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# THE EFFECTS OF ETHANOL ON RECRUITING, AUGMENTING AND RETICULAR ACTIVATION RESPONSE THRESHOLDS

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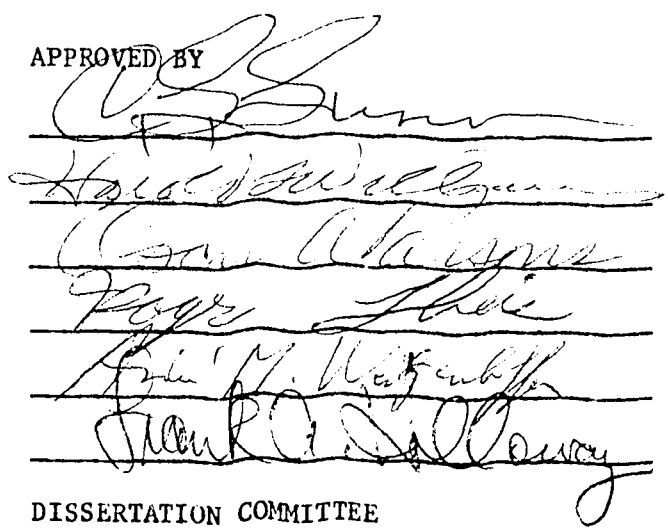
THE EFFECTS OF ETHANOL ON RECRUITING, AUGMENTING

AND RETICULAR ACTIVATION RESPONSE THRESHOLDS

APPROVED BY



DISSERTATION COMMITTEE

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# EFFECTS OF ETHANOL ON RECRUITING, AUGMENTING AND RETICULAR ACTIVATION RESPONSE THRESHOLDS

## CHAPTER I

## INTRODUCTION

Ethanol is traditionally described as an anesthetic drug which depresses the central nervous system (CNS) in a non-specific manner. A contemporary pharmacology text cites ethanol as a "primary and continuous depressant of the CNS" (Ritchie, 1965). Although it is a solvent, a dehydrating agent and an astringent, ethanol is more commonly used as the principle ingredient of an infinite variety of beverages imbibed by man. Consumption in sufficient volumes produces the psychological and physiological state of intoxication whose common names and descriptions are as varied as the drinks which produce it. Few people are unable to describe the symptoms of intoxication whether drawn from personal experience or observation of others.

Notwithstanding these anecdotal descriptions, little is known about the effects of ethanol on the CNS. Its chemical properties are widely known; its neurophysiological properties are not. Despite renewed interest in ethanol as a relatively safe general anesthetic (Dundee, 1970) there have been few systematic attempts to gain insight into its mysterious effects, as is the case with

many anesthetic agents. Considering the countless lives and millions of dollars lost annually to its acute and chronic effects this studied ignorance is unfortunate.

## Acute Effects of Ethanol

Unlike those of other alcohols, the metabolites of ethanol are thought to be relatively innocuous in the body. Sensitive analysis of human blood usually detects an endogenous blood ethanol concentration of 3-6 mg% (Mardones, 1963), the result of normal metabolism of foodstuffs.[1]

With continued ingestion of ethanol, blood concentration rises greatly above endogenous levels and at some concentration, probably idiosyncratic for each drinker, certain external signs of behavioral change and uncoordination mark entry into the intoxicated state. With increasing blood levels, the drinker passes through a predictable progression of psychological phases (Kalant, 1970). The interpretation of these changes by the drinker is a function of his personality and previous drinking experience, the social environment in which he imbibes and blood concentration. Table 1 summarizes some observed behavioral and neurological effects of ethanol.

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[1]The units of "mg%" will be used throughout this dissertation. 100 mg% equals 100 mg ethanol/100 ml plasma which is equivalent to 0.1% ethanol. Where various *in vitro* experiments are cited, the ethanol concentrations cited are converted to mg% as if the bathing solution was plasma. Thus, a 1% (weight/volume) solution approximates 1000 mg%. Specific gravity of ethanol is 0.789; 1% (v/v) solution is 0.789% (w/v) and a 1% (w/v) solution is equivalent to 1.24% (v/v).

Table 1

APPROXIMATE RISING ETHANOL BLOOD LEVELS AT WHICH SOME SIGNS AND SYMPTOMS COMMONLY APPEAR IN MAN

| Blood level (mg%) | Neurological signs | Disturbed function | Mental and skill disturbance |
|---|---|---|---|
| 21-40 | -- | Pain threshold<br>Finger-finger | Euphoria |
| 41-60 | Position nystagmus | Flicker fusion | Problem tests |
| 61-80 | Activation of EEG alpha waves | Romberg tests<br>Binocular fusion | -- |
| 81-100 | Decrease in frequency of EEG alpha waves | -- | Driving mild |
| 101-150 | Overt motor incoordination | -- | Driving medium |
| 151-200 | EEG slow waves episodes | -- | Driving severe |
| 201-300 | Amnesia | -- | -- |
| 301-400 | Comatose state | -- | -- |
| 401-500 | Respiratory arrest | -- | -- |

(Adapted from Mardones, 1963, p. 130.)

Although the exact blood ethanol concentration indicative of intoxication and drunkeness, as well as the medicolegal methods of its determination, are disputed, there is concensus that 200 mg% is associated with mild-to-moderate intoxication and a concentration of 250 mg% or greater with moderate-to-marked intoxication. Of those persons with a blood level of 300 mg%, 90% are intoxicated (Ritchie, 1965). Loss of consciousness may occur in the 400-500 mg% range although Dundee (1970) found that with rapid infusions surgical anesthesia (Stage 3) could be obtained at 200 mg%. The lethal concentration is between 500 and 800 mg%, death being caused by respiratory depression. With artificial respiration, blood levels of 700-1000 mg% have been obtained before cardiac arrest.

## Theories of Anesthetic Action

Until recently, physiological theories seeking to explain the CNS actions of ethanol have depended upon the general theory of anesthetic action. As an anesthetic ethanol has been lumped with the barbiturates and other central depressants as having a primary action on the ascending reticular activating system. According to Ritchie (1965) "electrophysiological studies suggest that ethanol, like other general anesthetics, exerts its depressant action upon a more primitive part of the brain, namely, the reticular activating system."

The monolithic reticular system to which these theories applied has dissolved in recent years into several subsystems having complementary and antagonistic actions. It has, therefore, become difficult to ascribe an anesthetic effect to a drug simply because it

depresses the reticular formation. It may be expected that a general depression of brain stem reticular areas would lead to the collapse of the several subsystems having various actions upon the remainder of the CNS. The result may not necessarily be monotonically (non-reversing) decreasing activation. The existence of non-monotonic effects is demonstrated by the appearance of Stage 1 and Stage 2 anesthesia, defined by a period of behavioral and reflex hyperexcitability at low doses of anesthetic agents. Ethanol may share these stages of excitation providing some justification for the popular use of ethanol as a "stimulant" without regard for its eventual depressive actions.

The implication that the ascending reticular activating system is the main site of anesthetic action is not based on conclusive evidence (Domino, 1955; King, 1956; Longo and Silverstein, 1958). Following the enunciation of the reticular activating system dogma (although Magoun hardly intended it as such) several investigators (French, et al, 1953; Arduini and Arduini, 1954) observed that evoked responses to peripheral stimulation recorded in midbrain reticular formation were depressed at low doses of anesthetics, particularly barbiturates, while evoked responses in specific pathways were not attenuated at these doses. Significantly higher doses were required before amplitude decreases in lemniscal pathways could be demonstrated. Since the reticular activating system was shown to be strongly implicated in the maintenance of consciousness, and hence, perception, this was taken as evidence that the reticular formation was the primary site of action of these drugs.

Arguments for the reticular formation as a preferential site of action for anesthetic drugs vary, but they are primarily concerned with certain morphological characteristics of the brain stem reticular areas. Given anatomical evidence (Ramon-Moliner and Nauta, 1966) that the substrate nuclei of the reticular activating system were mainly composed of comparatively small fibers, one could state as a hypothesis that neuronal metabolism in reticular areas was exquisitely sensitive to depression by anesthetic agents. Another argument, based on a synaptic effect (French, et al, 1953), asserted that most systems considered to be of reticular origin were composed of short multi-synaptic pathways, whereas the classical lemniscal pathways were comparatively pauscisynaptic. It was assumed that if anesthetic agents primarily effected synaptic action, the induced depression in a given system should be proportional to the number of synapses along its routes of reception and action. These arguments would then predict that wherever in the CNS morphological characteristics similar to those specified are found, similar depressive effects would be observed. Other such areas might include the non-specific nuclei of the thalamus as well as portions of the cortical mantle and hippocampus, where extensive arborization of component neurons provides high synaptic content per unit volume as well as small diameter fibers and dendrites.

These concepts predict that anesthetic drugs will cause blocking or alteration in conduction of neural information through the reticular formation. More recent studies indicate that while blocking of conduction through reticular nuclei by whatever means may be responsible for impairment of consciousness, the actions of

anesthetic agents are not wholly confined to reticular areas. For example, King (1956) hypothesized that block of conduction through the reticular formation and subsequent anesthesia was due to the preferential blocking of reticular interneurons. She found, however, that drugs acting solely on midbrain reticular interneurons (e.g., mephenesin) did not produce the same depression of reticular responses or decreases in cortical arousal to reticular stimulation as the general anesthetic agents (e.g., barbiturates and ether). She also found evidence for a differential action between reticular formation and cerebral cortex by demonstrating that recruiting response could be enhanced by low doses of general anesthetics while cortical arousal was depressed. Hence, the original hypothesis of Jasper (1954) that the diffuse thalamic system is the rostral extension of the midbrain activating system was not supported. King demonstrated subsequently (1957) that depression of reticular systems was not the only effect of anesthetic drugs. Doses higher than those required to initiate blocking of cortical arousal to reticular formation stimulation caused increased latency and decreased amplitude with an increase in recovery times in specific thalamic relay responses. This indicates that at higher doses barbiturates may exert a depressant action directly upon thalamic relay nuclei.

In studies of recovery functions of medial lemniscus, ventrobasal thalamus and reticular formation, Boyd and Merrit (1966) found effects similar to those observed by King (1957). Evoked potential latencies and recovery times were increased by barbiturates in thalamus and medial lemniscus and showed greatly increased lability in reticular

formation. They suggested that sedation and general anesthesia result from cumulative depressant effects exerted over the entire neuraxis including the cuneate, thalamic and reticular nuclei.

General theories of anesthesia advanced by early investigators (French, et al, 1953; Randt, et al, 1958) are incomplete since depressive effects have been shown to be widespread through the CNS. Drugs ultimately producing anesthesia also cause functional depression of the spinal cord (Wall, 1967; De Jong and Wagman, 1968; Richens, 1969a, 1969b), brain stem (Arduini and Arduini, 1954; Killam, 1962), thalamus (King, 1956; Boyd and Merrit, 1966) and cerebral cortex (Crawford, 1970).

Considering the variety of drugs capable of inducing anesthesia, the failure of a general theory is not surprising. A comprehensive theory of anesthetic action, other than one specifying ultimate reticular formation depression and loss of consciousness, may not be possible. It can be assumed that while many classes of drugs have similar end-point action (e.g., midbrain depression), the means by which they accomplish this end may be different depending on their different chemical properties.

Examination of the CNS effects of one drug, specifically ethanol, should begin with consideration of the possible actions any drug may have if the final result is anesthesia. It should also consider the known pharmacology of the drug under examination and assess what physiological properties it has in common with other drugs. Given this information it may then be possible to outline the expected actions of ethanol and design tests of these proposed actions. The task of the following chapters is to provide that information.

# CHAPTER II

# EFFECTS OF ETHANOL ON CNS ELECTROPHYSIOLOGY AND BEHAVIOR

## General Drug Effects on the CNS

In general, alterations in CNS functions caused by a drug are due to its presence in the extracellular or intracellular fluid or both. These effects may be one or more of the following: the drug may cause (1) an increase or (2) a decrease in the effect of a CNS transmitter agent on the post-synaptic membrane; the drug may (3) increase or (4) decrease the capability of the neural unit to convey a propagated electrochemical action potential over the extent of its surface.

Effects (1) and (2), alterations in the potential effect of a CNS transmitter, may result from both direct and indirect causes. Certain drugs may directly affect the post-synaptic membrane (e.g., curare at the muscle end-plate) or alter the amount of transmitter reaching the post-synaptic membrane (e.g., acetylcholinesterase). Changes in the potential effect of transmitters may also result indirectly from drug-induced blockade of certain metabolic steps in the production or degradation of transmitter agents at some remote site. These indirect effects are exemplified by the use of reserpine

to deplete and monoamine oxidase inhibitors to increase CNS stores of possible monoamine transmitter agents.

Alternatives (3) and (4), changes in action potential propagation, can also be elaborated to include drug-induced changes in the membrane resting potential. If the drug acts to increase resting potentials, causing hyperpolarization, the firing threshold for an action potential will be raised and the neuron will become less excitable. If the drug acts to decrease the resting potential, the firing threshold will be decreased and the neuron will be rendered more excitable. If the drug causes excessive depolarization, action potentials will be effectively abolished (cathodal block), and the neuron will be totally unexcitable.

These changes may result from the physical presence of the drug at the neural membrane, by the biochemical alterations of some of the membrane constituents, or by changing intracellular metabolic processes. The results of these changes will, in general, cause an increase or decrease in the level of inhibition or excitation operating on other neural units.

## Ethanol Effects on the CNS

### Physical and Metabolic Parameters of Ethanol

Several reviews of ethanol metabolism are available (Kalant, 1962; Mardones, 1963; Russel and Van Bruggen, 1964; Walgren, 1966). In the steady-state, ethanol is distributed through bodily tissues with approximately the same partition coefficients as water and exerts

no osmotic pressure on tissues (Huang, et al, 1957; Davson, et al, 1963; Pappenheimer and Heisey, 1963).

Distribution. Although Eggleton (1940a) suggested that equilibrium may not be reached for 1-1.25 hours with slow intravenous infusion, the use of measurement techniques which are assumed to be more sophisticated suggests that steady state concentrations are reached much more rapidly (Davson, et al, 1966). Ethanol concentration in brain water is at least 96% of plasma concentration within 3 minutes. At the same time the ratio of ethanol in cerebrospinal fluid to that in plasma is only 0.35. The difference in these ratios suggests that there may be a considerable difference in the diffusion velocity of ethanol through the "blood-brain" and "blood-liquor" barriers.

The existence of regional concentration differences in the CNS is also disputed. Recent work (Scherrer-Etienne and Posternak, 1963) suggests that concentration differences, resulting from regional differences in blood supply, are found only prior to the attainment of steady-state conditions. Meyer (1957), however, found evidence of persistent regional differences in concentration correlated with the extent of regional blood supply. He reported brain region to plasma concentration ratios of 0.69 for telencephalon; 0.55 for diencephalon, mesencephalon and cerebellum; and 0.43 for medulla 1-2 hours after infusion. Confirmation of these regional differences would lend support to an hypothesis of differential regional CNS susceptibility to ethanol based on concentration differences.

Blood clearance rates. In cats the rate of ethanol clearance from blood, a rough measure of metabolic rate for ethanol, is proportional to blood concentration over a reasonable range. Eggleton (1940b) found that clearance rates increased 30% for every 100 mg% increase in blood concentration and was 70-75 mg/kg/hr at 100 mg%, 120 at 200 mg% and 140-165 mg/kg/hr at 300 mg%. Under these conditions, 75 mg/kg/hr may roughly approximate 7.5 to 15 mg%/hr clearance.

Studies conducted on humans (Sidell and Pless, 1971) suggest that ethanol clearance rates in man may be independent of blood concentration and average about 19 mg%/hr.

Infusion concentrations. Investigations of animals have often used ethanol concentrations far beyond those which can be tolerated by humans. Lee (1962) found that intra-arterial infusions of ethanol in concentrations greater than 7.5% (w/v) damaged the "blood-brain" barrier. Since infusion was via the external carotid artery directly to the brain without the benefit of systemic dilution, the effects of normal intravenous procedures (e.g., through femoral vein) may be less serious. Nevertheless, it is certain that high concentrations of ethanol place the brain's normal protective mechanisms in jeopardy. Changes in response measures may be caused ordinarily by direct ethanol effects, but with high infusion concentrations they may also result from the invasion of nervous tissue by plasma constituents normally held impermeable.

In studies published as late as 1968 (Di Perri, et al, 1968), infusion concentrations of 20% were routinely used. In another study from the same group (Kakolewski and Himwich, 1968) concentrations

of 33 and 45% were used to reach doses of 4.4 and 6.0 gm/kg. Simple calculations show that these doses are equivalent to the direct intravenous infusion of 13.2 and 18.0 ounces, respectively, in a 70 kg man. It should be apparent that the use of such large quantities at these concentrations have no real significance other than demonstrating the lethality of ethanol using doses beyond those a human could ingest.

## Ethanol Effects at the Neuronal Level

Ethanol directly effects the neural membrane by causing alterations in the resting potential (excitability changes) or action potential (transmission changes). Further, it indirectly affects neural membrane functions by altering the utilization of adenosine phosphates. These substances provide the energy required for active transport of ions necessary to maintain the neuron resting potential and aid in its restoration following action potential passage.

