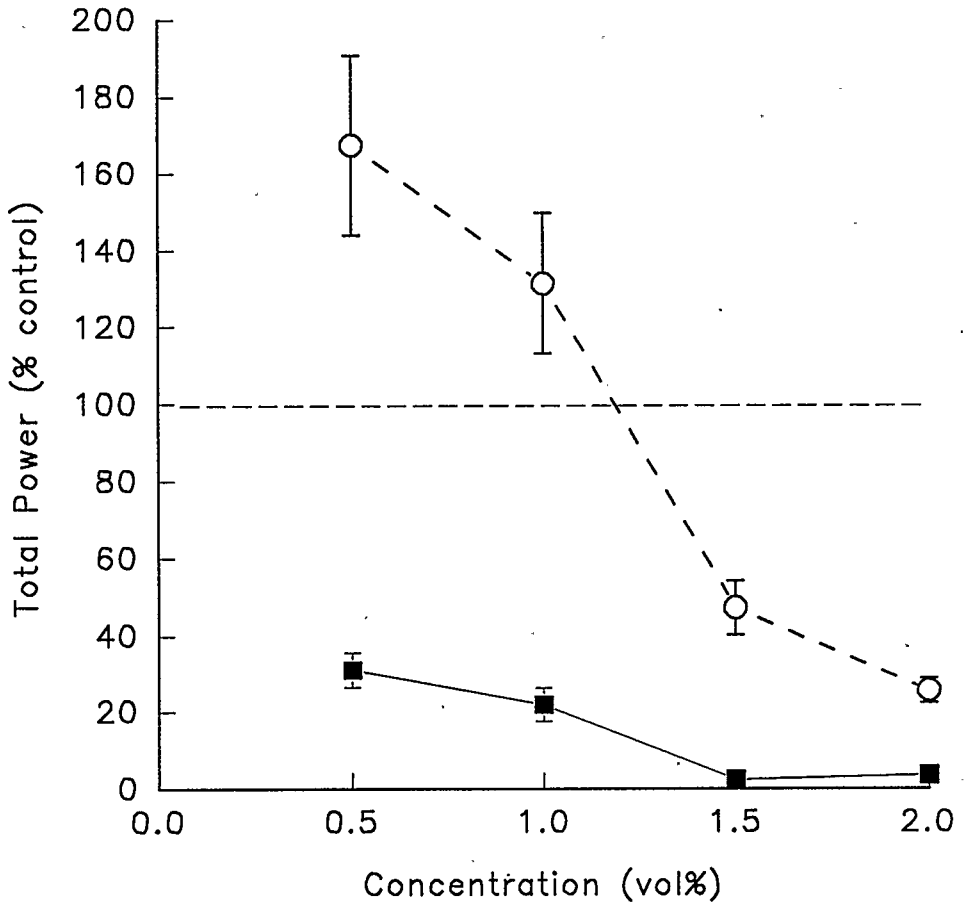

CA1
DENTATE
CTL
0.5 vol%
2.0 vol%
500 µV
1 Second

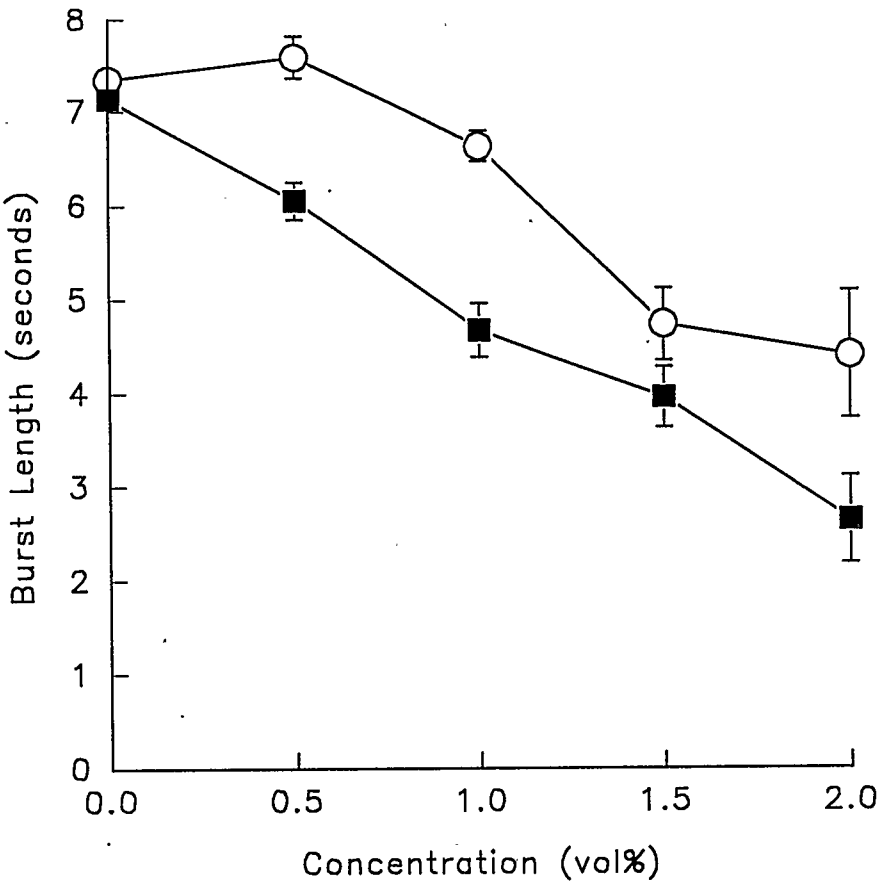
Figure 34. A graph summarizing the concentration-dependent effects of enflurane on the power of RPA recorded from CA1 (O) and dentate (■) neurons. Each point represents the mean (+/- S.E.M.) of 5 - 7 experiments.


200
180
160
140
120
100
80
60
40
20
0
Total Power (% control)
0.0
0.5
1.0
1.5
2.0
Concentration (vol%)

3.6.2 Burst Length

The overall effect, a reduction in RPA burst length, was similar in both neuronal populations, however, there were subtle differences (Figures 33 and 35). Dentate neurons appeared to be much more sensitive than CA1 neurons. At a concentration of 0.5 vol%, RPA burst length of CA1 pyramidal neurons was slightly increased. Although this increase was not statistically significant, the trend was evident in 3/5 experiments. In contrast, there was a significant decrease observed for dentate neurons ($n=6$; $p<0.0005$). At 1.0 vol%, there was a further decrease for dentate neurons which represented a 2.5 second reduction from control recordings whereas for CA1 neurons there was only a 0.7 second decrease. Maximal effects appeared to be reached on CA1 RPA at 1.5 vol% and for dentate RPA at 2.0 vol% with the burst length reaching a lower value at this concentration for dentate neurons. It must be pointed out that for many segments of RPA at concentrations of 1.5-2.0 vol% it was difficult to define a burst. That is, the burst/interburst pattern was obliterated such that there was no quiescent interburst periods. Consequently, only those epochs in which the pattern was not disrupted were used in the analysis. This was similar to the effects of pentobarbital at concentrations of 40 μM and higher.

Figure 35. A summary of the concentration-dependent effects of enflurane on the burst length of RPA recorded from CA1 (O) and dentate (■) neurons. Each point represents the mean (+/- S.E.M.) of 5 - 7 experiments.


Burst Length (seconds)
0
1
2
3
4
5
6
7
8
0.0
0.5
1.0
1.5
2.0
Concentration (vol%)

3.6.3 Peak Frequency

The effects of enflurane on RPA peak frequency were similar to those effects observed with the other volatile anaesthetics. For both CA1 and dentate neurons there was a concentration-dependent decrease in the mean peak frequency (Figures 36 and 37). Dentate neurons appeared to be more sensitive since a significant effect occurred at 0.5 vol% ($n=6$; $p<0.0005$) whereas for CA1 neurons it did not occur until 1.0 vol% ($n=5$; $p<0.0005$). The maximum effect for both neuronal populations appeared to be reached at a concentration of 1.5 vol% with the CA1 attaining the lowest frequencies.

3.6.4 Reversibility/Irreversibility

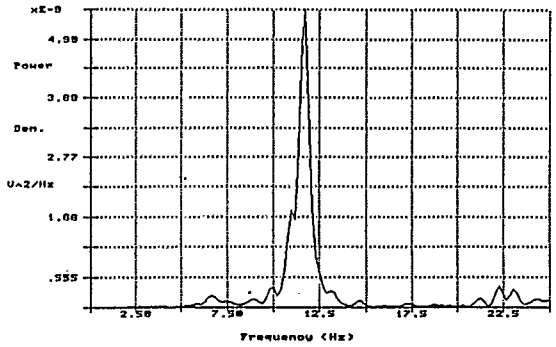
RPA burst length for both CA1 and dentate neurons returned to near contol values (Figure 38). For CA1 neurons, however, the burst length of the wash (8.0 ± 0.3 sec) was slightly longer than the control (7.3 ± 0.1 sec; $n=4$; $p<0.025$). This rebound effect also occurred for the total power of the activity which was 251 +/- 64% of control ($n=4$) (Figure 38). In contrast, the return in the total power for dentate neurons was less than any other volatile anaesthetic. The activity returned to only 65.2 +/- 8.4% of control values (Figure 38). But, the return in the peak frequency was closer to control values for dentate RPA than it was for CA1 RPA. The frequency of the wash of the CA1 activity (9.3 ± 0.4 Hz) was 2.5 Hz lower than the control (11.8 ± 0.3 Hz) whereas the

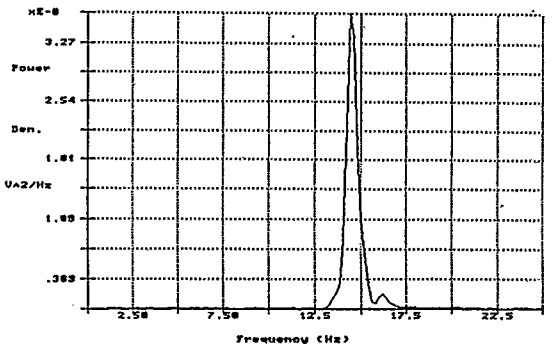
Figure 36. Example power spectra of RPA recorded from CA1 and dentate neurons at different concentrations of enflurane. The peak of the spectra shifts to the left (i.e. decrease) for both neuronal populations at 0.5 and 2.0 vol%. Notice that the spectral power density of the peak of the spectra is increased, relative to control, for the CA1 whereas it is depressed in the dentate (compare ordinates to control). At 2.0 vol% the spectral power density of the peak is depressed for both neuronal populations.

