THE UNIVERSITY OF CALGARY

THE EFFECTS OF PENTOBARBITAL ON IMMATURE HIPPOCAMPAL SLICES

BY

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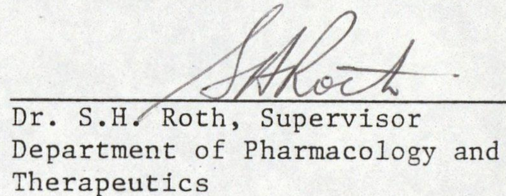

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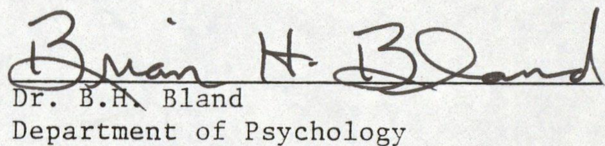

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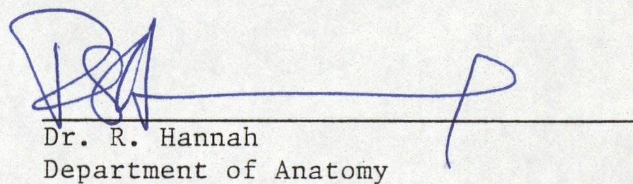

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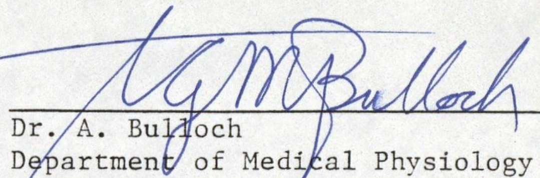

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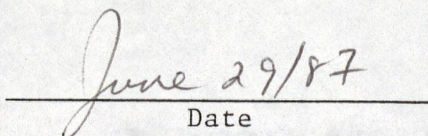

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ABSTRACT

There is a lack of information concerning the actions of neuroactive, pharmacological agents on the immature CNS. It has been shown that immature animals exhibit an increased sensitivity to barbiturates, however, a well defined quantitative study of dose-response relationships has not been reported. The purpose of the present thesis was to develop a model system to examine the physiological and pharmacological responses of the immature CNS to pentobarbital using electrophysiological techniques. The rat *in vitro* hippocampal slice was chosen as the preparation for this study.

Differences were noted between mature (2-3 months postnatal) and immature (14-16 days postnatal) animals for both control and drug studies. Control input-output relationships for mature preparations were stable over a three hour period, whereas for immature preparations this relationship was variable. Stimulus-response relationships for both mature and immature preparations had similar profiles, however, for immature preparations this relationship decreased more rapidly as compared to mature preparations. That is, over a shorter period of time a larger stimulus input was necessary to evoke a constant postsynaptic output for the immature preparations. Bursting activity was recorded from immature slices, suggesting that inhibitory synaptic circuitry was not completely developed in the immature hippocampus.

Pentobarbital produced biphasic actions on mature population spike responses; at lower concentrations (0.04 mM to 0.1 mM) an increase in activity occurred, at higher concentrations (0.2 mM to 0.4 mM) a decrease in activity was observed. In contrast, pentobarbital, at all

concentrations studied (0.02 mM to 0.4 mM) was found to only decrease population spike amplitudes in immature preparations. For both mature and immature preparations, pentobarbital was found to produce a reversible decrease in the second positive component of the field potential that had not been previously reported. On input-output curves from mature preparations, pentobarbital, at low concentrations (0.04 mM to 0.08 mM), produced an increase in EPSP slope and population spike amplitude. At higher concentrations (0.2 mM to 0.4 mM) a decrease in both EPSP slope and population spike amplitude was observed. Bursting activity in immature preparations was unaffected in the presence of pentobarbital.

The results of the present thesis demonstrated that pentobarbital had significantly different effects on immature hippocampal slice preparations as compared to mature preparations. These results are in agreement with previous *in vivo* studies that demonstrated an increased sensitivity in immature animals to the depressant effects of pentobarbital. An *in vitro* preparation can, therefore, be used to investigate the differences seen in response between the mature and immature CNS to neuroactive agents.

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I. INTRODUCTION

A. Drug Effects on the Immature Central Nervous System

It is not uncommon for therapeutic agents to be administered throughout the developmental period ranging from gestation to maturation. This results in both pre and postnatal exposure to pharmacological agents. A question that arises is: how does the immature central nervous system (CNS) respond to these drugs and how does this response differ from that of the mature system? There is very little information available to answer this question and well defined quantitative studies of dose-response relationships have not been reported.

Many pharmacological agents administered during pregnancy can readily cross the placenta and have adverse effects on the neonate (Finnegan, 1976). In a report by O'Connor et al. (1981), the newborn of a woman who had been taking the antipsychotic agent, chlorpromazine, throughout pregnancy exhibited many neurological signs including excessive crying, irritability and spastic movements of upper limbs, all of which persisted for nine months. In addition to apparent neurological abnormalities produced by many agents, more subtle behavioral deficits can result. For example, pups from female rats given chlorpromazine at various stages in pregnancy demonstrated significantly slower maze learning skills when compared to controls (Hoffeld and Webster, 1965). In another study, Abel and Sherwin (1983) found that offspring from rats given alcohol throughout gestation performed less effectively than controls on a shock avoidance task.

These investigators also analyzed neuroanatomical differences and found that the hippocampi of treated pups had reduced dendritic structures (Abel and Sherwin, 1983). In two studies reported by Hannah et al. (1982a,b), it was found that perinatal exposure to chlorpromazine and phenobarbital resulted in a significant decrease in the number of Purkinje cells and alterations in vasculogenesis in the cerebellum of rats.

In addition to prenatal (in utero) exposure, infants and children are often prescribed or indirectly exposed to a number of neuroactive agents to which they respond very differently as compared to adults (Nyhan, 1961). It has been reported that the antiepileptic agent sodium valproate induces a comatose state more commonly in children than in adults (Janssen et al., 1985). These authors also reported a case of sodium valproate overdose in an infant where neuronal damage occurred in a number of brain areas including the inferior olive, thalamus, dentate region, purkinje cells and cerebral cortex. The CNS stimulant, nicotine, when administered postnatally to rats was found to decrease serotonin levels in the cerebellum and cerebral hemispheres in animals 12 and 22 days old as compared to animals 60 days old (Hudson et al., 1974). Catecholamine synthesis, storage and degradation develop later in maturation, and as a result, agents which affect these transmitter systems can produce greater effects in the immature system (Mirkin, 1970). As a result, neonates demonstrate a greater sensitivity to the drug reserpine which depletes catecholamines, and this sensitivity decreases with increasing age (Mirkin, 1970). The effects of the antipsychotic agent, haloperidol, were compared on mature and immature dogs (Himwich and Davis, 1974). In the adults, haloperidol was found to

decrease dopamine levels and produce behavioral changes, whereas in the young animals dopamine levels remained constant. In the same study, it was also reported that differences in amino acid content, following administration of haloperidol, existed between the mature and immature animals. Another class of neuroactive drugs, antihistamines, have been shown to produce a number of effects on the CNS which include, antiserotonergic actions, inhibition of dopamine uptake and blockade of cholinergic muscarinic and alpha adrenergic receptors (Schuller and Turkewitz, 1986). Of particular interest, it was pointed out that in children the most common side effect from these agents was stimulation compared to physiological depression reported for adults.

A number of drugs can accumulate in breast milk, and as a result, neonates can be exposed unintentionally to a variety of neuroactive agents that could have detrimental effects. For example, drowsiness in a nursing infant of an epileptic mother who had been taking phenobarbital was reported by Ananth (1978). In the same report it was noted that diazepam, reported to distribute into breast milk, also caused central depression in a nursing infant. Haloperidol and the related drug penfluridol were found to cause behavioral abnormalities in nursing rat pups which included impaired acquisition of a conditioned avoidance response (Ananth, 1978).

In a review by Pruitt (1983) the problems involved with treating neonates pharmacologically for drug withdrawal as a result of _in utero_ drug exposure were discussed. To treat narcotic withdrawal in the neonate, the opiate, paregoric, has been used; however, seizures and CNS depression were described as adverse side effects. Diazepam was also noted in this report to be an agent used to treat withdrawal symptoms in

the neonate, however, this agent caused late-onset seizures in the newborn.

What becomes apparent from the above observations is that the immature CNS responds very differently than the mature to neuroactive pharmacological agents. The reasons given for these differences, in some cases, have been related to specific mechanisms; for example, altered neuroanatomical structures (Abel and Sherwin, 1983) and differences in neurotransmitter levels (Hudson et al., 1974). However, for most reported observations a well defined explanation has not been provided. What is required is a better understanding of the physiological and pharmacological responses that underlie the differences in sensitivity seen between the mature and immature CNS in response to neuroactive agents.

B. Development

1. Central nervous system

The event of neurulation marks the initiation of nervous system development in the embryo (Purves and Lichtman, 1985). This involves the formation of the neural groove in the ectoderm. As the groove widens, the neural plate is created and the neural folds, ridges at the margin of the neural plate, are formed. The neural folds fuse to create the neural tube. The spinal cord and brain develop from the neural tube and its anterior widening portions respectively. For development of the brain, three distinct swellings become apparent at the anterior end of the neural tube: the forebrain, midbrain and hindbrain vesicles. These

regions give rise to the cerebral hemispheres, the midbrain and hindbrain in the adult animal.

The differentiation of these areas results from cell division occurring at the inner surface of the wall of the neural tube, termed the ventricular zone. As cells begin to enter their mitotic phase they draw in their peripheral processes and move to the inner surface of the ventricular zone. Following mitosis the peripheral processes reform and the cells move to the outer region of the ventricular zone, the intermediate zone. From this point, cells that have lost their capacity to further divide migrate superficially to their final destinations in the brain (Cowan, 1979). During this migratory period considerable cell growth takes place as reported as an increase in surface area during elongation of cells to bipolar shapes. In addition, migrating neurons are associated with radially oriented fibers, radial glia, which have been suggested to guide the moving neurons to their final destination within the brain. In the cerebral cortex, the first neurons to arise migrate the shortest distance to remain in the deeper layers of the cortical plate. Later forming neurons migrate past these cells to occupy the more superficial layers. This type of neuroembryogenesis has been termed the "inside-out" pattern of development (Rakic et al., 1974).

2. Hippocampus

(a) Anatomical development of hippocampus

The hippocampus is a part of the archicortex of the brain,

intermediate in evolutionary development between the paleocortex and neocortex. It is a well organized structure that together with the dentate gyrus forms an S-shaped structure termed the hippocampus derived from the sea horse or Ammon's (ram) horn (Shepherd, 1979).

Neuronogenesis in the developing hippocampus, resulting from cell proliferation in the lateral ventricle, has been well described due to the neuronal orderliness and well defined boundries of this cortical structure. There exist very ordered patterns of neuronogenesis in the hippocampus and these patterns of development are different for different cell populations. Using ^{3}H thymidine labelling techniques the birthdates and patterns of migration of cells has been studied in a number of different animals (see Angevine, 1975). Initial studies done in the mouse demonstrated that the first neurons arise on embryonic day 10, located in the molecular and hilar regions of the dentate gyrus and in the superficial layers of the pyramidal cell region. By embryonic day 18 neuronogenesis has ended in these regions, while in the granule cell layer of the dentate gyrus cell production has begun and continues onto postnatal day 20 (Angevine, 1965). At birth then, neuronogenesis in the pyramidal cell region is completed, while in the dentate region granule cells continue to be produced postnatally. This postnatal development of granule cells has also been described for other brain regions (Altman and Das, 1966; Altman, 1966). Another difference between these two cell types in hippocampal neuronogenesis is that the pyramidal areas develop in an "inside-out" pattern, whereas the granule cell layer develops in an "outside-in" pattern.

This very specific organization of development was later described for the rat hippocampus (Hine and Das, 1974; Schlessinger et al., 1978).

In the rat, the development of the hippocampus is the same as described for the mouse, but occurs 4 - 6 days later. In the pyramidal cell region neuronogenesis begins on embryonic day 16 and ends by embryonic day 20. Neurons in the CA3 region are the first of the pyramidal cell population to appear and neuronogenesis in the CA2 region ends before that in the CA1 and CA3 areas. In addition, large interneurons in the strata oriens, radiatum and lacunosum-moleculare appear 1 to 2 days before neuronogenesis begins in the pyramidal region. This "sandwich gradient" form of development is characteristic for the developing hippocampus (Bayer, 1980). It is also seen in the dentate gyrus where the granule cells appear after larger cells in the hilus and molecular layer. In the dentate gyrus, cells in the hilar region appear at embryonic day 15 while labelled cells in the granule layer are seen on embryonic day 20 to 22 and again neuronogenesis in this area continues into the third week postnatal (Schlessinger et al., 1975). The first cells to be formed in the dentate region probably arise from the ventricular zone while the cells that are produced later in this region come from a proliferative center in the hilus (Gaarskjaer, 1985).

There is a considerable amount of postnatal neuronogenesis occurring in the hippocampus. Small cells in the Ammonic strata oriens, radiatum and lancunosum moleculare are formed during the second week postnatal, and in the dentate gyrus the granule cell population increases by 85% postnatally in rats (Bayer and Altman, 1974). A cell count done in the dentate gyrus 3 months after birth demonstrated a six-fold increase in the number of differentiated granule cells (Altman and Das, 1965). The differentiation of this cell type, in terms of synapse formation, was found to reach adult levels by day 25 postnatal,

yet complex spines as seen in mature animals were still not apparent at this age (Crain et al., 1973; Cotman et al., 1973). In relation to these studies it has also been reported that there is an increase and decrease in the number of polyribosomes that parallels the time of synapse formation in the dentate gyrus (Steward and Falk, 1985).

Neuronogenesis takes place both prenatally and postnatally in the developing hippocampus (Hine and Das, 1974). The extent of pre and postnatal neuronogenesis varies with different cell types. Differentiation also occurs to a great deal postnatally. This has been discussed above for the dentate region, and in the following section postnatal differentiation of pyramidal cells will be considered.

(b) Physiological development of the CA1 pyramidal region

There have been a number of studies done on the physiological responses of the developing hippocampus. One of the first was done on kitten hippocampus (Purpura et al., 1968). Intracellular recordings in response to fornix stimulation from kittens 1 to 18 days postnatal revealed that inhibitory postsynaptic potentials (IPSP) activity was the most predominate type of synaptic response observed in the youngest animals. This inhibitory activity was found to reach adult form by the end of the first postnatal week, whereas excitatory postsynaptic potentials (EPSP) activity did not become effective in producing cell discharges until the second to third week postnatal. This study was carried out on kittens anesthetized with pentobarbital. Subsequently, an *in vitro* study was done on developing kitten hippocampal neurons (Schwartzkroin and Altshuler, 1977). CA1 pyramidal cell responses to

orthodromic stimulation from the stratum radiatum revealed a complex EPSP-IPSP sequence. In agreement with the earlier study it was reported that IPSP activity was the synaptic event seen most in response to stimulation. In addition to this, however, cell bursting, an increase in EPSP potentiation, increased cell spiking triggered by orthodromic stimulation and an increase in baseline noise perhaps due to excitatory input onto dendritic processes were all reported for the younger animals. In this study, therefore, it appeared that in addition to potent IPSP activity, excitatory actions were also playing an active role in the immature hippocampus.

In vitro studies on developing hippocampus were than carried out in the rabbit and rat preparations. One common finding that became apparent from these investigations was that of late development of inhibitory activity. Postnatal development of the rabbit CA1 area was followed anatomically and electrophysiologically in the pyramidal cell layer (Schwartzkroin et al., 1982; Schwartzkroin, 1982). From the anatomical study it was found that symmetric, axosomatic synapses, believed to mediate inhibition on pyramidal cells, were not apparent until 2 weeks postnatal (this had also been reported for kitten hippocampus, Purpura, 1968). The electrophysiological data revealed that IPSP activity was also not apparent until the second week postnatal. Together these studies demonstrated that inhibition was not present in the immature rabbit hippocampus until the second postnatal week.

In another study on immature rabbit hippocampal slices, spontaneous and stimulus evoked spreading depression (SD) was reported for the CA1 and CA3 regions (Haglund and Schwartzkroin, 1984). It was found that in

the immature animals SD occurred more frequently than seen in mature animals, and that the CA1 region was more susceptible to SD episodes than the CA3 region. It was suggested that due to the absence of mature IPSPs SD episodes occur in the immature slice with the CA1 area lagging in development of inhibitory systems as compared to the CA3 region. Evidence for late development of inhibition in the CA1 pyramidal region has also been described for rats (Harris and Teyler, 1983). When looking at paired-pulse inhibition, which is indicative of recurrent inhibition on pyramidal cells, it was found that paired-pulse inhibition was not observed before postnatal day 6. In addition, spontaneous background firing activity, described as a developmental form of seizure activity, was observed. Again these results indicate that in immature rat hippocampal slices there is a late development of inhibitory innervation on CA1 pyramidal cells.

To further characterize the mechanisms underlying this phenomenon, Mueller et al. (1983) investigated the actions of GABA on immature rabbit slice preparations. They found that GABA produced a depolarizing response at the dendrites and a hyperpolarizing response at the cell body on mature slices. In the immature slices, however, only a depolarizing response could be seen independent of the site of application. In a follow up study using the GABA hyperpolarizing agonist, 4,5,6,7-tetrahydroisozazolo[5,4-C]pyridine (THIP), and the GABA depolarizing antagonist, bicuculline, the development of inhibitory activity was further studied (Mueller et al., 1984). THIP was found to produce a depolarization at the cell body region and bicuculline was found to be effective in blocking the depolarizing response in the immature preparations. It was hypothesized that two different GABA

receptors, one coupled to a depolarizing ionophore (permeable not only to Cl, but to Na and Ca as well), and one coupled to a hyperpolarizing ionophore, exist in the mature preparation on the dendritic and cell body regions respectively. In the immature the depolarizing receptors appear to be located over the entire cell, and with maturation, hyperpolarizing receptors develop on the soma and depolarizing receptors remain in the dendritic region. The GABA synthesizing enzyme glutamic acid decarboxylase (GAD) was examined in relation to synaptic development of inhibitory interneurons in the developing rabbit hippocampus (Kunkel et al., 1986). Interneurons are present very early in development, yet as described above, IPSP activity does not become apparent until later in development. In the study by Kunkel et al. (1986), using immunocytochemical techniques, the question of whether or not GABA was being produced in these interneurons in immature animals was investigated. GAD was found to be present in the youngest animals studied (8 days postnatal), yet the synaptic profiles (synaptic vesicles, pre and postsynaptic densities) were not as well developed as those seen for the mature animals. This suggests that in the younger animals an immature form of inhibitory synapses may underlie the late development of inhibition reported.

Another mechanism may be involved in these differences seen in synaptic events. Na,K-ATPase activity was investigated in immature rabbit hippocampus (Haglund et al., 1985), and was found to be higher in the CA2/CA3 region earlier in development as compared to the CA1 area. It was suggested that due to a lack of complete Na,K-ATPase activity, extracellular K levels could not be regulated resulting in an increase in excitatory activity. These findings could explain a mechanism for

the seizure activity in immature hippocampus, and also the differences in susceptibility to SD episodes between the CA3 and CA1 region.

An additional factor that may play a role in these differences between the mature and immature hippocampus is the activity of second messenger systems. In a study by Nicolletti et al. (1986), it was reported that in immature rat hippocampal slices, phosphatidylinositol 4,5 biphosphate (PIP2) hydrolysis was stimulated by a number of excitatory amino acids; however, by day 24 postnatal this activation ceased. It was also noted in this study that stimulation of PIP2 hydrolysis by NE was lower in the immature preparation and increased to adult levels by day 35 postnatal. Following hydrolysis of PIP2, intracellular Ca is released and protein kinase C is stimulated resulting in alterations of cellular responses (see Berridge, 1985). Either inhibition or enhancement of second messenger intracellular reactions in the immature hippocampus may be involved in a number of different processes such as synaptogenesis and receptor maturation.

From the above discussion it becomes apparent that the immature hippocampus is physiologically very different from the mature hippocampus. In rat and rabbit preparations it is well documented that a late development of inhibitory activity occurs during the maturation of the system. Given that such physiological differences exist between the mature and immature hippocampus, one would expect a unique or different response of the immature preparation to pharmacological agents.

C. Pentobarbital

1. Effects on mature preparations

Barbiturates produce a spectrum of effects ranging from convulsant to anesthetic actions. Pentobarbital, a prototype barbiturate, possesses anticonvulsant properties, but is most effective as a sedative-hypnotic and general anesthetic agent (Harvey, 1985). Postsynaptic, presynaptic and nonsynaptic sites have all been identified as being modified by pentobarbital and a number of different mechanisms have been postulated to underlie its actions.

Depression of synaptic transmission has been the most cited mode of action for anesthetics. It was suggested that this depression resulted from a nonspecific block of impulse conduction in axons. It has been demonstrated, however, that synaptic depression is observed in concentrations of drug that are not sufficient to block impulse conduction in axons (Richards, 1983). The actions of pentobarbital, as well as other general anesthetics, have been proposed to be due to more specific effects on chemical transmission.

At postsynaptic sites pentobarbital has been shown to produce specific effects on IPSPs (Judge, 1983). From _in vivo_ studies on hippocampus, pentobarbital was found to hyperpolarize hippocampal neurons, increase the half decay time and amplitude of the IPSP, increase the inhibitory period following orthodromic stimulation and increase paired-pulse inhibition intervals (Nicoll et al., 1975; Wolf and Hass, 1977). _In vitro_ studies confirmed these earlier _in vivo_ findings and provided more information as to the possible site of drug

action. In CA1 pyramidal cells pentobarbital was again shown to enhance IPSP activity, but it was found to have a greater effect on the dendritic IPSP than the somal IPSP. The drug effects were blocked by bicuculline and picrotoxin indicating GABA receptors as the site of action (Alger and Nicoll, 1982a,b). Other _in vitro_ studies demonstrated that pentobarbital prolonged and potentiated IPSPs and it was suggested that this was due to an increase in the Cl channel opentime (Collinridge et al., 1984; Dingledine and Korn, 1985). An increase in Cl conductance in rat spinal cord cultures was seen in the presence of pentobarbital and the anticonvulsant barbiturate phenobarbital. The concentration of phenobarbital necessary to elicit the conductance increase, however, was much higher than that for pentobarbital (Shulz and MacDonald, 1981). This increase in Cl conductance has been shown to be due to an increase in the mean opentime of the Cl channel (see Simmonds, 1983). By studying membrane current variance it was demonstrated that the Cl conductance that is increased by pentobarbital appears to be the same as that seen in response to GABA stimulation (Barker and Mathers, 1981). The effects of pentobarbital on different channel types have also been studied on invertebrate models because of the advantage of possessing simplified neuronal systems. In Aplysia neurons, pentobarbital decreased the amplitude, time to peak and half decay time of a Cl dependent IPSP that was elicited by acetylcholine (ACh), (Adams et al., 1982). It was hypothesized that this was due to the drug blocking the open Cl channel. In addition, ACh excitatory responses mediated by Na ions were also depressed while K dependent inhibitory responses were unaffected by drug exposure (Judge, 1983).