Axonal effects. Gallego (1948) explained the low dose excitation effects found by early investigators (Gibbs, et al, 1937) by showing that in "low" concentrations ethanol exerts a depolarizing action on the resting potential of frog neurons. At high concentrations (4600 mg%) axonal transmission is effectively suppressed by cathodal block resulting from excessive depolarization. Gallego concluded that these changes in neural excitability were sufficient to explain CNS excitatory effects despite the high concentrations required.

However, subsequent studies, using voltage clamp techniques on isolated squid and lobster axon (Moore, et al, 1964; Moore, 1966; Houch, 1969), show that changes in the propagated action potential are

induced at concentrations considerably lower than those required for alterations in axonal resting potentials. Moore, et al (1964) found that 3% ethanol (2367 mg%) decreased sodium and potassium ion conductance across the axonal membrane by 18 and 20%, respectively, and 6% solutions doubled these changes. Nevertheless, Moore (1966) and Houch (1966) concluded that the minimum concentrations (1180 mg%) required for the induction of action potential changes was indicative of relative axonal resistance to ethanol compared with other areas of the neural membrane (e.g., the synapse). Furthermore, Houch (1969) suggests that these data also show that ethanol has separate effects on action potential and resting potential generating mechanisms, but these effects cannot be held to account for CNS changes at lower, non-lethal concentrations.

Although there is no direct comparison of these data with mammalian brain neurons available, cardiac nerve fibers have often been employed as a reasonable analogue. Gimeno (1959) found no significant change in several measured parameters of action potentials conducted through rat atrial nerves. At the concentration used, 480 mg%, the only effect observed was a 10% reduction in action potential duration.

Several anesthetics produce changes similar to those of ethanol in neural membrane conductances. Since these agents have narcotizing activities proportional to their lipid solubilities (Overton-Meyer hypothesis) or thermodynamic activities (Larrabee and Posternal, 1952; McIlwain, 1966), it is possible that ethanol affects the neural membrane by its presence: i.e., interaction of ethanol with components of the membrane lipid layer may cause inhibition of ionic conductances

during an action potential. Such a reaction must take place on the exterior surface of the membrane because conductance changes are not observed when ethanol is introduced into the medium perfusing only the interior of the squid axon (Israel, 1970).

Effects on membrane active transport. The capacity of the neural membrane to generate an action potential depends upon its ability to undergo rapid, short-lived changes in its conductance for sodium and potassium. This ability is apparently decreased by ethanol according to the above data. Action potential generation also depends upon the existence of active (metabolically energized) transport mechanisms. These transport mechanisms enable the neuron to move sodium and potassium back across the membrane, against their electrochemical gradients, in order to reaccumulate resting, steady state concentrations of these ions.

Ethanol is known to inhibit active transport mechanisms for potassium throughout the body, at least in the 400-700 mg% range (Israel-Jacard and Kalant, 1965). This suggests, indirectly, that ethanol depresses the CNS generally by inhibiting the active reaccumulation of potassium. Additional indirect evidence of ethanol's interference with active transport within the CNS has been provided by Wallgren (1963). He reported that a 400 mg% concentration of ethanol in the solution bathing isolated cortical slabs caused a 50% reduction in creatine phosphate (phosphocreatines) breakdown during electrical stimulation of the slab. The phosphocreatines in normal brain exist in equilibrium with adenosine phosphates which serve as the energy producing substrate for active transport. In the unstimulated brain,

this equilibrium is maintained in the presence of ethanol which indicates that the drug is not interfering with normal metabolic production of adenosine triphosphate. Wallgren concluded, therefore, that ethanol interferes with the utilization, rather than synthesis, of adenosine triphosphate. Hence, ethanol induces a blockade of the active transport mechanisms within the neural membrane which serve to reaccumulate sodium and potassium after its depolarization by an action potential. This would result in decreased amplitude or complete suppression of a closely following action potential. Decreased amplitude of the action potential would result in decreased release of synaptic transmitter at the pre-synaptic membrane--causing an indirect but effective depression of synaptic transmission.

Direct examination of the adenosine phosphate systems in vitro substantiates Wallgren's conclusion that ethanol inhibits their utilization. Sun and Samorajski (1970) examined adenosine triphosphate enzyme (ATPase) activities in synaptosomes derived from ultra-centrifugation of guinea pig cerebral cortex. They found that ethanol almost specifically reduced sodium-potassium ATPase activity with a 50% reduction at 2000 mg% and complete abolition at 5500 mg%.

Effects at the synapse and neuromuscular junction. Only the sympathetic pre- to post-ganglionic synapse has been examined in detail. Larrabee and Posternak (1952) found that ethanol (and urethan), in contrast to other anesthetic agents, exerted a greater depressant action on direct fiber transmission than on synaptic activity. Conversely, ethanol facilitated synaptic transmission at "low" concentrations (1000-2000 mg%). These data contrast with those of Moore (1966) and

Houch (1969) who found the axon of squid and lobster to be relatively resistant to ethanol effects--they concluded that the synapse would be more sensitive. One explanation of this difference may be that the cholinergic sympathetic system shares the ethanol-induced facilitation of peripheral cholinergic systems suggested below. Another may be that the class of fibers represented by sympathetic nerves are different in certain characteristics when compared to squid and lobster axon.

At the neuromuscular junction several investigators (Gage, 1965; Inhou and Frank, 1967; Okada, 1967) have shown that ethanol potentiates cholinergic transmission by causing increases in pre-synaptic excitability, thereby causing the release of larger quantities of acetylcholine, as well as increasing post-synaptic (end-plate) excitability. Gage (1965) found increased pre-synaptic excitability at concentrations equivalent to 37 mg% with no accompanying change in post-synaptic events. At doses equivalent to 500-800 mg% both pre- and post-synaptic excitabilities are increased (Gage, 1965); complete block of transmission occurs at 3000 mg% (Okada, 1967). Since they could not demonstrate similar facilitatory effects at central cholinergic synapses, Inhou and Frank (1967) concluded that any general theory of ethanol's anesthetic action based on cholinergic effects is unwarranted.

Studies in isolated cortex (Kalant and Grose, 1967; Kalant, et al, 1967) also indicate a lack of similarity between the effects of ethanol on peripheral and central cholinergic systems. Upon electrical stimulation, acetylcholine is spontaneously liberated by isolated slices of cerebral cortex. On the basis of neuromuscular junction data, this liberation should increase under the influence of ethanol. However,

ethanol in concentrations equivalent to 500 mg% reduced liberation of acetylcholine by 20%. This result suggests that ethanol may inhibit release of acetylcholine centrally and lead to a dimunition of transmission at central cholinergic synapses.

Summary of ethanol effects at the neuronal level. Ethanol causes a specific decrease in sodium and potassium ion conductances across the axonal membrane and depresses active transport mechanisms necessary for the regeneration of normal resting potentials. However, these effects occur only at ethanol concentrations known to be lethal. Furthermore, direct effects on membrane resting potential occur at even higher concentrations. Potentiation of cholinergic transmitter release is found at peripheral synapses within the range of non-lethal concentrations. Similar information has not been reported for adrenergic systems. The effect of ethanol on central cholinergic systems may be to inhibit rather than potentiate transmitter release.

These data suggest that membrane resting potentials are relatively immune to acute ethanol effects, that the mechanisms responsible for action potential production and subsequent membrane recovery are less immune, and that the synapse is probably the area of the neural membrane most susceptible to ethanol.

## Effects of Ethanol on Reflexes

Studies of the effects of ethanol on reflexes have focused predominantly on those mediated by the spinal cord. Megirian (1958) found that either during rising blood ethanol concentrations or after an apparent steady-state had been achieved, ethanol exerted a selective

effect, depressing polysynaptic reflexes to a greater extent than monosynaptic reflexes. Pentothal exerted a similar action but chloroform and thiopentothal showed no selective action.

Supraspinal modulation of polysynaptic reflexes is found to be depressed at ethanol concentrations lower than those required for alteration of isolated mono- and polysynaptic reflexes (Machne, 1950; Megirian, 1958; Ishido, 1962; Kalant, 1970). These investigators found evidence for a biphasic action of ethanol with relative excitation of polysynaptic reflexes at low doses and pure depression of both mono- and polysynaptic reflexes at higher doses. Severance of supraspinal connections abolished these biphasic effects causing mono- and polysynaptic reflexes to be equally depressed at all doses. Ishido (1962) showed that selective facilitation or inhibition of polysynaptic spinal reflexes, elicited by appropriate stimulation of the midbrain reticular formation, was abolished before ethanol induced changes in non-reticular influenced reflexes were observed.

Modulation of spinal reflexes by descending fibers arising from supraspinal nuclei is apparently mediated by post-synaptic inhibition and facilitation of interneurons and by pre-synaptic inhibition of primary afferent fibers (Lundberg, 1967; Brodal, 1969). Inhibition of spinal and trigeminal reflexes by orbital-frontal cortex stimulation (Sauerland, Nakamura and Clemente, 1967) is mediated by post-synaptic inhibitory mechanisms (Nakamura, et al, 1967). Ethanol reduces the extent of cortically induced inhibition on these reflexes--an ethanol-induced inhibition of inhibition--with a maximum observed reduction at 67 mg% (Sauerland, Knauss and Clemente, 1967). Sauerland concluded

that reduction of orbital-frontal inhibition results from depression of the medullary reticular formation.

In contrast, ethanol increases pre-synaptic inhibition of trigeminal sensory neurons (Sauerland, 1970; Sauerland, et al, 1970) and primary afferent spinal fibers (Miyahara, 1966) over a blood ethanol concentration range of 50-250 mg%. When descending fiber systems arising from supraspinal nuclei are severed, ethanol-induced enhancement of pre-synaptic inhibition is abolished.

These data suggest that ethanol-induced depression of spinal reflexes in the intact animal, with supraspinal modulatory mechanisms operative, must be primarily a function of increased pre-synaptic inhibition. Furthermore, they provide a source for the increased excitation observed at low concentrations. The reduction of inhibition derived from orbital-frontal cortex begins at concentrations as low as 25 mg% but enhancement of pre-synaptic inhibition requires somewhat higher concentrations. Increased excitation may occur within a concentration range bounded by these effects due to a decline in descending post-synaptic inhibition before pre-synaptic inhibition increases sufficiently to compensate for the loss. At higher concentrations, increased pre-synaptic inhibition leads to the depression of spinal reflexes and loss of sensory transmission through the cord. Hence, a transient period of reflex excitability is followed by reflex depression and deepening analgesia. Loss of cutaneous sensation (Sauerland, 1967) may be attributed to increased pre-synaptic inhibition of primary afferents as sensory information reaches the terminal arborizations of first-order sensory neurons.

Summary of ethanol effects on reflexes. Ethanol exerts certain selective actions on spinal reflexes, depressing polysynaptic reflexes to a greater extent than monosynaptic reflexes. Biphasic effects, with relative excitation at low doses, are a function of descending modulatory systems which primarily mediate inhibition. Ethanol apparently exerts a selective pharmacological blockade of these supraspinal influences, reducing the strength of descending post-synaptic inhibition of spinal interneurons while enhancing pre-synaptic inhibition of primary afferent pathways. Suppression of post-synaptic inhibition at ethanol concentrations lower than those required for potentiation of pre-synaptic inhibition may account for biphasic phenomena. It may be assumed that interference with modulatory controls is primarily a function of ethanol's direct effects on supraspinal nuclei while strictly depressive effects on spinal reflexes result from direct depression of the spinal cord.

All effects noted here occur well within the range of non-lethal ethanol concentrations. The range wherein biphasic phenomena have been observed is in good agreement with the range required for low dose cortical activation effects discussed below.

## Effects of Ethanol at Supraspinal Levels

Effects on spontaneous electrical activity. There is evidence that at low doses ethanol activates spontaneous cortical activity. Horsey and Akert (1953), studying immobolized, acute cats, observed a transitory stage of prolonged activation at blood concentrations below 50 mg%. With doses in the 500 mg/kg range (estimated blood

concentration: 60 mg%) and during the initial stages of infusion, Akabane, et al (1964) found signs of slight, transient activation of cortical and hippocampal spontaneous electrical activity. With higher intravenous doses, Akabane found increased high and low voltage slow wave activity with a decrease in spindle frequency.

Attempting to elucidate the mechanism whereby ethanol affects spontaneous electrical activity, Akabane, et al (1964) used intracarotid and intravertebral injections of ethanol and observed spontaneous activity and autonomic changes before and after midbrain transection. Intracarotid ethanol (50-100 mg/kg) produced slow wave activity in the cortex and hippocampus accompanied by a transient decline in systemic blood pressure. No signs of cortical activation were seen. After transection at the intercollicular level, all effects were the same during identical infusions, except that no change in blood pressure was observed. Injecting 30-50 mg/kg intravertebrally, they observed transient activation of cortex and hippocampus, followed by slow waves; blood pressure transiently declined 40 mm Hg and returned to control levels as spontaneous electrical activity reverted to baseline patterns. With midbrain transection, no activation was observed but all other effects were the same. Akabane concluded that ethanol depresses the CNS rostral to midpontine areas, thus causing transient activation of the midpontine and bulbar reticular formation, then depresses the latter regions.

Studies of spontaneous electrical activity in man (Gibbs, et al, 1937; Doctor, et al, 1966) also show biphasic effects, with signs of cortical activation at low doses. In humans, alpha band (8-13 Hz)

amplitude increases with shifts in the predominant frequency. Doctor, et al (1966) observed an increase in the 8-9 Hz alpha band with either no change or a decrease in activity in higher alpha bands. These changes were observed simultaneously with improved performance on a vigilance task; maximum effects could be obtained with a concentration of 26 mg%.

Low dose activation is also suggested by changes in cortical blood flow. Hadji-Dimo, et al (1968) observed transient but significant increases in cortical blood flow accompanied by an increase in the electroencephalograph frequency index with blood concentrations of 50 mg%. Both cortical blood flow and frequency index decreased at higher concentrations (130 mg%). They interpret these data as evidence of increased functional activity of the cerebral cortex at low doses. Similar effects could not be demonstrated with pentobarbital.

The effects of ethanol can also be differentiated from barbiturates through observation of drug induced changes in spontaneous electrical activity in the red nucleus. Gogalak, et al (1969) observed that barbiturates elicit a 4-30 Hz rhythm in red nucleus of rabbits, and that the frequency decreased linearly with increasing barbiturate blood concentration. Ethanol (and urethan) potentiated the action of barbiturates at the cortex but were antagonistic to bartiturate-elicited waves in red nucleus. Ethanol increased the frequency in proportion to concentration and counteracted the slowing elicited by barbiturates. All drugs usually classified as hypnotic-narcotic and, therefore, potential anesthetics, elicited regular waves.

These investigators agree that few depressive effects, at least as indicated by cortical slowing, are seen below 100 mg%. At higher concentrations there is an increasing frequency of slow-wave spindles, followed by repeated bursts of theta (4-8 Hz) and delta (less than 4 Hz) waves which will finally coalesce to diffuse slow wave activity as a comatose state is entered. Spontaneous activity in subcortical and limbic structures usually parallels changes in cortical activity (Story, et al, 1961; Akabane, et al, 1964) but no systematic examination of these areas has been made.

Effects on CNS evoked responses. Ethanol is generally agreed to cause decreasing amplitudes in the sensory evoked response with increasing concentration (Kalant, 1970). Such a change might be a function of several factors including changes in transmission properties of the axon, changes in transfer properties of the synapse, or indirect changes in these functions caused by abnormal changes in the physiological parameters of tissues surrounding neural elements.

Direct changes in transmission caused by the deterioration of axonal conduction, either by the reduction of resting potentials or changes in conductance properties, do occur. However, as shown in the preceeding sections, such changes occur only at ethanol concentrations higher than those compatible with life. Direct examination of the conduction properties of peripheral nerves, which may be assumed to reflect possible alterations in axonal transmission, shows no significant changes in conduction velocities up to 130 mg% (Peiris, et al, 1966).

Changes in the normal physiology of the CNS do not seem to be a tenable alternative either. In fact, cortical oxygenation may be increased at low doses of ethanol (Hadji-Dimo, et al, 1968). At high concentrations there is every sign, however, that normal physiology is adversely affected as is the case with most general anesthetics (Nagai, 1963).

Although ethanol apparently exerts biphasic effects on certain spinal reflexes and spontaneous electrical activity, only a monophasic effect is seen with sensory evoked potentials. The lack of biphasic action led Nakai (1964) to discriminate between ethanol and barbiturates as anesthetics since only the latter increased sensory evoked potential amplitudes at low doses. With ethanol, amplitudes dropped 10 to 30% below control with doses of 200-400 mg/kg. Similar results were found with chronic, unrestrained animals (Nakai, et al, 1966). In both sets of experiments urethan produced the same monotonic effects as ethanol while chlorpromazine and chloralose produced some biphasic effects.

Interestingly, urethan also exerts effects similar to those of ethanol on the sympathetic ganglion (Larabee and Posternak, 1952) and red nucleus (Gogolak, et al, 1969) as described in previous sections. Here, Nakai, et al (1966) also found that urethan has a similar effect upon evoked potentials. Since urethan is the carbamic acid ester of ethanol these similarities in action might be accounted for by some similarity in chemical structure.