CA1

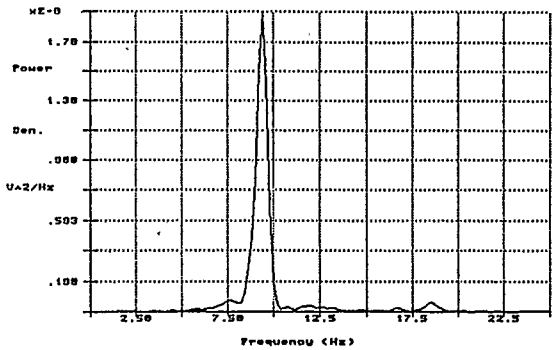
DENTATE

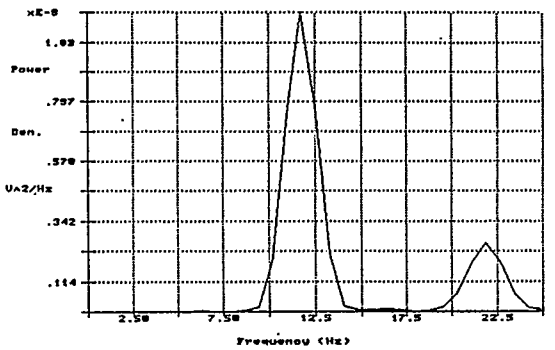
CTL



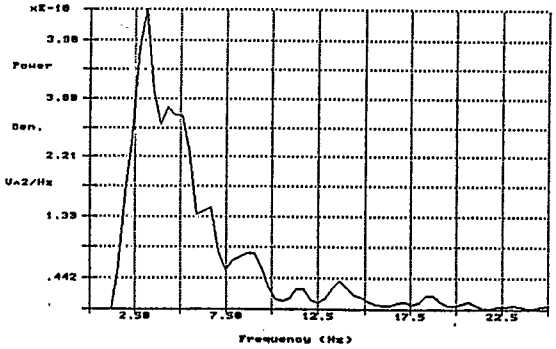


0.5 vol%





2.0 vol%



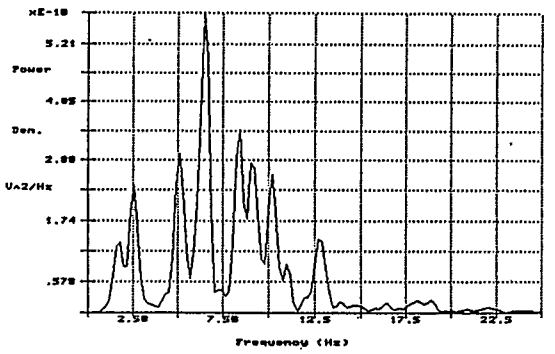


Figure 37. A graph summarizing the concentration-dependent effects of enflurane on the peak frequency of RPA recorded from the CA1 (O) and dentate (■) neuronal populations. Each point represents the mean (+/- S.E.M.) of at 5 - 7 experiments.

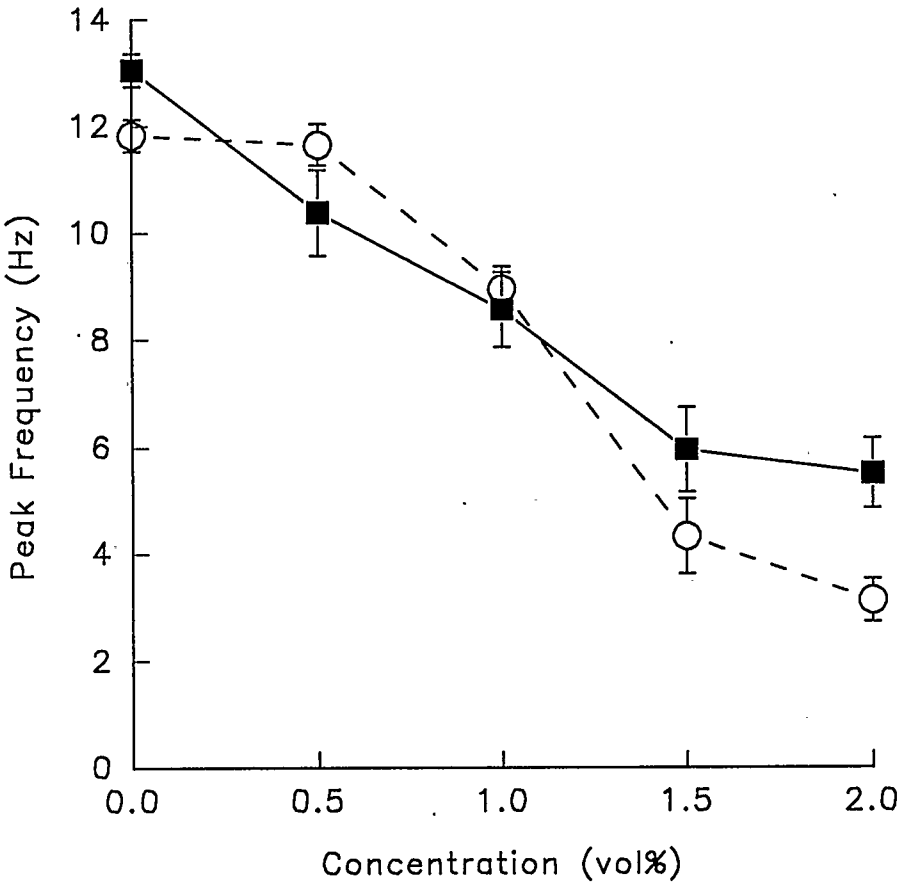
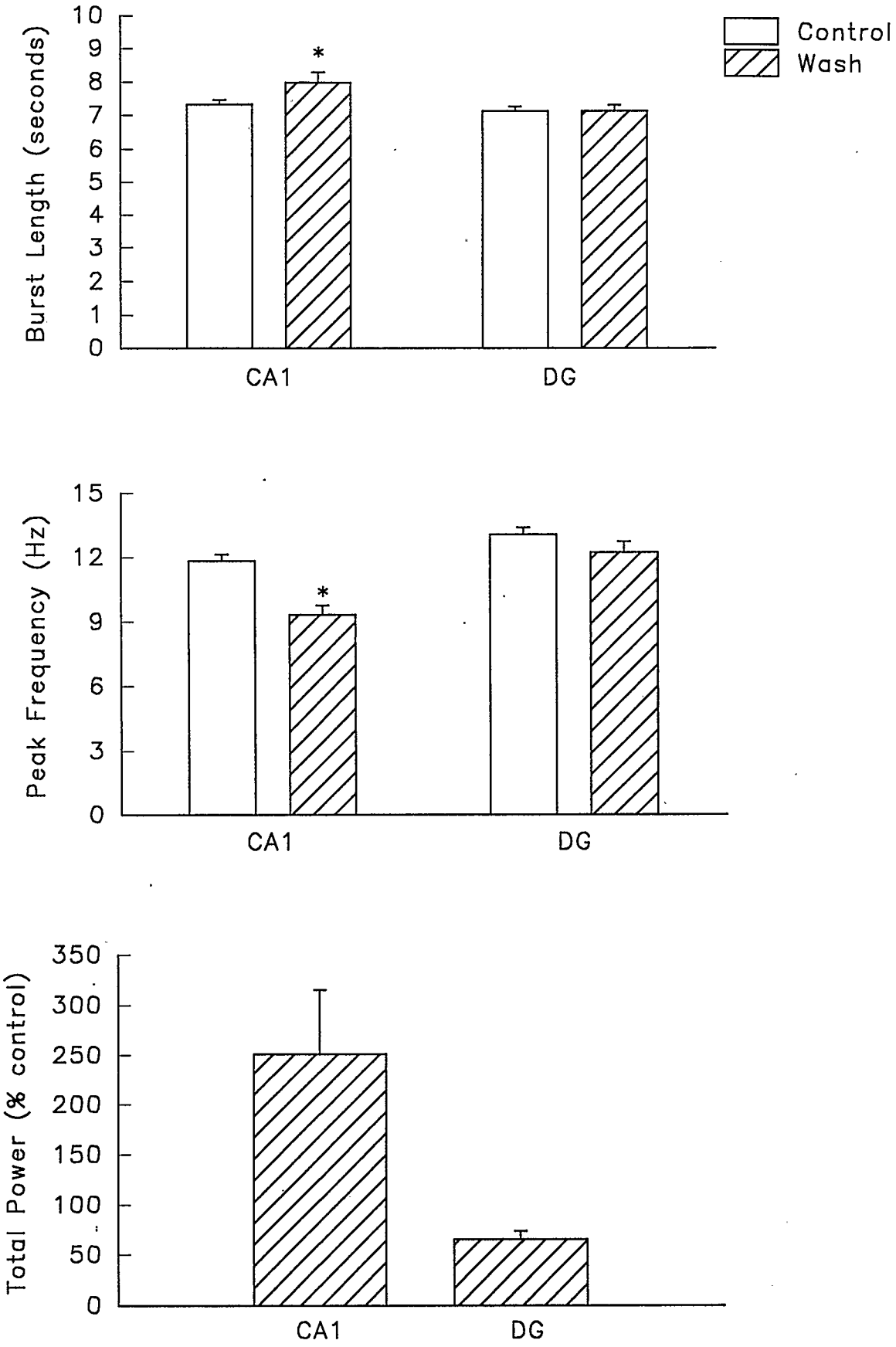

14
12
10
8
6
4
2
0
Peak Frequency (Hz)
0.0
0.5
1.0
1.5
2.0
Concentration (vol%)

Figure 38. The average values for burst length, peak frequency and power in comparison to their respective controls after washout of enflurane. Each bar represents the mean (+/- S.E.M.) of 5 - 7 experiments and * refers to a signficant difference ($p<0.05$) between the means of the wash and control.