Pentobarbital has also been shown to induce changes in K

conductances (O'Bierne et al., 1987; Carlen et al., 1985). A study on the *in vitro* hippocampal preparation has revealed that there is an increase in the Ca dependent K conductance that mediates neuronal after-hyperpolarizations and the late component of the IPSP in response to sedative concentrations of pentobarbital, 10 uM to 30 uM (O'Bierne et al., 1987). At higher concentrations, 100 uM, an increase in the GABA mediated IPSP at the dendritic region was observed, and it was suggested that this may reflect anesthetic actions (Carlen et al., 1985). In addition, Ca spikes were abolished by pentobarbital suggesting that the increase in K conductance resulted from an increased release of intracellular Ca (Carlen et al., 1985).

The effects of barbiturates on the synthesis, release and brain levels of a number of different transmitters have been studied both *in vivo* and in *in vitro* systems (Richter and Holtman, 1982). As reviewed by Richter and Holtman (1982), a number of studies have demonstrated that pentobarbital causes a decrease in turnover of ACh, serotonin (5-HT), and GABA. For ACh and 5-HT the decrease was suggested to reflect a decrease in activity of these neuronal types, while for GABA the decrease reflected a compensatory inhibition produced by pentobarbital. Transmitter actions on postsynaptic membranes has also been investigated. Anesthetic concentrations of pentobarbital, 100 uM to 300 uM, were found to depress the excitatory glutamate response seen in olfactory cortical cells. This action occurred in the presence of high Mg suggesting a postsynaptic site of action (Richards and Smaje, 1976). Cerebral cortical neurons were found to be depressed in response to norepinephrine (NE) and 5-HT in cats anesthetized with pentobarbital, whereas in animals anesthetized with N_2O these transmitters were found

to increase activity on the same cells (Johnson, 1969). In invertebrate preparations, pentobarbital was shown to selectively depress excitatory postsynaptic potentials, EPSPs (Barker and Gainer, 1973). These EPSPs reflected glutamate, ACh and Na dependent excitation. In addition, pentobarbital was found to be without effect on IPSP activity resulting from GABA and dopamine mediated by K and Cl conductances.

Actions of pentobarbital on presynaptic transmitter responses have also been reported (Richards, 1983). In rat olfactory cortical slices pentobarbital was found to inhibit aspartate and taurine release and enhanced the GABA inhibition of release of these amino acids (Collins, 1980). These actions were not blocked by picrotoxin demonstrating that some action other than GABA inhibition of release was taking place. In the cat spinal monosynaptic pathway, pentobarbital caused a decrease in the mean quantum content, indicative of presynaptic transmitter release, while there was no change produced in the postsynaptic "unit" EPSP (Weakly, 1969). In frog spinal pathway, pentobarbital was found to cause a depolarization of primary afferents similar to that seen with GABA (Nicoll, 1975). These afferents mediate presynaptic inhibition in sensory pathways. This effect was blocked by picrotoxin indicating that these effects were mediated at GABA receptors.

A mechanism that may underlie this presynaptic inhibition is the blockage of Ca currents seen in the presence of pentobarbital. In mouse brain synaptosomes, pentobarbital inhibited Ca accumulation in response to K depolarization (Harris and Stokes, 1982). In addition, there was an increase in Ca efflux in the presence of the drug suggesting that the decreased accumulation was in part due to this efflux. In culture preparations of hippocampal and spinal neurons, low concentrations of

pentobarbital, 10 uM, inhibited the triggering of Ca spikes (Owen et al., 1986). In another study on mouse spinal cord cultures, MacDonald et al. (1986) found that pentobarbital inhibited Ca dependent action potentials in the presence of cesium, which blocks K channels, demonstrating that the inhibition of Ca currents was not due to an inward K current block, but due to an effect on the Ca channel itself. Interestingly, pentobarbital effects on Ca currents were seen at higher concentrations of drug than GABA mimetic effects. It was suggested that GABA responses at lower concentrations may be responsible for anticonvulsant actions while Ca effects at higher concentrations may reflect anesthetic actions.

It becomes apparent from the above studies that pentobarbital produces a number of actions on neurons that appear to be acting on selective sites. This point is exemplified when one looks at the stereoselective effects of this agent. The R (+) isomer has been associated with excitatory effects, while the S (-) isomer has been associated with depressant effects (Ho and Harris, 1981). The (+) isomer increases CNS excitability at lower concentrations and depresses it at higher concentrations, while the (-) isomer produces depression independent of concentration (Barker and Mathers, 1981). In a study by Roth et al. (1986) the (+) isomer was found to be more effective on an isolated sensory neuron and on hippocampal slice preparations than the (-) isomer. In addition, both an increase and a decrease on the same synaptic pathway was produced in a concentration dependent way and differential effects were observed on specific pathways within the hippocampus (MacIver and Roth, 1987a). From this it was concluded that

pentobarbital produces selective actions at specific membrane recognition sites.

Barbiturates may also act at nonsynaptic sites of the neuronal membrane. This could involve altering threshold depolarization for action potential generation and/or changing resting conductance of the membrane, thereby altering electrotonic conduction. In support of this, studies have shown that barbiturates cause an increase in resting membrane conductance (see Richards, 1983).

2. Effects on immature preparations

Studies have demonstrated that younger animals are much more sensitive to the depressent actions of barbiturates than adult animals (Mirkin, 1970). This increased sensitivity appears to be inherent in the immature CNS.

The concentration-response relationship of pentobarbital on induced sleeptime was compared between immature (1-20 days postnatal) and mature (15 weeks postnatal) rats (Bianchine and Ferguson, 1967). Immature animals were found to be more sensitive to drug effects. For example, newborn, 5 day and 10 day old animals were found to be 3, 2 and 1.5 times more sensitive as compared to adult animals. When brain levels of pentobarbital were compared no difference in content existed that could explain the increased sensitivity observed. This suggested that the increased sensitivity was due to an increased sensitivity of the immature CNS itself. Similar results were reported on the effects of the barbiturate, hexobarbitone, on mature and immature animals (Kalser et al., 1968). Again, the immature were found to be much more sensitive

to the drug induced sleep than the mature animals. In addition, the metabolic rate of immature animals was found to be much lower, however, the difference was not great enough to explain the increased sensitivity to hexobarbitone. This again suggested that the differences in response observed were due to an increased sensitivity of the immature CNS.

In a later study, the effects of pentobarbital were investigated directly on the immature CNS in the dentate region of the hippocampus *in vivo* (Wilson and Racine, 1985). Pentobarbital was reported to increase paired-pulse depression in both mature and immature (14 days postnatal) rats, but for the younger animals this depression was greatly enhanced. It was suggested that this increase in depression resulted from an increased sensitivity of the K mediated afterhyperpolarization and the Cl mediated, GABAergic recurrent inhibition in the immature CNS.

These studies demonstrate that in the whole animal the immature are much more sensitive to the actions of barbiturates, and that the CNS appears to be the site that underlies this increased sensitivity. The actual sites of action on a specific synaptic pathway and the effective concentrations at these sites, however, have not been investigated. This type of information could be gained from an *in vitro* study on a well defined system of the immature CNS.

D. Hippocampal Brain Slice as a Model System

The synaptic circuitry of the hippocampal formation including afferents, efferents and intrinsic connections have been well characterized as described in Grays Anatomy (1980). The hippocampus receives afferent input from a number of different sources. Fibers from

the lateral part of the entorhinal cortex enter the hippocampus through the perforant path to synapse on cells in the dentate gyrus and pyramidal cells at the level of the molecular layer. Afferents from the medial entorhinal cortex and commissural fibers from the contralateral hippocampus enter through the alveus to make contacts in the stratum oriens. Other commissural and septal inputs enter the hippocampus via the fornix. Efferent fibers projecting to the septum, thalamus, hypothalamus and contralateral hippocampus leave via the fimbria to travel through the fornix. Septal inputs to the hippocampus are cholinergic while perforant path synapses are glutaminergic (Shepherd, 1979). In addition, 5-HT fibers from the raphe nuclei, noradrenergic fibers from the locus coeruleus and dopaminergic fibers from the midbrain also project onto the dentate and pyramidal cell regions. These transmitters have been reported to decrease neuronal activity in the hippocampus (Storm-Mathisen, 1977; Langmoen et al., 1981).

Within the hippocampus itself exist a synaptic chain consisting of three serial connections (see Figure 1). Input from the perforant path fibers synapse on granule cells of the dentate gyrus whose projections form the mossy fibers that synapse on the CA3 pyramidal cells. The axons of these cells bifurcate, one branch leaving the hippocampus via the fimbria while the other contributes to the Schaffer collateral fibers which synapses on the apical dendrites of the CA1 pyramidal neurons. Axons from the CA1 neurons leave the hippocampus via the alveus and fimbria to project to other brain regions as described above (Andersen, 1982). Both the mossy fiber synaptic contact onto CA3 pyramidal cells and the Schaffer collateral synapses on CA1 pyramidal neurons are excitatory (Storm-Mathisen, 1977). For the dentate granule

cells and CA1 pyramidal cells inhibition is mediated by GABA via recurrent collaterals and basket cell activation (Schwartzkroin and Knowles, 1983; Wilson and Racine, 1985).

E. Purpose of Study

There is a lack of information regarding the actions of neuroactive agents on the immature CNS. Studies have described behavioral and anatomical effects of drugs on the young, however, little physiological data has been reported. The purpose of the present study was to examine the physiological and pharmacological responses of the immature CNS in the presence of a neuroactive drug using electrophysiological techniques.

F. Hypothesis

The working hypothesis was: The immature CNS is more susceptible than the mature CNS to neuroactive drugs and this can be demonstrated in an *in vitro* preparation.

G. Specific Objectives

1. To establish viable rat brain slice preparations of mature (2-3 months postnatal) and immature (14-16 days postnatal) hippocampus.
2. To examine and compare the response characteristics of the mature and immature preparations to electrical stimulation using extracellular recording techniques.

3. To examine the effects of pentobarbital on both mature and immature preparations, and to generate and compare concentration response curves of pentobarbital on both.

H. Rationale

1. Hippocampal brain slice as a model system

The hippocampal brain slice was chosen for this study because of the many advantages it offers. When compared to cell culture and *in vivo* preparations, the slice falls somewhere in between possessing the better attributes of both (Dingledine et al., 1980). The electrophysiological responses have been well characterized and appear to be similar to those reported *in vivo* (Teyler, 1984).

For this study an *in vitro* preparation offers a number of advantages over an *in vivo* preparation. For example, visualization of distinct fiber pathways allows for specific positioning of stimulating and recording electrodes. There is also less variability with the *in vitro* preparation, for example, one can maintain constant pO_2, pH and temperature (Schwartzkroin, 1981). The blood brain barrier has been eliminated, thereby allowing for more ways to interfere experimentally with the slice than would be possible with the intact brain (Andersen and Langmoen, 1980). In addition, by using an *in vitro* preparation numerous sites of drug action are eliminated (e.g. systemic sites of drug metabolism), therefore, known concentrations of drug can easily be applied. This final point provides the greatest advantage offered by an *in vitro* preparation for this study due to the extremely different

metabolic reactions in the whole animal between mature and immature systems.

2. Choice of drug for this study

Pentobarbital has been well studied in the central nervous system, and in particular its effects have been examined on the hippocampus of mature animals. It is a prototype barbiturate that has been widely used and is administered to infants and children as a sedative to treat a number of disorders (Harvey, 1985). In addition, pentobarbital has been shown to produce different effects on mature and immature hippocampus *in vivo* (Wilson and Racine, 1985).

Pentobarbital produces a number of effects on neuronal inhibitory systems. Given that these inhibitory systems are not completely developed in the immature rat hippocampus, it is appropriate to use pentobarbital in this study to observe the differential responses between the mature and immature CNS.

II. METHODS

A. Dissection/Preparation

Rats were first exposed to diethyl ether until unconscious, then rapidly weighed. The heart was stopped by a blow to the back of the thorax to minimize bleeding for the remainder of the dissection. Using scissors, the scalp was removed and an opening was made up the midline of the skull. In mature animals rongeurs were used to remove the overlying cranium taking care to leave the dura intact. In immature animals, because the skull was quite pliable, small scissors were used to cut completely around the lateral border of the skull. The cut out area was then easily removed using tweezers, again leaving the dura intact. Using a scalpel the dura was removed and the olfactory nerves were severed. A sharpened, stainless steel spatula was then used to remove the brain rostral to the cerebellum and to transfer it to a petri dish containing cold, preoxygenated artificial cerebral spinal fluid (ACSF) solution (see: Materials for ACSF composition). The brain was cut sagitally down the midline, and the hemisphere that had incurred the least damage during the dissection was transferred to moistened filter paper. To expose the hippocampal-dentate formation, the connections to the septal and temporal poles of the hippocampus were severed and the ventral surface was freed by removing the thalamus. The hippocampus was then gently separated from the overlying neocortex at the surface of the alveus along the floor of the lateral ventricle. The entire hippocampus was placed on the stage of a tissue chopper (Univ. of Calgary, Technical Services), moistened with ACSF and positioned with a small brush so that

the tissue could be cut in alignment with fibers on the surface of the alveus. Transverse slices (400 uM) taken from the middle third of the hippocampus were transferred to a petri dish containing ACSF. Six to eight slices were placed in the recording chamber using a wide mouth glass pipette to minimize mechanical damage. The slices were maintained at a gas/liquid interface on a nylon mesh screen at a constant temperature of 35° C in a tissue chamber designed and fabricated by Dr. Per Andersen. The tissue was exposed to warm, humidified oxygen and carbon-dioxide (95/5% carbogen) while the lower surface was submersed in pre-warmed, oxygenated ACSF.

The total time for the dissection was 5 - 8 minutes and for the majority of this time the hippocampal tissue was maintained in ice-cold ACSF. After being placed in the tissue chamber, slices were left to stabilize for 1½ hours before recording.

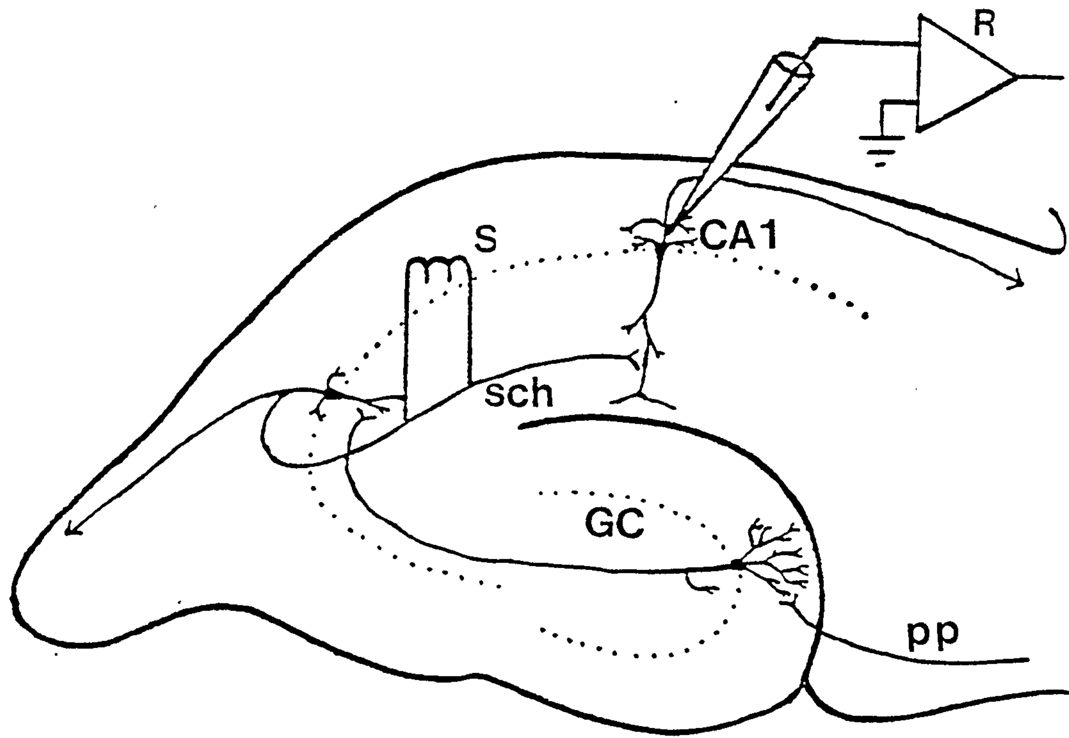
B. Stimulation and Response Parameters

Bipolar metal stimulating electrodes were kindly provided by Dr. Q. Pittman (Univ. of Calgary). The electrodes were made from staiblohm 650 wire coated with H Formvar, size 008 (California Fine Wire Company). They were constructed by coating two pieces of wire in Insulex (Cardinal Industries), polishing the ends and gluing the wires together with Insulex. Extracellular recording electrodes were made from fiber filled glass micropipettes (Kwik-Fil glass capillaries, #1B100F W-P Instruments, Inc., New Haven, Conn.). The micropipettes were pulled using a Narishige (Japan) model 5 pipette puller and back-filled with 2M NaCl; tips were broken off to provide resistances of 2 to 10 Mohm.

Stimulating electrodes were placed in the stratum radiatum, recording electrodes in the stratum pyramidal (area CA1) to record orthodromically evoked field potentials (Figure 1). Both stimulating and recording electrodes were mounted on Narishige (Japan) micromanipulators. Stimulating electrodes were positioned on slices under manual control. Recording electrodes were coupled to the micromanipulator by a Burleigh PZ 550 Inchworm Controller (Burleigh Instruments Inc., Fishers, New York). The electrodes were manually positioned above the tissue and using the Burleigh were slowly advanced until they were in contact with the top of the slice. They were then lowered in 2 uM steps to a depth of 70 - 90 uM from the surface.

Single stimulus pulses ranging from 5 - 26 volts with a duration of 0.25 msec were delivered at a rate of 0.1 Hz using a Grass S-48B stimulator and stimulus isolation unit (Grass SIU 5A). Stimulus intensity was varied to generate input-output relationships between field excitatory postsynaptic potentials (EPSP) and population spikes (PS), termed E-S curves in earlier studies (Andersen et al., 1980). In addition, input-output relationships of stimulus strength and field PS (stimulus-response curves) were produced as had been previously described (Schiff and Somjen, 1985). Signals were amplified (x100) and filtered (0.1 Hz to 10 KHz, bandpass) using a Grass P15B preamplifier. They were further amplified (x20) and DC offset was adjusted to optimize the input requirements of a Digital Equipment Corp. PDP 11/23 minicomputer. This allowed for the storage of signals up to 20 mV in size. For larger field potentials the gain at the second level of amplification was reset to eliminate saturation of the signal into the computer. The waveforms were digitized, using a 12 bit A/D converter with a

Figure 1 - Diagram showing the placement of recording (R) and stimulating (S) electrodes to record orthodromically evoked field potentials in the CA1 pyramidal region. Fiber pathways are labelled as pp - perforant path, GC - granule cell layer and sch - Schaffer collaterals.


R
S
CA1
sch
GC
pp

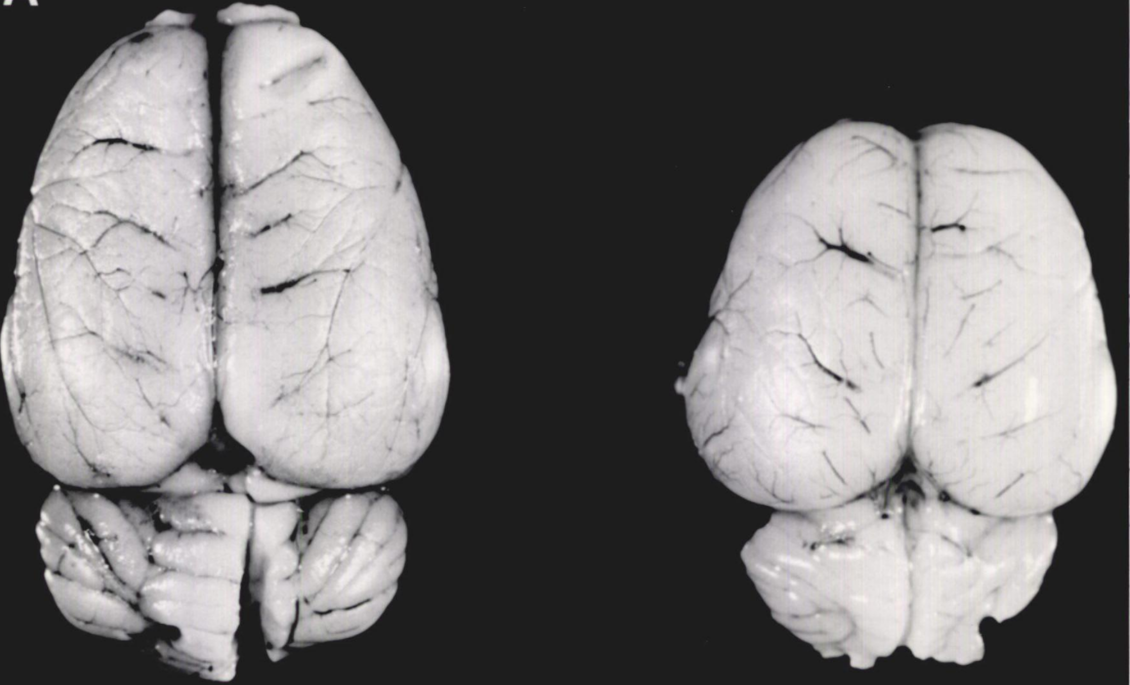
resolution of 40 usec. Throughout the experiment responses were viewed on a Tektronix digital oscilloscope (Tektronix D13, 5D10 waveform and digitizer). All data were stored in the computer for later analysis.

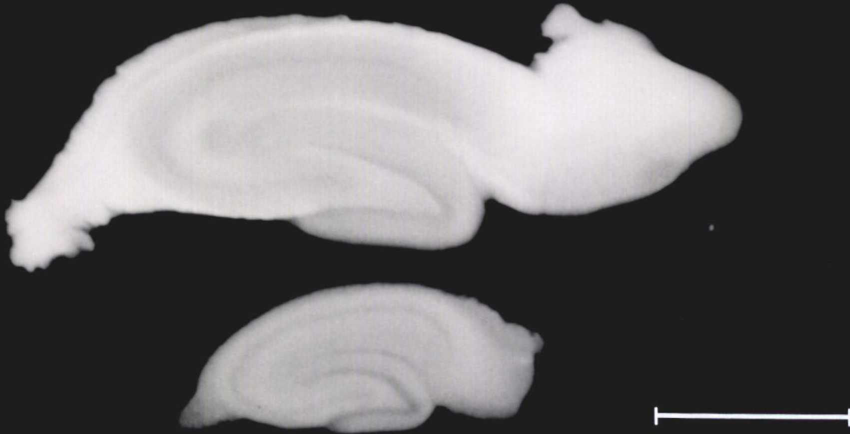
C. Experimental Procedure

Slices were prepared from mature (2 - 3 months, 150 - 300 gm) and immature (14 - 16 days postnatal, 28 - 42 gm) Sprague-Dawley rats (Figure 2). They were left to stabilize following dissection for 1½ hr before recording began. This appeared to be the minimum amount of equilibration time possible prior to recording for obtaining quality responses. In addition, keeping the experimental time to a minimum was crucial due to the possibility of rapid degradative reactions in the immature slices (see: Discussion). During this time the slices were perfused with ACSF at a rate of 1 ml/3 min.