Attempts to show regional differences in ethanol effects by the Himwich group using evoked responses have not produced any consistent

conclusions (Dravid, et al, 1963; Schweigerdt, et al, 1965; Himwich, et al, 1966; Di Perri, et al, 1968). Their findings have been confounded by intracortical differences in ethanol effect (Schweigerdt, et al, 1965), but they have generally concluded that association cortex has the greatest sensitivity to ethanol followed by midbrain reticular formation, then specific cortex. On the basis of these results, Himwich, et al (1966) have espoused the hypothesis that ethanol depression is a function of the number of synapses in a sensory pathway, with multisynaptic systems (e.g., association cortex) showing the greatest depression.

However, more conclusive data from other studies indicate that if decreases in evoked response amplitude reflect depression of functional capacity, then the order of sensitivity for reticular formation and specific projection cortex should be reversed. Using auditory and visual evoked responses with concurrent stimulation of the reticular formation, Nakai (Takaori, et al, 1966; Nakai and Domino, 1969) showed that alterations in the cortical evoked response caused by reticular "inhibition" or "facilitation" were unchanged with doses of ethanol up to 1600 mg/kg. Using a reticular area producing "inhibition", Takaori, et al (1966) found abolition of reticular influences with pentobarbital, chlorpromazine and chloralose but retention of "inhibition" with ethanol and urethan.

Nakai and Domino (1969) observed reticular formation facilitated evoked responses elicited by optic tract stimulation and found that the non-facilitated response decreased in amplitude with increasing ethanol doses. However, at all doses (200-1600 mg/kg) reticular

stimulation returned the visual response to undrugged control amplitudes. Pentobarbital, in contrast, directly depressed the reticular formation as shown by the decreasing amplitude of the facilitated response parallel with a decrease in the non-facilitated response.

Closer examination of the ethanol altered visual evoked response shows that the first positive wave, known to indicate the arrival of pre-synaptic radiation volleys, is unchanged over the dose range employed by Nakai. Similar results were found with pentothal and chlorpromazine in the same study and by others (Evarts, et al, 1961; Schoolman and Evarts, 1959). There is some argument about waves of intermediate latency, but the final positive peak is known to be of post-synaptic origin (Chang and Kaada, 1950). Sharp attenuation of this peak strongly implicates the cortex in ethanol-induced alteration of the visual evoked response: reticular stimulation returns the final peak to control amplitude (within 10%) with ethanol but not with a barbiturate.

There are, however, some inconsistencies with this interpretation in the literature. Story, et al (1961) found decreases in the amplitude of visual evoked responses recorded at the cortex at a concentration of 250 mg% as might be expected from the above data. This study also found that augmenting and recruiting response amplitudes were decreased. However, increases were observed in the amplitude of the direct cortical and interhippocampal responses. Unfortunately, no systematic conclusions can be drawn from this study because no response was observed at a concentration of less than 200 mg% and all responses were observed on the declining concentration curve after a

very high initial dose. Additionally, both augmenting and recruiting responses were elicited by paired shocks rather than repetitive stimulation which, by definition, is required for these responses.

Summary of ethanol effects at supraspinal levels. Spontaneous electrical activity is relatively resistant to ethanol with depressive signs observed only at blood concentrations above 100 mg%. Some signs of activation are observed at low doses, however. Evoked response studies show that CNS depression may be proportional to the number of synapses in the tested pathway, but the reticular formation does not necessarily fit this pattern. The functional integrity of the reticular formation appears to be more resistant to the effects of ethanol than cortical mechanisms although there is some evidence that reticular evoked responses are depressed without loss of ascending reticular influences. Thus far, the weight of the evidence for low and intermediate doses indicates greatest sensitivity at the cortical level.

## Effects of Ethanol on Behavior

The primary concern of this section is to review the possible effects of ethanol on human behavior at low and intermediate doses. Several reviews and monographs are available for more complete data (Jellinek and McFarland, 1940; Mardones, 1963; Goldberg, 1966; Kalant, 1970).

It is well accepted that moderate to high doses depress most all tests of dexterity, information processing and mentation (Kalant, 1970). The results of some tests, however, suggest that there is a slight but significant improvement in performance at low concentrations.

Doctor, et al (1966) found that subjects maintained control levels of performance on a vigilance task over a range of 26-64 mg%. Usually there is a fall in vigilance performance proportional to the duration of testing as shown by study of undrugged subjects. When the same subjects were tested with ethanol, performance showed a transient, but much smaller, decrease. As blood concentrations declined to 26 mg%, performance showed steady improvement. These changes were paralleled by marked changes in alpha power distribution in the electroencephalograph (see above, "Effects on spontaneous electrical activity"). Additional signs of increased performance at low doses were found by Carpenter (1961) on various tests of attention, memory and mathematical problem solving.

In an attempt to discriminate the effects of ethanol on different mental operations, Hamilton and Copeman (1970) placed subjects in a complex tracking task while requiring them to monitor and react to brief stimuli placed about the periphery of their visual field. Under low doses of ethanol, performance on the tracking task improved over that of control subjects, but detection of peripheral stimuli was impaired. They concluded that ethanol caused an increase in "attentional bias" towards the central task, and therefore caused better performance, but the added bias reduced the total information processing capability of the subject.

Ethanol, in low doses, has been thought to produce a state of tranquilization demonstrated by the reduction of selective emotional responses to previously evocative words (Coopersmith, 1964), reduction of "anxiety" produced by conditioned stimuli (Forney and Hughes, 1963)

and synergistic potentiation of a known tranquilizer (Goldberg, 1966). With the observation of increased rapid-eye-movement in quiet (non-sleeping) subjects, Doctor, et al (1966) proposed the following theory of low dose ethanol action: (1) the initial effect is tranquilization as shown by electroencephalographic changes, followed by (2) a release of ideational processes suggested by rapid eye movement increases and (3) this increased mental activity contributes to sustained vigilance task performance.

The observation that ethanol in low doses produces a euphoric state accompanied by increased performance on some tasks suggests a state of inhibition on mentational processes so long as the task is relatively simple. When the task is complicated by confusing extraneous stimuli, performance declines at a rate faster than that for non-drugged subjects (Forney, et al, 1964; Hughes and Forney, 1963).

While low concentrations may be associated with a state characterized as tranquilization, a decrease in performance in real-life situations may not necessarily follow. A study of the relationship between blood ethanol concentration and driving accidents shows that the probability of being in a reportable accident may decrease at low concentrations. Dale (1963), after exhaustive study of vehicle accident reports, found, contrary to the usual expectation, that the probability of being involved in an accident declined with concentrations in the 20-30 mg% range and only approached that of the non-drinking driver again at 40 mg%. At higher concentrations, accident probability increased rapidly until at 100 mg% drivers were 6-7 times more likely to be involved in an accident.

These studies suggest a certain basis for the layman's impression of ethanol's "stimulating" character. Anecdotal evidence suggests that there is a decrease in socially-derived inhibition in the lowest state of intoxication shown by increased disregard for social conventions and the consequences of their violation.

## General Summary of Ethanol Effects on the CNS

Although it has received scant attention in the literature, the most important effect of ethanol on the CNS may be the extent to which it distorts normal modulatory control over afferent sensory transmission and information processing, in general. This distortion is the result of ethanol's disruption of descending inhibitory processes which arise from structures rostral to the superior colliculus. The hypothesis stated above, concerning the discrepancy between concentrations required for inhibition of descending inhibition from orbital cortex and that required for enhancement of pre-synaptic inhibition, may well be the primary basis for low dose CNS excitation.

While there are direct effects on areas of the neural membrane besides synaptic regions, these effects are only manifested at concentrations considerably higher than those observed in acute intoxication. The original supposition by Gallego (1948) that low dose effects are primarily a function of resting potential alterations is found to be entirely unrealistic.

Although ethanol exerts specific, predictable effects on certain aspects of behavior, particularly those usually defined as "cognition," the physiological mechanisms whereby such alterations

are made manifest are relatively unknown and are the subject of continuous, but thus far, unproductive, debate. Deeper understanding of the means by which ethanol alters normal modulatory functions may ultimately provide viable explanations for behavioral phenomena.

## Evaluation of Ethanol Effects on CNS Modulatory Mechanisms

In the first section of this chapter it was suggested that the effects of a general CNS-active drug consisted of one or more of the following alternatives: the drug may (1) increase or (2) decrease the effect of a CNS transmitter agent on the post-synaptic membrane; the drug may (3) increase or (4) decrease the capability of the neural unit to convey a propagated electrochemical action potential over the extent of its surface. These alternatives suggest that the actions of a general drug may be classified as having a primary mode of action on either synaptic or axonal functions.

Considering ethanol specifically, the preceding review of its known CNS effects demonstrates that alternatives (3) or (4) are not likely to be the primary modes of action. Although ethanol has been shown to alter certain characteristics of action potential generation, such alterations occur only at concentrations incompatible with life. Alterations in axonal conduction may contribute to the depression observed at high doses, but they cannot account for the changes in CNS functions observed at low doses.

A reasonable hypothesis, therefore, is that the primary mode of ethanol involves alteration of synaptic function. Whether ethanol exerts direct or indirect actions on synaptic functions remains moot.

however, the rapid onset of ethanol-induced changes suggests that the drug has a primary action at the synapse.

Although the synapse serves primarily to mediate the transfer of information from neuron to neuron in the form of action potentials, it also enables the modulation of that information. The complex operations of the synapse involve the summation of excitatory and inhibitory potentials present at the post-synaptic membrane. An action potential is triggered and information is transferred only when that summation yields a total potential exceeding a certain threshold. The process of modulation involves an increase or decrease in the amount of inhibition placed on the post-synaptic membrane.

When these processes are summated over a pool of neurons, the amount and quality of information transmitted through that pool is a function of the total amount of inhibition and excitation present within the pool. Hence, factors which alter the modulatory capability of the CNS may do so by increasing or decreasing the overall level of inhibition present in the CNS. Such factors may not necessarily alter the transmission of information but rather change certain of its characteristics. At the behavioral level these characteristics may include alterations in the preset priorities or perceptive thresholds allowed for certain classes of information.

Experimental evidence reviewed in the preceding sections suggests that the CNS actions of ethanol may be roughly dichotomized into low dose effects on modulatory mechanisms and high dose effects on the process of information transmission itself. Investigations on the spinal cord (Machne, 1950; Megirian, 1958; Ishido, 1962) demonstrate

that low dose facilitation of certain reflexes is a function of descending modulatory systems while direct depression of spinal reflexes is found only at higher doses. Similarly, observations of spontaneous cortical electrical activity show changes at low doses which may be interpreted as increased excitation (Horsey and Akert, 1953; Akabane, et al, 1964; Doctor, et al, 1966) and changes at high doses which can only be interpreted as evidence of CNS depression. Additionally, examination of the effects of ethanol on behavioral tasks (Hamilton and Copeman, 1970) suggests that there may be a facilitation of certain aspects of information handling but that such improvement is offset by a loss of modulatory capacities which serve to order the priorities of responses to afferent information. At high doses, behavioral tests demonstrate strictly depressive effects (Kalant, 1970).

Although these investigations provide support for the conclusion that ethanol alters modulatory functions at low doses and, hence, the integration of information within the CNS, this support is indirect. None of these investigations have attempted to examine directly the effects of ethanol on CNS modulation. If this conclusion is warranted, these studies also do not fully examine the low range of ethanol concentrations wherein the alteration of modulatory functions may be more evident.

Direct support for this conclusion may be obtained by examination of a wide range of ethanol concentrations and their effect upon CNS systems thought to be predominantly responsible for mediating modulation of CNS afferent pathways.

## Purpose of this Dissertation

The primary purpose of this dissertation is to provide some explanation of the CNS and behavioral changes defining the intoxicated state in man. It will seek that information by direct examination of neurophysiological systems thought to be responsible for the modulation of CNS inhibition and excitation. The experiment will measure changes in the excitability of these systems with the expectation of interpreting these changes as evidence for direct effects on CNS modulatory mechanisms.

This study will examine the effects of ethanol on the characteristic responses of three well-documented integrative systems. These are the recruiting response of the non-specific thalamic system, the augmenting response of the specific thalamic afferent system, and the cortical desynchrony, cardiovascular pressor and skin resistance responses of the reticular activating system.

The non-specific thalamic system exerts primarily inhibitory controls over the rostral CNS particularly directed towards the cerebral cortex (Andersen and Andersson, 1968). The reticular activating system provides excitatory controls over the same rostral areas as well as descending influences through the level of the spinal cord (Brodal, 1969). Observation of changes in three different expressions of reticular activation will provide information about both the ascending and descending portions of this system. Examination of the specific thalamic afferent system will provide information about the integration of information in a system whose function is primarily the transmission of that information to the cerebral cortex.

Specifically, this study will examine the effects of increasing blood ethanol concentrations on the stimulation threshold of each of these five responses. Observation of threshold changes induced by ethanol should provide information about the excitability of these systems. If changes are observed in the threshold for any of these responses, they may be interpreted as suggesting increases or decreases in the potential amount of modulatory capability available in the intoxicated state. Observation of these changes may then lead to more precise explanations of the source of the alterations in information handling and integrating functions known to be a part of the definition of ethanol intoxication.

# CHAPTER III

## METHODS AND PROCEDURES

Experiments were performed on 42 cats of either sex, weighing between 1.7 and 4.2 kg. All animals were obtained in good health from licensed sources and maintained prior to use in the Laboratory Animal Facility of the University of Oklahoma Medical Center licensed under Federal regulation for animal care.

### Surgical Procedures

Animals were prepared under diethyl ether general anesthesia initiated in an airtight box. When the animal was fully anesthetized, it was transferred to the surgical table where anesthesia was maintained by an ether impregnated mask. An endotracheal catheter was inserted to maintain an unobstructed airway, and anesthesia was maintained thereafter with a bottle vaporizer. The femoral artery and vein on the same side were cannulated and all surgical and pressure sites were widely infiltrated with local anesthetic (Xylocaine, 1%). Ether anesthesia was then discontinued and the animals were immobilized with intravenous gallamine triethiodide (Flaxadil; 1 mg/kg initially and every 45 minutes thereafter). As paralysis became evident the animal was placed on a positive pressure non-rebreathing respirator circuit through the endotracheal catheter derived from a Harvard large animal

respirator. Final respirator parameters fell within a volume range of 75-150 cc at 15-25 cycles per minute.

It should be noted that, contrary to usual procedures, general anesthesia was discontinued prior to completion of surgery and immobilization. Immediately after the animals were placed on the table, endotracheal, arterial and venous catheters were inserted and extensive local anesthetization secured. Thereafter, ether was discontinued, and the animals' health was routinely ascertained by visual observation during partial recovery from general anesthesia. Only then were they immobilized and surgical preparation completed. With this procedure, prolonged depression resulting from general anesthesia was minimized to a period averaging 20 minutes (minimum 12 minutes) from the time of first exposure to ether. Continuous observation of blood pressure and skin resistance (discussed below) during surgery led to the conclusion that local anesthesia was complete: no autonomic activation suggestive of pain was noted.

## Electrode Placement

After the animals were placed in a Kopf stereotaxic apparatus, wide surgical exposure of the midline skull was made. Musculature and periosteum were elevated and retracted, the skull was dried and the border of the incision packed with warmed beeswax. Stainless steel screws with silver-soldered Nichrome wire leads were then inserted through stereotaxically guided burr holes over the anterior cruciate gyrus, bilaterally, and the left medial suprasylvian gyrus. Additional beeswax was used to completely isolate the screws from surrounding tissues.

Bipolar concentric depth electrodes (In Vivo Systems, SNE-100, 100 mm) were placed stereotaxically using two carriers through burr holes previously located with a pilot electrode in a third carrier. Depth electrodes were placed in the intermediate hippocampus (HP), mesencephalic reticular formation (RF), ventrolateral nucleus of the thalamus (VL) and either centre median (CM) or reuniens (RE) midline thalamic nuclei; all placements were on the same side.

## Experimental Procedures

### Experimental Protocol

The experimental protocol consisted of determining the stimulation current intensity required for the elicitation of a "threshold" response for each of five different CNS responses under control conditions and at each of five succeeding doses of ethanol. The responses observed were (1) the recruiting response elicited by stimulation of non-specific thalamus (n. reuniens or n. centre median); (2) augmenting response to stimulation of specific thalamus (n. ventrolateralis); and three manifestations of "reticular activation"; (3) the cortical desynchronization; (4) cardiovascular pressor; and (5) skin resistance responses elicited by stimulation of the mesencephalic reticular formation.

### Stimulation and Monitoring Techniques

Stimulation of n. reuniens or n. centre median (8/sec, 0.5 msec duration), n. ventrolateralis (8/sec, 0.5 msec) and reticular formation (200/sec, 0.05 msec) for the elicitation of recruiting,

augmenting and reticular activation responses, respectively, was produced by a Nuclear-Chicago constant current square wave stimulator (Mod. 7150). Stimulation voltage and wave-form were observed on an oscilloscope via the stimulator's monitor output but not systematically recorded.