Control
Wash
Burst Length (seconds)
10
9
8
7
6
5
4
3
2
1
0
*
CA1
DG
Peak Frequency (Hz)
15
12
9
6
3
0
*
CA1
DG
Total Power (% control)
350
300
250
200
150
100
50
0
CA1
DG

frequency of the activity for dentate RPA (12.2 ± 0.5 Hz) returned to within 0.9 Hz of control (13.1 ± 0.3 Hz) (Figure 38).

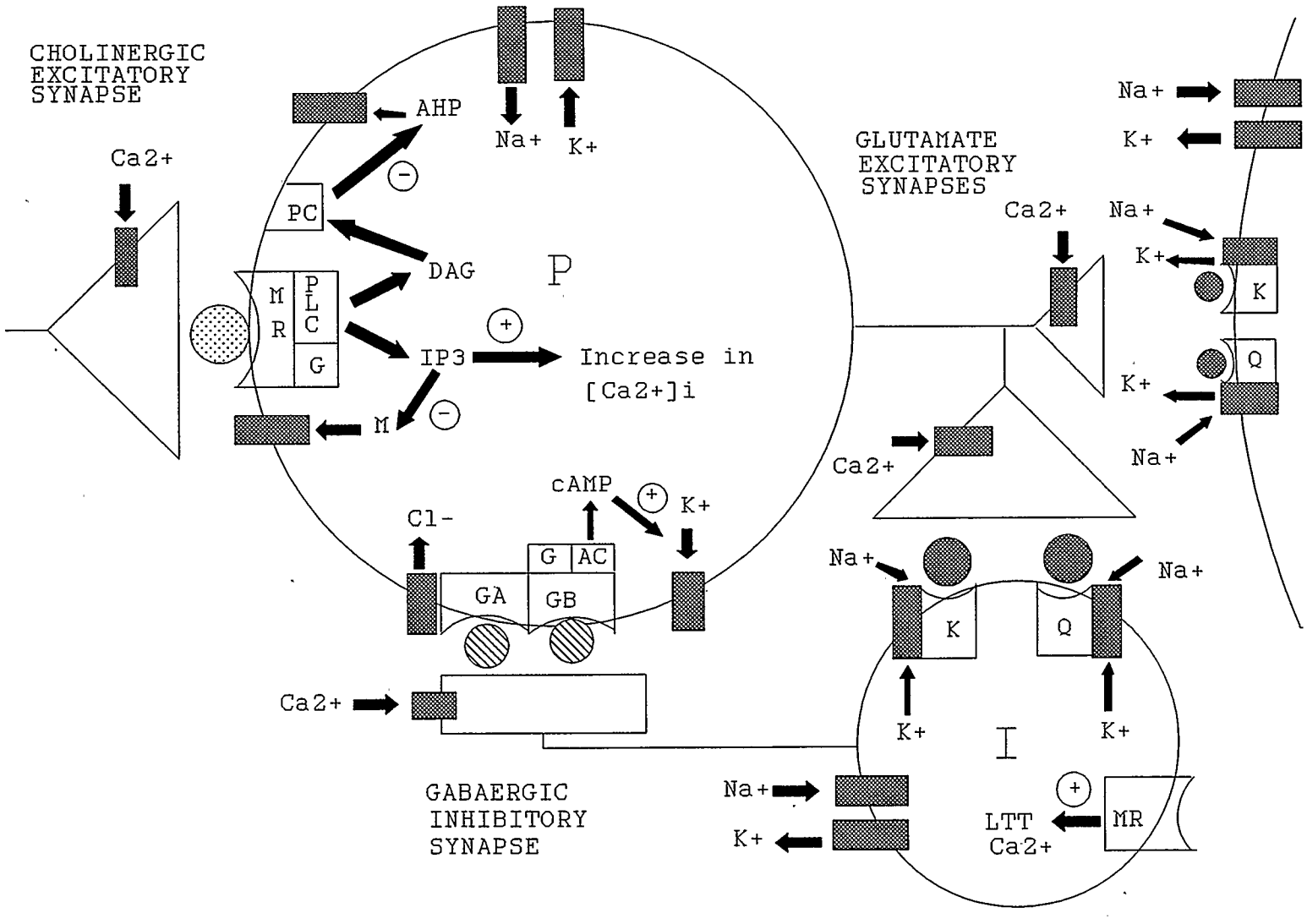
DISCUSSION

This study provides further documentation of the differential actions of three classes of general anaesthetics (i.e. alcohol, barbituate and volatiles) on the mammalian central nervous system. The following DISCUSSION will examine the possible cellular mechanisms of carbachol-induced RPA which will be used as a 'model' to discuss the anaesthetic effects. The differential actions of the anaesthetics will be summarized and related to the multiple site and/or mechanism hypothesis. The possible cellular actions of the anaesthetics on RPA will also be discussed in the context of the multiple site and/or mechanism hypothesis. In addition, the usefulness of RPA for studying anaesthetic actions on the mammalian central nervous system will be evaluated.

4.1 Possible Cellular Mechanisms of Carbachol-Induced RPA

There are few data about carbachol-induced RPA from which a model can be proposed. However, it is known that the generation of RPA requires a synaptic network and that the circuit present in the CA1 or the dentate are sufficient to produce RPA (MacVicar and Tse 1989; Konopacki et al., 1987). Thus, the traditional concept of a hippocampal circuit present in Ammon's horn and fascia dentata was used as a framework and is diagramed in Figure 39, (adapted from Schwartzkroin et al., 1990). This diagram incorporates cholinergic effects on hippocampal neurons and what is currently known about

Figure 39. A schematic of a local *in vitro* hippocampal neuronal circuit. Included are the known cellular mechanisms of carbachol-induced RPA. P refers to the principal cell type (i.e. either a CA1 pyramidal or dentate granule neuron) and I refers to an interneuron (all interneurons were grouped into this representation). The following symbols were used: -▷ are excitatory synapses; -□ is an inhibitory synapse; ● - glutamate neurotransmitter ; ◍ - GABA neurotransmitter; ⊛ - carbachol; MR - muscarinic receptor; G - G-protein; PLC - phospholipase C; PC - protein kinase C; ▮ - ion channel; AHP - the calcium-activated potassium current known as the afterhyperpolarization; M - the potassium current known as the M-current; [Ca2+]i - intracellular calcium concentration; GA -$GABA_A$ receptor; GB - $GABA_B$ receptor; AC - adenylate cyclase; K - kainate subtype of the glutamate receptor; Q - quisqualate subtype of the glutamate receptor; LTT Ca2+ - low threshold transient calcium currrent (i.e. T-type calcium current); ⊕ - enhancement or increase; ⊖ - depression or decrease.


CHOLINERGIC
EXCITATORY
SYNAPSE
Ca2+
AHP
Na+
K+
PC
DAG
M
R
PLC
G
IP3
Increase in
[Ca2+]i
M
P
cAMP
K+
Cl-
G
AC
GA
GB
Ca2+
GLUTAMATE
EXCITATORY
SYNAPSES
Na+
K+
Ca2+
K
Q
Na+
K+
Ca2+
Na+
Na+
K
Q
Na+
K+
K+
I
GABAERGIC
INHIBITORY
SYNAPSE
Na+
K+
LTT
Ca2+
MR

carbachol-induced RPA. This schematic is not intended to be a comprehensive representation of the electrophysiolgy, biochemistry or anatomy of the hippocampus but rather is presented so that it is possible to visualize sites of anaesthetic action that are relevant to this model system.

In the scheme presented in Figure 39, the principal output cells (i.e. CA1 pyramidal and dentate granule neurons) of each hippocampal region receive excitatory cholinergic projections and then send excitatory glutaminergic projections to the next cell group. As previously discussed, activation of muscarinic receptors has a number of specific electrophysiological and biochemical effects on hippocampal neurons. The main cholinergic effects on hippocampal neurons (very generally speaking) can be summarized as follows: (1) at the receptor level, signal transduction occurs via a phosphoinositide second messenger system which is mediated through a G-protein (2) an increase in membrane resistance and depolarization at the cellular level due to blockade of various potassium conductances (i.e. I_M and I_{AHP}) (3) a reduction of inhibiton (i.e. a reduction in the potency of inhibitory synaptic potentials). Presumably, some or all of these effects could contribute to the generation of RPA. In addition, glutamate synapses of the non-NMDA type also appear to play a role in the generation of this activity but probably at the level of the interneuron (MacVicar and Tse, 1989). A collateral of the principal cell concurrently excites local

interneurons which in turn send inhibitory GABAergic connections to the prinicipal cell that excited them (i.e. recurrent inhibition). Heynen and Bilkey (1991) found that simultaneous blockade of GABAergic IPSPs and excitation by glutamate could produce activity similar to that induced by carbachol which suggests that GABAergic synapses are also involved. Thus, the neural network that generates this activity appears to involve two major excitatory synapses and one inhibitory synapse: acetylcholine, glutamate and GABA.