Following this equilibration period the perfusion rate was increased to 2 ml/min and was maintained at this rate for the remainder of the experiment. Stimulating and recording electrodes were positioned as previously described and remained stationary for the duration of the experiment. Once a response could be found a stimulus strength that would produce a half maximal response was maintained for 30 - 40 minutes. After this period, if the response appeared to be stable, control data was collected by varying stimulus strength in 0.5 volt steps going from a stimulus that produced a subthreshold response to one that produced a maximal response. Drug solutions were then substituted for ACSF as the perfusate for 30 minutes, after which the recording protocol was repeated. ACSF was again perfused through until the

Figure 2 - A) Photographs of brains from mature (2 months postnatal) and immature (16 days postnatal) rats x 4.5. B) Hippocampal slice preparations from the brains shown in A. Calibration: 1.5 mm.


A


B

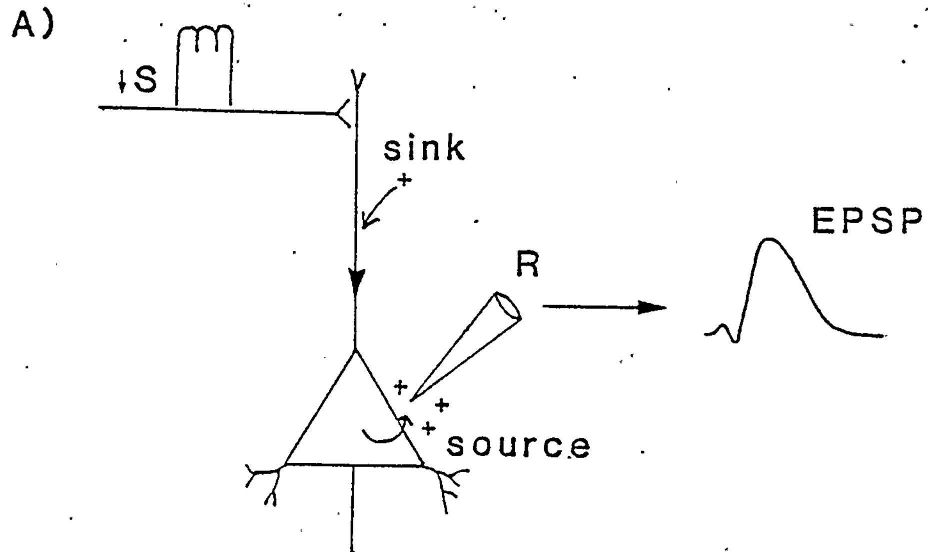
response recovered from drug effects. At this time data was collected and, if possible, another (usually higher) concentration would be evaluated. In the immature preparation, usually only 1 concentration per experiment could be performed due to lack of complete washout and/or decaying response. In the mature preparations, 2 and sometimes 3 doses could be evaluated in one experiment.

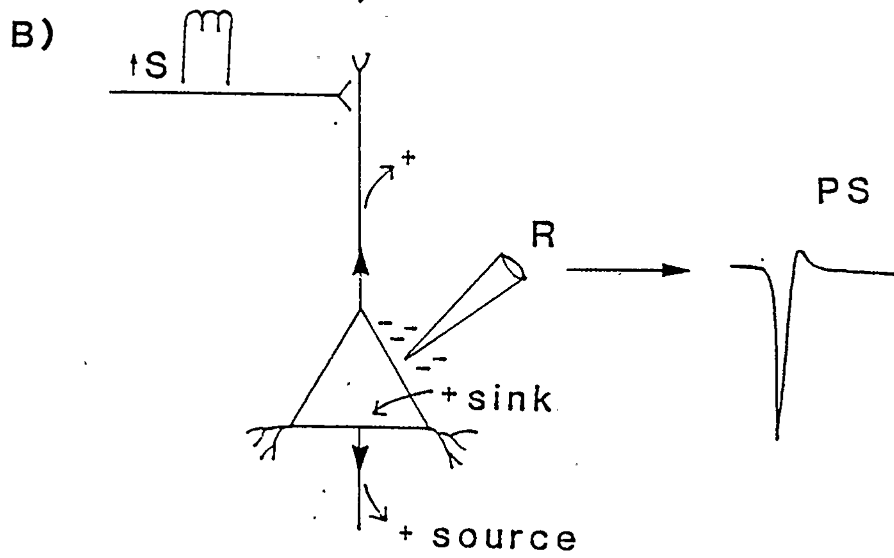
D. Analysis

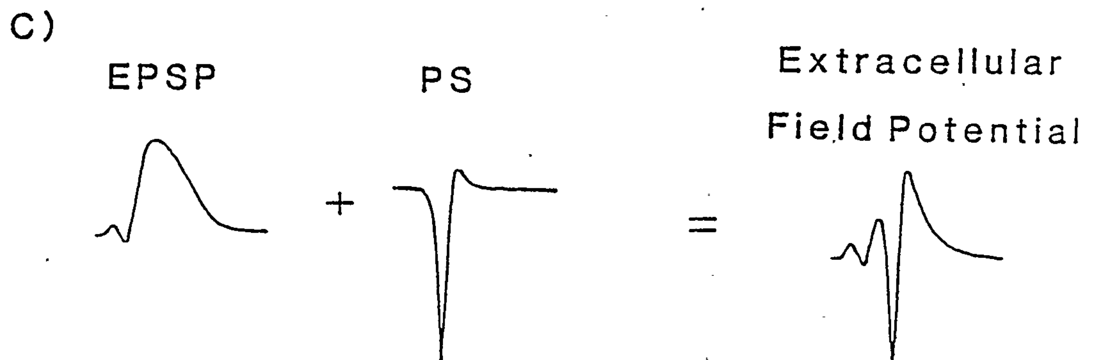
Following orthodromic stimulation CA1 pyramidal cells produce a field potential consisting of a positive going wave, the EPSP and a negative going wave, the PS (Skrede and Westgaard, 1971; Andersen et al., 1971; Figure 3). These waveforms are produced from the summed charge movement of ions across the membranes in a sink-source distribution. At subthreshold stimulus strengths a depolarization occurs at the dendritic region resulting in an influx of Na and Ca ions producing a sink phenomenon that moves out across the somal membrane producing a positive going wave (field EPSP), at threshold positively charged ions move into the cell body region resulting in depolarization and discharge of the cell producing a negative going wave (field PS). The sum of these two waveforms produces the field potential, thus the relationship of drug action with synaptic and/or postsynaptic sites can be evaluated.

The amplitude of population spikes (mV) were measured from the first positive peak, threshold, to the peak negativity. Slope of EPSP's (V/sec) were determined by linear regression through data points from 20% to 80% of maximum value from the first positive going waveform. In

Figure 3 - The generation of field potentials in response to orthodromic stimulation. A) at low stimulus strengths (S) depolarization at the dendritic region results in an outward flow of positively charged ions across the somal membrane, recorded (R) as the field EPSP, B) at higher stimulus strengths positively charged ions move into the somal region producing the field PS, C) the sum of these of two waveforms produces the field potential response.


A)
↓S
sink
+
EPSP
R
+
+
+
source


B)
↑S
+
PS
R
+ sink
+ source


C)
EPSP
PS
Extracellular
Field Potential
+
=

a few experiments the amplitude of the PS was measured from the peak of the second positive going waveform to peak negativity in addition to a threshold PS measurement.

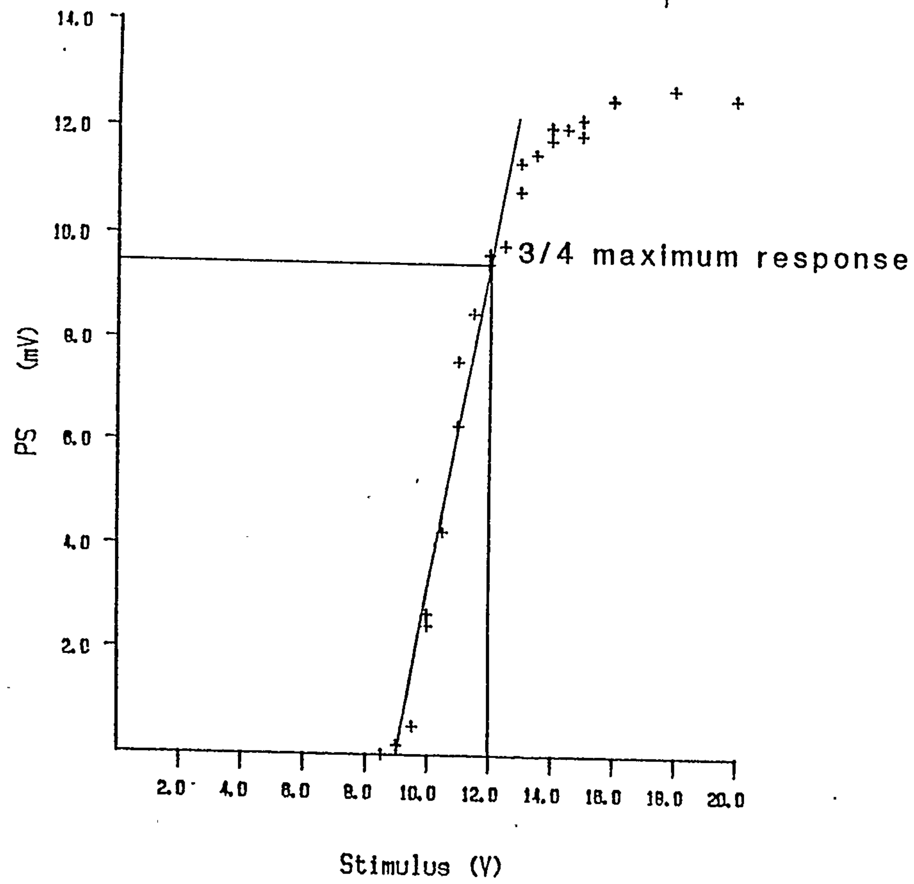
Data were analyzed under UNIX on a PDP 11/23 minicomputer. Programs for data acquisition and analysis were developed in this laboratory with the exception of some standard UNIX utilities for numerical management and statistics.

Synaptic versus postsynaptic input-output curves were generated by plotting the slope of the EPSP (dV/dt) against PS amplitude (mV). Stimulus-response curves were constructed by plotting stimulus strength (volts) against PS amplitude (mV).

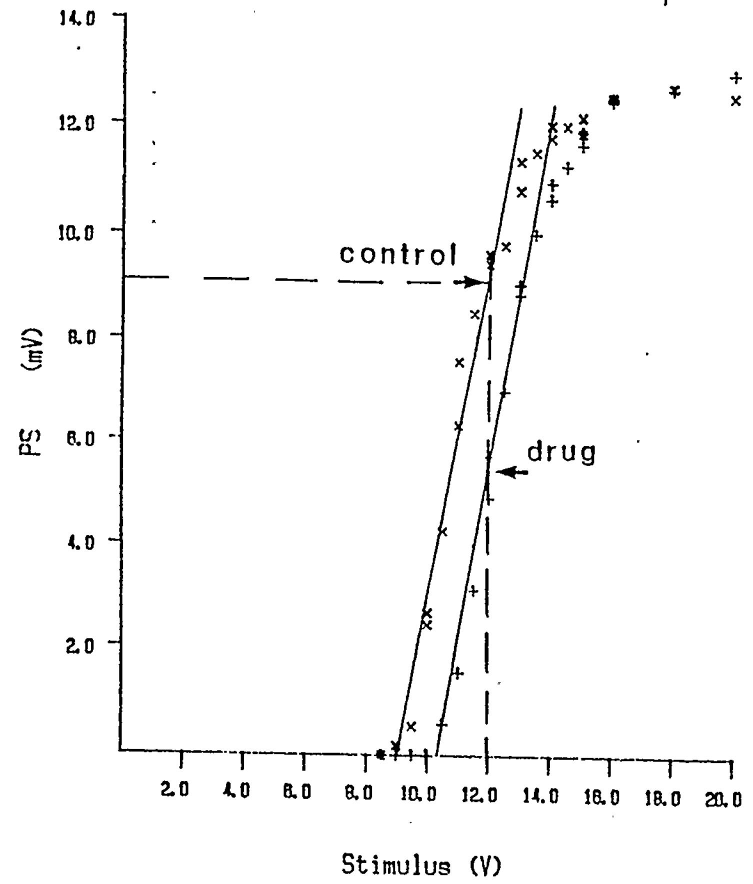
Data from the stimulus-response curves were used to generate the data points on the concentration-response curves (Figure 4). Control data from the stimulus-response curves were analyzed using linear regression from 20% to 80% of maximum PS values. The 75% of maximum PS response was calculated and the stimulus strength at that level was determined from the line generated. Drug and wash values were determined at the same stimulus strength for a given experiment following linear regression through data points from 20% to 80% of maximum response. These values were divided by the control value and were expressed as response percent of control. This percentage was then plotted against drug concentration to generate the concentration-response curve. A minimum of 5 determinations at each concentration were averaged and each point represents the mean +/- the standard error of the mean (SEM). Data points for both mature and immature responses displayed normal distributions and similar degrees of variance, therefore, statistical comparisons between mature and immature

Figure 4 - Analysis of data points from stimulus-response curves. A) Linear regression from 20% to 80% of maximum PS value was done on control data points to generate the line drawn through the data points. The 3/4 maximum response level was calculated and the stimulus strength at that level was determined; shown in this relationship at 12 volts. B) Linear regression from 20% to 80% of maximum PS value was done on drug data points to generate the line drawn through the data points. A comparison of drug to control values (% response of control) was done at the stimulus level, 12 volts, that had been determined from the control data line. Points for comparison are indicated by arrows.

A)



B)



responses were done using a 2 tailed, independent Student's t test. All calculations were done on a TI-56 calculator.

E. Materials

Adult, male, Sprague-Dawley rats were obtained from Charles River, Inc. (Quebec) and Biosciences (Univ. of Calgary). Immature animals were kindly provided by Dr. B. MacVicar (Univ. of Calgary). Litters were culled to 6 pups soon after birth, which resulted in larger animals due to optimal nutrition. Only 1 to 2 animals were used from each litter.

Laboratory grade diethyl ether was obtained from Fisher Scientific, Ltd. (Edmonton, Alta.). Sodium pentobarbital was obtained from BDH Chemicals (Ontario) and carbogen was acquired from Medigas, Ltd. (Calgary, Alta.).

The ACSF solution had the following composition (in mM): NaCl - 124, KCL - 5, NaH_2PO_4 - 1.25, $MgSO_4$ - 2, $CaCl_2$ - 2, $NaHCO_3$ - 26, and glucose - 10 (Schwartzkroin and Altshuler, 1977). All chemicals were reagent grade and were obtained from Fisher. ACSF solutions were made fresh for each experiment and had a pH of 7.4 when taken directly from the recording chamber.

III. RESULTS

A. Control Data

1. Field potential responses

A characteristic field potential, as shown in Figure 3, was recorded from the CA1 cell body layer of both mature and immature preparations in response to orthodromic stimulation of stratum radiatum inputs (Figure 5).

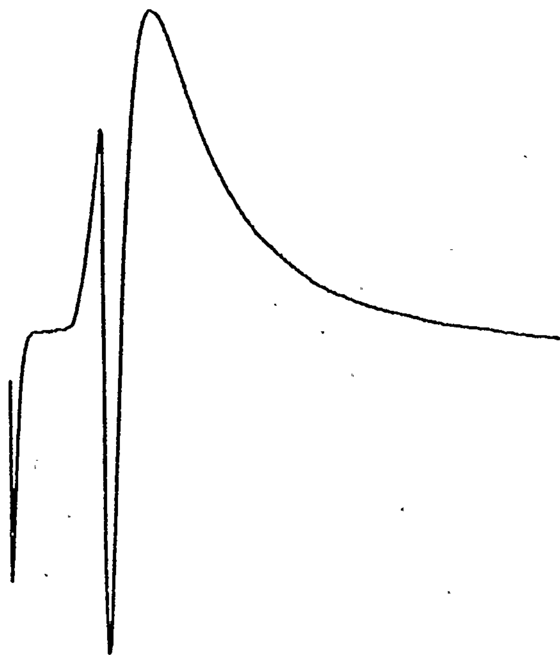
At specific stimulus strengths a variety of postsynaptic output responses (PS amplitude) were recorded from preparation to preparation. In addition, there was no correlation between postsynaptic output size and age of the preparation studied. As shown in Figure 5, field potentials generated from the CA1 pyramidal cell region for mature and immature preparations had similar profiles. When looking at unlabelled field potentials from mature and immature preparations no estimation of age could be made.

2. Input-output relationships

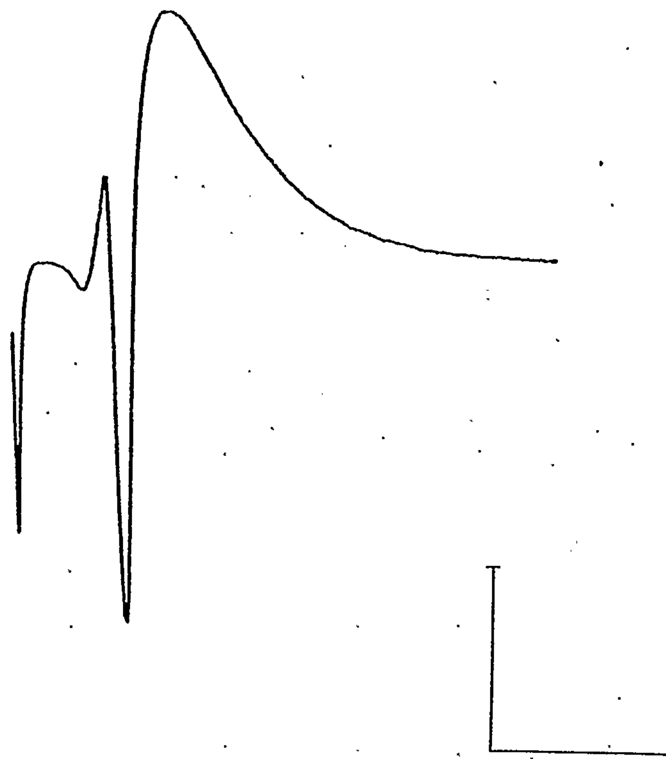
EPSP slope (dV/dt) and PS amplitude (mV) were measured at different stimulus strengths. These two parameters were plotted against one another to generate a curve that described the relationship between synaptic input (EPSP slope) and postsynaptic output (PS amplitude). As previously described (MacIver and Roth, 1987a), by examining changes of this relationship in the presence of drug (i.e. the direction of shift

Figure 5 - Control field potential recordings from orthodromically evoked responses in mature and immature CA1 pyramidal cells. Calibration: 5 mV and 10 ms.

Mature CA1



Immature CA1



in the curve) a prediction can be made concerning the drug effects on postsynaptic excitability and/or synaptic function.

Figure 6 illustrates this relationship for a mature slice preparation generated over ½ hour time intervals. For immature preparations, input-output relationships were found to be highly variable as compared to mature preparations, displaying a high degree of scatter of the data points (Figure 7). Due to this variability drug effects were not assessed on input-output relationships for immature preparations, consequently a comparison of drug effects on input-output relationships between mature and immature preparations was not done.

3. Stimulus-response relationships

PS amplitudes were measured at different stimulus strengths. These two parameters, stimulus strength (volts) and PS amplitude (mV) were plotted out to generate a stimulus (volts) - response (PS amplitude, mV) curve. For mature and immature preparations control stimulus-response curves were similar in shape (Figures 8 and 9). At low stimulus strengths (below threshold) no PS was elicited, as stimulus strength was increased (at threshold) a small PS was evoked. Increasing the stimulus beyond this level resulted in a very rapid rise in PS amplitude which represented an increase in the number of cells of the population discharging. At higher stimulus strengths a further recruitment of cells did not occur and the PS amplitude reached a plateau level. In Figure 8 the mature preparation reached a plateau response level at 15 mV, while for the immature preparation (Figure 9) a plateau was reached

Figure 6 - Control input-out curve showing the relationship between synaptic input (EPSP slope) and postsynaptic output (PS amplitude) over a 1½ hour time period for a mature preparation. Symbols: (+) ½ hour, (X) 1 hour and (□) 1½ hours following onset of stimulation.