Spontaneous and evoked potentials were led from cortical and depth electrodes through coaxial cables to a Grass Model VI electroencephalograph for immediate writeout and, from the pen driver amplifier of the polygraph, through an attenuation network, to an Ampex FR-1200 FM tape recorder. Cortical potentials were recorded monopolarly relative to a remote electrode consisting of either a stainless steel screw imbedded in the frontal sinus or a heavy guage needle buried in the neck musculature. Depending on experimental conditions either of these references may have been used to which a lead to the stereotaxic frame was occasionally added. Spontaneous and evoked activity was observed during the course of the experiments by inspection of polygraph records and by display on an on-line Tektronix RM 564 4-channel oscilloscope and a Fabri-Tek 1052 signal averager.

Electroencephalograph amplifier settings were adjusted during early monitoring and these settings were maintained as consistently as possible through the later experiment. The master high pass filter was usually maintained at 35 Hz, individual channel low and high pass filters were set at 1 and 70 Hz, respectively.

Tape recording began automatically at three hours after the end of general anesthesia and continued until the experiment was terminated. Recorder bandpass at the 1 7/3 ips tape speed used was

0-325 Hz. An associated time code generator (Milgo TCG-100) provided precise time calibration for the experimental protocol and, through its graphic output, a time marker for the polygraphs.

Blood pressure, Lead II electrocardiograph and skin resistance were continuously monitored on a Grass Model IV polygraph. Blood pressure was derived from the femoral artery cannula and recorded via a Sanborne P23d pressure transducer and DC preamplifier. Skin resistance was derived from an active lead (silver-silver chloride disc electrode) placed on the large footpad of the forelimb contralateral to reticular stimulation and recorded with a DC preamplifier using the PGR bridge. Skin resistance was referred to the inactive lead used for electroencephalograph recording.

Throughout the surgical procedure and the course of the experiment arterial blood samples were drawn and assayed for pH, $pO_2$, and $pCO_2$ with an IL Model 310 pH/Blood Gas Analyzer and for hematocrit using standard techniques. During the surgical procedure and ether blow-off period, respirator parameters were adjusted until repeated blood samples fell within normal ranges for the cat (pH, 7.35-7.40; $pO_2$, 90-95 mm Hg; $pCO_2$, 25-30 mm Hg). Core temperature was monitored with Yellow Springs Telethermometer and rectal probe and was maintained at 37.0-38.0° C with an overhead infrared lamp.

## Ethanol Infusion and Measurement

Absolute ethanol was mixed in 7% (w/v) solution of lactated Ringer's (Hartmann's) solution. The mixture was administered through the femoral vein catheter by a Harvard infusion pump at a constant

rate of 4.52 ml/min in accumulative doses of 100, 200, 400, 800, and 1600 mg/kg based on the animal's weight immediately prior to surgery. After initial infusion of 100 mg/kg the second dose level was obtained by a second infusion of 100 ml/kg giving a total of 200 ml/kg. The 400 mg/kg dose level was obtained by the infusion of an additional 200 mg/kg and this procedure was repeated for subsequent doses. Infusion periods were calculated at the beginning of the experiment and timed precisely. Immediately before infusion commenced, the catheter was cleared by drawing the saline it contained into a syringe until venous blood filled the catheter to its junction with the ethanol supply at a three-way stopcock. The infusion pump was then started and timing began when blood cleared the catheter. At the end of the infusion period the catheter was again flushed through with saline and sealed.

Ten minutes after the end of ethanol infusion arterial blood samples were drawn into separate micropipettes for blood gas analysis, hematocrit determination and ethanol concentration measurement. The sampling was completed in 15-20 seconds. Duplicate blood samples for ethanol determination were always taken. A second complete blood sample was drawn at the end of all threshold measurements; the next ethanol dose proceeded immediately thereafter.

Blood samples for ethanol determination were taken in previously florided micropipettes and centrifuged to separate plasma within an hour of its collection. The separated plasma was then frozen for later gas chromatographic analysis.

The resulting measurements of ethanol concentration were averaged over pre- and post-threshold measurement samples and the mean recorded as the blood concentration for all measurements at that dose. Experiments in which irregularities were found between ethanol measurements and dose level were discarded.

In some experiments an additional dose of 3200 mg/kg was given after completion of the regular protocol. This dose was given as a 20% solution (w/v) at the same rate of infusion used for lower doses. Threshold and ethanol measurement procedures were the same.

## Response Threshold Measurement and Analysis

Control recording. Periodic monitoring of spontaneous electrical and autonomic activity began immediately after the completion of surgery. Control recording for both spontaneous background and thresholds began at the end of a 3 hour ether blow-off period. Pilot experiments demonstrated that the traditional 2 hour blow-off period was insufficient for complete recovery from general anesthesia.

Response measurement. Stimulation current intensities necessary to elicit threshold recruiting, augmenting and cortical desynchronization were derived from the cortical polygraph record. Additionally, reticular stimulation current intensities required for the elicitation of a threshold cardiovascular pressor response and skin resistance response were obtained. Stimulation for recruiting and augmenting consisted of a 10-sec train with a minimum inter-train interval of 30-sec. Reticular stimulation was a 10-sec train repeated at no less than 60-sec intervals.

Thresholds were obtained by repeated stimulation beginning with an estimated subthreshold current intensity and continuing with constant increments until threshold and suprathreshold response were observed. In all conditions thresholds were obtained in the following order: recruiting, augmenting and reticular activation. Reticular stimulation was increased until cortical and autonomic thresholds were obtained.

Threshold criteria. The completion of 7 pilot experiments demonstrated that (1) no simple technique of visual observation gave reliable threshold information and that (2) variability in threshold measurements within the same experiment could be reduced by stimulating only when spontaneous cortical electrical activity could be interpreted as "drowsy." Such activity consisted of a pattern of low-voltage fast activity mixed with slow-wave activity with occasional spindling. This pattern naturally resulted if local anesthesia, core temperature and adequate respiration was rigidly maintained.

Therefore, based on the results of pilot experiments, a set of criteria was established for background spontaneous electrical activity and each evoked response threshold. These criteria consisted of the minimum characteristics required to be observed during a 10-sec stimulation period before that stimulation current was designated as threshold intensity. A complete statement of these criteria is included as Appendix I. Satisfaction of these criteria is denoted in the following chapter by "criterion background" and "criterion threshold."

While an attempt was made to provide criteria for an all-or-none response threshold, no animal exhibited a clearly defined threshold

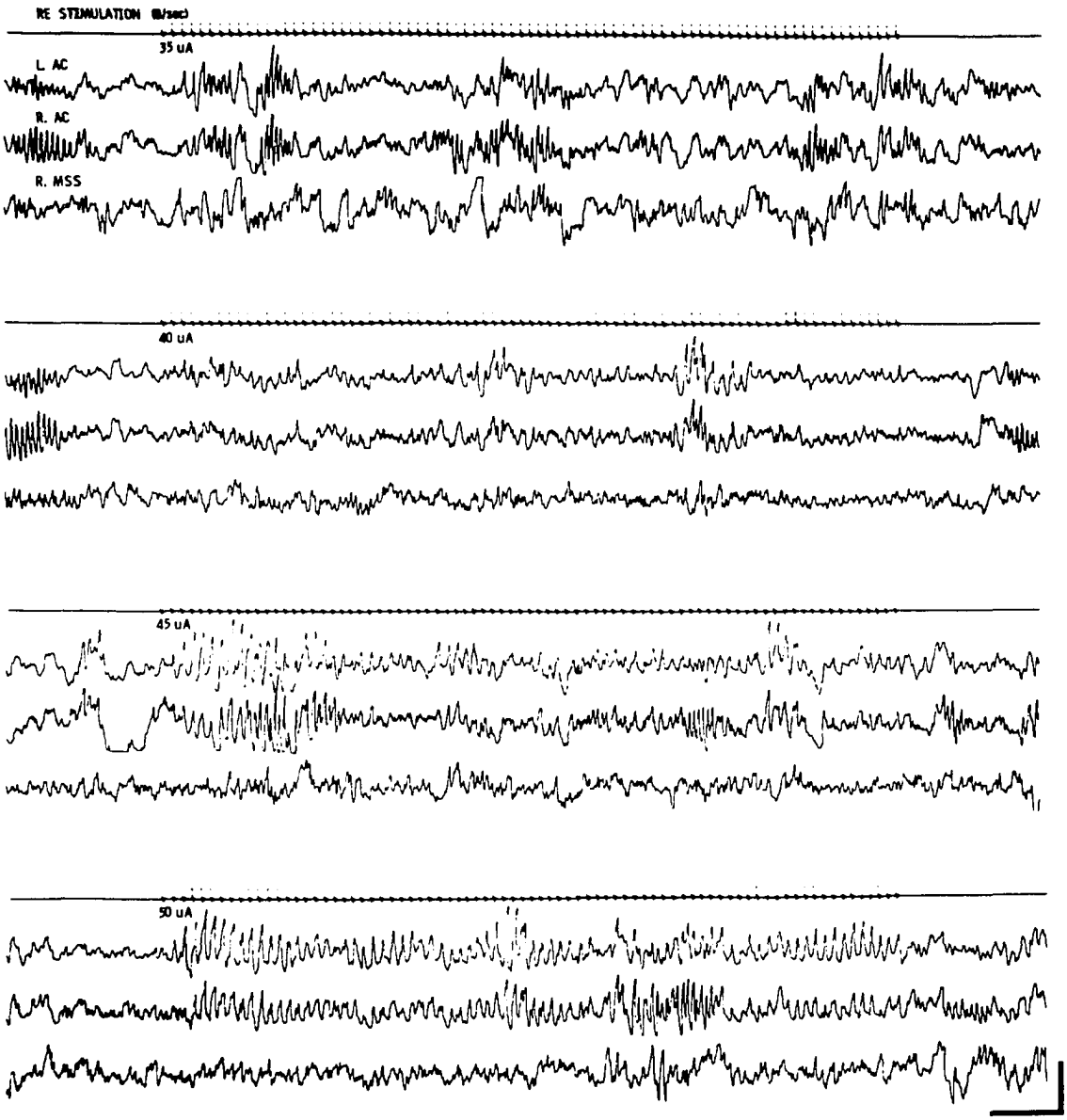
for any response. The criteria established for response thresholds were to some extent arbitrary; they, nevertheless, provided an exact operational definition of threshold for each response and yielded a basis for repeatable, standardized scoring of electrical and autonomic records.

Threshold determination. Threshold determinations for both control and ethanol conditions consisted of at least two complete runs through an ascending series of current intensity increments. Once threshold had been adequately measured in the first control stimulation run, current increments could be reduced as the estimated threshold was approached. For recruiting and all reticular activation response the minimum increment was usually 5 μA; for the augmenting response discriminations in the elicited response could often be made with increments of 2-3 μA. Typical threshold determinations for each of the five responses are illustrated in Figures 1 through 5.

Statistical analyses. Threshold current intensities required for the elicitation of criterion response thresholds were obtained for two independent stimulation runs during control and 5 ethanol dose conditions. Threshold currents derived from the two runs were averaged and the mean recorded as the absolute current threshold for each condition. With the mean control threshold current defined as 100%, threshold currents obtained during ethanol dose conditions were transformed to percent of control response to enable analyses between doses and responses. This transformation generated data points essentially independent of differences in absolute thresholds among animals.

Figure 1. Determination of Recruiting Response Threshold.

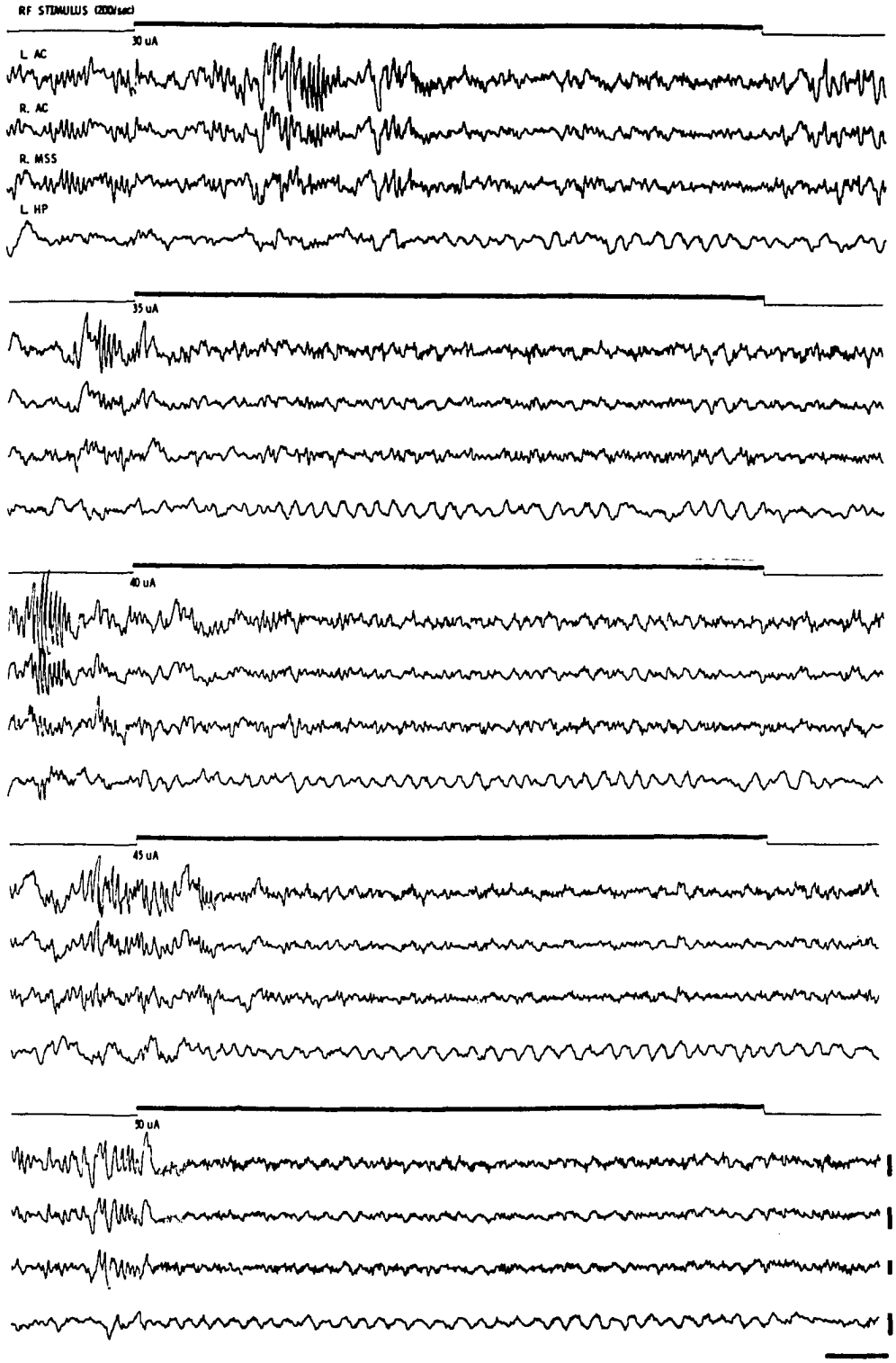
Stimulation of n. reuniens (RE) resulted in a threshold recruiting response at 45 μA (8/sec, 0.5 msec duration) in this experiment. Spontaneous spindles were clearly observed before and during the stimulus period at 35 μA, particularly in left and right anterior cruciate channels (L AC and R AC). At 40 μA there were brief periods of repeated responses during the 1st, 5th and 7th seconds of stimulation but of a duration insufficient to meet criteria. Threshold criteria were met in L AC at 45 μA with stimulus following in R AC. Recruiting was more developed in response to 50 μA and several repeated responses may be seen in the right medial suprasylvian channel (R MSS). Calibration, 200 μV and 1 sec. In this and all other illustrations, cortical activity is recorded monopolarly with cortical negativity upwards.




THRESHOLD DETERMINATION: RECRUITING RESPONSE

Figure 2. Determination of Cortical Desynchrony Response Threshold.