It is presently unkown how carbachol initiates population activity. Since experimental studies are sparce, possible explanations are highly speculative. Some insight may be gained from a computer model of carbachol-induced RPA, generated by *in vitro* pyramidal neurons of the CA3 region, that was presented by Traub, Buzsaki and Miles (1991). This model generated similar activity provided that (1) the afterhyperpolarization (I_{AHP}) conductance was decreased (2) EPSP conductances were reduced (3) IPSPs were blocked and (4) the CA3 pyramidal cells were depolarized. All these actions are consistent with the effects of carbachol on hippocampal neurons (Halliwell, 1990). In an earlier study, also using computer simulations, Traub and Dingledine (1990) found that population activity could arise as the I_{AHP} decreased which resulted in the firing of a small number of cells in response to spontaneous EPSPs. In addition, decreasing the I_{AHP} amplitude in their computer simulation increased the frequency

of the population bursts. This observation was supported experimentally by the finding that reductions in the I_{AHP} amplitude by phorbol esters in *in vitro* CA3 pyramidal neurons led to a decrease in the interburst interval of population bursts (Chamberlain and Dingledine , 1989). Further experimental support comes from a study by Schneiderman (1986) who reported that hippocampal slices perfused with low concentrations of pencillin, which blocks $GABA_A$ receptors, can produce synchronous synaptic potentials. This present study found that the threshold carbachol concentration for generating RPA was somewhere between 12.5 and 25 μM. According to the computer model, this threshold concentration could be the concentration that sufficiently reduces the I_{AHP} and $GABA_A$ mediated IPSPs thereby producing RPA.

Madison et al. (1987) investigated cholinergic actions on the I_{AHP} of *in vitro* hippocampal CA1 pyramidal cells and found that carbachol concentrations of 30 μM completely abolished the I_{AHP}. This concentration is close to the threshold concentration for induction of RPA which, combined with Heynen and Bilkey (1991) observation, lends further support to the computer based hypothesis. However, frequency modulation of RPA occurred at concentrations from 25 to 75 μM which suggests that the I_{AHP} may not be involved in the carbachol dependent frequency modulation. This leads one to propose that the cellular mechanisms that control frequency are different from those that intitiate RPA.

The control of RPA frequency may involve a number of different cellular mechanisms. Madison et al. (1987) found that I_M was less sensitive, by a factor of 10, than I_{AHP} to inhibition by carbachol. Progressive inhibition of I_M by carbachol may account for the frequency modulation since reduction of the M-current increases the ability of neurons to fire repetitively in response to a depolarization (Halliwell and Adams, 1982). Alternatively, Fraser and MacVicar (1991a) found a low threshold transient calcium current (LTT or T-type current) in lacunosum-moleculare (L-M) interneurons which was increased by bath applications of carbachol (see Figure 2 for location of this stratum of the hippocampus). They suggested that enhancement of this T-type Ca^{2+} current in L-M interneurons could produce repetitive bursting. This suggestion was based on an observation that was made by Llinas and Jahnsen (1982) who found that the presence of this current in thalamic neurons imparted the ability of those neurons to burst in a rhythmic manner. Furthermore, the frequency of burst firing could be controlled by manipulating the membrane potential (Llinas and Jahnsen, 1982). In the hippocampus, L-M interneurons could produce a rhythmic variation of inhibition of pyramidal cells thereby producing rhythmic activity in pyramidal neurons (Fraser and MacVicar, 1991). In support of the hypothesis that the T-type calcium channel is involved in RPA, MacVicar and Tse (1989) reported that blockade of calcium channels by inorganic cations (i.e. Cd^{2+}, Co^{2+}, Mn^{2+}) abolished

RPA recorded intracellularly. In addition, Fraser and MacVicar (1991b) found that ethosuxamide, a blocker of T-type Ca^{2+} currents, blocked carbachol-induced RPA of *in vitro* CA1 pyramidal neurons. Thus, frequency modulation of RPA by carbachol may be explained by such a mechanism, but this hypothesis remains to be rigorously tested. It must be emphasized that other, yet unknown, mechanisms may also a play role in RPA and/or frequency modulation and generation.

Carbachol also increased the length of a single burst of RPA in a concentration-dependent manner. As discussed above, GABAergic synapses appear to play a role in RPA. However, precisely what role they play is not clear. Fraser and MacVicar (1991a) suggested that GABAergic input may terminate a single burst of RPA. According to their hypothesis, an increase in burst length is readily explainable. As stated previously, cholinergic agonists produce a disinhibitory action on hippocampal neurons. Muller and Misgeld (1989) found that carbachol depressed GABA-mediated inhibition. Hence, increasing concentrations of carbachol could produce progressively greater reductions in GABA-mediated inhibition thereby allowing RPA to continue for longer time periods.

4.2 The Profile of Anaesthetic Effects on RPA

The nonspecificty of anaesthetics and similarity of effects produced on membrane lipids led to a 'unitary theory' of anaesthesia (Roth, 1980; Roth, 1988). These theories

suggested that a common mechanism and site of action existed for all general anaesthetics which implied that anaesthetics would produce similar and nonselective effects on neuronal activity (Roth, 1980; Roth, 1988). The anaesthetics studied in this thesis produced differential effects on RPA. Tables 2 and 3 provide a descriptive summary of these effects on the CA1 and dentate neuronal populations, respectively. A number of features are striking about these profiles. Firstly, each anaesthetic produced a unique overall profile of effects when compared to the other compounds. Secondly, a single anaesthetic produced different effects depending on the neuronal population that was studied.

Differences between anaesthetics were evident for all three parameters of RPA. Ethanol and isoflurane depressed the power of RPA in both neuronal populations whereas pentobarbital, halothane and enflurane had both excitatory and depressant actions. It was interesting to note that isoflurane and enflurane, two structural isomers, had different effects on power. Ethanol and pentobarbital both increased the burst length whereas the volatile anaesthetics, for the most part, shortened it although there were subtle differences amongst the volatiles. Pentobarbital had a unique action in that at a certain concentration it obliterated the burst/interburst pattern. The most common effect of the compounds was on the peak frequency of RPA. All anaesthetics decreased peak frequency except ethanol which had the dual

Table 2. Summary of anaesthetic effects on carbachol-induced activity recorded from CA1 pyramidal neurons.

ANESTHETIC	TOTAL POWER	BURST LENGTH	PEAK FREQUENCY
Ethanol	depression	increased: pattern unaltered	biphasic: increase/ decrease
Pentobarbital	biphasic: excitation/ depression	increased: pattern abolished	decrease
Halothane	biphasic: excitation/ depresion	small decrease: pattern unaltered	decrease
Isoflurane	depression	decrease: pattern unaltered	decrease
Enflurane	biphasic: excitation/ depression	decrease: some pattern disruption	decrease

Table 3. Summary of anaesthetic effects on carbachol-induced activity recorded from dentate granule neurons.

ANESTHETIC	TOTAL POWER	BURST LENGTH	PEAK FREQUENCY
Ethanol	depression	increase: pattern unaltered	biphasic: increase/ decrease
Pentobarbital	depression	no effect: pattern abolished	decrease
Halothane	depression	small decrease: pattern unaltered	decrease
Isoflurane	depression	slight increase/ decrease: pattern unaltered	decrease
Enflurane	depression	decrease: some pattern disruption	decrease

action of increasing frequency at low concentrations and then decreasing it at higher concentrations. These differential actions between the anaesthetics are not compatible with a 'unitary theory'.

With the exception of isoflurane and ethanol, the effects of the other anaesthetics were dependent upon the neuronal population that was studied. The most prominent effect was the excitatory action that was observed on the CA1 pyramidal neurons in contrast to the depression that was observed on the dentate granule neurons. However, even though significant differences between the two populations were not evident for ethanol and isoflurane there were differences in sensitivities. There is no evidence which suggests that CA1 and dentate neurons generate RPA by different mechanisms. This casts doubt on the possibility that the differential actions arose because the anaesthetics, having a common mechanism and site of action, were acting upon two different mechanisms of generation of RPA. The differences in effects produced by a single anaesthetic on the two neuronal populations suggest that anaesthetics can exert selective actions which are also not compatible with a 'unitary theory'.

Since the effects of the anaesthetics are not compatible with a common site and mechanism of action this suggests that the differential actions arose as a result of the anaesthetics acting via multiple sites and/or mechanisms. This implies that no single cellular action or set of actions, common to

all the anaesthetics, exists which would be able to explain the different effects that were observed on RPA. The next section will discuss the cellular actions of anaesthetics that may explain the effects on RPA and show that the anaesthetics do not share common cellular actions.

4.3 The Possible Cellular Effects of Anaesthetics on RPA

It must be emphasized that the cellular effects included were limited to those effects that were demonstrated on hippocampal neurons at concentrations that were used in the present study. The reader is encouraged to refer to the 'model' of RPA (Figure 39) throughout the discussion. The anaesthetics will first be discussed in their respective classes which will then be followed by a comparison of the cellular effects as a group.