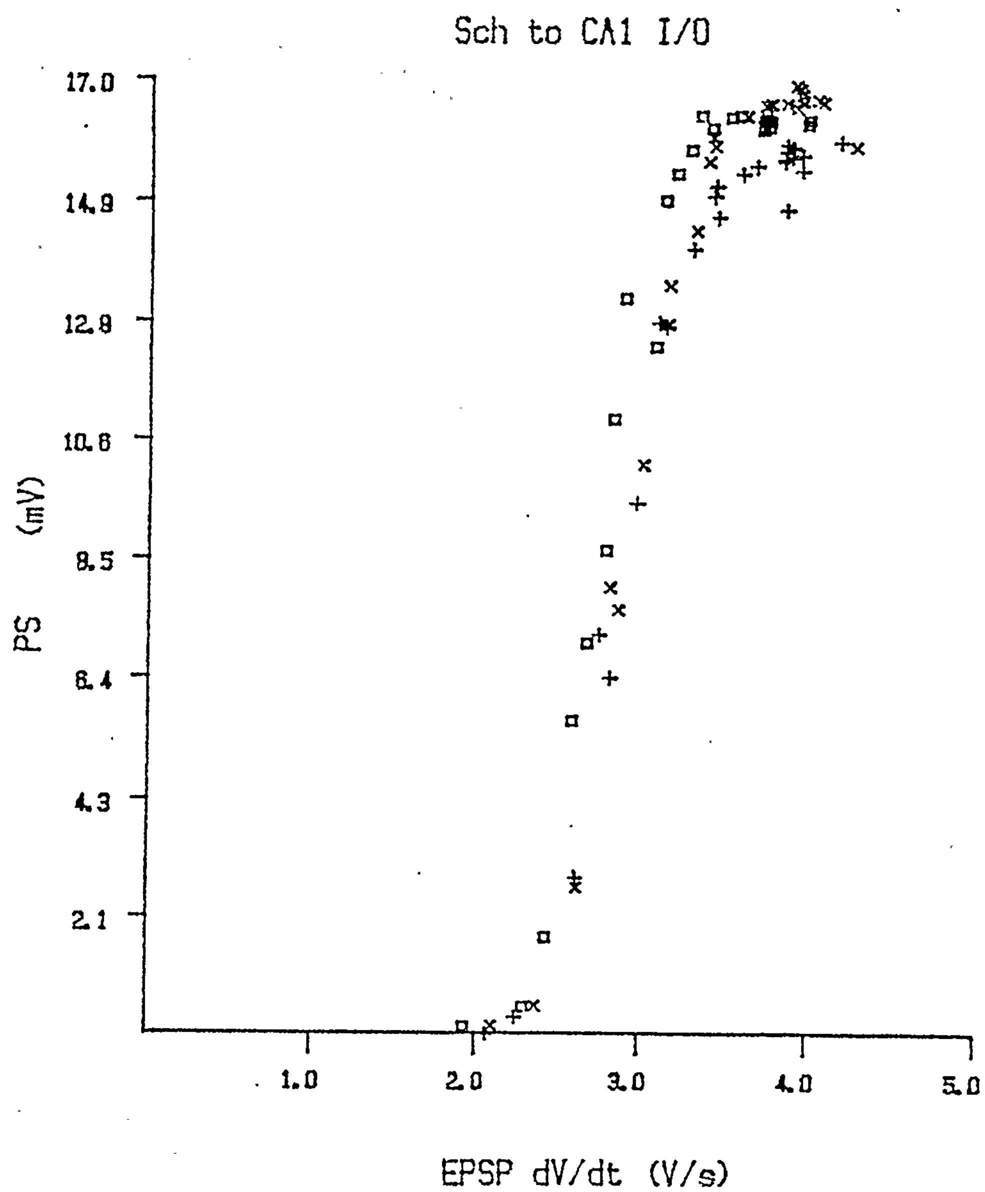
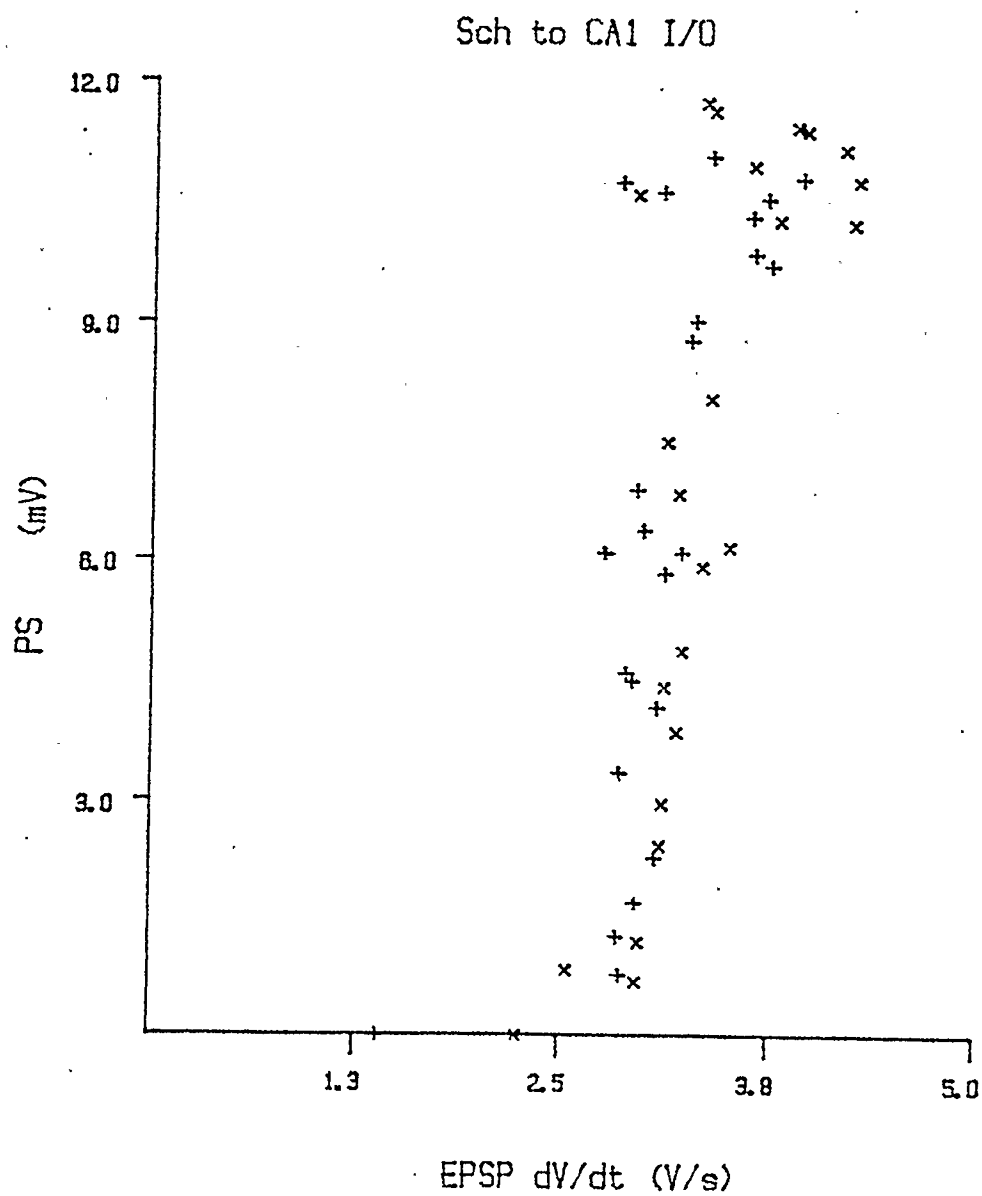

Sch to CA1 I/O
17.0
14.9
12.8
10.6
8.5
6.4
4.3
2.1
PS (mV)
1.0
2.0
3.0
4.0
5.0
EPSP dV/dt (V/s)

Figure 7 - Control input-output curve showing the relationship between synaptic input (EPSP slope) and postsynaptic output (PS amplitude) over a 1½ hour time period for an immature preparation. Symbols: (+) ½ hour and (X) 1½ hours following onset of stimulation.



Sch to CA1 I/O
12.0
9.0
6.0
3.0
PS (mV)
1.3
2.5
3.8
5.0
EPSP dV/dt (V/s)

Figure 8 - Control stimulus-response curve showing the relationship between stimulus strength (V) and population spike amplitude (mV) over a 1½ hour time period for a mature preparation. Symbols: (+) ½ hour, (X) 1 hour and (□) 1½ hours following onset of stimulation.

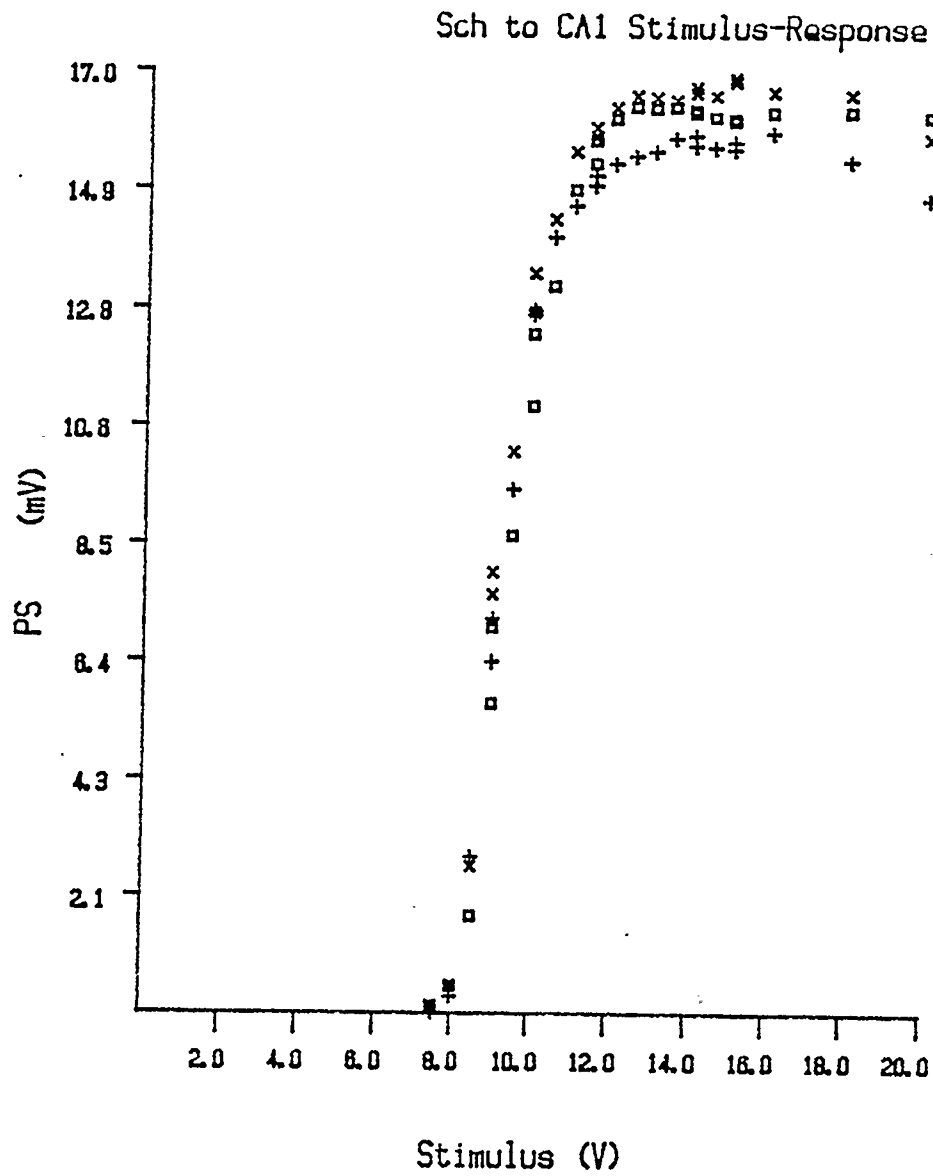
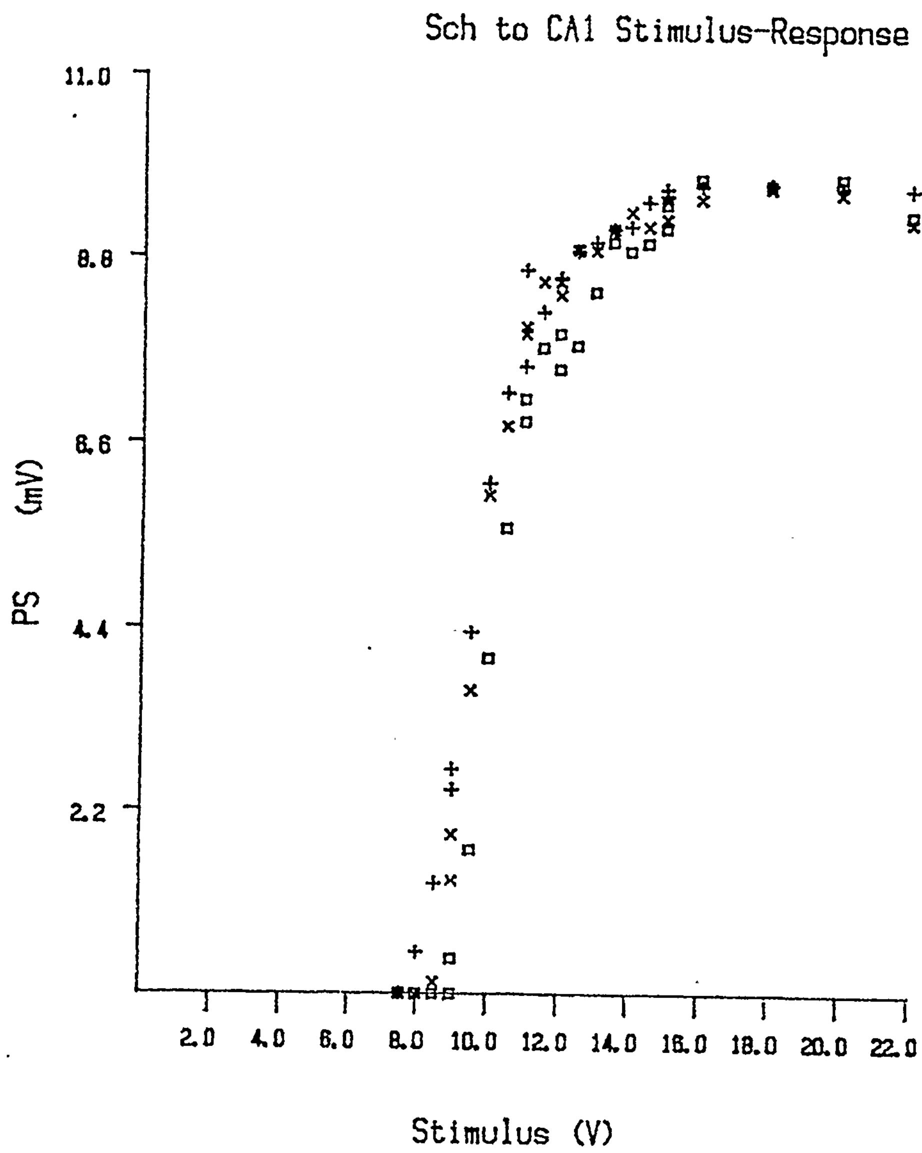

Sch to CA1 Stimulus-Response
17.0
14.9
12.8
10.8
8.5
6.4
4.3
2.1
PS (mV)
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
Stimulus (V)

Figure 9 - Control stimulus-response curve showing the relationship between stimulus strength (V) and population spike amplitude (mV) over a 1½ hour time period for an immature preparation. Symbols: (+) ½ hour, (X) 1 hour and (□) 1½ hours following onset of stimulation.


Sch to CA1 Stimulus-Response
11.0
8.8
6.6
4.4
2.2
PS (mV)
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
Stimulus (V)

at 9 mV. From these figures it can be seen that the immature response (PS amplitude) was smaller than the mature, but as discussed above postsynaptic response size was variable between preparations.

Three stimulus-response curves were generated at 30 minute intervals (over a 1½ hour time period) as shown in Figures 8 and 9. The three curves on each figure are superimposed demonstrating that the preparations remained stable for at least 1½ hours. Responses for immature slices began to decrease after this 1½ hour period (data not shown) and, as a result, drug studies were conducted within this time frame for the younger animals.

4. Bursting activity

A major difference between the two experimental groups was that of bursting activity exhibited by the immature preparations (Figure 10). This was seen in approximately 60% of the immature slices and observed for all ages tested in this group (14, 15 and 16 days postnatal). This bursting activity was similar to that described previously by other investigators for immature rat, rabbit and kitten hippocampal slice preparations (see: Introduction). These findings suggest that the immature preparations used in this study lacked complete development of inhibitory circuitry.

Figure 10 - Bursting activity from two separate immature (16 day postnatal) preparations.

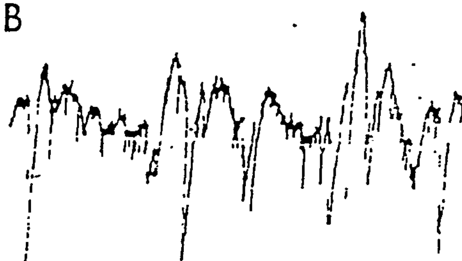
Preparation 1: A represents baseline recording, B and C are recordings during bursting activity. Calibration: A,B - 1.25 s and 0.8 mV, C - 25 ms and 0.8 mV.

Preparation 2: A represents baseline recording, B recording during bursting activity. Calibrations: A,B - 0.5 s and 0.8 mV.

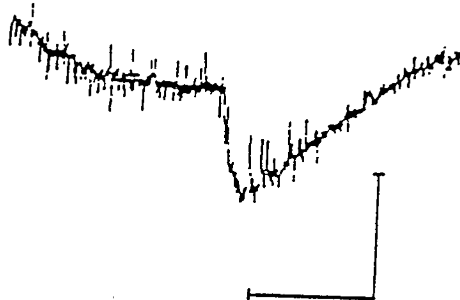
1)

A

B



C



2)

A

B

B. Drug Responses

1. Effects on field potential and stimulus-response relationships

(a) Mature preparations

Pentobarbital produced concentration dependent effects on CA1 pyramidal cell field potentials as had been previously reported for mature hippocampal preparations (Roth et al., 1986). Biphasic drug actions were observed; at lower concentrations (0.04 mM to 0.1 mM) an increase in activity occurred, while at higher concentrations (0.2 mM to 0.4 mM) a decrease in activity was observed. This excitation and depression could be seen as a shifting of the stimulus-response curve to the left indicating excitation or to the right representing depression.

As shown in Figure 11, 0.04 mM pentobarbital slightly increased the PS amplitude at all stimulus strengths above threshold. It can be seen in Figure 12, that 0.08 mM pentobarbital caused a further increase in PS amplitude at all stimulus strengths above threshold. This effect was irreversible as can be seen by a further shift to the left of the wash stimulus-response curve. Pentobarbital at 0.1 mM also increased the postsynaptic response at all stimulus levels above threshold as shown in Figure 13. This effect was reversible as indicated by the superimposed curves for control and wash data. At higher concentrations, pentobarbital (0.2 mM, Figure 14) caused a decrease in PS amplitude at

Figure 11 - A) The effects of pentobarbital and washout on evoked field potential responses of mature CA1 pyramidal cells. Sweeps show control (C) response before drug, in the presence of pentobarbital (0.04 mM) and washout (W) following drug exposure. Calibration: 5 mV and 10 ms. B) Stimulus-response curves from the same population of cells as above demonstrating the effect of pentobarbital on the relationship between population spike amplitude (PS) and stimulus strength. Symbols: (X) control, (+) 0.04 mM pentobarbital, and (□) washout.

A)

C

0.04

W

B)

Sch to CA1 Stimulus-Response

11.0

8.8

6.6

4.4

2.2

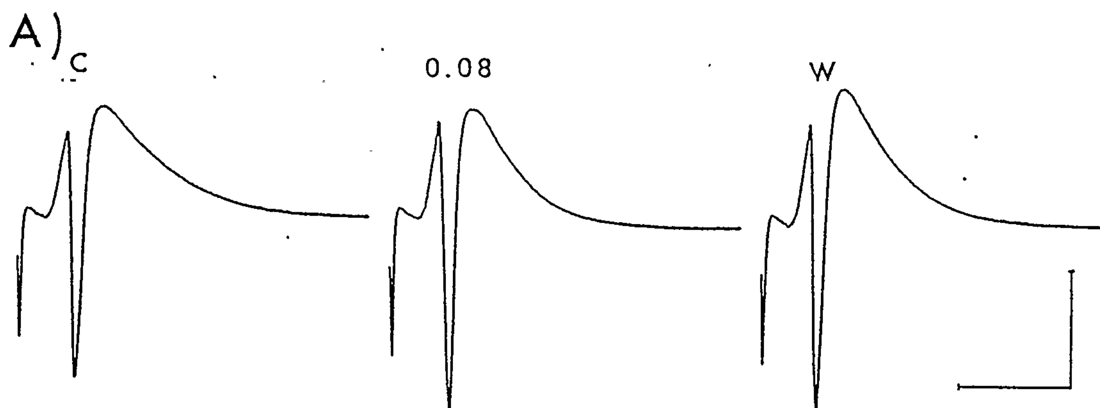
PS (mV)

2.0 4.0 6.0 8.0 10.0 12.0 14.0 16.0 18.0 20.0

Stimulus (V)

Figure 12 - B) Effects of pentobarbital and washout on evoked field potential responses of mature CA1 pyramidal cells. Sweeps show control (C) response before drug, in the presence of pentobarbital (0.08 mM) and washout (W) following drug exposure. Calibration: 5 mV and 10 ms. B) Stimulus-response curves from the same population of cells as above demonstrating the effect of pentobarbital on the relationship between population spike amplitude (PS) and stimulus strength. Symbols: (+) control, (X) 0.08 mM pentobarbital, and (□) washout.

A)



B)

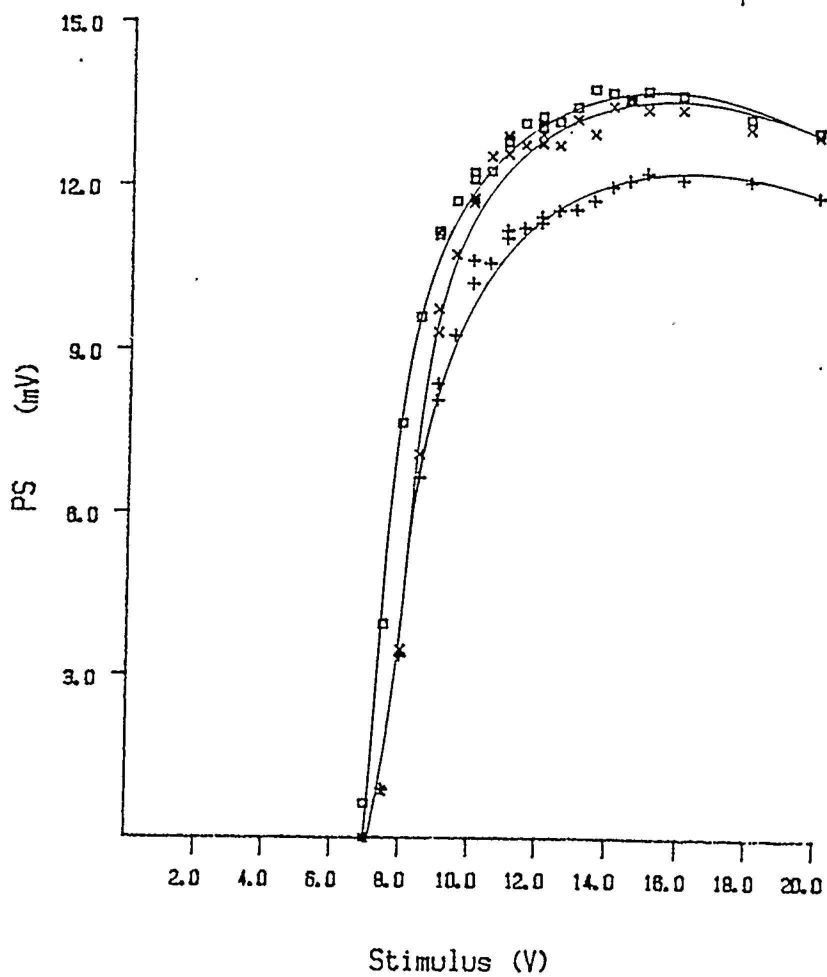
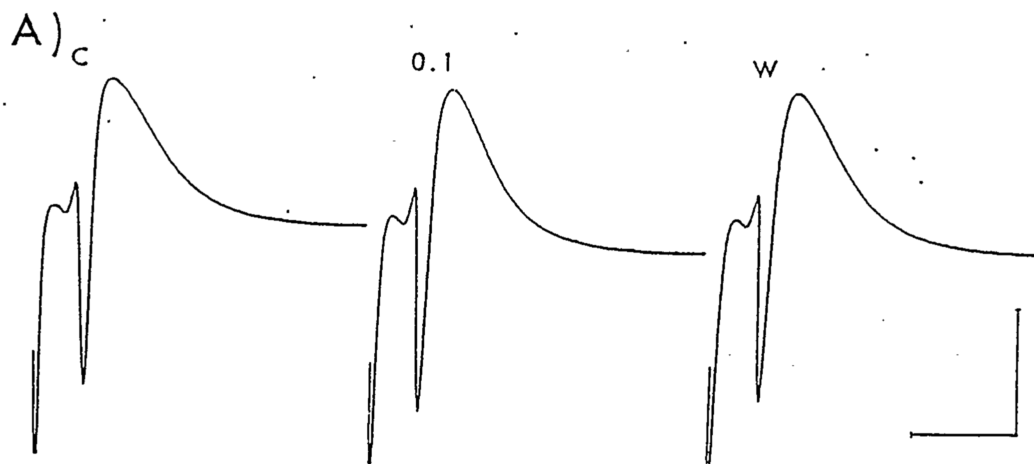


Figure 13 - A) The effects of pentobarbital and washout on evoked field potential responses of mature CA1 pyramidal cells. Sweeps show control (C) response before drug, in the presence of pentobarbital (0.1 mM) and washout (W) following drug exposure. Calibration: 5 mV and 10 ms. B) Stimulus-response curves from the same population of cells as above demonstrating the effect of pentobarbital on the relationship between population spike amplitude (PS) and stimulus strength. Symbols: (X) control, (+) 0.1 mM pentobarbital, and (□) washout.