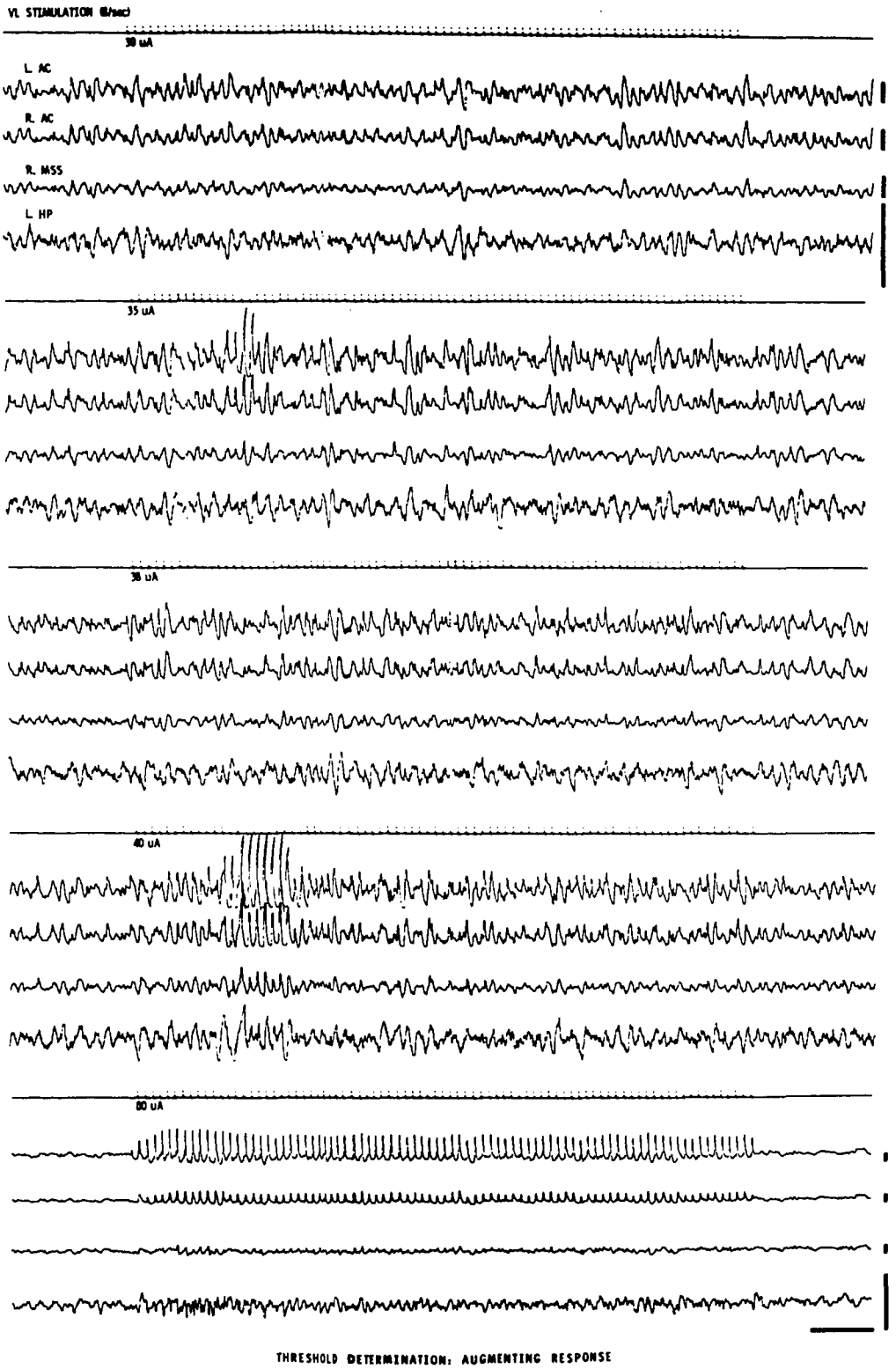
In this experiment, a criterion threshold cortical desynchrony response, elicited by stimulation (200/sec, 0.05 msec duration) of the mesencephalic reticular formation (RF), was obtained at 50 μA. Hippocampal (L HP) theta occurred in the latter half of the stimulus train at 30 μA concurrent with some signs of cortical desynchronization. With increasing current intensities cortical activity in L AC, R AC and R MSS became progressively more desynchronized and hippocampal theta became more synchronous. At 45 μA stimulation was initiated during a spontaneous spindle which subsequently was reduced in amplitude but it maintained synchrony. At threshold current, 50 μA, a spindle was blocked within 0.25 seconds of stimulus onset. The single negative slow wave recorded in cortical channels near the beginning of stimulation was often associated with stimulus onset at threshold and suprathreshold current intensities and was assumed to be mostly artifactual. Calibration, 100 μV and 1 sec.




THRESHOLD DETERMINATION: RETICULAR ACTIVATION - CORTICAL DESYNCHRONY

Figure 3. Determination of Augmenting Response Threshold.

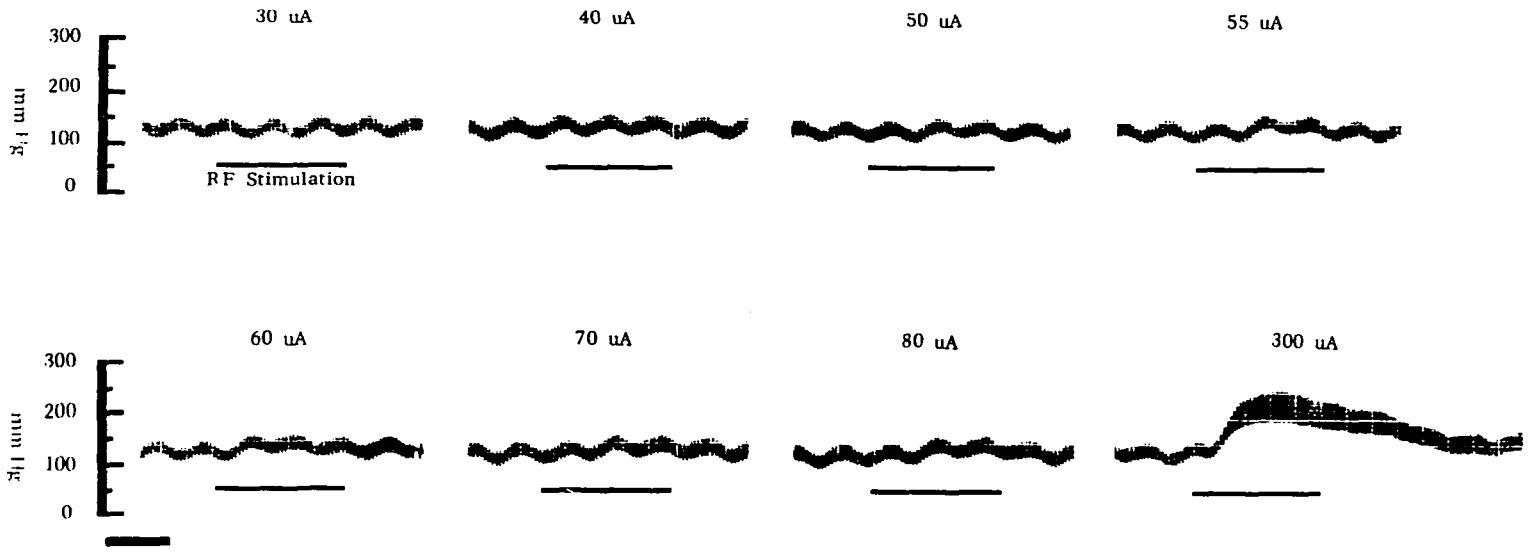
Stimulation (8/sec, 0.5 msec duration) of the left n. ventro-lateralis (VL) of the specific thalamus at 40 μA elicited a threshold augmenting response in this experiment. No change in cortical activity was evident at 30 μA but at 35 μA there was a brief episode of high amplitude frequency specific activity during the 2nd second of the stimulus train. The threshold augmenting response, obtained at 40 μA, consisted of approximately 2 seconds of repeated waves having maximal amplitude in L AC, ipsilateral to the stimulated site in the thalamus. No parallel changes in activity were seen in L HP. The last set of traces (note reduced amplification) shows an augmenting response elicited in the same experiment by a current intensity twice threshold. At such suprathreshold currents, the response rose to near maximal amplitude within a few repetitions, then declined slightly and remained at a relatively constant amplitude for the duration of the stimulus train. Cortical negativity upwards, calibration, 200 μV and 1 sec.




THRESHOLD DETERMINATION: AUGMENTING RESPONSE

Figure 4. Determination of Cardiovascular Pressor Response Threshold.

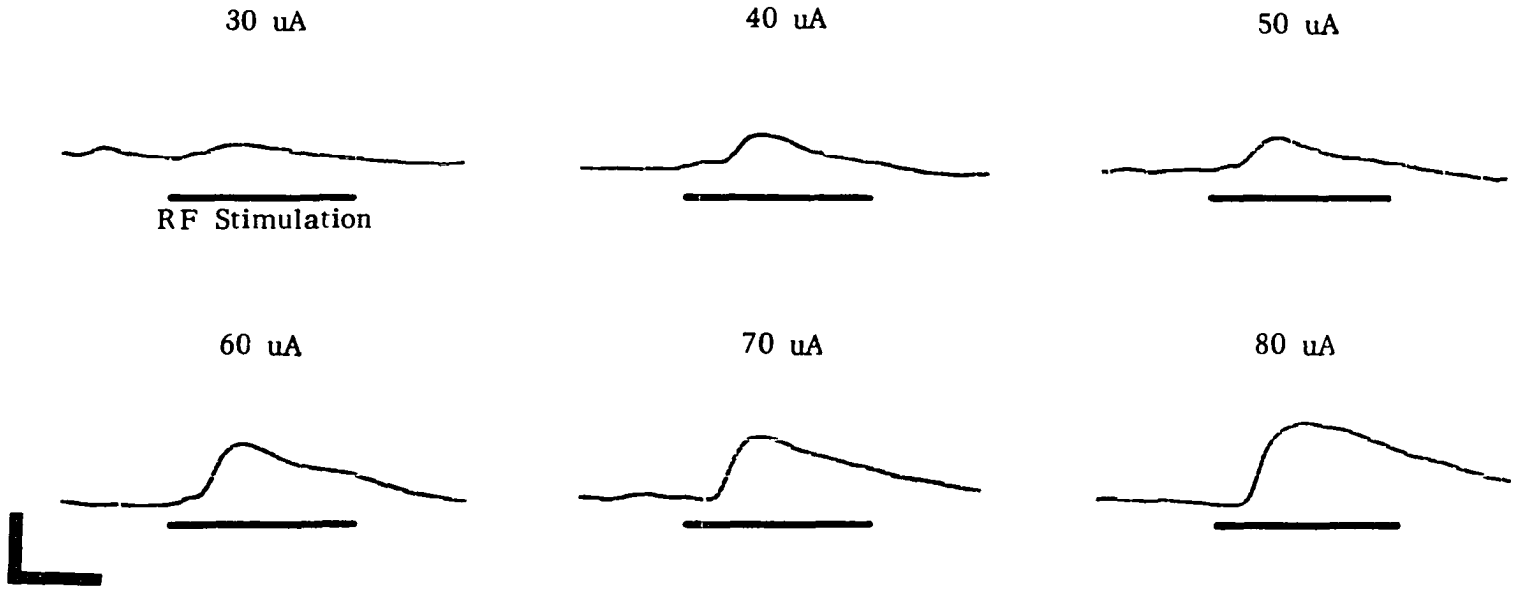
Stimulation (200/sec, 0.05 msec duration) of the mesencephalic reticular formation (RF) elicited a phasic increase in systemic blood pressure. In this experiment a criterion (10 mm HG increase in systolic pressure) response was obtained at 55 μA. Amplitude of the pressor response was proportional to the applied current intensity and with sufficient suprathreshold intensities, responses of more than 100 mm Hg could be elicited. Time calibration, 5 seconds.




THRESHOLD DETERMINATION: RETICULAR ACTIVATION - CARDIOVASCULAR PRESSOR RESPONSE

Figure 5. Determination of Skin Resistance Response Threshold.

Reticular formation (RF) stimulation (200/sec, 0.05 msec duration) elicited a phasic decrease in skin resistance as recorded from the forepaw contralateral to the site of midbrain stimulation. In this experiment a criterion (50 ohm decrease) skin resistance response is obtained at 50 μA. As shown by the response recordings taken at 30 through 80 μA, amplitude of the response was proportional to the stimulus current intensity. In many experiments, as shown here at 40, 50 and 60 μA, a brief, low amplitude response of shorter latency preceded the major response at near-threshold currents. A decrease in resistance is indicated by an upward deflection of the polygraph; calibration, 100 ohms and 5 seconds.




THRESHOLD DETERMINATION: RETICULAR ACTIVATION - SKIN RESISTANCE RESPONSE

All statistical analyses reported in the following chapter employed non-parametric tests. The Wilcoxen matched pair-signed ranks test (Siegal, 1956) was used to test the significance of the following: (1) differences between response thresholds measured under control conditions and those measured under each of the five ethanol conditions, (2) threshold differences between doses for each response, and (3) threshold differences between responses at each dose. Additionally, ethanol effects on each response over all doses was tested with the Friedman 2-way analysis of variance (Siegal, 1956).

## Termination of Experiments

An experiment was terminated at the end of the protocol or upon observation of physiological deterioration of the animal based upon any one of several criteria: (1) death to respiratory arrest during anesthesia or cardiac arrest at any time, (2) observation of continuous, synchronous high voltage slow waves in the electrocorticogram indicative of cortical depression, (3) unresponsiveness to stimulation to the limit of non-injurious current intensity, (4) observation of high voltage, hypersynchronous slow waves elicited by reticular stimulation indicative of cortical or brain stem depression (Prince and Sazner, 1966), (5) inability to maintain normal blood gas parameters.

## Verification of Electrode Placements

Functional control over electrode placement was maintained during the course of the experiments. Thalamic electrodes were placed for maximal response at an arbitrary suprathreshold current intensity. The reticular formation placement was not altered if initial trials

showed consistent cortical and autonomic responses at suprathreshold currents.

Upon completion of the experiment the animal was killed with an overdose of pentobarbital; the brain extending to the bulb carefully removed and placed in formalin. After several days the brain was removed from formalin, rinsed, blocked and returned to formalin for additional hardening.

Unstained slides were prepared of 50 μ coronal sections taken at the deepest electrode penetration. The slides were transilluminated and projected; the location of the electrode tip was closely estimated. Electrode placements and their verification were made with reference to Jasper and Ajmone-Marsan (1956). The estimated coordinates were then transferred to a tracing of the appropriate atlas plate.

## Control Experiments

Threshold stability over time. In order to assess the stability of response thresholds over time, experiments were conducted in which no infusions were made. Threshold determinations according to the above procedure were made beginning at two hours post-anesthesia and repeated at one-half hour intervals thereafter over a period of 4-6 hours. Periodic blood samples were obtained and analyzed for blood gas concentrations and hematocrit.

Threshold stability with high infusion volumes. Experiments were conducted to test the effects of the 800 and 1600 mg/kg infusion volumes on response thresholds. In these experiments normal procedures were followed except that the first three doses were omitted and

ethanol-free diluent substituted in the infusion. The infusion volumes used were weight adjusted to give doses of 800 and 1600 mg/kg, without the normally intervening low dose infusions.

# CHAPTER IV

# EXPERIMENTAL RESULTS

## Subject Fate

Forty-two animals were successfully carried through the surgical procedure. The first seven served as pilot experiments used to test various aspects of the experimental protocol and establish criteria for threshold measurements. Since many of these pilot experiments were incomplete according to the later definition of experimental criteria, they were not included in the analyses reported below.

The experimental protocol was completed in 12 experiments; 8 additional experiments were conducted as controls for stability of response thresholds over time (4 experiments) and the effects of high diluent volumes (4 experiments). The remaining 15 experiments failed to meet all criteria of experimental controls in post-experimental analysis of records.

The data derived from each experiment consisted of a dose-response threshold profile for each of five responses. Each profile was compiled by the measurement of the threshold for each response without ethanol (control measurement) and at each of five dose levels of ethanol giving 6 measurement conditions and a total of 30 threshold determinations in each experiment. During final analysis of polygraph records derived from the 12 ethanol experiments, it was found that one

threshold determination in each of three experiments and two determinations in a fourth experiment were unsatisfactory. Since these experiments met all other criteria the incomplete profiles were discarded and the remaining complete profiles obtained in these experiments retained. Because of these deletions the final n for statistical tests was reduced from 12 to 11 for pressor and skin resistance response thresholds and to 10 for augmenting response thresholds. Those tests used to examine inter-response threshold differences used the minimum n of 10.

## Ethanol Blood Concentrations and Clearance Rates

Intravenous infusion of ethanol in doses of 100, 200, 400, 800, and 1600 mg/kg produced mean blood concentrations (measured at 10 minutes after the end of each infusion) of 15.6, 23.6, 48.9, 98.5 and 214.0 mg%, respectively (Table 2). A mean blood concentration for the threshold measurement period at each dose was derived by assuming the decrease in concentrations between the first and second measurements to be a linear function of time and determining the mean of these values. The calculated ratios of first post-infusion measurement ($BS_1$ in Table 2) to infused dose ranged from 0.118 to 0.156 but they were sufficiently consistent to suggest that a rough estimate of the expected blood level produced by a given dose is 13% of that dose in mg/kg.