4.3.1 Ethanol

This study demonstrated that ethanol produced concentration-dependent effects on carbachol-induced RPA of *in vitro* hippocampal neurons. The effects of ethanol on the two neuronal populations (CA1 pyramidal and dentate granule) at low concentrations were very similar suggesting that similar mechanisms and/or sites of action were involved. Ethanol produced biphasic effects on frequency, increased the length of RPA and depressed the power of the activity. In rats, impairments in motor tasks have been reported to occur at

blood concentrations of 33 mM which correspond well to the effects on peak frequency (Gibbins, Kalant and Blanc, 1968). A differential sensitivity emerged at higher concentrations (i.e. 80 mM); CA1 RPA was abolished while RPA could still be recorded from dentate neurons. This concentration approximately corresponds to the concentration required to produce anaesthesia in rats which is 100 mM (Carlen, Zhang and Cullen, 1991).

Recent attention has been focused on the effects of ethanol on excitatory amino acid synapses. Lovinger, White and Weight (1989) reported that ethanol selectively reduced the NMDA-activiated current of *in vitro* hippocampal neurons, the IC_{50} for this effect was approximately 30 mM. However, MacVicar and Tse (1989) demonstrated that only glutamate receptors of the non-NMDA type were involved in the generation of carbachol-induced RPA. According to Lovinger et al. (1989), the effects of ethanol on the kainate-activated current, at low concentations, was minimal (i.e. only a 18% reduction at 50 mM). Therefore, it would appear that ethanol has another major action, in addition to the inhibition of the NMDA current, on *in vitro* hippocampal neurons that could account for the observed effects on RPA.

It also has been reported that ethanol can potentiate GABA-activated chloride currents. Nishio and Narahashi (1990) reported that 30 mM ethanol could increase the transient GABA-activated current by 20% and the effect was concentration-

dependent up to 300 mM. In contrast, Aguayo (1990) found that 10 mM ethanol potentiated the peak amplitude by a factor of 65% but there was no further increase above this concentration. An evaluation of Nishio and Narahasi's (1990) data shows that the concentration-dependent effect was small, only a further 10% increase at a concentration of 300 mM. Based on these results, enhancement of GABAergic transmission may be contribute to the effects of ethanol on RPA.

In contrast, Siggins, Pittman and French (1987) studied the effects of ethanol on synaptic transmission of *in vitro* hippocampal neurons. They failed to find an enhancement of GABA-mediated IPSPs. Instead their most consistent finding was a depression of both EPSPs and IPSPs. Depression of GABA responses and/or excitatory synaptic transmission by ethanol was also observed by MacIver and Roth (1987) in the crayfish stretch receptor neuron and Gruol (1982) in cultured mammalian spinal cord neurons. It appears that excitatory synaptic transmission is consistently altered and therefore plays a role whereas the effects on inhibitory transmission is controversial and yet to be determined. If GABAergic inhibition terminates a single burst of RPA, then a depression of inhibitory transmission would explain the observed increase in the burst length of RPA.

Carlen et al. (1991) has shown that concentrations as low as 20 mM can enhance the I_{AHP} which, as discussed above, may play a role in carbachol-induced RPA. However, since

concentrations of carbachol used to elicit RPA in this study are sufficient to completely block the I_{AHP}, it does not seem likely that this effect of ethanol could account for the observed effects. This suggestion is supported by Moore, Madamba and Siggins (1990) who found that ethanol concentrations of 22-44 mM did not affect the I_{AHP} nor did it affect a variety of other potassium conductances such as I_A and I_Q. However, ethanol did reduce the amplitude of the M-current at depolarized membrane potentials. They suggested that this could explain the excitatory responses of *in vivo and in vitro* hippocampal neurons to low ethanol concentrations (Mancillas, Siggins and Bloom, 1986; Siggins et al., 1987). Although the role of the M-current in carbachol-induced RPA is unknown, this selective effect of ethanol may explain the increase in frequency of RPA that was observed at 20 mM. The sudden decrease in frequency of RPA at an ethanol concentration of 30 mM may be explained by other actions of ethanol on hippocampal neurons which might play a more prominent role at this concentration thereby counteracting the effect on the M-current.

Calcium channels have also been implicated as a target for ethanol action. Twombly, Herman, Kye and Narahashi (1990) using cultured neuroblastoma cells found that ethanol signficantly inhibited a calcium current labeled type 1 (analogous to the low threshold transient calcium current or T-type) at low concentrations (30 mM). Higher concentrations

(i.e. 100 mM) significantly inhibited the type 2 calcium current (analogous to the high threshold long-lasting calcium current or L-type). As stated above, the T-type Ca^{2+} current has been hypothesized to be involved in carbachol-induced RPA (see Figure 39). Inhibition of the T-type current could contribute to ethanol's action at low concentrations whereas blockade of RPA at 80 mM may be due to an inhibition of the T and L-type calcium current. If this is the case, then RPA should also be abolished in dentate granule neurons. One possiblity for this selective effect is that there is a differential distribution of calcium channels between the two regions. This suggestion is supported experimentally by a study conducted by Fisher, Gray and Johnston (1990). They found that of the three voltage-dependent calcium channels (T, N and L) CA1 neurons had the following proportions: 22%, 48% and 30%, respectively. In contrast, dentate granule neurons had a different distribution of T, N and L-type calcium channels: 10%, 83% and 7%, respectively. According to these findings, 80 mM ethanol would block a higher proportion of calcium channels in CA1 pyramidal as compared to dentate granule neurons which could account for the differential sensitivity.

4.3.2 Pentobarbital

In rats, pentobarbital concentrations of approximately 30 μM are associated with sedation while anaesthetic levels have

been reported to occur from 60 to 100 μM (O'Beirne, Gurevich and Carlen, 1987; MacIver and Roth, 1987b). Pentobarbital produced concentration-dependent effects on carbachol-induced RPA within this range. The overall profile of effects on the CA1 were different from those on the dentate neuronal population. In the CA1, there was a biphasic effect on power (i.e. enhancement at low concentrations followed by depression at higher concentrations), a decrease in frequency and an increase in the burst length at low concentrations followed by abolishment of the burst/interburst pattern at higher concentrations. Only a depression in power was observed for dentate neurons. The other effects of pentobarbital on dentate neurons included a disruption of the burst/interburst pattern and a decrease in the frequency of RPA. A differential sensitivity was also observed, pentobarbital abolished RPA in the dentate at a concentration of 80 μM but not in the CA1.

It is well established that pentobarbital enhances GABA-mediated inhibitory synaptic transmission at anaesthetic concentrations (Olsen, Fischer and Dunwiddie, 1986). These studies have shown that: (1) GABA binding to the ionophore is increased in the presence of pentobarbital (Willow and Johnston, 1980; Willow and Johnston 1981; Asano and Ogaswarara, 1981; Bureau and Olsen, 1990) and (2) GABA-mediated IPSPs are augmented in the presence of pentobarbital by prolonging the mean open time of chloride channels (Schulz

and MacDonald, 1981; Jackson, Lecar, Mathers and Barker, 1982; Wong, Leeb-Lundberg, Teichberg and Olsen, 1984; Segal and Barker , 1984; Gage and Robertson, 1985; O'Beirne et al., 1987; MacDonald, Twyman, Rogers and Weddle, 1988; Mody, Tanelian and MacIver, 1991). Anaesthetic concentrations of pentobarbital depressed the power, disrupted the burst pattern, and decreased the frequency of RPA in both the CA1 and dentate neuronal populations. This suggests that enhancement of GABA-mediated inhibition, via an interneuron (see Figure 39), could account for some or all of these effects at anaesthetic concentrations.

Pentobarbital at a concentration of 80 μM reversibly abolished RPA in the dentate but not the CA1. This differential sensitivity may be explained by examining the molecular biology of the GABA/benzodiazepine ionophore. Cloning of this ionophore from rat brains has revealed that it is composed of a family of polypeptides with the following designations: α_1, α_2, α_4, β_1, β_2, β_3, γ_2 and δ (Olsen and Tobin 1990). Each of these polypeptides is structurally capable of forming chloride channels and each contains binding sites for GABA, pentobarbital and picrotoxin (Olsen and Tobin, 1990). Bureau and Olsen (1990) demonstrated that the ability of pentobarbital to enhance [^{3}H]muscimol binding was different depending upon the peptide. Furthermore, a differential expression of these peptides between the CA1 and dentate of the rat hippocampus has been demonstrated (Shivers, Killisch,

Sontheimer, Kohler, Schofield and Seeburg, 1989; Olsen and Tobin 1990). These two observations suggest that the relative proportions of these subunits in a particular GABA/benzodiazepine ionophore will determine the efficacy of pentobarbital (Olsen and Tobin, 1990; Bureau and Olsen, 1990). This is supported by the observation that pentobarbital enhances [^{3}H] muscimol binding to a greater degree in the dentate as compared to the CA1 (Olsen, Sapp, Bureau, Turner and Kokka, 1991). Pentobarbital may have abolished RPA in the dentate because of differences in the subunit composition of the GABA/benzodiazepine ionophore, compared to the CA1.