A)
C
0.1
W

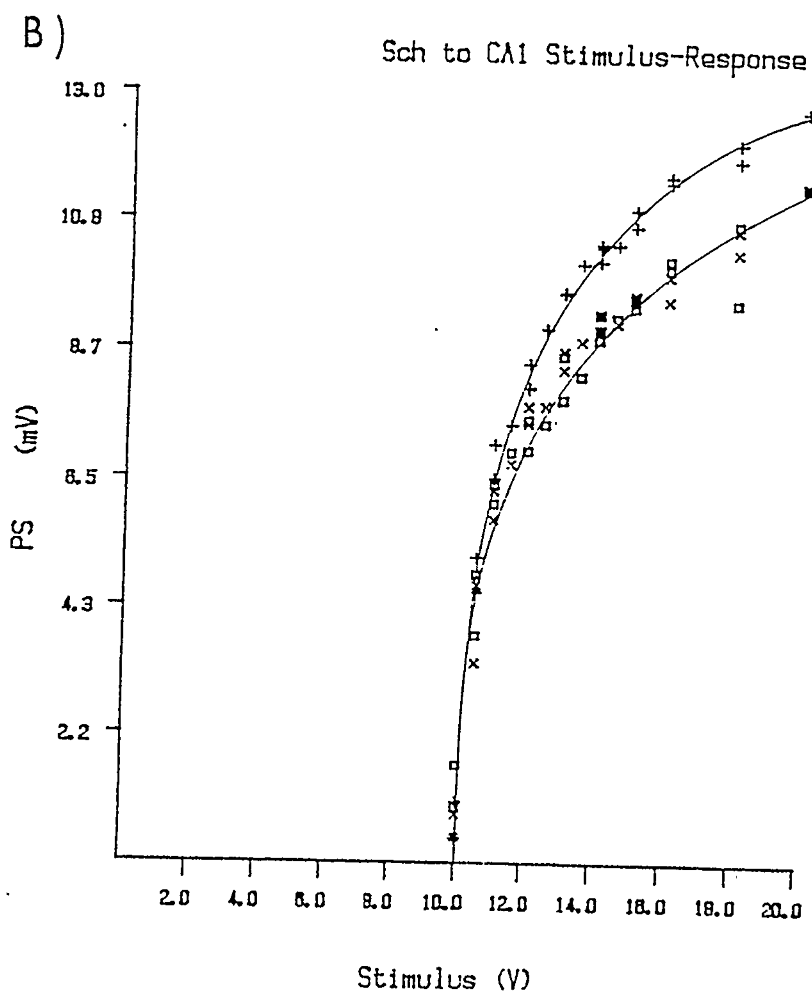
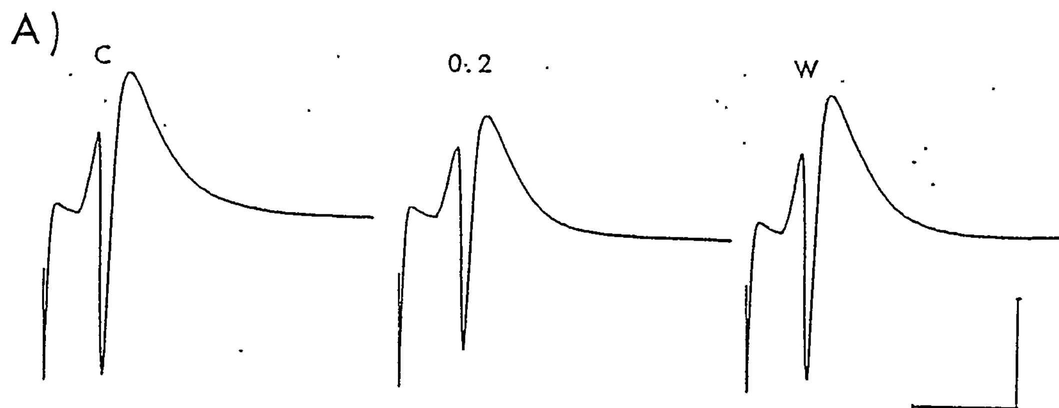
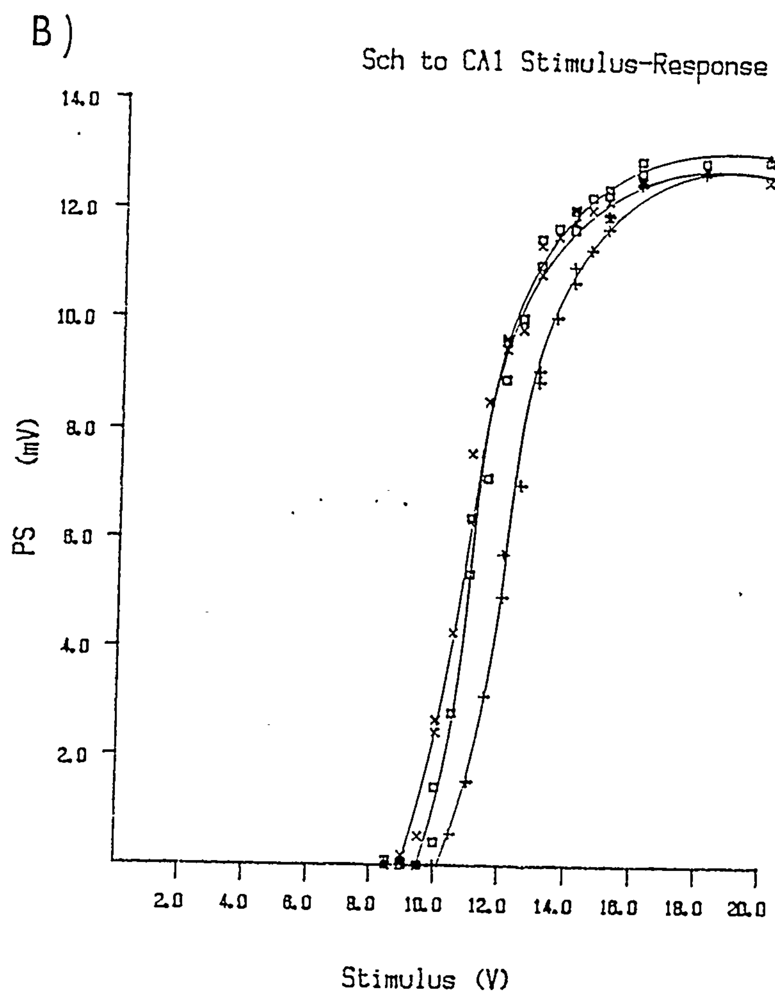

B)
Sch to CA1 Stimulus-Response
13.0
10.8
8.7
6.5
4.3
2.2
PS (mV)
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
Stimulus (V)

Figure 14 - A) The effects of pentobarbital and washout on evoked field potential responses of mature CA1 pyramidal cells. Sweeps show control (C) response before drug, in the presence of pentobarbital (0.2 mM) and washout (W) following drug exposure. Calibration: 5 mV and 10 ms. B) Stimulus-response curves from the same population of cells as above demonstrating the effect of pentobarbital on the relationship between population spike amplitude (PS) and stimulus strength. Symbols: (X) control, (+) 0.2 mM pentobarbital, and (□) washout.


A)
C
0.2
W


B)
Sch to CA1 Stimulus-Response
PS (mV)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
Stimulus (V)

threshold and at higher stimulus strengths, however, at the highest stimulus strengths (producing the plateau PS response) no difference was seen between control and drug responses. Drug effects were apparent within 10 to 15 minutes after drug perfusion began and maximum effect was attained 20 to 30 minutes following onset of drug perfusion.

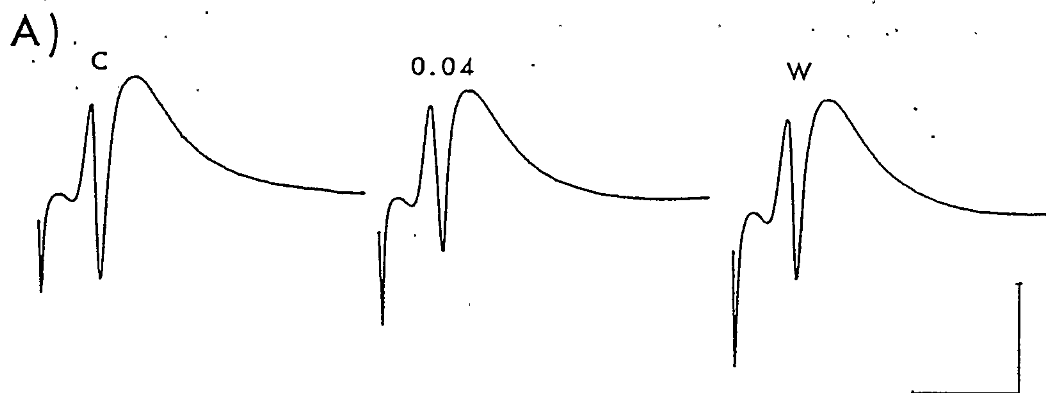
(b) Immature preparations

Concentration dependent effects of pentobarbital were also observed on immature field potential responses. In contrast to the biphasic effects (enhancement/depression) seen in mature preparations, only a decrease in activity was observed for immature slices. This depression, seen as a decrease in PS amplitude, was noted at all concentrations studied (0.02 mM to 0.4 mM).

In Figure 15, 0.04 mM pentobarbital produced a decrease in PS amplitude indicated by a shift to the right of the control curve at threshold and stimulus strengths up to 16 volts. At higher stimulus strengths, however, drug responses were slightly increased over control values. This effect was partially reversible, as can be seen at stimulus strengths of 14 volts and higher, indicated by a shift to the left for the wash curve. Figure 16 illustrates that higher concentrations of pentobarbital, 0.08 mM, produced a further decrease in postsynaptic output. In this experiment an irreversible decrease was seen at all levels of stimulus strength. In Figure 17, 0.1 mM pentobarbital produced an irreversible, biphasic effect. At threshold and low stimulus strengths (8-14 volts), a decrease in PS amplitude was seen, at high stimulus strengths (14.5 - 16 volts) no change in response

Figure 15 - A) The effects of pentobarbital and washout on evoked field potential responses of immature CA1 pyramidal cells. Sweeps show control (C) response before drug, in the presence of pentobarbital (0.04 mM) and washout (W) following drug exposure. Calibration: 5 mV and 10 ms. B) Stimulus-response curves from the same population of cells as above demonstrating the effect of pentobarbital on the relationship between population spike amplitude (PS) and stimulus strength. Symbols: (+) control, (X) 0.04 mM pentobarbital, and (□) washout.

A)



B)

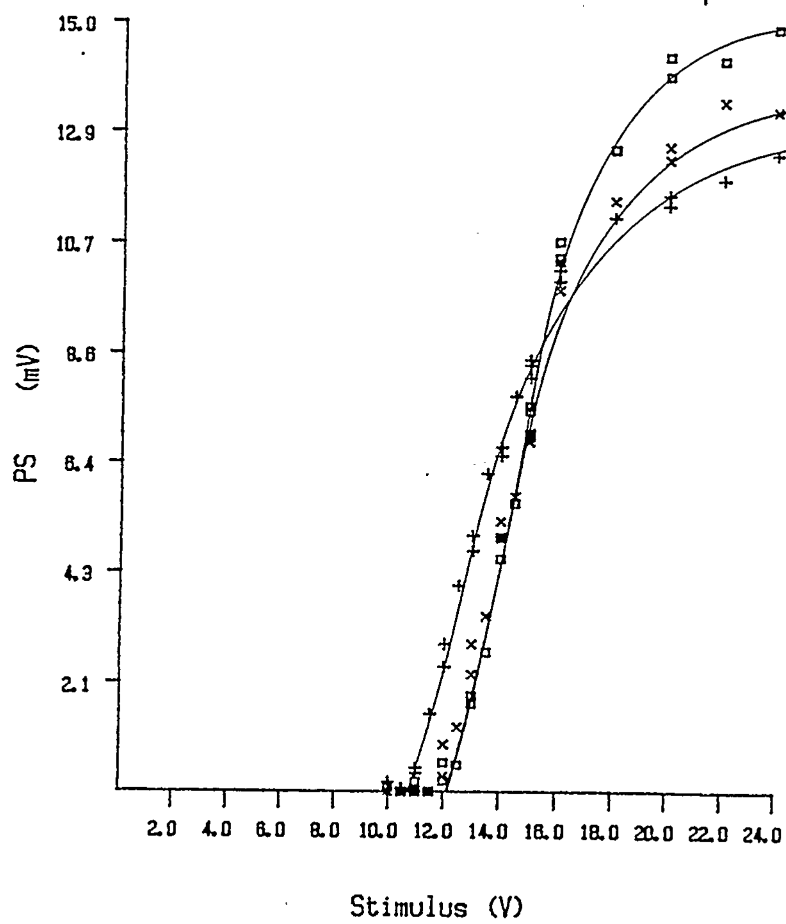
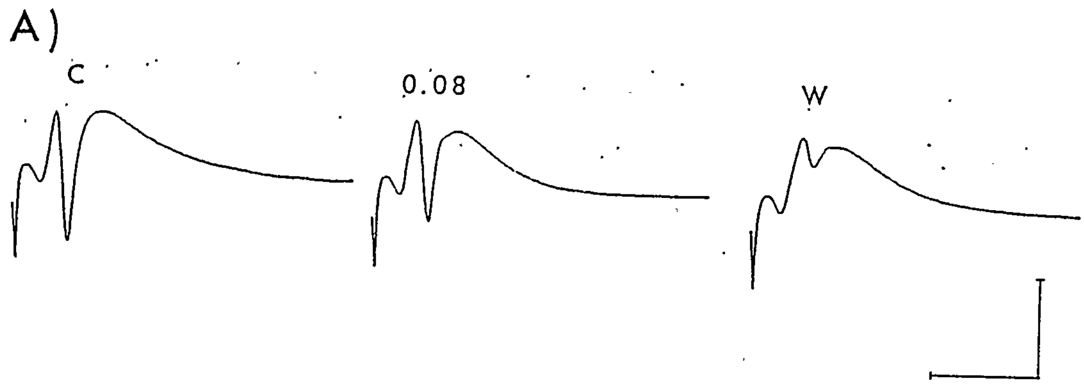


Figure 16 - A) The effects of pentobarbital and washout on evoked field potential responses of immature CA1 pyramidal cells. Sweeps show control (C) response before drug, in the presence of pentobarbital (0.08 mM) and washout (W) following drug exposure. Calibration: 5 mV and 10 ms. B) Stimulus-response curves from the same population of cells as above demonstrating the effect of pentobarbital on the relationship between population spike amplitude (PS) and stimulus strength. Symbols (+) control, (X) 0.08 mM pentobarbital, and (□) washout.

A)



B)

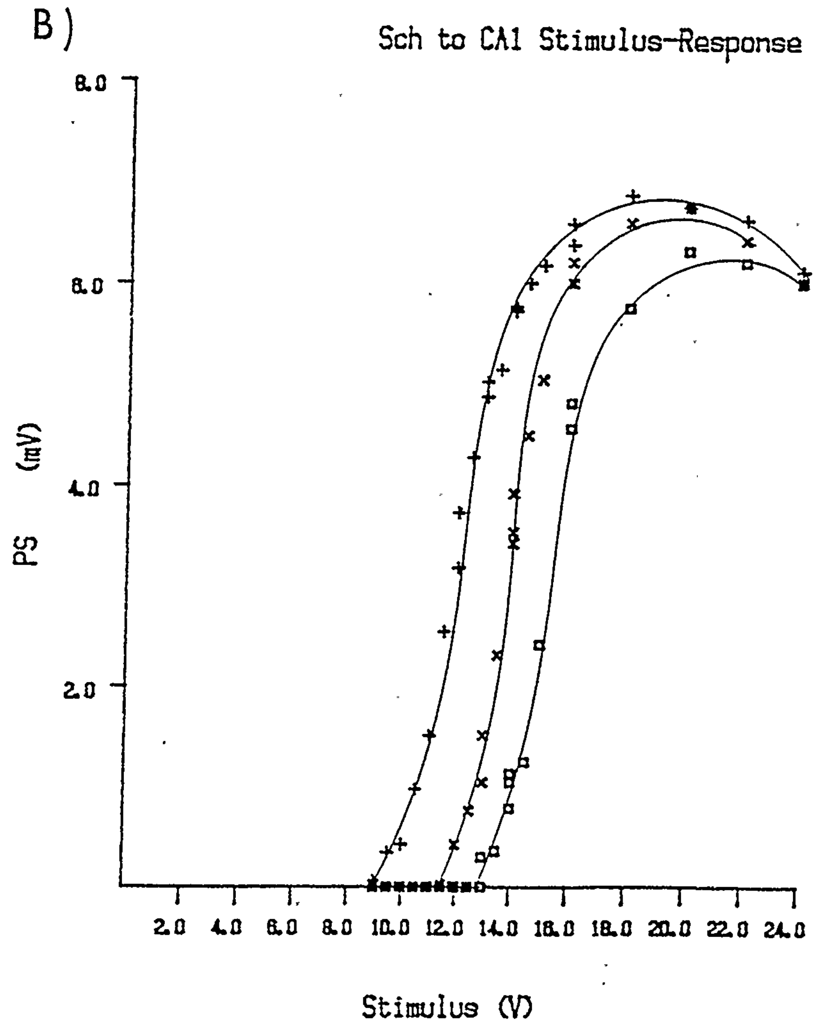
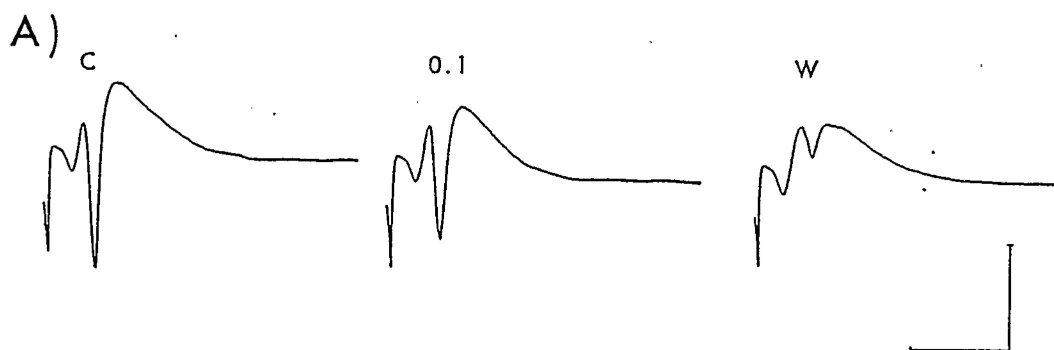
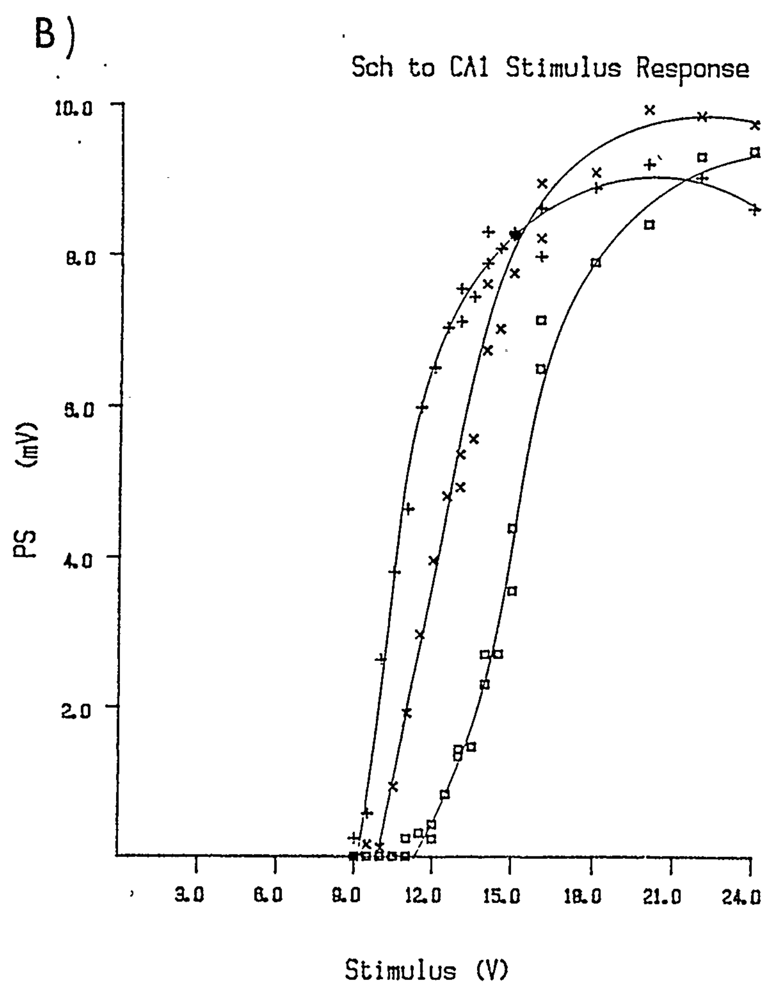


Figure 17 - A) The effects of pentobarbital and washout on evoked field potential responses of immature CA1 pyramidal cells. Sweeps show control (C) response before drug, in the presence of pentobarbital (0.1 mM) and washout (W) following drug exposure. Calibration: 5 mV and 10 ms. B) Stimulus-response curves from the same population of cells as above demonstrating the effect of pentobarbital on the relationship between population spike amplitude (PS) and stimulus strength. Symbols (+) control, (X) 0.1 mM pentobarbital, and (□) washout.


A)
C
0.1
W


B)
Sch to CA1 Stimulus Response
PS (mV)
10.0
8.0
6.0
4.0
2.0
3.0
6.0
9.0
12.0
15.0
18.0
21.0
24.0
Stimulus (V)

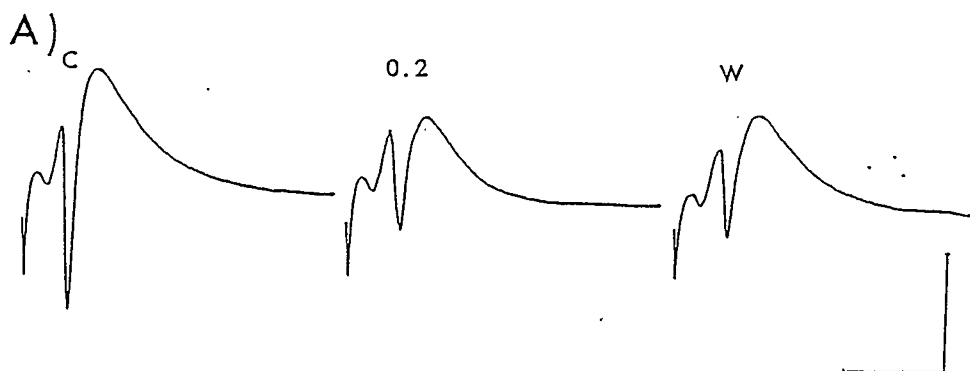
was seen, and at higher stimulus strengths (18 - 24 volts) an increase in PS amplitude occurred. Exposure to higher concentrations of pentobarbital produced a further decrease in postsynaptic output. In Figure 18, 0.2 mM pentobarbital produced a further decrease in PS amplitude, again this response was biphasic in profile and drug effects were irreversible.