Despite precise calibration of dosages and careful timing of blood sampling there was considerable variability among animals in the blood levels produced by a given dose. As shown by the standard

Table 2

SUMMARY OF BLOOD ETHANOL CONCENTRATIONS (BEC)
DERIVED FROM ARTERIAL BLOOD SAMPLES (BS)

| Dose (mg/kg) | $BS_1$ BEC (mg%) | $BS_2$ BEC (mg%) | Mean BEC (mg%) | Elapsed Time ($BS_1$-$BS_2$) (min) | Clearance Rate (mg%/hr) | Correlation ($BS_1$ x Rate) (r) | Ratio $BS_1$/Dose |
|---|---|---|---|---|---|---|---|
| 100 | 15.6[a]<br>1.4[b] | 9.9<br>1.4 | 12.8 | 15.0<br>1.2 | 23.0 | 0.774[d] | 0.156 |
| 200 | 23.6<br>1.8 | 17.0<br>1.5 | 20.4 | 14.9<br>1.2 | 28.3 | 0.538[e] | 0.118 |
| 400 | 48.9<br>2.6 | 40.5<br>2.7 | 44.7 | 13.0<br>0.7 | 36.5 | 0.330 | 0.122 |
| 800 | 98.5<br>5.5 | 83.9<br>4.2 | 91.2 | 16.1<br>1.5 | 53.5[c] | 0.609[e] | 0.123 |
| 1600 | 214.0<br>9.7 | 166.0<br>4.2 | 190.0 | 18.3<br>1.0 | 172.0[c] | 0.462 | 0.123 |

[a] Mean blood concentration (n=13).

[b] Standard error of mean.

[c] Clearance rate at 1600 mg/kg greater than 800 mg/kg ($t$ = 3.06, $df$ = 12, $p<.01$).

[d] $p<.01$

[e] $p<.05$

errors (Table 2) associated with the blood concentration means, the variability in resultant blood levels is apparently an increasing function of infused dose.

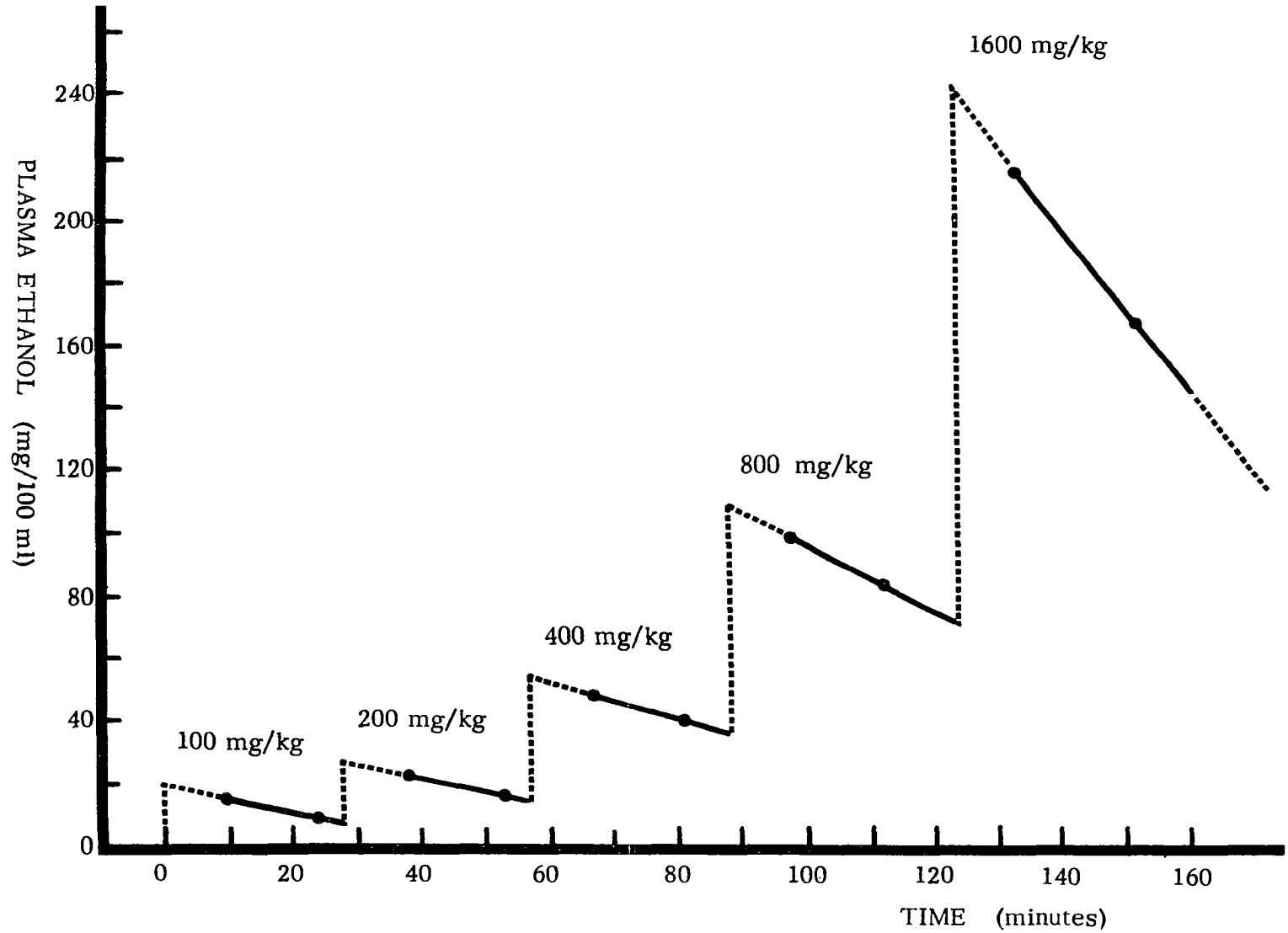
Ethanol blood clearance rates were calculated for each dose as the ratio of decrease in blood concentration between the two measurements ($BS_1 - BS_2$) to the time between measurements (Elapsed Time). These data are summarized in Table 2 and a mean blood ethanol concentration curve derived from all available experiments ($\underline{n} = 13$) is shown as Figure 6.

These data strongly implied the existence of a first-order relationship between clearance rates and blood concentration, expecially since the calculated clearance rate increased by a factor of 7.5 between the lowest and highest doses. This possibility was explored by statistically comparing clearance rates between contiguous doses; these tests showed that clearance rates differed significantly only between 800 and 1600 mg/kg (correlated $\underline{t} = 3.06$, $\underline{df} = 12$, $p<.01$). This relationship was further tested by computing Pearson $\underline{r}$ correlations between the calculated clearance rate and first post-infusion blood sample concentration ($BS_1$) at each dose. These correlations were significant for the 100, 200 and 800 mg/kg doses (Table 2, Correlation $BS_1$ x Rate).

Comparison of measured blood gas values with ethanol concentrations did not indicate any changes in blood chemistry which could be obviously attributed to the effects of ethanol. Since respirator parameters were carefully adjusted during the experiments to maintain normal values, this lack of effect could not be verified. There were

Figure 6. Mean Blood Ethanol Concentrations over Duration of Experiment.

This graph illustrates the mean blood concentrations profile produced by five succeeding doses of ethanol derived from 13 experiments. The dashed line shows the extrapolated peak ethanol concentration assumed at the end of infusion. Ethanol measurements were made on duplicate blood samples with one set obtained at 10 minutes after the end of infusion, the second at the conclusion of all measurements at a given dose level. The declining slope of the blood ethanol profile is a rough measure of ethanol metabolism and as may be seen, the slope becomes greater at each dose. The profile is derived from data summarized in Table 2.




Mean Blood Ethanol Concentration over Experimental Period

no changes in hematocrit values associated with increasing ethanol dosages other than a small and transient decrease at the highest dose which may have resulted from systemic dilution by high volume infusions (See below, "Control Experiments").

## Effect of Ethanol on Spontaneous Electrical Activity

Ethanol had little apparent effect on spontaneous electrical activity recorded from cortical and hippocampal electrodes. Figure 7 illustrates typical epochs from a single experiment recorded at each dose immediately after the end of infusion. Those changes in spontaneous activity observed were associated primarily with the lowest and highest doses.

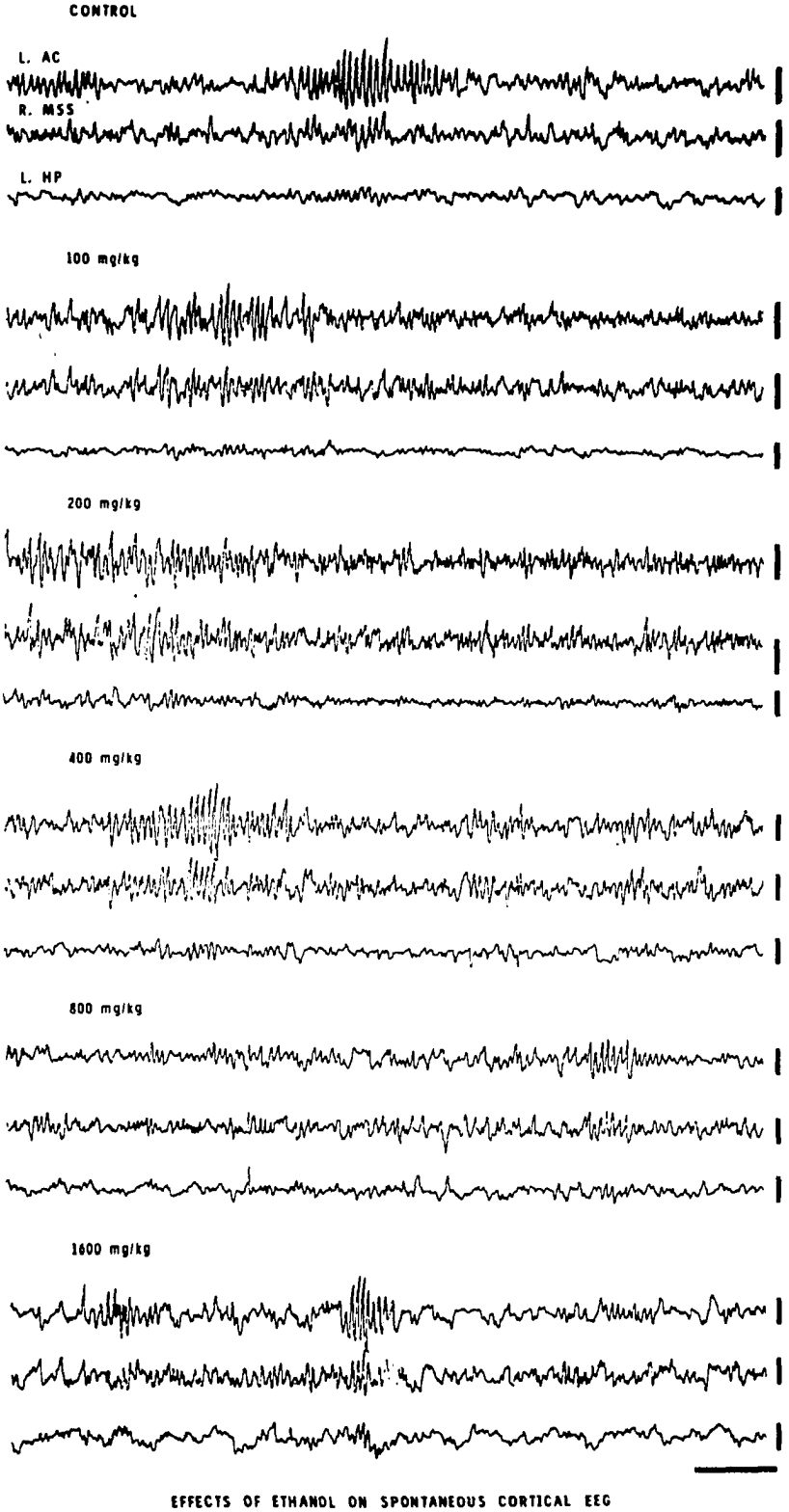
### Low Dose Effects

At 100 mg/kg there was generally increased variability in cortical activity consisting of an increased number of sustained periods of low-voltage fast activity. At this dose, criterion background was more difficult to maintain and transient periods of electrical activation, indicated by cortical desynchrony and hippocampal theta (4-7 Hz), were accompanied by increased variability in blood pressure and skin resistance. Under most circumstances there was excellent correlation between the observation of increased cortical activation and increased autonomic lability.

With increased periods of cortical desynchrony at low doses there was a corresponding decrease in the frequency of spontaneous cortical spindles and those observed at these doses were usually of shorter duration. Hippocampal theta was not reliably observed in all

Figure 7. Effects of Ethanol on Spontaneous Electrical Activity.

This illustration shows 10-second epochs extracted from the polygraph record of a typical experiment. The periods shown occurred immediately after the end of ethanol infusion at each dose. The epoch recorded under control conditions was taken at a time midway between the first and second set of control measurements. At the lowest dose (100 and 200 mg/kg) cortical spindle activity was less frequent and of shorter duration; hippocampal (L HP) activity was of lower amplitude and of higher frequency. At 1600 mg/kg the cortical record is slightly slower than that recorded during control conditions and the hippocampus shows considerably lower frequencies. Calibration, 100 μV and 1 second.




EFFECTS OF ETHANOL ON SPONTANEOUS CORTICAL EEG

experiments but when seen it was always contemporaneous with periods of intense cortical activation. As shown in Figure 7, the only changes observed in the hippocampus while criterion cortical background was maintained was a slight increase in fast activity and a corresponding decrease in slow-wave amplitude.

In two experiments these effects were exaggerated to such an extent that determination of reticular activation thresholds was impossible and threshold measurements obtained in these experiments at higher doses had to be discarded. In one experiment, illustrated in Figure 8, a period of spontaneous cortical activation and hippocampal theta began during measurement of augmenting response threshold and continued for several minutes. In these experiments the initial period of activation was prolonged and followed by recurrent periods of activation of decreasing duration. In the experiment illustrated (Figure 8), this pattern continued through the measurement period of the succeeding dose.

## High Dose Effects

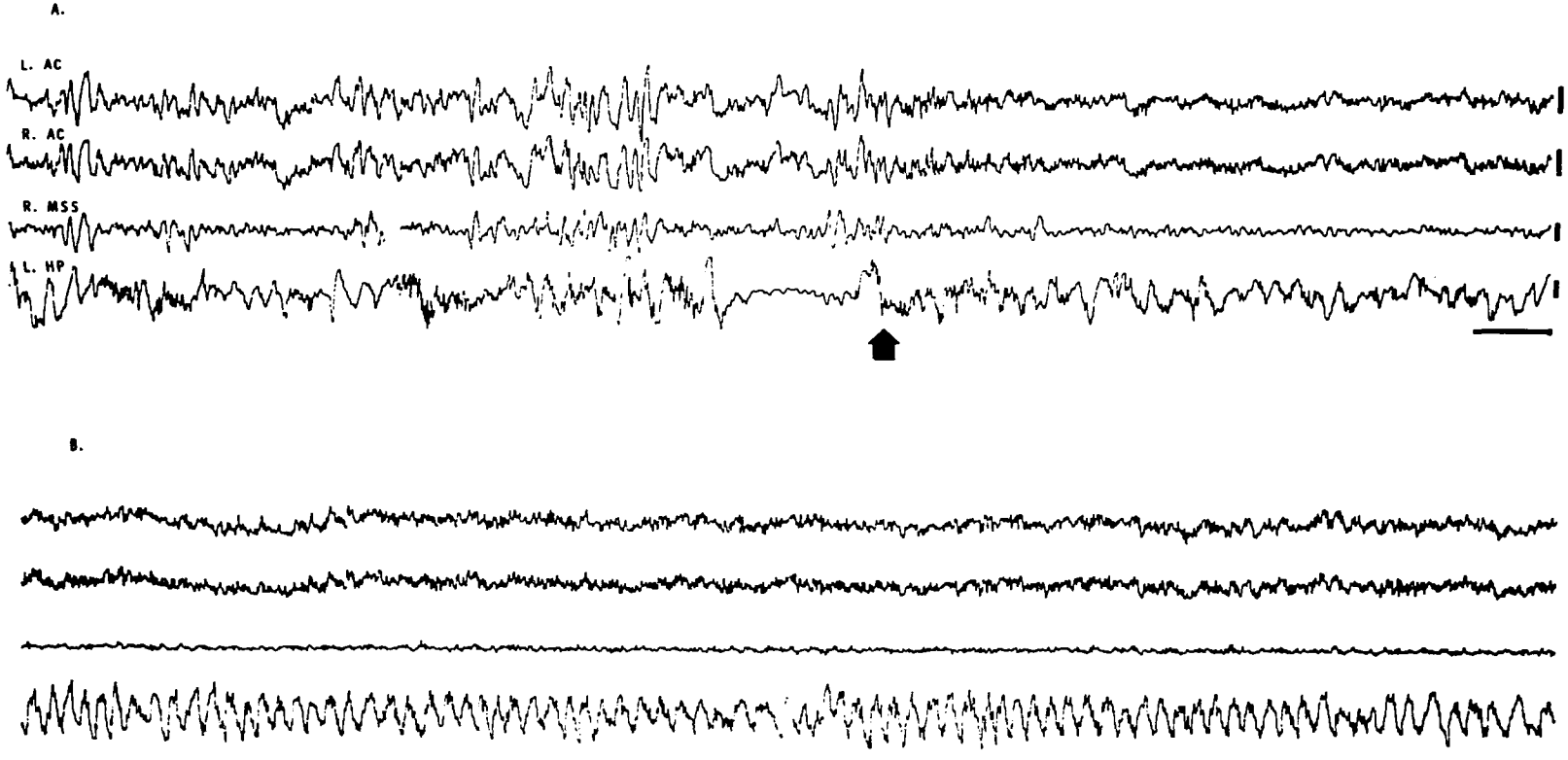
Generally, spontaneous activity recorded from the cortex changed slowly over the intermediate doses with the only apparent effects being an increase in slow wave amplitude and a decrease in faster activity in proportion to the dose. These effects are more evident at the 1600 mg/kg dose where much of the cortical fast activity seen at lower doses was no longer distinguishable. In most experiments a period of obvious, prolonged slowing was observed concurrently with the latter part of the infusion. However, cortical activity always

Figure 8. Spontaneous Electrical Activation at 100 mg/kg.