There are suprisingly few studies that have shown effects on hippocampal neurons at concentrations that are similar to those examined in this study. Excitatory effects of pentobarbital on CA1 RPA were observed to occur at pentobarbital concentrations as low as 20 μM. This effect has also been observed by a few other investigators (Dunwiddie et al., 1986; MacIver and Roth 1987). Several lines of evidence suggest that pentobarbital's enhancement of GABA-mediated inhibition occurs at concentrations that are higher than the concentration that increased RPA power of CA1 neurons in this study. For example, enhancement of [^{3}H]muscimol binding to various polypeptide subunits of the GABA/benzodizepine ionophore by pentobarbital was observed at concentrations above 30 μM (Bureau and Olsen, 1990). O'Beirne et al. (1987) demonstrated that pentobarbital did not augment focally

pressure-ejected GABA responses in CA1 pyramidal neurons at 10 μM but did at 100 μM. In addition, Iadarola, Fanelli, McNamara and Wilson (1985) concluded that concentrations of at least 50 μM were required to produce a robust enhancement of recurrent inhibition in CA1 pyramidal neurons. Since a significant enhancment of GABA-mediated inhibition occurs at pentobarbital concentrations around 50 μM it appears that there may be actions, other than an enhancment of inhibition, which may explain the excitatory response.

O'Beirne et al. (1987) also reported that concentrations as low as 10 μM could hyperpolarize the membrane (by 1.9 mV) and decrease the frequency of spontaneously firing *in vitro* CA1 neurons. Furthermore, this effect was dependent upon the concentration with the hyperpolarization reaching 3.9 mV at pentobarbital concentrations of 100 μM. Hyperpolarization has also been reported by Nicoll and Madison (1982) at anaesthetic concentrations and both investigators postulated that it could be due to an increase in the conductance of a potassium current thereby decreasing neuronal excitability. However, depending upon the activity of the neuron, hyperpolarization can lead to increased neuronal excitability. MacVicar and Tse (1989) found that depolarizing the membrane of CA3 pyramdial neurons decreased the amplitude of carbachol-induced RPA while an intracellular injection of hyperpolarizing current increased the amplitude. In neither case was the frequency of the membrane potential oscillations affected. Theta activity

recorded intracellularly from *in vivo* CA1 pyramidal and dentate granule neurons also exhibit this behaviour in response to depolarizing and hyperpolarizing current injections (Nunez et al., 1987; Munoz et al., 1990). Presumably, this behaviour occurs because the membrane potential oscillations are of synaptic origin (i.e. EPSPs) and are not due to intrinsic ionic conductances (MacVicar and Tse, 1989). Hence, a hyperpolarization shifts the membrane potential away from the EPSP reversal potential thereby increasing the amplitude (MacVicar and Tse, 1989). On the other hand, intrinsic ionic conductances modulate the frequency of neuronal firing in response to depolarizing (increase in frequency) and hyperpolarizing (decrease in frequency) current injections (Llinas and Jahnsen, 1982). Spontaneously firing hippocampal neurons alter their frequency in response to changes in membrane potential which suggests their activity is governed by intrinsic ionic conductances (Munoz et al., 1990; Schwartzkroin, 1977). Therefore, the effects of membrane potential changes on neuronal activity appears to depend upon the mechanism of that neuronal activity; synaptic origin or intrinsic ionic conductances.

Based on these observations, one could propose that the excitatory response of the CA1 neuronal population was due to a hyperpolarization that was produced by pentobarbital. According to this hypothesis, this would increase the amplitude of RPA by shifting the membrane potential away from

the EPSP reversal potential. However, this leaves two discrepancies: the depression at higher concentrations and the lack of an excitatory response by the dentate granule neuronal population. As alluded to above, significant enhancement of GABAergic inhibition seems to occur around 50 μM which corresponds to the depression that was observed in the CA1. This enchancment could overshadow the excitatory reponse. On the other hand, since dentate granule neurons are more sensitive to pentobarbital than CA1 pyramidal neurons, a lower pentobarbital concentration would be necessary to produce enhanced GABA responses. This may mask the excitatory effect of pentobarbital in the dentate.

4.3.3 Volatile Anaesthetics

Halothane, isoflurane and enflurane also produced concentration-dependent effects on carbachol-induced RPA at concentrations that were relevant to anaesthesia in the whole animal. In the rat, the minimum alveolar concentration (MAC) for these anaesthetics (volume %) are, respectively: 1.03, 1.5 and 2.2 (Roth and Miller, 1986). All three anaesthetics produced very similar effects at or near MAC: depression in power, decrease in frequency and the burst length of carbachol-induced RPA of both the CA1 and dentate neuronal populations.

Effects on RPA were also observable at low subanaesthetic concentrations. Halothane and enflurane had excitatory

effects on the power of CA1 neurons whereas isoflurane only produced depression. The frequency was altered by all three anaesthetics but the burst length of RPA was not, it was relatively insensitive to halothane and enflurane at low concentrations. The power and frequency of dentate neurons were also altered by all three anaesthetics. However, differences were apparent on the burst length: isoflurane had biphasic effects whereas enflurane reduced it and halothane had small effects. Since studies examining the effects of anaesthetics on hippocampal neurons are scant, observations from other preparations will be used to augment the following discussion. In addition, since data showing effects at subanaesthetic concentrations are also scarce most of the discussion will be limited to concentrations near MAC. However, data at low concentrations will be cited whenever possible.

There is evidence which suggests that anaesthetics can disrupt the muscarinic receptor complex. These studies have revealed that although the binding of carbachol to the receptor is not altered by anaesthetics, the coupling of the muscarinic receptor to its G-protein is inhibited by anaesthetics (Aronstam et al., 1986; Dennison et al., 1987; Anthony et al., 1989). Despite the fact that the consequences of this effect on neuronal activity is not known, a disruption of the G-protein may contribute to the anaesthetic effects on RPA.

It is well documented that volatile anaesthetics depress excitatory synaptic transmission (Pocock and Richards, 1991). In the rat hippocampus, volatile anaesthetics can depress the amplitude of electrically evoked field EPSPs of both the CA1 and dentate neuronal populations at MAC (Richards and White, 1975; MacIver and Roth, 1988; Miu and Puil, 1989; MacIver and Kendig, 1991) explain the depression of power that was observed. Miu and Puil (1989) found that isoflurane at a concentration of 100 μM could depress the CA1 EPSP amplitude to 70% of control. At this concentration, the RPA power of CA1 neurons was close to 40% of control. MacIver and Roth (1988) reported that the EC_{50} for depression of CA1 population spikes by halothane and enflurane were approximately 400 and 900 μM, respectively. RPA of both CA1 and dentate neurons was found to be completely abolished at concentrations much lower than these. A possible explanation for the lower anaesthetic concentrations that were found to be effective on RPA is that electrically evoked population EPSPs are different from rhythmic EPSPs generated by carbachol. Evidence for this suggestion comes from a study conducted by Wheal and Miller (1980) which found that the excitatory responses elicited by electrical stimulation of the perforant path was blocked by a glutamate but not by a muscarinic antagonist. This finding demonstrates that the cholinergic pathway was not activated by electrical stimulation of the perforant path. This result may extend to the activation of the stratum radiatum and stratum

oriens synaptic inputs. The relevance of this finding becomes apparent when one considers a study by Puil and El-Beheiry (1990) who reported that the cholinergic responses of neurons were more sensitive to anaesthetics than were the glutaminergic responses. For example, the EC_{50} for isoflurane depression of depolarization evoked by acetylcholine was 0.9 MAC whereas it was 1.9 MAC for glutamate. The relative insensitivity of glutamate-mediated neuronal responses combined with the lack of involvement of the cholinergic pathway may be a reason for the higher concentrations that were necessary to depress EPSP amplitudes.

A depression of EPSP amplitude, however, may not lead to a decrease in frequency. The time course of an EPSP should determine the frequency of RPA. Hence, decreases in RPA frequency could be the result of an increase in the EPSP rise time or decay time. MacIver and Roth (1988) found that there were changes in the slope of the evoked EPSP of *in vitro* hippocampal neurons. Although EPSP rise times as a function of anaesthetic concentrations were not included in their analysis, enflurane and isoflurane were reported to decrease the rate of rise of the EPSP. Increases in the rise times of EPSPs by isoflurane has also been reported by El-Beheiry and Puil (1989) in rat somatosensory cortex slices. They explicitly analyzed the relationship between rise time and anaesthetic concentration and found that a significant increase was attained at 180 μM whereas a significant increase

in the decay time occurred at approximately 50 μM. This latter concentration corresponds to the decrease in frequency of CA1 RPA which was found to be significant at approximately 60 μM. This suggests that the rate of decay of the EPSP may be more important than the rate of rise in the depression of RPA frequency at low concentrations, at least for isoflurane, although both may eventually contribute to the decrease in frequency. Unfortunately, equivalent data with halothane and enflurane is unavailable at this time.

Neurons possess multiple types of calcium currents (Miller, 1987). In the hippocampus, Krnjevic and Puil (1988) found that halothane (0.5 vol%) inhibited slow inward currents, presumably calcium because they were recorded in the presence of TTX and TEA, recorded from CA1 neurons. However, the study failed to discriminate between the different types of currents. Takenoshita and Steinbach (1991) studied the effects of anaesthetics on L-, T, and N-type calcium currents in dorsal root ganglion neurons. This study found that halothane and isoflurane blocked low threshold transient (T or LTT) calcium currents at lower concentrations than it blocked the L- and N-type currents. The approximate EC_{50} for inhibition of the T-type calcium current by halothane was 100 μM whereas it was 1 mM for the L- and N-type currrents. As discussed above, T-type calcium currents have been hypothesized to be involved in RPA (see Figure 39). Ethosuxamide, an antiepileptic drug which blocks T-type

currents, has been shown to depress the frequency and amplitude of carbachol-induced oscillations of CA1 neurons (Fraser and MacVicar, 1991b). Given these observations, it follows that the decrease in frequency, as well as power, of RPA may be due to inhibition of the T-type calcium current by the volatile anaesthetics.