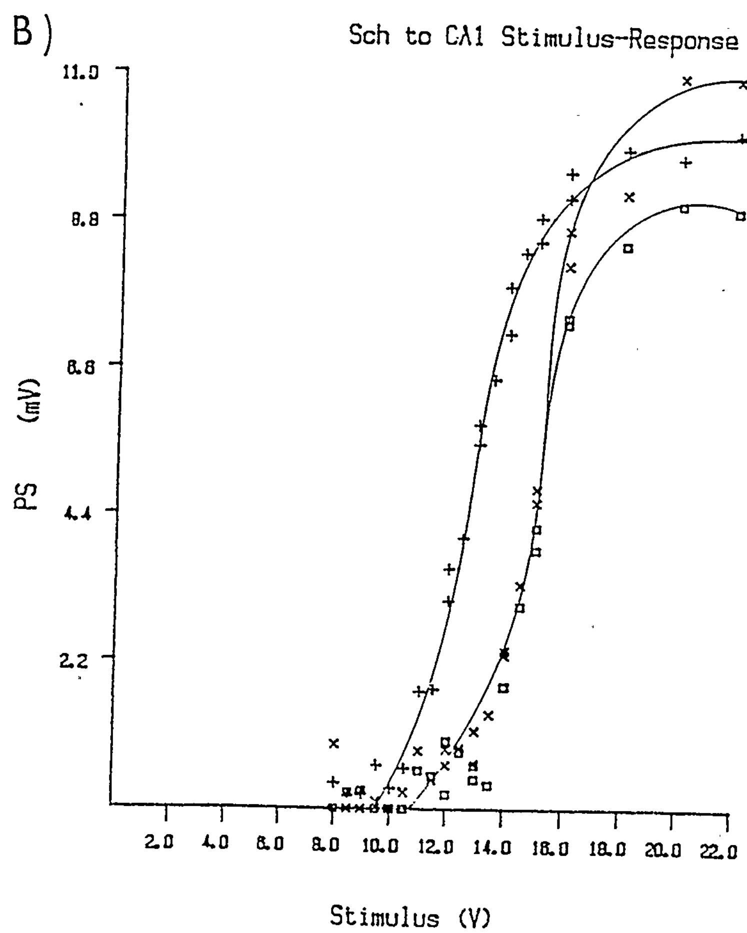
In addition to the decrease in activity seen with all drug concentrations for immature preparations other differences were also noted in the presence of drug between mature and immature slices. First, for the immature slices, recovery of response was very difficult to obtain, as described for Figures 16, 17 and 18. At all concentrations studied, however, washout of drug effects were seen for at least one experiment. The amount of decrease recorded in PS amplitude in experiments when recovery occurred was similar to the amount of decrease in PS amplitude seen in experiments where washout was not achieved. Second, for the immature preparations drug effects were found to be dependent on stimulus strength. This was shown in Figures 15, 17 and 18 as a biphasic response of PS amplitudes in the presence of drug. This is in contrast to the changes in the stimulus-response relationships for the mature preparations, where a parallel shift of curves in the presence of drug occurred, indicating an enhancement or depression at all stimulus levels.

(c) Effects on second positive component of field potentials

Another effect of pentobarbital seen on both the mature and

Figure 18 - A) The effects of pentobarbital and washout on evoked field potential responses of immature CA1 pyramidal cells. Sweeps show control (C) response before drug, in the presence of pentobarbital (0.2 mM) and washout (W) following drug exposure. Calibration: 5 mV and 10 ms. B) Stimulus-response curves from the same population of cells as above demonstrating the effect of pentobarbital on the relationship between population spike amplitude (PS) and stimulus strength. Symbols: (+) control, (X) 0.2 mM pentobarbital, and (□) washout.


A)
C
0.2
W


B)
Sch to CA1 Stimulus-Response
11.0
8.8
6.6
4.4
2.2
PS (mV)
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
Stimulus (V)

immature field potentials was that of a decrease in the second positive component of the field potential (Figure 19). This component of the field potential may represent a number of changes occurring in the cell population, including, activation of K currents and Cl currents due to recurrent inhibition following cell discharge, source phenomenon produced from the PS sink and the remainder of the field EPSP. The pentobarbital induced decrease was recorded in 9 out of 10 preparations for both mature and immature slices. It did not appear to be concentration dependent (see raw data sweeps for Figures 11 - 18) but was observed at all concentrations studied.

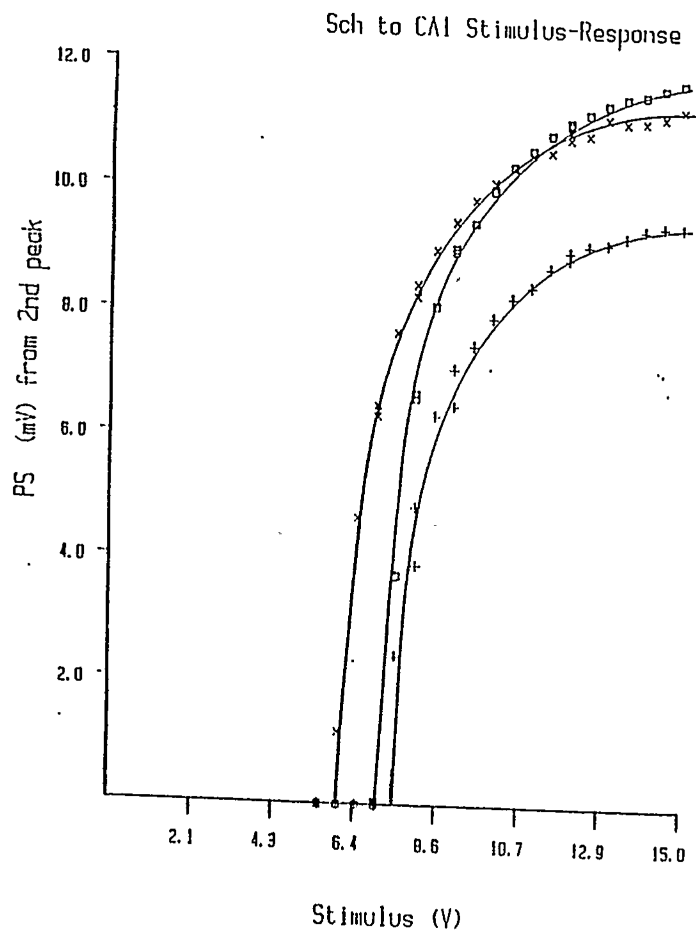
2. Effects on mature input-output relationships

Because stable input-output relationships could be obtained for mature preparations, drug effects on this relationship were examined to gain more information on the mechanisms of action of pentobarbital on mature preparations.

Low concentrations of pentobarbital (0.04 to 0.08 mM) produced an increase in both EPSP slope and PS amplitude (Figure 20). Input-output curves demonstrated that at these lower concentrations an increase in synaptic input accounted for the enhancement of response at lower stimulus strengths. This can be seen as a shift to the right on the input-output curves for drug data. At higher stimulus strengths an increase in post-synaptic output (PS amplitude) in addition to increased synaptic input (EPSP slope) was observed.

At higher concentrations of pentobarbital (0.1 mM to 0.4 mM) a decrease in EPSP slope was observed. This can be seen in Figure 21

Figure 19 - The effects of pentobarbital and washout on the second positive component of a mature CA1 region field potential. On the right is the field potential before, during and after exposure to pentobarbital (0.4 mM). On the left is the stimulus-response relationship as measured from the second positive component of the field potential. Arrowheads indicate the portion of the field potential measured as the second positive component of the waveform. Calibrations: 5 mV and 10 ms.


Sch to CA1 Stimulus-Response
12.0
10.0
8.0
6.0
4.0
2.0
PS (mV) from 2nd peak
2.1
4.3
6.4
8.6
10.7
12.9
15.0
Stimulus (V)

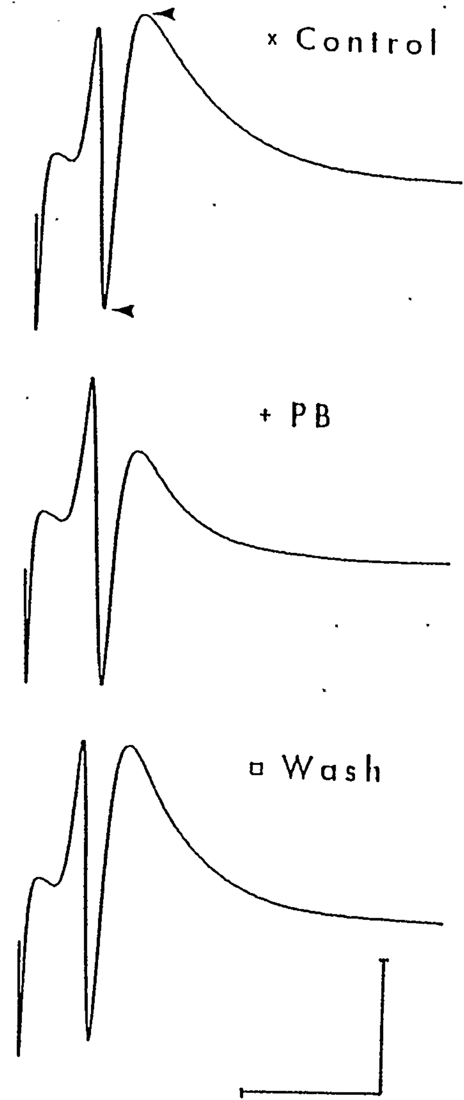
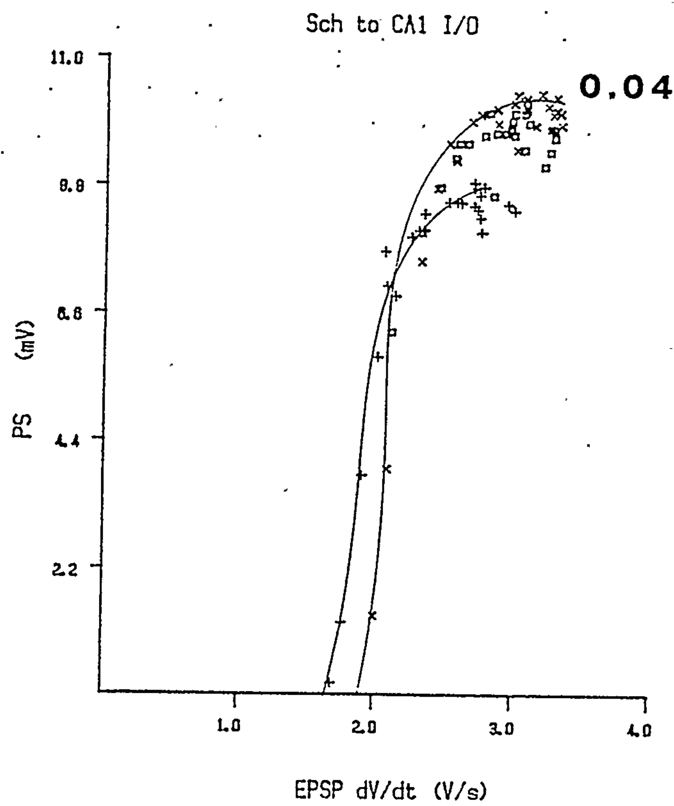
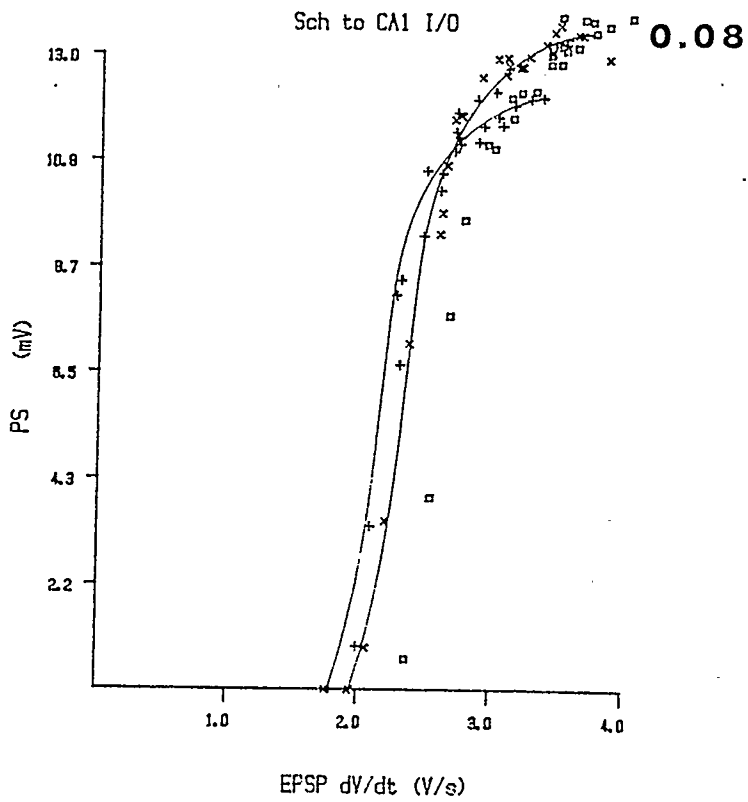

× Control
+ PB
□ Wash

Figure 20 - Input-output curves from mature preparations showing the effects of low doses of pentobarbital on the relationship between EPSP slope and PS amplitude. Symbols: (+) control, (X) pentobarbital, and (□) washout. Lines are drawn through control and drug data. Concentrations shown are in mM.


Sch to CA1 I/O
0.04
11.0
8.8
6.6
4.4
2.2
PS (mV)
1.0
2.0
3.0
4.0
EPSP dV/dt (V/s)


Sch to CA1 I/O
0.08
13.0
10.8
8.7
6.5
4.3
2.2
PS (mV)
1.0
2.0
3.0
4.0
EPSP dV/dt (V/s)

where drug data curves are shifted to the left of the control curves. At 0.1 mM pentobarbital, an increase in PS amplitude was seen at all stimulus strengths, whereas at 0.4 mM pentobarbital, a decrease in postsynaptic output accompanied the decrease in synaptic input.

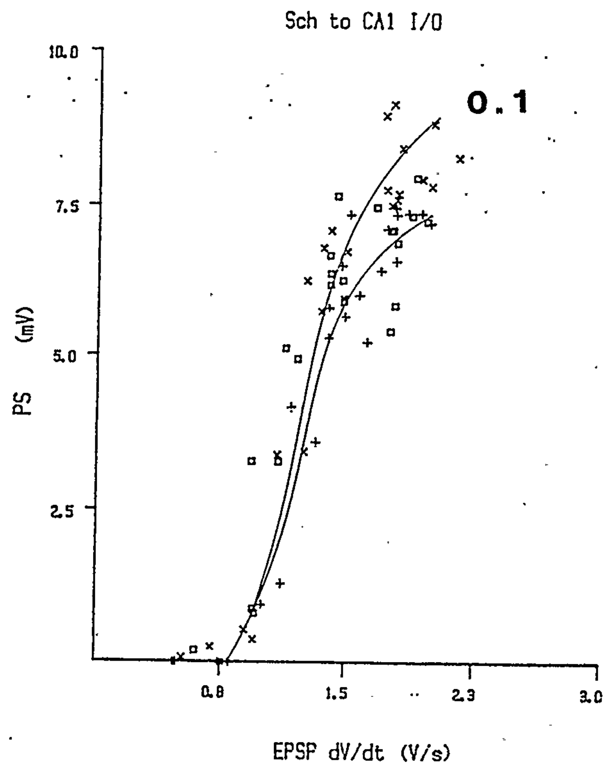
3. Effects on bursting activity

Bursting activity recorded in immature preparations was found to continue in the presence of pentobarbital. In Figure 22A (top trace), bursting activity before drug exposure is superimposed on a stored field potential. In Figure 22B, in the presence of 0.1 mM pentobarbital bursting activity (superimposed on the same stored field potential as in A) continued. In Figure 22C, at the end of the experiment following the washout period, bursting activity (shown on a slower time scale) was still recorded.

4. Concentration-response relationships

As described in Methods, data points for concentration-response curves were generated from stimulus-response relationships. Because this relationship for immature preparations in the presence of drug was different at different stimulus strengths, a comparison was done between drug concentration and response (% of control) from control and drug data at maximum, 3/4 maximum, ½ maximum and ¼ maximum response for four concentrations of pentobarbital (Figure 23). At maximum control response, it was shown that at higher drug concentrations (0.2 mM pentobarbital) there was little or no change in response to drug

Figure 21 - Input-output curves from mature preparations showing the effects of high doses of pentobarbital on the relationship between EPSP slope and PS amplitude. Symbols: top - (+) control, (X) pentobarbital, (□) washout and bottom - (X) control, (+) pentobarbital, and (□) washout. Lines are drawn through control and drug data. Concentrations shown are in mM.


Sch to CA1 I/O
0.1
10.0
7.5
5.0
2.5
PS (mV)
0.8
1.5
2.3
3.0
EPSP dV/dt (V/s)

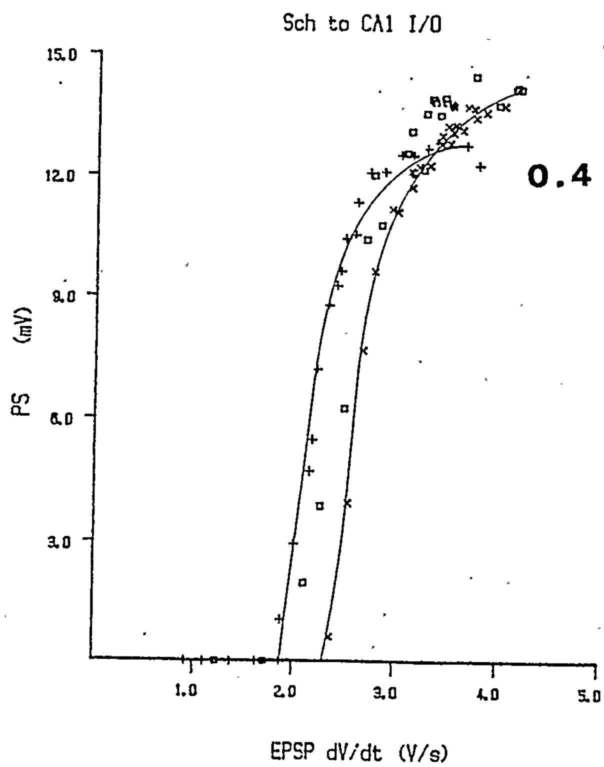

Sch to CA1 I/O
0.4
15.0
12.0
9.0
6.0
3.0
PS (mV)
1.0
2.0
3.0
4.0
5.0
EPSP dV/dt (V/s)

Figure 22 - Bursting activity from an immature (14 days postnatal) preparation in the presence of pentobarbital.

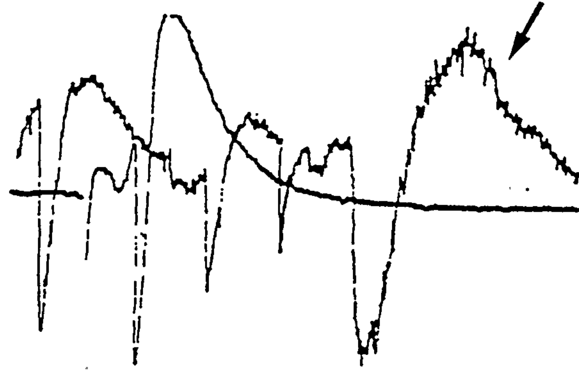
A - Bursting activity before drug exposure superimposed on a stored control field potential. Calibrations: 0.5 s and 0.8 mV for bursting activity, 10 ms and 5 mV for field potential.

B - Bursting activity in the presence of 0.1 mM pentobarbital. Bursting activity trace is superimposed on same field potential as in A. Calibrations are same as in A.

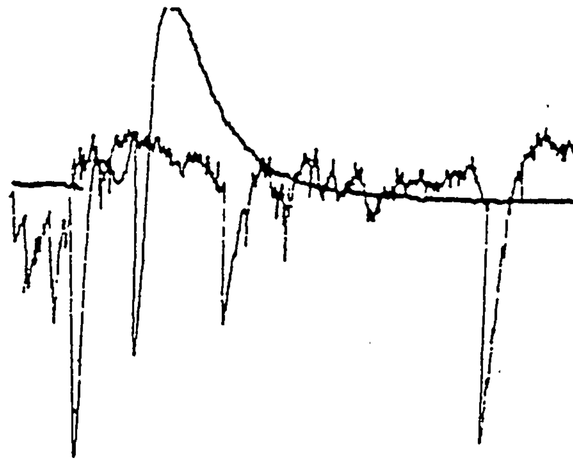
C - Bursting activity following washout period. Calibrations: 1.25 s and 0.8 mV.

A)

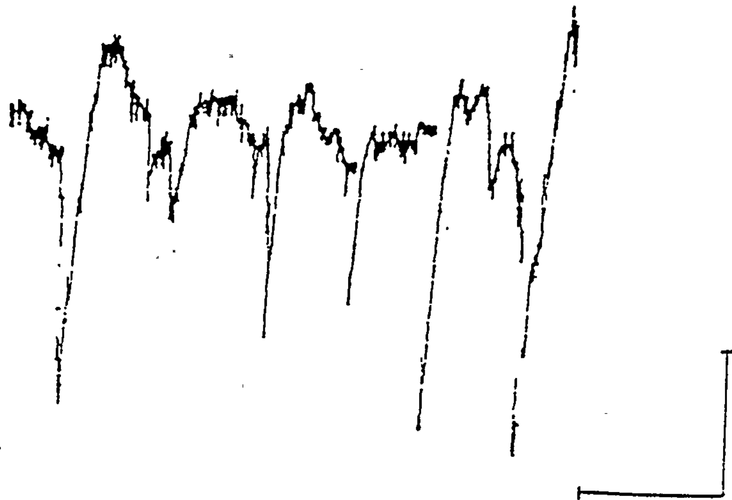
Bursting Activity



B)



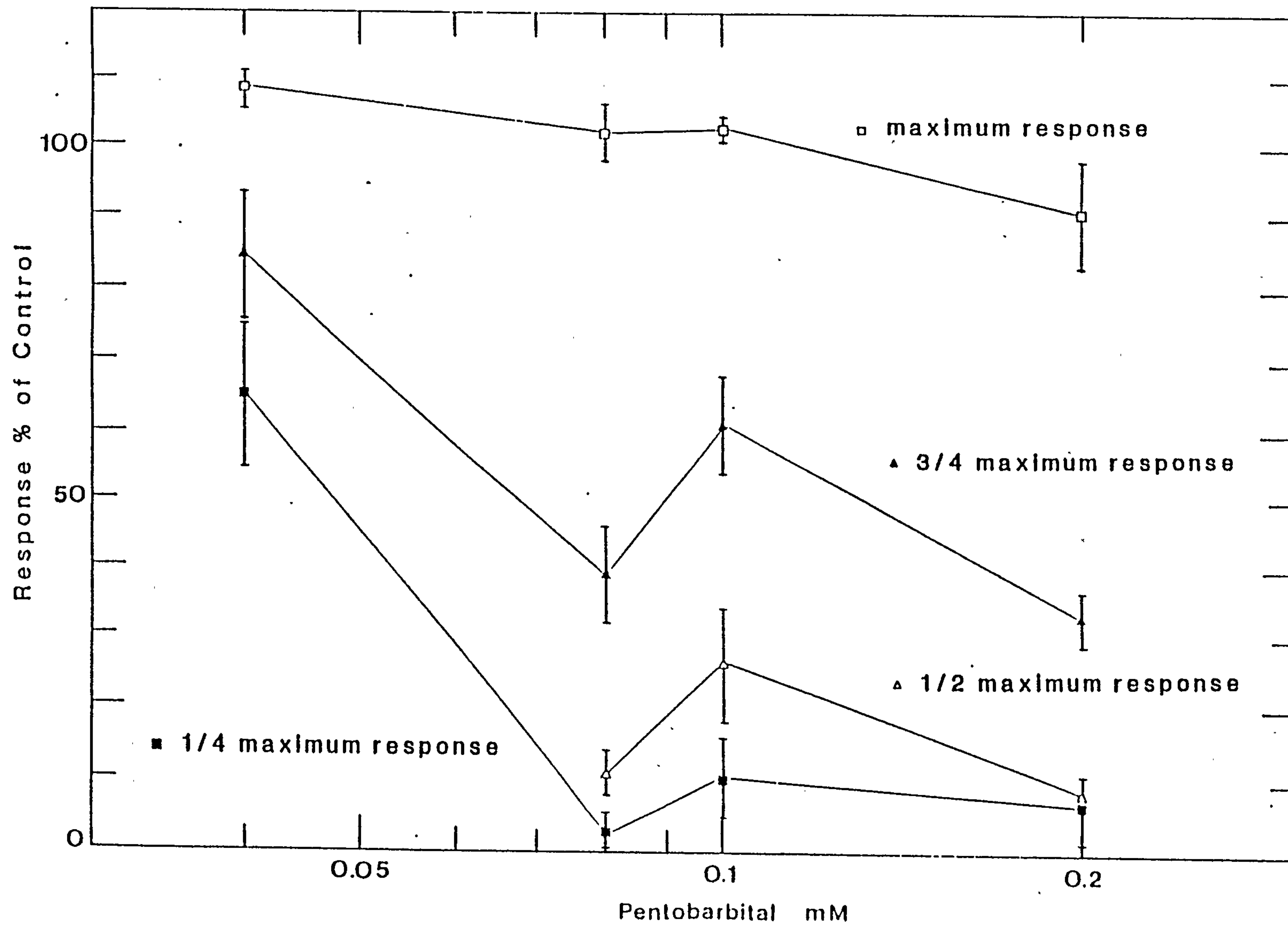
C)



exposure. At the ½ maximum and ¼ maximum levels, the population spike amplitude was nearly abolished at lower concentrations (0.08 mM pentobarbital). At the 3/4 maximum response level, pentobarbital was shown to produce an effect that could be analyzed at higher concentrations. As a result, the 3/4 maximum response level was chosen for comparison on the stimulus-response curves for both the mature and immature preparations.