This illustration shows the beginning of a prolonged period of spontaneous activation which began during the measurement of augmenting threshold at 100 mg/kg.

A. Activation, beginning at arrow, is preceded by a brief period of partial activation and proceeds to become more intense as indicated by decreased amplitudes and increased frequencies in cortical activity (L AC, R AC and R MSS). The hippocampus (L HP), as yet shows only a slight increase in synchrony.

B. Six minutes later activation was still continuing. Suprasylvian activity (R MSS) was much reduced in amplitude and hippocampal activity had progressed to hypersynchronous theta. This initial period of activation was continued for a total of nine minutes followed by recurrent periods of less intense activation of decreasing duration. Calibration, 100 μV and 1 second.




SPONTANEOUS CORTICAL DESYNCHRONY AT 100 mg/kg

reverted to normal background patterns before the beginning of threshold measurements. In many cases, slowing was more evident in the hippocampus where the most evident frequency decreased to 2-3 Hz for a longer period. Frank cortical spindling episodes were decreased in frequency and duration during the measurement period.

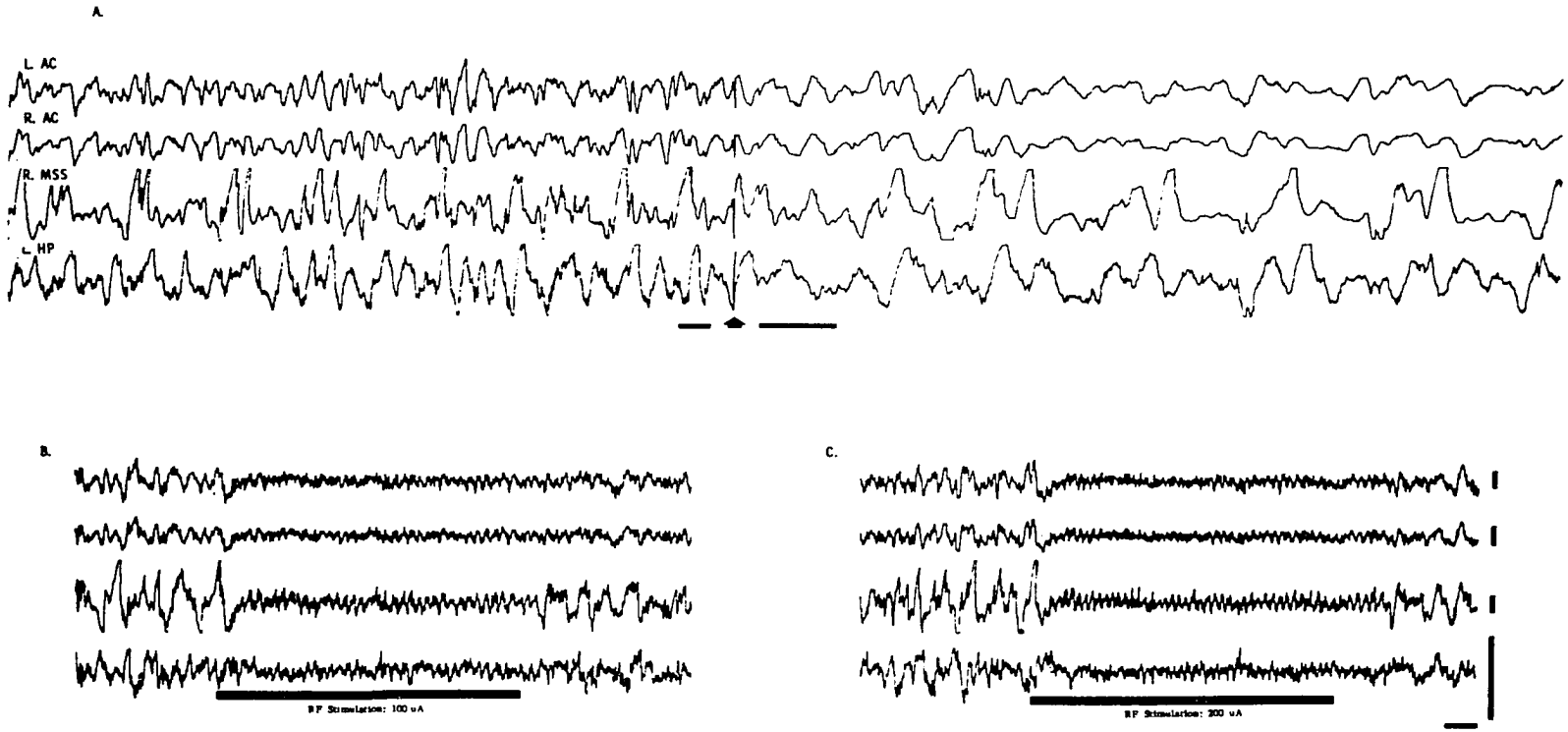
In some experiments, a dose of 3200 mg/kg was given after completion of the regular protocol. This dose invariably produced electrical signs of severe CNS depression indicated by delta range (less than 4 Hz) slow waves and periods of frank suppression of both cortical and hippocampal electrical activity. Figure 9 shows record samples taken from an experiment in which this dose produced moderate-to-severe CNS depression. The cortical desynchrony response is still obtainable but at a current intensity twice control threshold. This dose resulted in blood concentrations ranging to 450 mg% and its infusion was followed by severe hypotension and a marked increase in basal skin resistance. One animal died shortly after the end of infusion, another was maintained for 1-2 minutes with external cardiac massage until a spontaneous heart beat returned, but cortical activity remained completely suppressed until the animal was sacrificed. Some signs of recovery were seen in other animals which were always associated with maintenance of a blood pressure adequate for tissue perfusion.

In general, spontaneous electrical activity proved to be relatively unaffected by ethanol doses used within the regular protocol. Those effects observed consisted of increased cortical and hippocampal activation accompanied by increased autonomic lability at 100 mg/kg

Figure 9. Effects of a 3200 mg/kg Ethanol Dose on Spontaneous Electrical Activity and Cortical Desynchrony Response.

A. Both cortical and hippocampal spontaneous activity is reduced to random delta waves although there is some retention of faster frequencies in the hippocampus. Note expanded time scale beginning at arrow. This pattern is suggestive of severe cortical depression.

B and C. Results of midbrain reticular stimulation (200/sec, 0.05 msec duration) shortly following A. In this experiment, control cortical desynchrony was obtained at 50 μA. At 3200 mg/kg, criterion response was still obtainable at 100 μA. Calibration, 100 μV and 1 second.




SPONTANEOUS EEG AND RF STIMULATION EFFECTS AT 3200 mg/kg

and electrical signs interpreted as slight CNS depression at 1600 mg/kg.

## Effects of Ethanol on Evoked CNS Responses

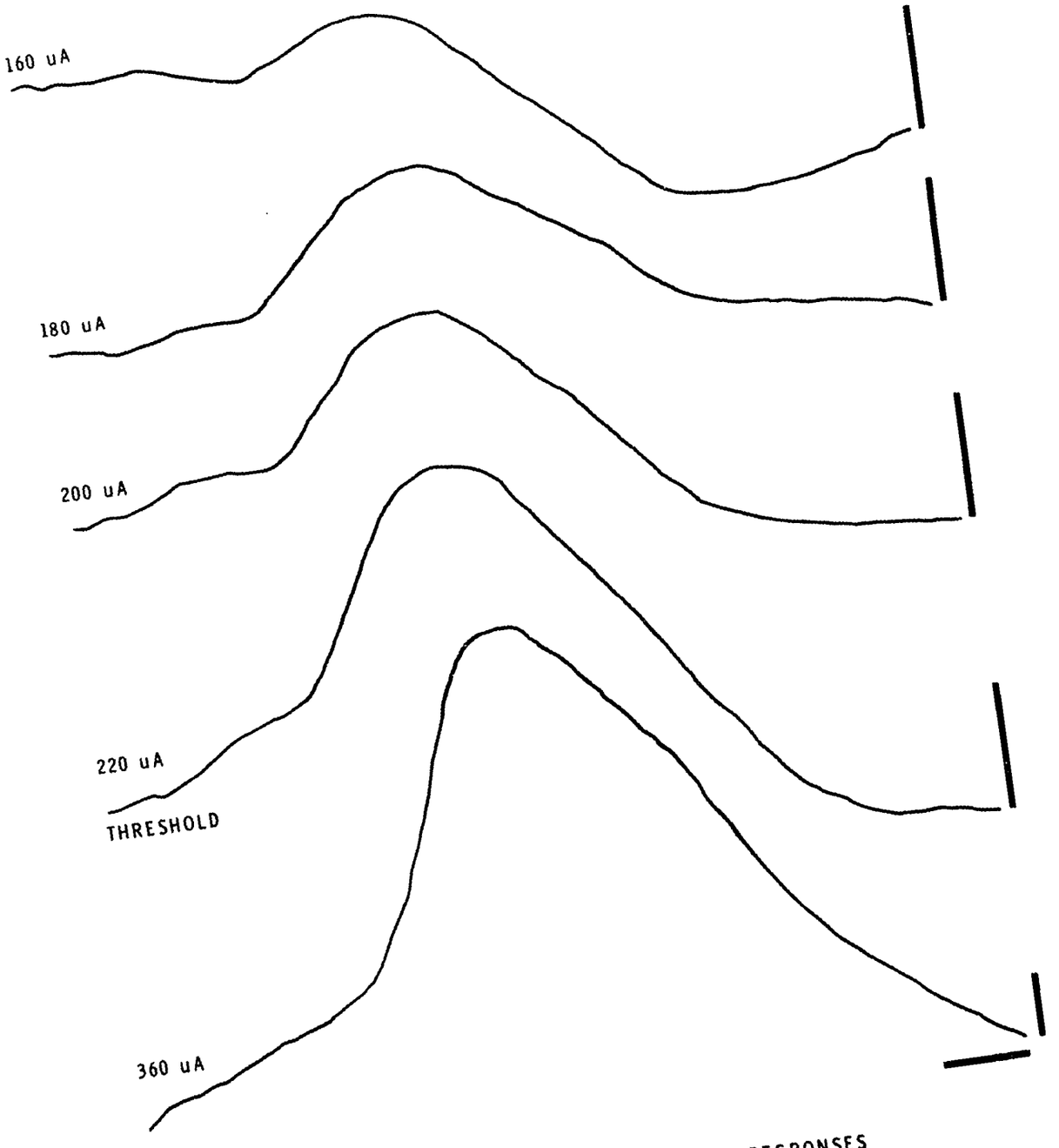
### Recruiting Responses

Control responses. Latency and waveform characteristics of suprathreshold recruiting responses recorded from the cortex were similar to earlier descriptions (Morrison and Dempsey, 1943; Jasper, 1954; Ajmone-Marsan, 1958). They were primarily surface-negative cortical events with peak latencies of 30-50 msec rising to maximal amplitude by the ninth or tenth stimulus of the train. Suprathreshold responses had greatest amplitude over the sensorimotor cortex (anterior cruciate gyrus) ipsilateral to the stimulated thalamic site. No apparent differences between cortical responses elicited by stimulation of n. reuniens or n. centre median were observed. In many experiments recruiting-like activity was observed in the hippocampus. These responses incremented at a considerably faster rate than those on the cortex and often reached maximal amplitude with the second stimulus of the train (Figure 11). Review of all experiments in which recruiting responses were elicited showed that hippocampal recruiting was only observed in response to stimulation of centre median, comparable responses were not elicited by stimulation of reuniens (compare Figure 1 to Figure 11).

Threshold recruiting (Figure 1) exhibited variable amplitude over the cortical recording sites but was usually first clearly observed in the ipsilateral sensorimotor area regardless of thalamic stimulation site. Computer summation of the cortical recordings (Figure 10)

Figure 10. CAT Amplification of Recruiting Responses.

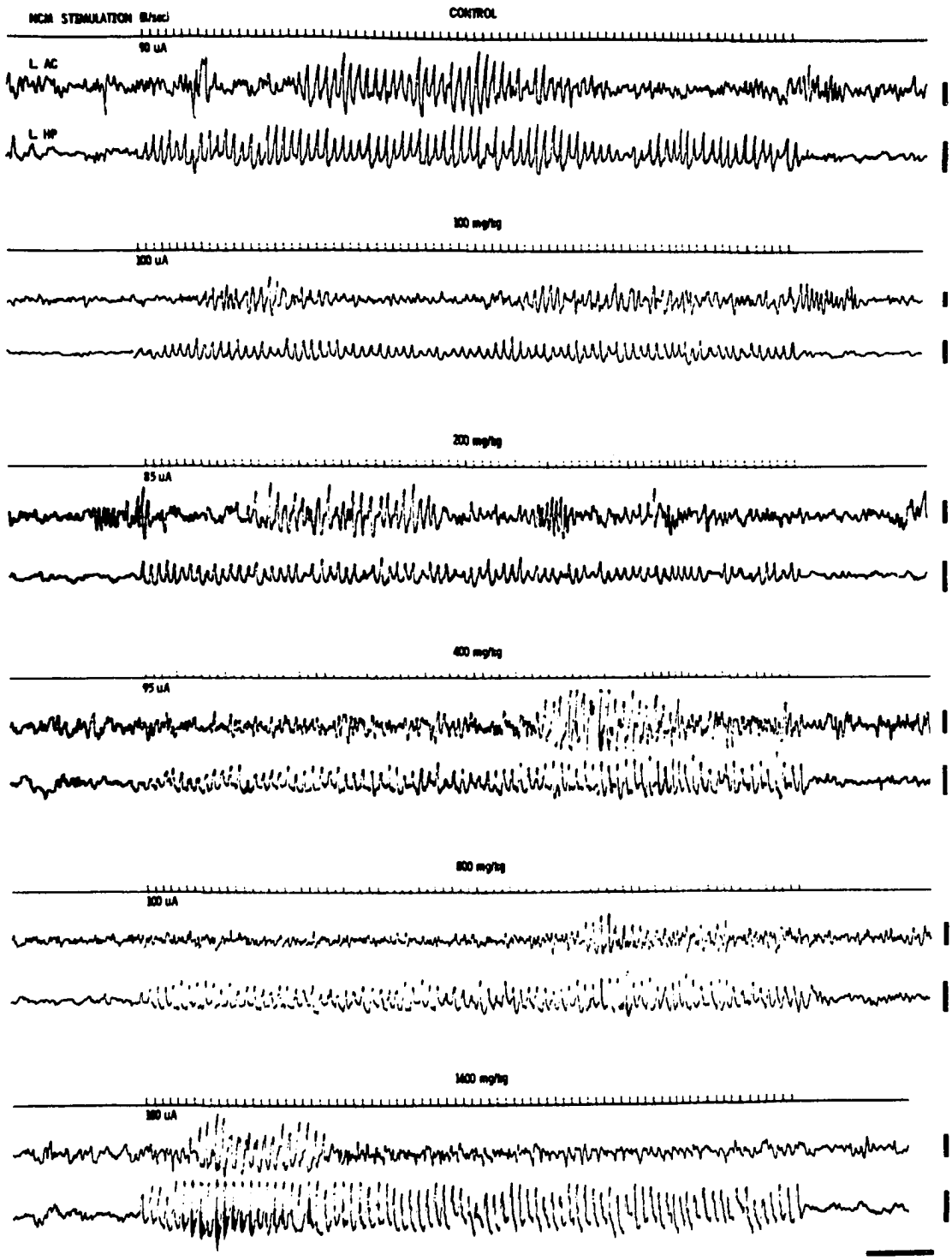
Sub-threshold responses could be amplified by summation with a computer of average transients (CAT). The illustration shows the waveform at various current intensities which resulted from the summation of the evoked responses to each stimulus of the train (80 responses). In this experiment, recruiting threshold was 220 μA. Sub-threshold responses have lower amplitude but show latency characteristics comparable to those of threshold and supra-threshold responses. Calibration, 50 μV and 10 msec, cortical negativity upwards.




CAT AMPLIFICATION OF RECRUITING RESPONSES

Figure 11. Effects of Ethanol on Recruiting Response Threshold.

In this experiment, stimulation (8/sec, 0.5 msec duration) of n. centre median (CM) of the non-specific thalamic system elicited a threshold recruiting response at 90 μA under control conditions. Recruiting-like activity may be observed in hippocampus (L HP); this pattern occurred only in response to NCM stimulation. Recruiting threshold was increased to 100 μA at 100 mg/kg and decreased to 85 μA at 200 mg/kg. At higher doses the threshold is again elevated above that obtained under control conditions. Calibration, 100 μV and 1 second.