The study by Krnjevic and Puil (1988) also found that in some CA1 neurons halothane augmented a calcium current at low concentrations. This calcium current was activated by step depolarizations from a holding potential of -80 mV. This current is known as the T-type current (Miller, 1987). As discussed above, this current appears to be a target for halothane. These observations provide an explanation for why halothane did not produce an excitatory effect on dentate neurons. Fisher et al. (1990) reported that of the three voltage-activated calcium channels 22% were of the T-type in CA1 neurons whereas only 10% were in dentate neurons. The lack of an excitatory response in the dentate may have been that too few of the T-type channels were present to allow an appreciable response. Although there are no data available for enflurane, the T-type current may also be enhanced at low enflurane concentrations thereby also explaining the excitatory response.

An interesting implication of the Takenoshita and Steinbach (1991) study is that depression of neuronal activity by anaesthetics occurs at concentrations less than those

required to decrease neuronal neurotransmitter release. N-type calcium currents are thought to be specifically responsible for neurotransmitter release (Miller, 1987). Anaesthetics have been shown to depress neurotransmitter release at clinically relevant concentrations (Richards and Pocock, 1991). In the present study, depression of RPA occurred at concentrations that were sufficient to inhibit the T-type calcium current but that were much lower than those required to inhibit the N- or L-type currents (see data Takenoshita and Steinbach, 1991). This suggests that a decrease in neurotransmitter release may not have been an effect that contributed to the anaesthetic depression of RPA. This is supported by the observation that volatile anaesthetics do not suppress muscarinic receptor-mediated secretion of norepinephrine and dopamine in PC12 neurons (Kress, Muller, Eisert, Gilge, Tas and Koschel, 1991).

A few studies have demonstrated that approximately 1 MAC halothane can prolong the time constant of decay of inhibitory postsynaptic currents in CA1 neurons (Gage and Robertson, 1985; Mody et al., 1991). This effect may be due to a modulation of the GABA ionophore since volatile anaesthetics have been found to enhance GABA-mediated currents in dorsal root ganglion neurons (Nakahiro et al., 1989). Contrary to these observations, Yoshimura et al. (1985) and Fujiwara et al. (1988) found that IPSPs were depressed in the presence of halothane, isoflurane, and enflurane in CA1 neurons. Thus, it

is not clear at this time whether or not enhanced inhibition could have contributed to the anaesthetic effects on RPA.

4.3.4 A Comparison of the Possible Cellular Effects of Anaesthetics on RPA

Both ethanol and the volatiles appeared to depress calcium currents at concentrations that produced anaesthesia in the whole animal. However, of the three voltage-dependent calcium currents that exist in hippocampal neurons (L-, N- and T-types) ethanol depressed the T- and L-types whereas halothane and isoflurane only depressed the T-type. Halothane, in some neurons, could actually potentiate the T-type current at low concentrations which may account for the excitatory effects. A similar effect may have occurred for enflurane whereas a lack of such an effect may explain why isoflurane did not produce excitation. Unfortunately, data for enflurane and isoflurane are not available and thus these suggestions are highly speculative. In contrast to the volatiles, potentiation of calcium currents were not observed with ethanol which coincides with the observation that ethanol did not produce an excitatory effect on RPA power. Pentobarbital has only been found to depress calcium currents at very high concentrations (i.e. 200 μM) which suggests that this effect did not play a role in the alteration of RPA (Werz and MacDonald, 1985).

GABA-mediated inhibition is consistently enhanced by

pentobarbital at anaesthetic (i.e. 50 μM) concentrations. However, the effects of ethanol and the volatile anaesthetics on the GABA/benzodiazepine ionophore are controversial. Some studies reported an enhancement while others reported a depression. Thus, increased inhibiton appears to play a role in the pentobarbital-induced alteration of RPA whereas it is not clear if such an effect is involved on RPA for the other anaesthetics.

Alterations of RPA could not be explained by a depression of synaptic transmission by all the anaesthetics. It is difficult to relate the results of previous studies to the effects on RPA since it is a muscarinic response whereas previous studies were probably investigating glutamate responses. Despite this difference, halothane, isoflurane, enflurane and ethanol have been shown to depress EPSPs at low concentrations. However, concentrations of pentobarbital beyond those that were used in this present study do not appear to depress EPSPs (O'Beirne et al., 1986).

It has been hypothesized that anaesthetics could depress neuronal function by altering the resting membrane properties of neurons (Nicoll and Madison, 1982). The only anaesthetic that has such an effect is pentobarbital which produces a hyperpolarization at concentrations similar to those used in this study. Although Nicoll et al. (1982) demonstrated a hyperpolarization by halothane on CA1 neurons they used very high concentrations (i.e. 1 mM). Fujiwara et al. (1988)

reported that halothane, isoflurane and enflurane at concentrations below 1.2 mM did not affect resting membrane properties of hippocampal neurons which suggests that a change in resting membrane properties may not account for the anaesthetic effects on RPA. This suggestion can also be extended to ethanol since Siggins et al. (1987) also reported that ethanol did not affect resting membrane properties of hippocampal neurons.

There have been other reported cellular actions of anaesthetics that could account for the effects on RPA that may be unique to a particular agent or agents. Ethanol has also been shown to depress a potassium current known as the M-current (I_M) which, to date, has not been shown to be affected by other anaesthetics. This may explain why ethanol had an excitatory effect on RPA frequency whereas the other anaesthetics had a depressant effect. In addition, the volatile anaesthetics can disrupt the coupling between the muscarinic receptor and its G-protein. This also is an effect that has not been reported to occur for pentobarbital or ethanol and may be unique to the volatiles.

It becomes apparent that a single anaesthetic has multiple cellular actions that could account for the alterations of RPA that were observed in this study. Moreover, a paticular anaesthetic appeared to have a unique set of actions which may explain some of the alterations of RPA. No single common action or set of actions emerged that

could explain the effects of the anaesthetics. This is consistent with the hypothesis that the differential effects on RPA arose as a result of the anaesthetics acting via multiple sites and/or mechanisms.

4.4 Carbachol-Induced RPA as a Model System

A number of features must be considered in order to evaluate the usefulness of carbachol-induced RPA in studying the actions of anaesthetics: (1) the sensitivity of RPA to the anaesthetics (2) the similarities between the *in vitro* and the *in vivo* potencies and (3) the similarity of the *in vitro* effects to the whole animal.

The sensitivity of the model can be determined by comparing the EC_{50}s to the clinically effective concentrations. Table 4 provides a summary of this comparison. These data show that the EC_{50} concentrations *in vitro* are lower than those required to produce anaesthesia. This suggests that RPA is sensitive enough to be used as a model to study anaesthetic actions on the CNS. Brazil, Raux, Yudell and Minneman (1987) compared the concentrations of halothane *in vivo* to *in vitro* in rats. They found MAC to be 1.05 vol%, which agrees with previous investigations, and that all the animals stopped responding to a tail pinch at a concentration of 1.25 vol%. GC analysis revealed that the brain tissue concentration of rats exposed to 1.25 vol% halothane could be attained in the *in vitro* hippocampal slice at 0.8-0.9 vol%. This suggests

Table 4. Anaesthetic concentrations (in mM) required to produce a 50% reduction in total power and peak frequency of RPA recorded from CA1 and dentate (DG) neurons in relation to clinically effective concentrations.*

ANAESTHETIC	TOTAL POWER		PEAK FREQUENCY		CLINICAL CONCENTRATION
	CA1	DG	CA1	DG	
ETHANOL	50	19.2	54.8	73.1	100
ENFLURANE	.076	.03+	.072	.074	.52
PENTOBARBITAL	.06	.051	.04	.05	.08
ISOFLURANE	.105	.05	.18	.18	.34
HALOTHANE	.195	.035	.165	.165	.24

* Data from Mody et al., 1988; Carlen et al., 1991; MacIver and Roth, 1987.

+ For enflurane, the half-maximal concentration could not be determined for the total power of dentate neurons since it was below 0.5 vol% (i.e. volatile anaesthetic concentrations in the ACSF were not determined below the lowest vaporizer setting), therefore the concentration was estimated.

that MAC *in vitro* may actually be lower which may account for the discrepancy between the EC_{50} concentrations and the clinically effective concentration of halothane. Although similar comparisons have not been made for isoflurane and enflurane, a similar argument may be valid.

Since anaesthetic potencies are linearly correlated with lipid solubility, a plot of the anaesthetic EC_{50} v:s its respective partition coefficient should also produce a linear correlation. However, no such correlation was found to exist for RPA (data not shown). This suggests that lipid solubility alone does not account for the effects on RPA. However, a plot of the anaesthetic EC_{50} and *in vivo* potencies (Figures 40 and 41) revealed a strong correlation for the effects on RPA frequency (R_{CA1}=0.97; $R_{DENTATE}$=0.98). The order of *in vitro* potencies were as follows (descending order): pentobarbital > enflurane > halothane > isoflurane > ethanol. *In vivo* potencies are as follows (descending order): pentobarbital > halothane > isoflurane > enflurane > ethanol. The finding that enflurane was more potent than halothane and isoflurane was very unexpected. If enflurane was eliminated the order of potencies would be identical. At this point there is no clear explanation for this anomalous finding. One must be cautious in making any interpretations since the water/gas partition coefficient of enflurane (0.78) is considerably lower than for isoflurane (1.08) or halothane (1.20) (data from Roth and Miller, 1986). This would result in a lower concentration of

Figure 40 - Correlation between *in vivo* potency and the anaesthetic concentration required to produce a 50% reduction of RPA peak frequency recorded from CA1 pyramdial neurons.