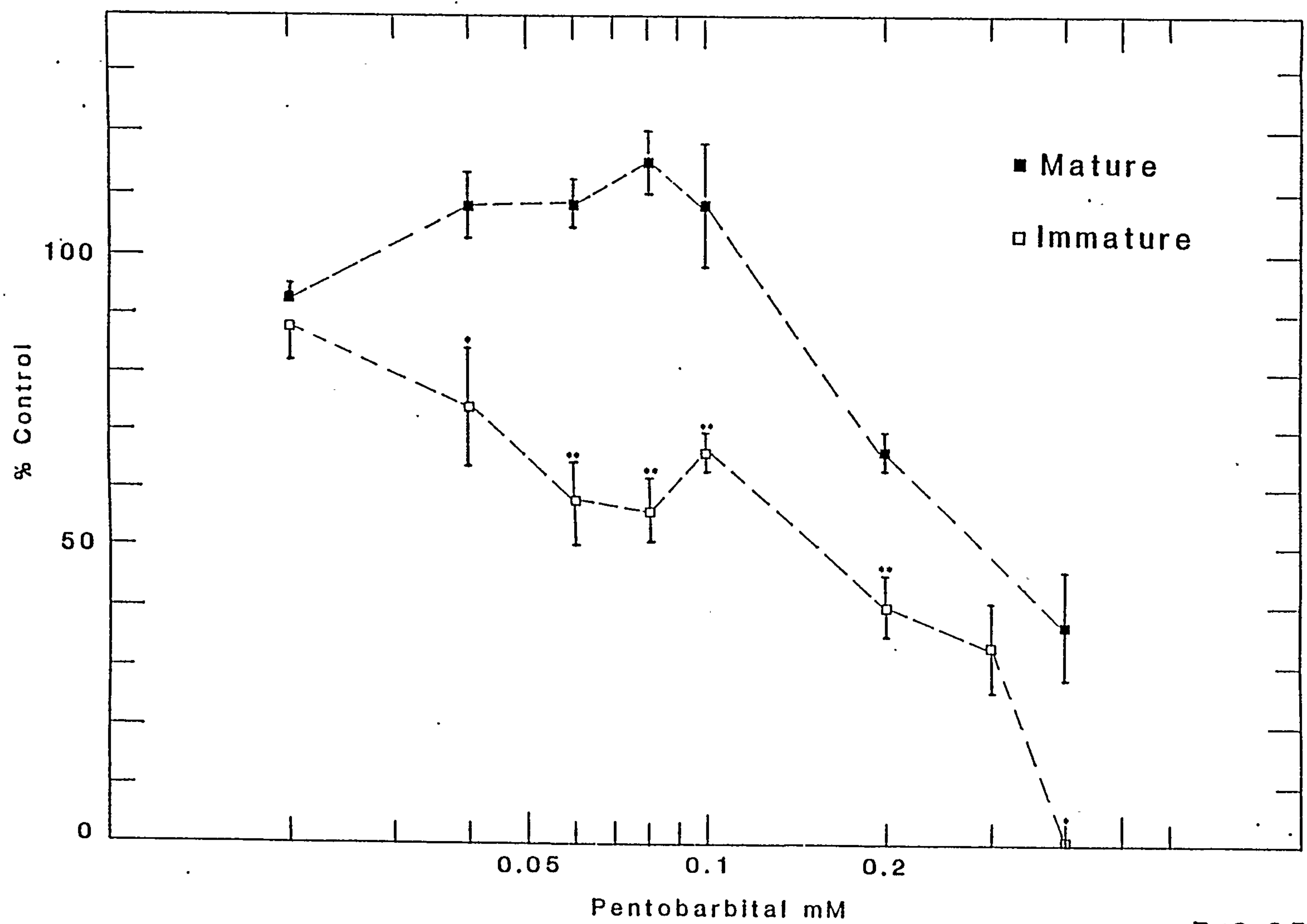
The actions of pentobarbital at 8 different concentrations were examined on immature preparations. At 7 of these concentrations a comparison was done with responses from mature preparations. Combined concentration-response curves are shown in Figure 24. The differences between the two age groups in response to pentobarbital is evident from this figure. At 6 different concentrations, ranging from 0.04 mM to 0.4 mM, the immature responses were found to be significantly lower ($P < 0.05$ and $P < 0.01$) than those seen for mature preparations.

Figure 23 - Concentration-response curves for pentobarbital on immature preparations. Population spike amplitudes (response % of control) were determined from stimulus response curves at (□) maximum, (▲) 3/4 maximum, (△) ½ maximum, and (■) ¼ maximum response. Each point represents the mean +/- S.E.M. of at least four experiments.



maximum response
3/4 maximum response
1/2 maximum response
1/4 maximum response
Response % of Control
100
50
0
0.05
0.1
0.2
Pentobarbital mM

Figure 24 - Concentration-response curves for pentobarbital on mature (■) and immature (□) preparations. The response, population spike amplitude, was expressed as % control. Each point represents the mean +/- S.E.M. of at least five experiments taken at 3/4 maximum response.


■ Mature
□ Immature
100
50
0
% Control
0.05
0.1
0.2
Pentobarbital mM
• P<0.05
•• P<0.01

IV. DISCUSSION

In this study a comparison of the effects of the neuroactive agent, pentobarbital, was investigated on *in vitro* preparations from mature and immature rat hippocampus. It was difficult to obtain stable recordings in animals younger than 14 days postnatal due to the mechanical difficulty of stimulating and recording from too small of a preparation, therefore, animals 14 - 16 days postnatal were chosen for the immature studies.

A. Control Data

1. Field potential responses

Orthodromically evoked field potentials from the CA1 pyramidal region were found to be indistinguishable between mature and immature preparations. In addition, immature preparations were often capable of generating as large or larger postsynaptic discharges (measured as population spike amplitude) as recorded in mature preparations. This indicates that in even youngest animals examined in this study (14 days postnatal), synaptic circuitry was developed enough to produce a coordinated, synchronous discharge from a population of cells in the CA1 pyramidal region.

2. Input-output relationships

When input-output relationships were examined at 30 minute time

intervals, differences between mature and immature preparations became apparent. The relationship of synaptic input to postsynaptic output (the input-output curve) was found to remain stable over a 3 hour period for mature preparations. For immature slices, however, this stability was not observed. Input-output curves collected at subsequent 30 minute intervals could not be superimposed, and for any given sample time the data points were quite scattered. This indicates that the coupling between synaptic input and postsynaptic output over a variety of stimulus strengths was variable in the immature preparations. An observation that may be related to this was reported by Schwartzkroin and Altshuler (1977) who observed a complex EPSP-IPSP sequence in response to orthodromic stimulation in immature kitten hippocampal slices. In the mature preparations a single EPSP-IPSP was recorded following stimulation, whereas in the immature, this initial synaptic event was followed by a second EPSP and IPSP that appeared to be due to different mechanisms than the initial synaptic events. A complex EPSP-IPSP response may result in fluctuations in the probability of evoking action potentials which would make the input-output relationship variable.

3. Stimulus-response relationships

There were also differences over time between stimulus-response relationships in mature and immature preparations. In contrast to the input-output curves, stimulus-response curves were found to be the same shape for both mature and immature preparations. The mature stimulus-response relationships remained stable for periods of up to 3

hours, while immature preparations were found to remain stable for only a maximum of 1½ hours. After 1½ hours, the response decreased as demonstrated by a further shift to the right for the stimulus-response curve. A possible explanation for the instability of the immature response may be that the metabolic activity of the immature system is much more sensitive to constant stimulus. In mature hippocampal slices, maintained high frequency stimulation causes a marked decrease in population spike amplitude, the decrease being dependent on stimulus frequency (Schurr et al., 1986). It was suggested that the decrease was due to an increased demand for necessary high energy substrates (e.g. adenylates) that were reduced in the slice preparations to between 1/3 to ½ their *in vivo* levels. The differences in metabolic rates between *in vitro* and *in vivo* hippocampus have been reviewed by Lipton and Whittingham (1984). A large increase in glucose consumption, a decrease in ion transport properties, and a decrease in protein synthesis have all been described for the *in vitro* preparation as compared to the *in vivo* hippocampus (Lipton and Whittingham, 1984). Metabolic activity in the hippocampus has been studied by examining 2-deoxy-D-glucose uptake (Meibach et al., 1981). In mature rats (30 days postnatal and older), activity was found to be highest in the stratum moleculare. In contrast, in immature rats (younger than 30 days postnatal) metabolic activity was found to be highest in the stratum pyramidale. Given that metabolic changes occur at different rates in different hippocampal regions with maturation, and that in slice preparations there is an increase in demand for an already decreased supply of metabolic substrates, the immature hippocampus could very easily be much more sensitive to the "damaging" effects of constant stimulation as

demonstrated by the instability of the immature stimulus-response relationships.

4. Bursting activity

The most striking difference between the mature and immature preparations was the bursting activity observed in immature slices. These results are in agreement with previous investigations on immature cat, rabbit and rat hippocampal slice preparations (Schwartzkroin and Altshuler, 1977; Haglund and Schwartzkroin, 1984; Harris and Teyler, 1983). This suggests, that in this study, inhibitory synaptic circuitry was not completely developed in the immature group (14 - 16 days postnatal). In addition, the bursting activity may have been endogenously generated by the pyramidal cells themselves. Bursting activity of this type has been described for both invertebrate and vertebrate neurons and appears to be due to slow inward currents carried by Na and/or Ca, and outward currents carried by K (Barker and Gainer, 1975; Eckert and Lux, 1976; Futamachi and Smith, 1982; Mathers and Barker, 1984). This bursting activity may also have had an effect on the rapid decay of response seen in immature preparations. The increase in activity resulting from bursting may produce an even greater demand for metabolic substrates and subsequently a more rapid decrease in response.

B. Drug Responses

1. Effects on field potentials and stimulus-response relationships

(a) Mature preparations

Population spike amplitudes from mature preparations increased in low concentrations of pentobarbital (0.04 mM to 0.1 mM) and decreased in higher concentrations (0.2 mM to 0.4 mM). This biphasic response was seen at all stimulus strengths tested and is in agreement with data previously reported (Roth et al., 1986). These effects also support the observations that barbiturates can produce a number of effects at selective sites on a single synaptic pathway. The increase in activity seen at lower concentrations of pentobarbital may be due to an increased release of intracellular Ca (Carlen et al., 1985; MacIver, 1987) which could cause an increased release of transmitter from presynaptic terminals. An increase in Cl conductances (Shulz and MacDonald, 1981; Simmonds, 1983; Barker and Mathers, 1981), an increase in K conductances (O' Bierne et al., 1987; Carlen et al., 1985) a decrease in presynaptic transmitter release (Collins, 1980; Weakly 1969; Nicoll, 1975) and a decrease in Ca spikes (Owen et al., 1986; MacDonald et al., 1986) could all be responsible for the decrease in response seen at higher concentrations. In addition, as shown in Figure 13, the enhancement in response seen with lower concentrations was at times difficult to reverse, whereas depression seen with higher concentrations was readily reversible. This phenomenon could be due to the differences in the

mechanisms that are responsible for the enhancement and depression. For example, if an increased release of intracellular Ca is responsible for the enhancement (MacIver, 1987), the stimulation of this response may produce a cascade of events (for example, stimulation of second messenger systems) that permanently modifies the cell following drug exposure. Mechanisms that underlie the decrease in activity (for example, an increase in Cl conductance, Barker and Mathers, 1981), however, may be easily reversed when the drug is washed away. This again supports the idea that pentobarbital produces selective actions at specific membrane sites (MacIver and Roth, 1987a).

(b) Immature preparations

Pentobarbital, at all concentrations studied (0.02 mM to 0.4 mM), was found to only decrease population spike amplitudes in immature preparations. In addition, the majority of drug effects were irreversible and in some experiments a further depression in response was seen with washout. These drug effects on the field potentials were found to be dependent on stimulus strength as shown in Figure 23. As shown in this concentration-response relationship, activation of more synaptic inputs can overcome the depressant effects of pentobarbital in these preparations. The fact that drug effects were irreversible and following washout a further depression occurred, suggests that the immature preparations were permanently modified by the drug. This irreversible depression, seen at all concentrations studied, clearly demonstrates that the immature preparations were more sensitive to the drug effects than the mature preparations.

(c) Effects on second positive component of field potentials

A consistent, reversible decrease in the second positive component of the field potential was recorded from both mature and immature preparations. This effect on the late component of the field potential has not been previously reported. This portion of the waveform may reflect the onset of recurrent inhibition due to increased Cl conductances, outward K currents stimulated by cell discharge and the remainder of the field EPSP. All of these have been reported to be altered by pentobarbital in concentrations comparable to the ones used in this study (Barker and Mathers, 1981; Carlen et al., 1985; Barker and Gainer, 1973). The cellular mechanisms underlying these changes are difficult to determine using extracellular recordings, therefore, these actions of pentobarbital should be further investigated using intracellular recording techniques. This would allow one to study the specific ionic conductance changes underlying the EPSP, AHPs and recurrent inhibition.

2. Effects on mature input-output relationships

Effects of pentobarbital were assessed on input-output relationships from mature preparations to gain more information as to possible sites of drug action as described by Andersen (1980) for E-S analysis. In agreement with a previous report (MacIver and Roth, 1987a) lower concentrations of pentobarbital (0.04 mM to 0.08 mM) were found to produce an increase in both EPSP slope and population spike amplitude.

This effect was dependent on the level of stimulus strength that was applied. At low levels of stimulation an enhancement in EPSP slope with a concomitant decrease in population spike amplitude was seen in the presence of drug. As had been discussed previously (MacIver, 1987) this may reflect an increase in release of intracellular Ca which would increase transmitter release accounting for the enhancement in EPSP slope, while increasing Ca levels could also stimulate Ca dependent K conductances, as described by Carlen et al. (1985) which could account for the decrease in postsynaptic output.

At higher concentrations, however, pentobarbital did not produce the same effects as previously described (MacIver and Roth 1987a). In the present study, pentobarbital (0.1 mM to 0.4 mM) decreased the EPSP slope as shown in Figure 21. At higher concentrations (0.4 mM) this decrease in EPSP slope was accompanied by a decrease in population spike amplitude. MacIver and Roth (1987a) found that the decrease in population spike amplitude was accompanied by an increase in EPSP values at higher concentrations of drug. This discrepancy in EPSP response may reflect differences in the experimental paradigms used between the two studies. In the above mentioned study of MacIver and Roth (1987a), paired-pulse stimulation was employed, whereas in the present study, field potentials in response to single stimulus pulses were examined. Perhaps paired-pulse stimulation, even in the presence of pentobarbital, produced a potentiation in EPSP responses while in the present study high concentrations of pentobarbital were able to depress single pulse synaptic input. This would be in agreement with previous reports where pentobarbital was found to selectively decrease EPSP activity on invertebrate neurons (Barker and Gainer, 1973) and inhibit presynaptic transmitter release (Weakly, 1969; Collins, 1980).

3. Effects on bursting activity

Pentobarbital, at all concentrations studied, was found to be without effect on bursting activity displayed by immature preparations. This finding raises some interesting points. Pentobarbital has been shown to enhance inhibitory activity in the hippocampus (Nicoll et al., 1975; Wolf and Haas, 1977; Alger and Nicoll, 1982a,b), therefore, it would be expected that pentobarbital would depress bursting activity by stimulating inhibitory pathways. A number of possibilities may explain the lack of effect of pentobarbital on bursting activity. First, a defiency in the Na/K pump activity has been suggested as a mechanism underlying the bursting and seizure activity described for immature slices (Haglund et al., 1985) and pentobarbital may not have an effect on this mechanism. Second, the inhibitory circuitry in the immature preparation may not be developed sufficiently to allow pentobarbital to produce an effect. Third, endogenously generated activity, due to slow inward Na and Ca currents, may be responsible for the bursting activity observed. Pentobarbital may not be effective in blocking these inward currents, therefore, not acting as an effective anticonvulsant agent. It would be of interest to investigate the effects of a specific anticonvulsant agent, such as phenobarbital, on the bursting activity observed in the immature slices.

4. Differences in sensitivity

This study demonstrated that pentobarbital had significantly different effects on immature hippocampal slice preparations at 6

concentrations as compared to mature preparations. This difference was seen as an increased sensitivity to the depressant effects produced by the drug. There are a number of mechanisms postulated for the actions of pentobarbital and many of these could be attributed to the differences in response between the two preparations.

The GABAergic mechanisms are not completely developed in the immature system. Iontophoresis of GABA in the immature rabbit hippocampus causes a depolarization at the cell body region while exposure to GABA in mature preparations causes a hyperpolarization at the cell body region (Mueller et al., 1983). Therefore, a difference in response to pentobarbital, which acts at this ionophore complex (Barker and Mathers, 1981), would be expected for the mature and immature preparations.

The increased sensitivity of the immature hippocampus could be due to enhancement of Ca dependent K conductances in the presence of pentobarbital that mediate neuronal afterhyperpolarizations (AHP), (Carlen et al., 1985). Schwartzkroin (1982) described the Ca dependent AHP for immature rabbit hippocampus having an "extremely long duration" as compared to that seen in the adult tissue. If exposure to pentobarbital further enhanced this AHP in the immature preparations, inhibition of cell discharge would occur resulting in a greater than expected depression for field potential responses.

Pentobarbital induced effects on Ca transport may be another mechanism involved in this increased sensitivity of the immature system. In tissue culture preparations pentobarbital has been shown to block Ca spike activity (Owen et al., 1986; MacDonald et al., 1986). Ca appears to play a role in the differentiation of electrical excitability in a

number of different cell types (Purves and Lichtman, 1985). In immature rabbit and cat hippocampus and kitten neocortex, action potentials have been described as having longer durations compared to mature responses (Purpura et al., 1965, 1968; Schwartzkroin, 1982). Perhaps an increased Ca conductance plays a role in the generation of wider spike potentials in the developing hippocampus. In immature rabbit hippocampus Ca spikes were elicited in the presence of tetrodotoxin demonstrating their presence in the developing system (Schwartzkroin, 1982). If calcium plays a major role in the cell spiking of the immature system, then blocking its actions could result in a greater decrease in response than seen in mature preparations.

In the immature rabbit CA1 region, Na/K-ATPase activity has been reported to not reach adult levels until two weeks postnatal (equivalent to 18 - 20 days postnatal in the rat, Haglund et al., 1985). A lack of regulatory mechanisms in the immature hippocampus may underlie the increased sensitivity seen with pentobarbital. If exposure to pentobarbital results in an alteration of intracellular and extracellular ionic concentrations, mechanisms such as the Na/K pump must be active to restore normal ionic distributions. If this cannot occur, exposure to pentobarbital could result in a greater and more permanent effect in immature preparations.

In addition to specific sites of action, effects at nonspecific sites should also be considered (Roth, 1979). Differences in membrane composition may exist between the mature and immature preparations and this could result in differences of drug-membrane interactions.

5. Conclusions

As can be seen a number of differences exist between the mature and immature preparations and any one of these alone or in concert with others could be responsible for the mechanisms that underlie the increased sensitivity seen in the immature CNS. The results presented here are in agreement with previous in vivo studies that demonstrated that immature animals were more sensitive to the actions of pentobarbital. (Bianchine and Ferguson, 1967; Kalser et al., 1968; Wilson and Racine, 1985). As shown in Table 1, immature rats demonstrate an increase in sensitivity to barbiturates ranging from 4 to 7.5 times that seen in mature animals, therefore, it can be recommended that the immature dose be reduced at least 1/4 of that prescribed for the mature.

This study demonstrated that an in vitro preparation can be used to show that the immature CNS responds differently to a neuroactive drug as compared to a mature CNS. By using an in vitro preparation one is allowed more ways to interfere experimentally with a specific synaptic pathway than would be possible with the intact brain. This system can, therefore, be used to investigate the proposed mechanisms suggested above to determine specifically the sites and mechanisms that are responsible for the differences seen in response between the mature and immature CNS to pharmacological agents.

Table 1. Comparison of the effects of barbiturates on mature and immature rats.

Response Parameter	Immature Age*	Immature Dose or Time	Mature Dose or Time	Sensitivity Ratio+	Reference
Drug induced sleep for 60 min.	5	40 mg/kg	200 mg/kg	5	Kalser et al., 1968
60% PS amplitude of test PS spike°	14	IPI** = 450 ms	IPI = 60 ms	7.5	Wilson and Racine, 1985
%60 PS amplitude of control response	14-16	0.07 mM	0.280 mM	4	Figure 24

* days postnatal

\+ Immature:Mature

° in the presence of 65 mg/kg pentobarbital

** interpulse interval

V. REFERENCES

Abel, E.L., Jacobson, S. and Sherwin, B.T. (1983). *In utero* alcohol exposure: functional and structural brain damage. Neurobeh. Tox. Teratol., 5: 363-366.

Adams, D.J., Gage, P.W. and Hamill, O.P. (1982). Inhibitory post-synaptic currents at Aplysia cholinergic synapses: effects of permeant anions and depressant drugs. Proc. R. Soc. Lond. B, 214: 335-350.

Alger, B.E. and Nicoll, R.A. (1982a). Feed-forward dendritic inhibition in rat hippocampal pyramidal cells studied *in vitro*. J. Physiol., 328: 105-123.

Alger, B.E. and Nicoll, R.A. (1982b). Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied *in vitro*. J. Physiol., 328: 125-141.

Altman, J. (1966). Autoradiographic and histological studies of postnatal neurogenesis II. J. Comp. Neurol., 128: 431-474.

Altman, J. and Das, G.D. (1966). Autoradiographic and histological studies of postnatal neurogenesis I. J. Comp. Neurol., 126: 337-390.

Altman, J. and Das, G.D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J. Comp. Neurol., 124: 319-336.

Ananth, J. (1978). Side effects in neonate from psychotropic agents excreted through breast-feeding. Am. J. Psychiatry. 135: 801-805.

Andersen, P. (1982). Synaptic control and modulation of hippocampal neurones. In: Advances in Biological Psychiatry. J. Mendlewicz and H.M. van Praag, eds. (Karger, Basel), pp. 40-55.

Andersen, P. and Langmoen, I.A. (1980). Intracellular studies on transmitter effects on neurones in isolated brain slices. Quarterly Rev. Biophys., 13: 1-18.

Andersen, P.A., Sundberg, S.H., Sveen, O., Swann, J.W. and Wigstrom, H. (1980). Possible mechanisms for long-lasting potentiation of synaptic transmission in hippocampal slices from guinea piga. J. Physiol., 302: 463-482.

Andersen, P., Bliss, T.V.P. and Skrede, K.K. (1971). Unit analysis of hippocampal population spikes. Exp. Brain Res., 13: 208-221.

Angevine, J.B., Jr. (1975). Development of the hippocampal region. In: The Hippocampus. R.L. Isaacson and K.H. Pribram, eds. (Plenum, New York), pp. 61-94.

Angevine, J.B., Jr. (1965). Time of neuron origin in the hippocampal region. Exp. Neurol. Suppl., 2: 1-70.

Barker, J.L. and Mathers, D.A. (1981). GABA receptors and the depressant action of pentobarbital. TINS, January pp. 10-13.

Barker, J.L. and Gainer, H. (1974). Studies on bursting pacemaker potential activity in molluscan neurons. I. Membrane properties and ionic contributions. Brain Res., 84: 461-477.

Barker, J.L. and Gainer, H. (1973). Pentobarbital: selective depression of excitatory postsynaptic potentials. Science, 182: 720-722.

Bayer, S.A. (1980). Development of the hippocampal region in the rat I. Neurogenesis examined with ^{3}H-Thymidine autoradiography. J. Comp. Neurol., 190: 87-114.

Bayer, S.A. and Altman, J. (1974). Hippocampal development in the rat: cytogenesis and the morphogenesis examined with autoradiography and low-level x-irradiation. J. Comp. Neurol., 158: 55-80.

Bernardo, L.S. and Prince, D.A. (1982). Dopamine action on hippocampal pyramidal cells. J. Neuroscience, 2: 415-423.

Berridge, M.J. (1985). The molecular basis of communication within the cell. Sci. Am., 253: 142-152.

Bianchine, J.R. and Ferguson, F.C., Jr. (1967). Acute toxicity and lethal brain concentrations of pentobarbital in young and adult albino rats. Proc. Soc. Exp. Biol. Med., 124: 1077-1079.

Biederman, J. and Jellinek, M.S. (1984). Psychopharmacology in children. New Eng. J. Med., 310: 968-972.

Carlen, P.L., Gurenvich, N., Davies, M.F., Blaxter, T.J. and O'Beirne, M. (1985). Enhanced neuronal K conductance: a possible common mechanism for sedative-hypnotic drug action. Can. J. Physiol. Pharmacol., 63: 831-837.

Collingridge, G.L., Gage, P.W. and Robertson, B. (1984). Inhibitory post-synaptic currents in rat hippocampal CA1 neurones. J. Physiol., 356: 551-564.

Collins, G.G.S. (1980). Release of endogenous amino acid neurotransmitter candidates from rat olfactory cortex: possible regulatory mechanisms and the effects of pentobarbitone. Brain Res., 190: 517-528.

Cotman, C., Taylor, D. and Lynch, G. (1973). Ultrastructural changes in synapses in the dentate gyrus of the rat during development. Brain Res., 63: 205-213.

Cowan, W.M. (1979). The development of the brain. Sci. Am., 241: 106-117.

Crain, B., Cotman, C., Taylor, D. and Lynch, G. (1973). A quantitative electron microscopic study of synaptogenesis in the dentate gyrus of the rat. Brain Res., 63: 195-204.

Dingledine, R. and Korn, S.J. (1985). Gama-aminobuteric acid uptake and the termination of inhibitory synaptic potentials in the rat hippocampal slice. J. Physiol., 366: 387-409.

Dingledine, R., Dodd, J. and Kelly, J.S. (1980). The in vitro brain slice as a useful neurophysiological preparation for intracellular recording. J. Neuro. Methods, 2: 323-362.

Eckert, R. and Lux, H.D. (1976). A voltage-sensitive persistent calcium conductance in neuronal somata of Helix. J. Physiol., 254: 129-151.

Finnegan, L. (1976). Clinical effects of pharmacologic agents on pregnancy, the fetus, and the neonate. Ann. New York Acad. Sci., 281: 74-89.

Futamachi, K. and Smith, T.G., Jr. (1982). Action of tetrodotoxin on pacemaker conductances in Aplysia neurons. Brain Res., 233: 424-430.

Gaarskjaer, F.B. (1985). The development of the dentate area and the hippocampal mossy fiber projection of the rat. J. Comp. Neurol., 241: 154-170.

Grays Anatomy (1980). P.L. Williams and R. Warwick, eds. (W.B. Saunders Co., Philadelphia), pp. 997-1002.

Haglund, M.M., Stahl, W.L., Kunkel, D.D. and Schwartzkroin, P.A. (1985). Developmental and regional differences in the localization of Na, K-ATPase activity in the rabbit hippocampus. Brain Res., 343: 198-203.

Haglund, M.M. and Schwartzkroin, P.A. (1984). Seizure-like spreading depression in immature rabbit hippocampus *in vitro*. Dev. Brain Res., 14: 51-59.

Hannah, R.S., Roth, S.H. and Spira, A.W. (1982a). The effects of chlorpromazine and phenobarbital on cerebellar Purkinje cells. Teratology, 26: 21-25.

Hannah, R.S., Roth, S.H. and Spira, A.W. (1982b). The effects of chlorpromazine and phenobarbital on vasculogenesis in the cerebellar cortex. Acta Neuropathologica (Berl), 57: 306-308.

Harris, A.R. and Stokes, J.A. (1982). Effects of a sedative and a convulsant barbiturate on synaptosomal calcium transport. Brain Res., 242: 157-163.

Harris, K.M. and Teyler, T.J. (1983). Evidence for late development of inhibition in area CA1 of the rat hippocampus. Brain Res., 339-343.

Harvey, D.C. (1985). Hypnotics and sedatives. In: The Pharmacological Basis of Therapeutics, G. Gilman, L. S. Goedman, T.W. Rall and F. Murad, eds. (Macmillan Publishing Co., New York), pp. 351-362.

Himwich, W.A. and Davis, J.M. (1974). Free amino acids in the developing brain as affected by drugs. In: Drugs and the Developing Brain. A. Vernadakis and N. Weiner, eds. (Plenum, New York), pp. 231-241.

Hine, R.J. and Das, G.D. (1974). Neuroembryogenesis in the hippocampal formation of the rat. Z. Anat. Entwickl. - Gesch., 144: 173-186.

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

Hoffeld, D.R. and Webster, R.L. (1965). Effect of injection of tranquilizing drugs during pregnancy on offspring. Nature, 205: 1070-1072.

Hudson, D.B., Merrill, B.J. and Sands, L.A. (1974). Effects of prenatal and postnatal nicotine administration on biochemical aspects of brain development. In: Drugs and the Developing Brain. A. Vernadakis and N. Weiner, eds. (Plenum, New York), pp. 243-256.

Janssen, F., Rambeck, B. and Schnabel, R. (1985). Acute valproate intoxication with fatal outcome in an infant. Neuroped., 16: 235-238.

Johnson, E.S., Roberts, M.H.T. and Straughan, D.W.. (1969). The responses of cortical neurones to monoamines under differing anaesthetic conditions. J. Physiol., 203: 261-280.

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

Kalser, S.C., Forbes, E. and Kunig, R. (1969). Relation of brain sensitivity and hepatic metabolism of hexobarbitone to dose-response relations in infant and young rats. J. Pharm. Pharmac., 21: 109-113.

Kunkel, D.D., Hendrickson, A.E., Wu, J.Y. and Schwartzkroin, P.A. (1986). Glutamic acid decarboxylase (GAD) immunocytochemistry of developing rabbit hippocampus. J. Neurosci., 6: 541-552.

Langmoen, I.A., Segal, M. and Andersen, P. (1981). Mechanisms of norepinephrine actions on hippocampal pyramidal cells _in vitro_. Brain Res., 208: 349-362.

Lipton, P. and Whittingham, T.S. (1984). Energy metabolism and brain slice function. In: Brain Slices. R. Dingledine, ed. (Plenum, New York), pp. 113-153.

MacDonald, R.L., Skerritt, J.H. and Werz, M.A. (1986). Barbiturate and benzodiazepine actions on mouse neurons in cell culture. In: Molecular and Cellular Mechanisms of Anaesthetics. S.H. Roth and K.W. Miller, eds. (Plenum, Boston), pp. 17-25.

MacIver, M.B. (1987). The Actions of General Anaesthetics on Synaptic Transmission in Hippocampal Slices. Ph.D. Thesis, University of Calgary, Calgary, Alta.

MacIver, M.B. and Roth, S.H. (1987a). Barbiturate effects on hippocampal excitatory synaptic responses are selective and pathway specific. Can J. Physiol. Pharmacol., 65: 385-394.

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

Mathers, D.A. and Barker, J.L. (1984). Spontaneous voltage and current fluctuations in tissue cultured mouse dorsal root ganglion cells. Brain Res., 293: 35-47.

Meibach, R.C., Ross, D.A., Cox, R.D. and Glick, S.D. (1981). The ontogeny of hippocampal energy metabolism. Brain Res., 204: 431-435.

Mirkin, B.L. (1970). Developmental Pharmacology. Ann. Rev. Pharmacol., 10: 255-272.

Mueller, A.L., Taube, J.S. and Schwartzkroin, P.A. (1984). Development of hyperpolarizing postsynaptic potentials and hyperpolarizing response to gamma-aminobutyric acid in rabbit hippocampus studied _in vitro_. J. Neurosci., 4: 860-687.

Mueller, A.L., Chesnut, R.M. and Schwartzkroin, P.A. (1983). Actions of GABA in developing rabbit hippocampus: an _in vitro_ study. Neurosci. Lett., 39: 193-198.

Nicoletti, F., Iadarola, M.J. Wreblewski, J.T. and Costa, E. (1986). Excitatory amino acid recognition sites coupled with insoitol phospholipid metabolism: developmental changes and interaction with $alpha_1$-adrenoceptors. Proc. Natl. Acad. Sci., 83: 1931-1935.

Nicoll, R.A. (1975). Presynaptic action of barbiturates in the frog spinal cord. Proc. Nat. Acad. Sci., 72: 1460-1463.

Nicoll, R.A., Eccles, J.C., Oshima, T. and Rubia, F. (1975). Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. Nature, 258: 625-627.

Nyhan, W.L. (1961). Toxcitiy of drugs in the neonatal period. J. Pedia., 59: 1-20.

O'Bierne, M., Gurevich, N. and Carlen, P.L. (1987). Pentobarbital inhibits hippocampal neurons by increasing potassium conductance. Can. J. Physiol. Pharmacol., 65: 36-51.

O'Connor, M., Johnson, G.H. and James, D.I. (1981). Intrauterine effect of phenothiazines. Med. J. Australia, 1: 416-417.

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

Pruitt, A.W. (1983). Neonatal drug withdrawal. Pediatrics, 72: 895-902.

Purpura, D.P., Prelevic, S. and Santini, M. (1968). Postsynaptic potentials and spike variations in the feline hippocampus during postnatal ontogenesis. Exp. Neurol., 22: 408-422.

Purpura, D.P., Shofer, R.J. and Scarff, T. (1965). Properties of synaptic activities and spike potentials of neurons in immature neocortex. J. Neurophysiol., 28: 925-942.

Purvis, D. and Lichtman, J.W. (1985). Principles of Neural Development. (Sinauer, Massachusetts), pp. 13-20, 48-49.

Rakic, P., Stensas, L.J. Sayre, E.P. and Sidman, R.L. (1974). Computer-aided three-dimensional reconstruction and quantitative analysis of cells from serial electron microscopic montages of foetal monkey brain. Nature, 250: 31-34.

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

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

Richter, A.R. and Holtman, J.R., Jr. (1982). Barbiturates: Their in vivo effects and potential biochemical mechanisms. Prog. Neurobiol., 18: 275-319.

Roth, S.H., Tan, K-S. and MacIver, M.B. (1986). Selective and differential effects of barbiturates on neuronal activity. In: Molecular and Cellular Mechanisms of Anaesthetics. S.H. Roth and K.W. Miller, eds. (Plenum, Boston) pp. 43-56.

Roth, S.H. (1979). Physical mechanisms of anaesthesia. Ann. Rev. Pharmacol. Toxicol., 19: 159-178.

Schuller, D.E. and Turkewitz, D. (1986). Adverse effects of anti-histamines. Postgrad. Med., 79: 75-86.

Schiff, S.J. and Somjen, G.G. (1985). The effects of temperature on synaptic transmission in hippocampal tissue slices. Brain Res., 345: 279-284.

Schlessinger, A.R., Cowan, W.M. and Swanson, L.W. (1978). The time of origin of neurons in Ammons horn and the associated retro-hippocampal fields. Anat. Embryol., 154: 153-173.

Schlessinger, A.R., Cowan, W.M. and Gottlieb, D.I. (1975). An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. J. Comp. Neurol., 159: 149-176.

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

Schurr, A., Reid, K.H. Tseng, M.T., Edmonds, H.L., Jr., West, C.A. and Rigor, B.M. (1986). Effect of electrical stimulation on the viability of the hippocampal slice preparation. Brain Res. Bull., 16: 299-301.

Schwartzkroin, P.A. and Knowles, P.A. (1983). Local interactions in the hippocampus. TINS, March, 6: 88-92.

Schwartzkroin, P.A., Kunkel, D.D. and Mathers, L.H. (1982). Development of rabbit hippocampus: anatomy. Dev. Brain. Res., 2: 453-468.

Schwartzkroin, P.A. (1982). Development of rabbit hippocampus: physiology. Dev. Brain Res., 2:469-486.

Schwartzkroin, P.A. (1981). To slice or not to slice. In: Electrophysiology of Isolated Mammalian CNS Preparations. G.A. Kerkut and H.V. Wheal, eds. (Academic Press, London), pp. 15-50.

Schwartzkroin, P.A. and Altschuler, R.J. (1977). Development of kitten hippocampal neurons. Brain Res., 134: 429-444.

Shephard, M.G. (1979). The Synaptic Organization of the Brain. (Oxford University Press, New York), pp. 308-337.

Simmonds, M.A. (1983). Multiple GABA receptors and associated regulatory sites. TINS, July: 279-281.

Skrede, K.K. and Westgaard, R.H. (1971). The transverse hippocampal slice: a well defined cortical structure maintained in vitro. Brain Res., 35: 589-593.

Steward O. and Falk, P.M. (1985). Polyribosomes under developing spine synapses: Growth specializations of dendrites at sites of synapto-genesis. J. Neurosci. Res., 13: 75-88.

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

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

Weakly, J.N. (1969). Effect of barbiturates on quantal synaptic transmission in spinal motoneurones. J. Physiol. (Lond), 204: 63-77.

Wilson, D.A. and Racine, R.J. (1985). Barbiturate-enhanced paired-pulse depression in neonatal rats. Neurosci. Lett., 56: 101-106.

Wolf, P. and Haas, H.L. (1977). Effects of diazepines and barbiturates on hippocampal recurrent inhibition. Arch. Pharmacol., 299: 211-218.

VI. APPENDIX

A. Effects of Chlorpromazine on Immature Hippocampal Slices

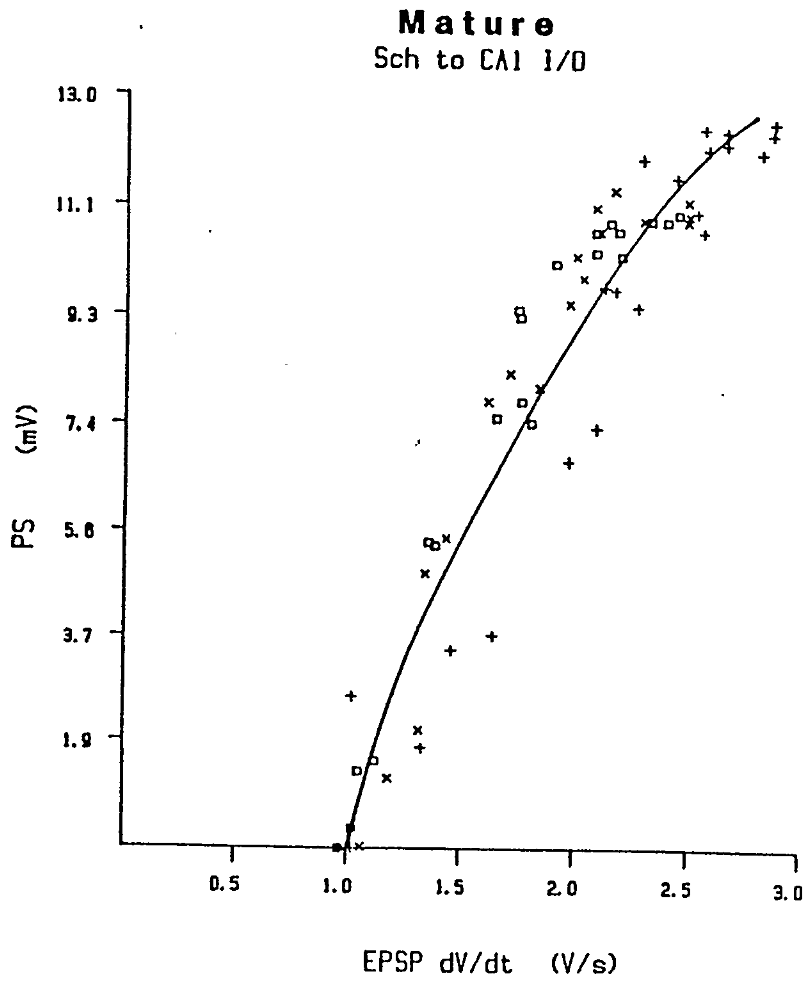
A number of therapeutic agents are administered postnatally to treat various behavioral disorders in infants and children. For example, phenothiazine medications are given to children to treat psychiatric disorders such as mental retardation, conduct disorders and stereotyped movement disorders (see Biederman, 1984). Chlorpromazine (CPZ), the prototype phenothiazine, produces marked learning deficits in animals treated 6 to 12 days postnatally (Hayaski and Tadokoro, 1981) and affects neuronal activity (MacIver and Roth, 1987b). As well, it has been demonstrated that cellular morphology is altered during development upon exposure to this agent (Hannah et al., 1982a,b).

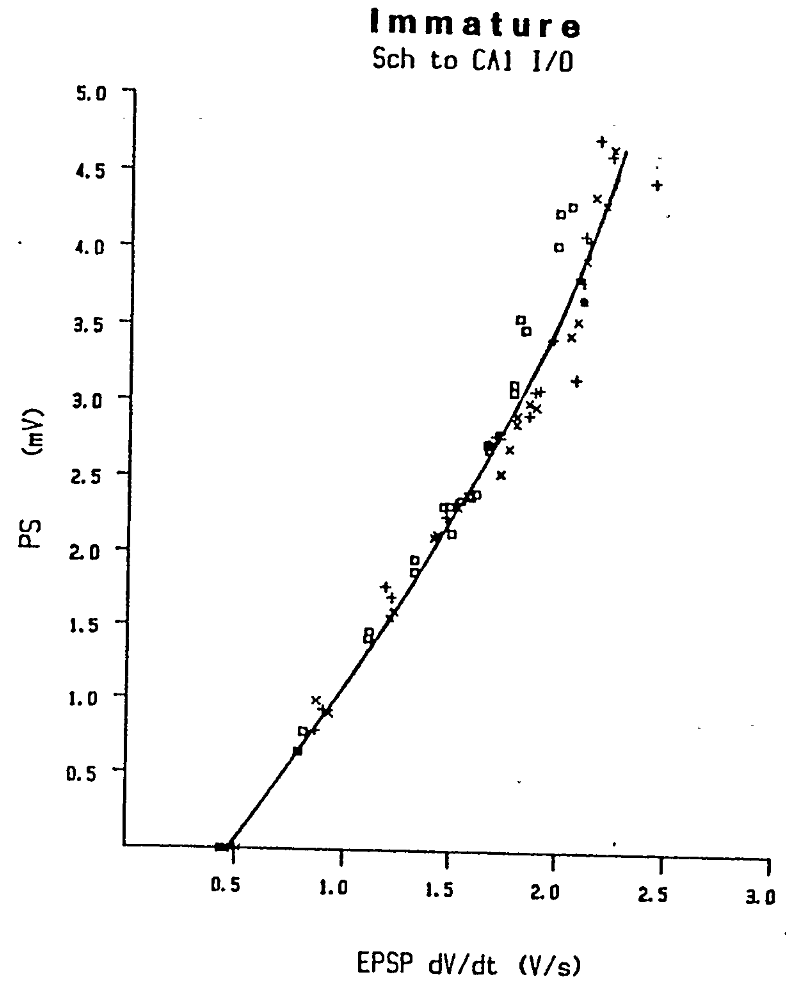
The purpose of the present study was to investigate the effects of CPZ on the immature CNS and to compare these to responses on the mature system.

Chlorpromazine, 1 to 100 uM applied by bath perfusion to both mature and immature slices, did not produce any effect on electrically evoked responses of either the CA1 pyramidal neurons or the dentate granule cells (Figure 25).

At the lower concentration (1 uM) this finding agrees with previous data that demonstrated the lack of effect of CPZ on resting membrane parameters in mature CA1 pyramidal cells (Bernardo and Prince, 1982). This absence of response to CPZ may reflect a low density of dopamine receptors. However, if the population of dopaminergic receptors is small, extracellular analysis may not possess the resolution required to

Figure 25 - Input-output curves from mature and immature preparations showing the effects of chlorpromazine (100 uM) on the relationship between EPSP slope and PS amplitude. Symbols: (+) control, (X) chlorpromazine and (□) washout. Lines are drawn through control points, demonstrating data points for chlorpromazine and washout are well within the range of control data.


Mature
Sch to CA1 I/O
13.0
11.1
9.3
7.4
5.6
3.7
1.9
PS (mV)
0.5
1.0
1.5
2.0
2.5
3.0
EPSP dV/dt (V/s)


Immature
Sch to CA1 I/O
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
PS (mV)
0.5
1.0
1.5
2.0
2.5
3.0
EPSP dV/dt (V/s)

detect subtle changes to CPZ.

The finding that CPZ at the higher concentrations (100 uM) had no effect was unexpected. At this concentration one would predict nonspecific effects to occur. The explanation for this lack of effect remains to be determined.