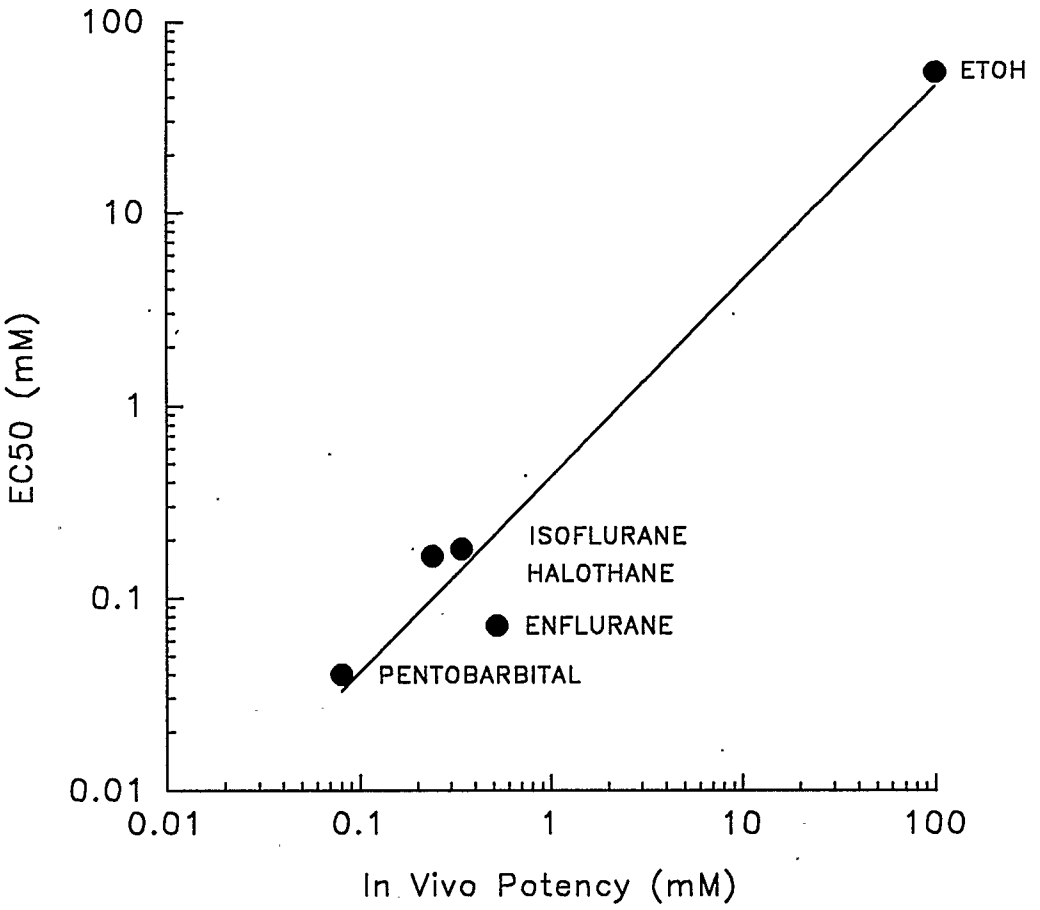
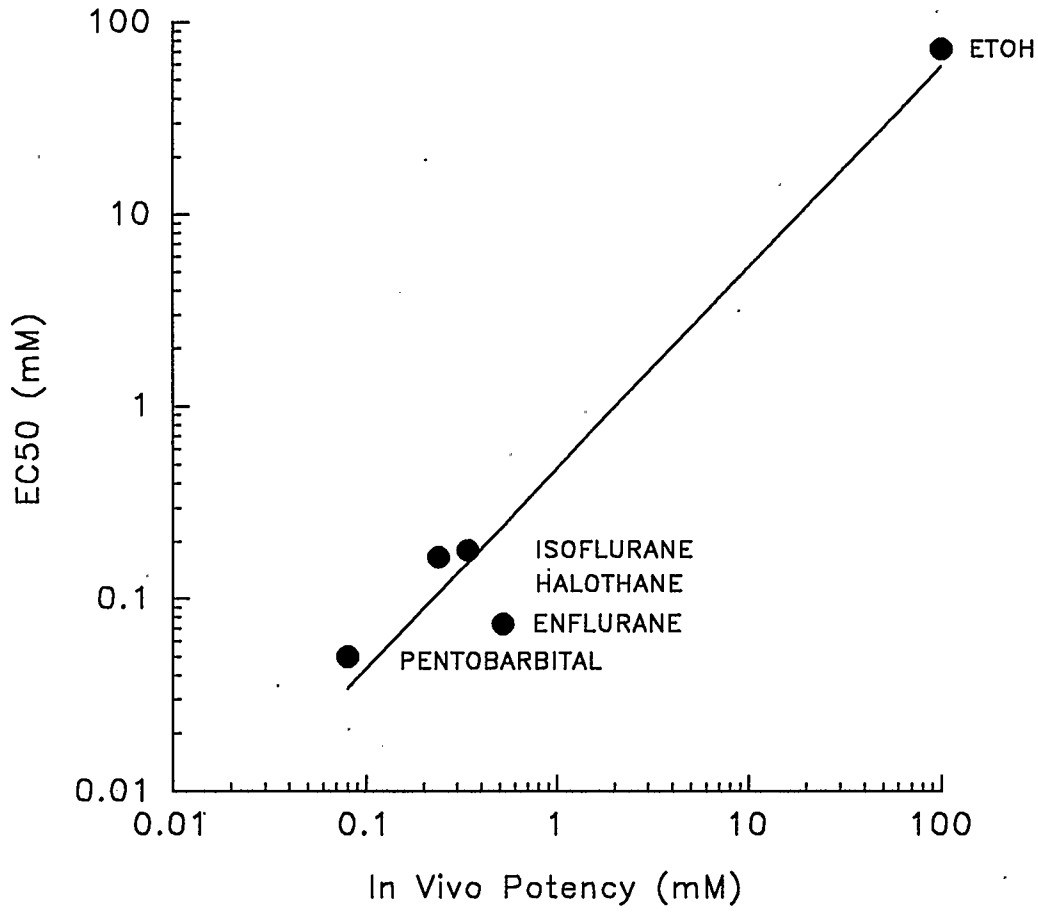

100
10
1
0.1
0.01
EC50 (mM)
0.01
0.1
1
10
100
In Vivo Potency (mM)
ETOH
ISOFLURANE
HALOTHANE
ENFLURANE
PENTOBARBITAL

Figure 41 - Correlation between *in vivo* potency and the anaesthetic concentration required to produce a 50% reduction of RPA peak frequency recorded from dentate granule neurons.


100
10
1
0.1
0.01
EC50 (mM)
ETOH
ISOFLURANE
HALOTHANE
ENFLURANE
PENTOBARBITAL
0.01
0.1
1
10
100
In Vivo Potency (mM)

enflurane in the ACSF relative to isoflurane and halothane which may explain the apparent discrepancy in the order of potencies.

There are virtually no sytematic investigations of anaesthetic effects on hippocampal theta activity in the whole animal. Code, Roth, Bland and Strunin (1988) found that halothane reduced the frequency of theta in dose-dependent manner but that was the extent of their investigation. McNaughton, Richardson and Gore (1986) reported that amylobarbital, a structural isomer of pentobarbital, also decreased the frequency theta, although in their experiments theta was induced by stimulation of the reticular formation. The *in vitro* results reported in this study agree with these previous findings. However, it must be pointed out that the results do not coincide exactly since RPA has a higher frequency than *in vivo* theta.

In humans, the effects of anaesthetics on EEG are similar to the results of this study. Halothane, isoflurane and enflurane all have been found to shift the power of the frequency spectrum from higher to lower frequencies in a dose-dependent manner (Findeiss et al., 1969; Levy, 1986; Sugiyama, Joh, Kiyomitsu, Niwa and Matsura, 1989; Yli-Hankala, Eskola and Klaukinen, 1989). This suggests that the anaesthetics are acting in a similar manner on carbachol-induced RPA of *in vitro* hippocampal neurons.

4.5 General Conclusions

Carbachol-induced rhythmical population activity of the *in vitro* hippocampal slice preparation is susceptible to general anaesthetics at concentrations well below those required for surgical anaesthesia in the whole animal. The anaesthetic agents that were studied produced differential actions on this activity which is not compatible with a 'unitary theory' of anaesthesia. These differential actions were observed when comparing anaesthetic effects on RPA generated by a single population of neurons (i.e. CA1 pyramidal or dentate granule neurons) and when the effects of a single anaesthetic agent were compared between the two populations of neurons. The differential actions of a single compound suggest that anaesthetics can exert selective actions which is also not compatible with a 'unitary theory'. Thus it appears that the anaesthetics altered RPA by acting via multiple sites and/or mechanisms of action. This hypothesis was supported by the argument that amongst the possible cellular effects that may have explained the alteration of RPA, at concentrations that produced effects in this present study, a common cellular effect or set of effects produced by the anaesthetics could not be found.

The susceptibility of RPA to the anaesthetics at clinically relevant concentrations suggest that this is a useful model for studying anaesthetic effects. This suggestion is further supported by the finding that, with the

exception of enflurane, the model displayed the appropriate order of potencies. Given these observations, it appears that this model may be relevant to the state of anaesthesia in the whole animal. Detailed studies on the membrane concentrations of the volatile anaesthetics should resolve the apparent discrepancy between the potency of enflurane in the *in vitro* model compared to the whole animal.

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