EFFECTS OF ETHANOL ON RECRUITING RESPONSE THRESHOLD

demonstrated the existence of recruiting-like activity elicited by sub-threshold stimulation. Usually, sustained recruiting was not elicited until current intensity reached some level at which the response suddenly appeared as a short "burst" with near maximal amplitude responses which was often delayed for 5-8 seconds from the onset of the stimulus train. In many experiments, and during most all measurements at low doses, this burst was not readily observed and threshold was determined only by the observation of frequency-specific activity.

If stimulation was purposely initiated when the spontaneous electrocorticogram was predominantly desynchronized, the response elicited was less prominent and had a much sharper waveform; under these circumstances "bursts" were never observed. However, if the resulting response was scored for frequency-specific activity rather than presence or absence of the typical recruiting pattern, thresholds determined during cortical activation were not different than those determined during criterion background. The mean control threshold current required for the elicitation of recruiting responses based on all experiments was 94.4 μA (range 20 to 180 μA).

Effects of ethanol on recruiting thresholds. Individual dose-response threshold profiles for recruiting response were generally of a peak-trough-peak configuration. At low doses threshold peaked at some level above control; at intermediate doses most profiles showed a decrease in threshold from this peak which approached or was lower than control threshold. At high doses almost all profiles showed a monotonic (non-reversing) rise. The initial peak usually occurred

at 100 or 200 mg/kg with the trough predominantly occurring at 400 mg/kg, although in some experiments thresholds at 800 mg/kg were slightly lower or at the same level. In all experiments, thresholds were elevated above control at the highest dose. Figure 11 shows recruiting thresholds under control and ethanol conditions in a typical experiment.

The mean dose-response threshold profile is shown in Figure 12, and is based on 12 ethanol experiments. The mean profile shows an extended peak over 100 and 200 mg/kg, a return to near control thresholds at 400 mg/kg followed by increasing thresholds above this intermediate dose. Table 3 summarizes mean thresholds and associated standard errors. Reference to this table shows that thresholds at 200 mg/kg had the greatest individual variability. Table 3 also summarizes statistical evaluation of the differences between ethanol condition thresholds and those obtained under control conditions. These tests showed that recruiting thresholds were significantly increased over control values at 100 mg/kg. They were not different than control at either 200 or 400 mg/kg, but they were again increased significantly at 800 and 1600 mg/kg. Analysis of overall drug effect was significant (Friedman, $\underline{df} = 4$, $p<.01$).

Results of the Wilcoxen test analysis are graphically illustrated in the inset to Figure 12. In this plot, thresholds not significantly different than control are plotted at baseline and means different than control on the basis of this test are plotted at their absolute values.

Figure 12. Dose-Response Threshold Profile for Recruiting.

The graph represents the mean dose-response threshold profile derived from 12 experiments. Vertical lines extending from each data point represent standard errors associated with the mean threshold at each dose (Table 3). Inset represents statistical evaluation (Wilcoxen) of difference between dose thresholds and control. Thresholds not significantly different than control are plotted at the 100 % baseline; those different than control are plotted at their absolute value.

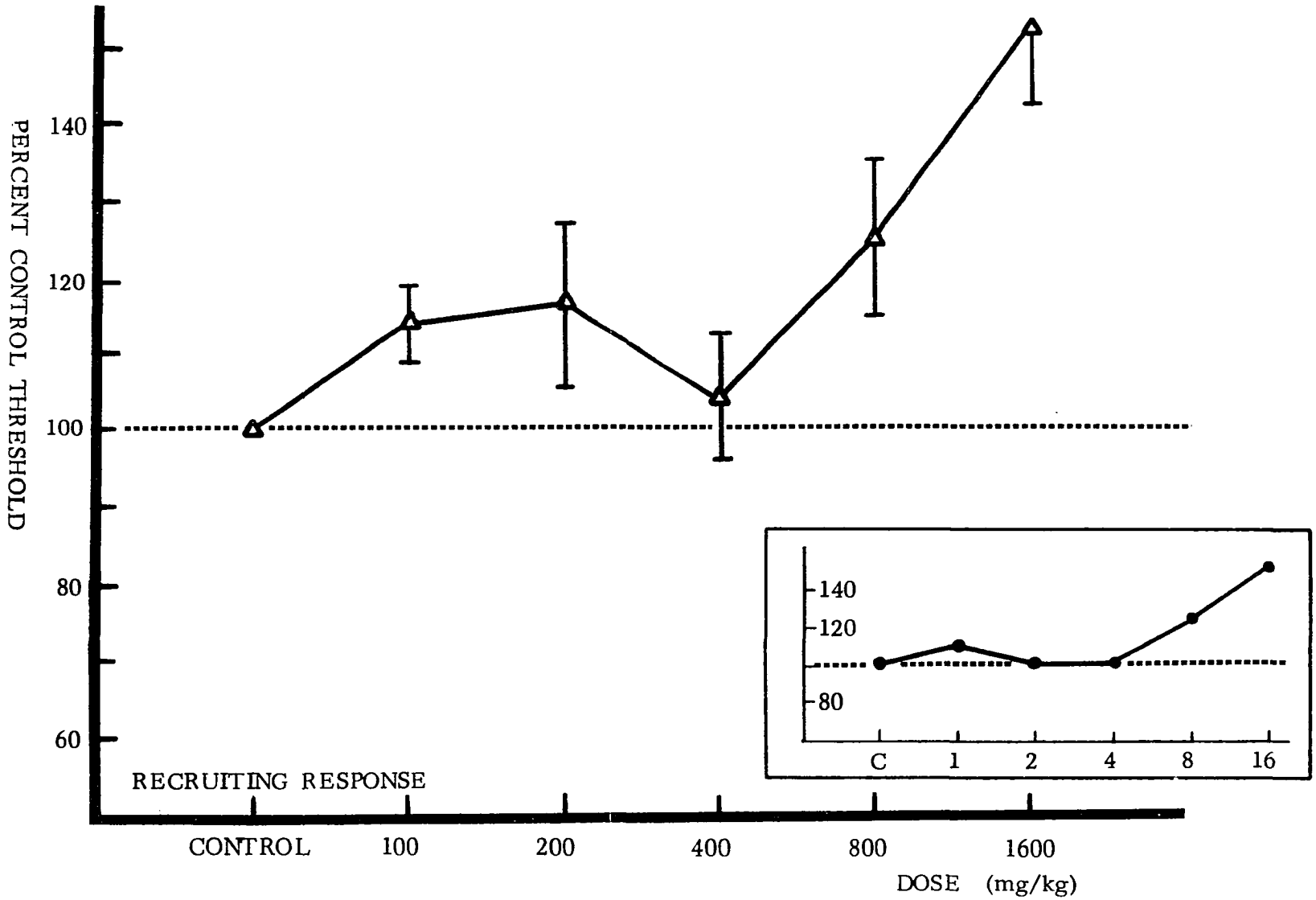

PERCENT CONTROL THRESHOLD
140
120
100
80
60
RECRUITING RESPONSE
CONTROL
100
200
400
800
1600
DOSE (mg/kg)
140
120
80
C
1
2
4
8
16

Table 3

RESPONSE THRESHOLD DATA AND RESULTS OF WILCOXEN TEST ANALYSIS OF DIFFERENCES BETWEEN CONTROL AND ETHANOL DOSE THRESHOLDS

| Response | Dose (mg/kg) 100 | 200 | 400 | 800 | 1600 |
|---|---|---|---|---|---|
| Recruiting (n=12) | 114.0[a] | 116.3 | 104.6 | 125.6 | 152.0 |
| | 5.9[b] | 10.7 | 8.2 | 10.2 | 9.5 |
| | .02[c] | -- | -- | .05 | .01 |
| Cortical Desynchrony (n=12) | 78.1 | 112.7 | 100.0 | 111.0 | 137.3 |
| | 4.0 | 12.6 | 6.6 | 7.8 | 11.5 |
| | .01 | -- | -- | -- | .02 |
| Augmenting (n=10) | 118.5 | 113.5 | 113.9 | 116.4 | 122.7 |
| | 8.0 | 4.0 | 4.1 | 4.3 | 5.6 |
| | .02 | .01 | .01 | .01 | .01 |
| Pressor (n=11) | 100.7 | 113.2 | 121.5 | 135.2 | 145.3 |
| | 3.8 | 6.1 | 5.4 | 12.5 | 12.3 |
| | -- | .05 | .01 | .01 | .01 |
| Skin Resistance (n=11) | 93.4 | 93.6 | 101.5 | 114.0 | 124.0 |
| | 7.1 | 9.7 | 6.5 | 8.9 | 8.3 |
| | -- | -- | -- | -- | -- |

[a]Mean threshold (% Control threshold).
[b]Standard error of mean.
[c]Significance level of difference between ethanol dose threshold and control (Wilcoxen).

An analysis of the significance of inter-dose threshold differences was made with the Wilcoxen test (Table 4). Results of these tests showed that thresholds at 1600 mg/kg were higher than all other doses; 800 mg/kg thresholds were higher than 400 mg/kg but there was no significant differences between 100, 200 and 800 mg/kg thresholds.

Overall, ethanol in low doses raised thresholds above those measured under control conditions; intermediate doses were associated with a return of thresholds to near control levels, and high doses caused an increase in thresholds above control that was proportional to dose.

Reticular Activation: Cortical Desynchrony Responses

Control responses. Suprathreshold cortical desynchronization, elicited by high frequency stimulation of the mesencephalic reticular formation was characterized by immediate activation of cortical spontaneous activity. This activation consisted of immediate suppression of slow wave activity and a general increase in frequency and a sharp reduction in overall amplitude in all cortical channels. Hippocampal activation, marked by the replacement of generally fast, low amplitude activity with theta, was not seen consistently. In many experiments no remarkable change was noted in the hippocampus; although histology demonstrated that all electrodes were within the usual boundaries of the intermediate hippocampus (A-2), no explanation can be given for this inconsistency other than that the recording electrodes were in the wrong place.

Table 4

RESULTS OF WILCOXEN TEST ANALYSIS OF RESPONSE THRESHOLD DIFFERENCES BETWEEN DOSES

| Response | Dose (mg/kg) | Dose (mg/kg) 200 | 400 | 800 | 1600 |
|---|---|---|---|---|---|
| Recruiting | 100 | -- | -- | -- | .01[a] |
| | 200 | | -- | -- | .01 |
| | 400 | | | .05 | .01 |
| | 800 | | | | .01 |
| Cortical Desynchrony | 100 | .05 | .01 | .01 | .01 |
| | 200 | | -- | -- | -- |
| | 400 | | | -- | .05 |
| | 800 | | | | .05 |
| Augmenting | 100 | -- | -- | -- | -- |
| | 200 | | -- | -- | -- |
| | 400 | | | -- | -- |
| | 800 | | | | -- |
| Pressor | 100 | -- | .01 | .01 | .01 |
| | 200 | | -- | .05 | .01 |
| | 400 | | | -- | .05 |
| | 800 | | | | -- |
| Skin Resistance | 100 | -- | -- | -- | .02 |
| | 200 | | -- | .05 | .05 |
| | 400 | | | -- | .02 |
| | 800 | | | | -- |

[a] Probability of test result less than stated value.

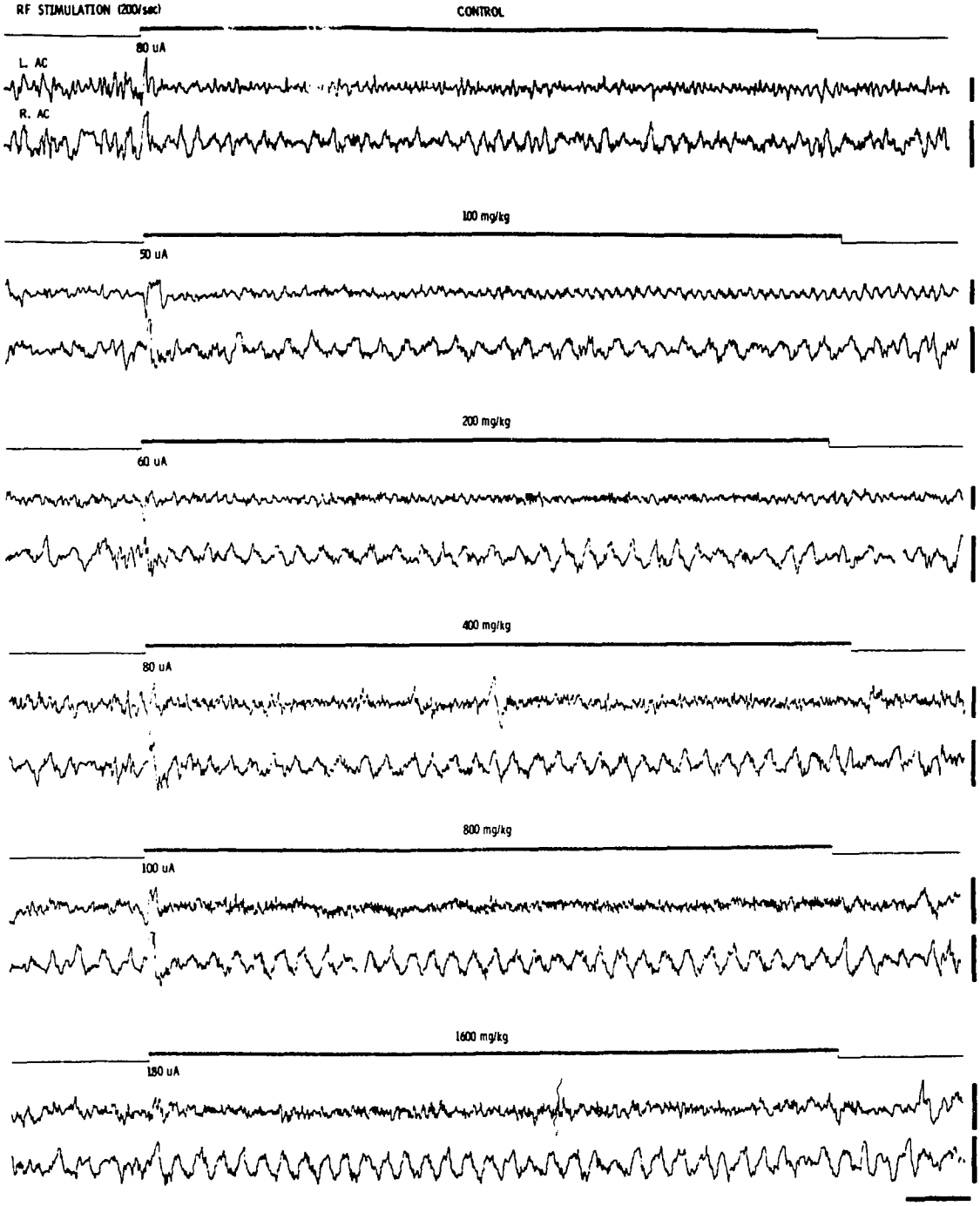
Against criterion background activity, suprathreshold stimulation resulted in a decrease in the amplitude of background activity and an increase in predominant frequencies well into the beta range (greater than 13 Hz). If stimulation was initiated in the middle of a cortical spindle, activity could be abruptly shifted from mid-spindle to desynchrony within 0.25-0.5 seconds.

Subthreshold cortical desynchrony responses (Figure 2) were usually marked by periods of desynchrony within the period of stimulation whose duration increased as threshold was approached. Reduction of mean amplitude, increase in mean frequency, decrease in latency of activation, and the duration of desynchrony continuing after offset of stimulation were all proportional to current intensity. In some experiments, where the absolute threshold was relatively low (e.g., 30 μA), the range of current intensity between that eliciting no response and that producing criterion threshold response was quite small. Mean control threshold current intensity was 58.8 μA with a range of 20 to 120 μA.

Effects of ethanol on cortical desynchrony thresholds. In almost all experiments, thresholds for cortical desynchrony decreased below control at 100 mg/kg. In some experiments this effect continued through the next dose but, overall, thresholds at the 200 mg/kg dose were extremely variable as indicated by the standard error of the mean of this dose (Table 3). The cortical desynchrony response showed a return of thresholds to control levels at the intermediate doses similar to that observed with the recruiting response, followed by a rise through the highest doses. Figure 13 illustrates cortical

Figure 13. Effects of Ethanol on Cortical Desynchrony Response Threshold.

Threshold cortical desynchrony elicited by high frequency stimulation (200/sec, 0.05 msec duration) of the mesencephalic reticular formation was obtained at 80 μA in this experiment. At 100 and 200 mg/kg criterion threshold was obtained at a much lower current intensity. Threshold was comparable to control at 400 mg/kg and then it increased rapidly at the two highest doses. Interestingly, hippocampal theta (L HP) becomes more prominent at higher doses. Calibration, 100 μV and 1 second.




EFFECTS OF ETHANOL ON RETICULAR ACTIVATION - CORTICAL DESYNCHRONY THRESHOLD

desynchrony thresholds recorded in a typical experiment under control and ethanol conditions.

Comparison of the mean dose-response threshold profiles for cortical desynchrony (Figure 14) and recruiting (Figure 12) shows that at 100 mg/kg mean threshold for desynchrony is decreased to 78% of control while mean recruiting threshold is elevated to 114% of control. At 400 mg/kg both mean response thresholds have returned to near control levels (desynchrony, 100%; recruiting, 104.6% of control). Mean thresholds and standard errors are summarized in Table 3.

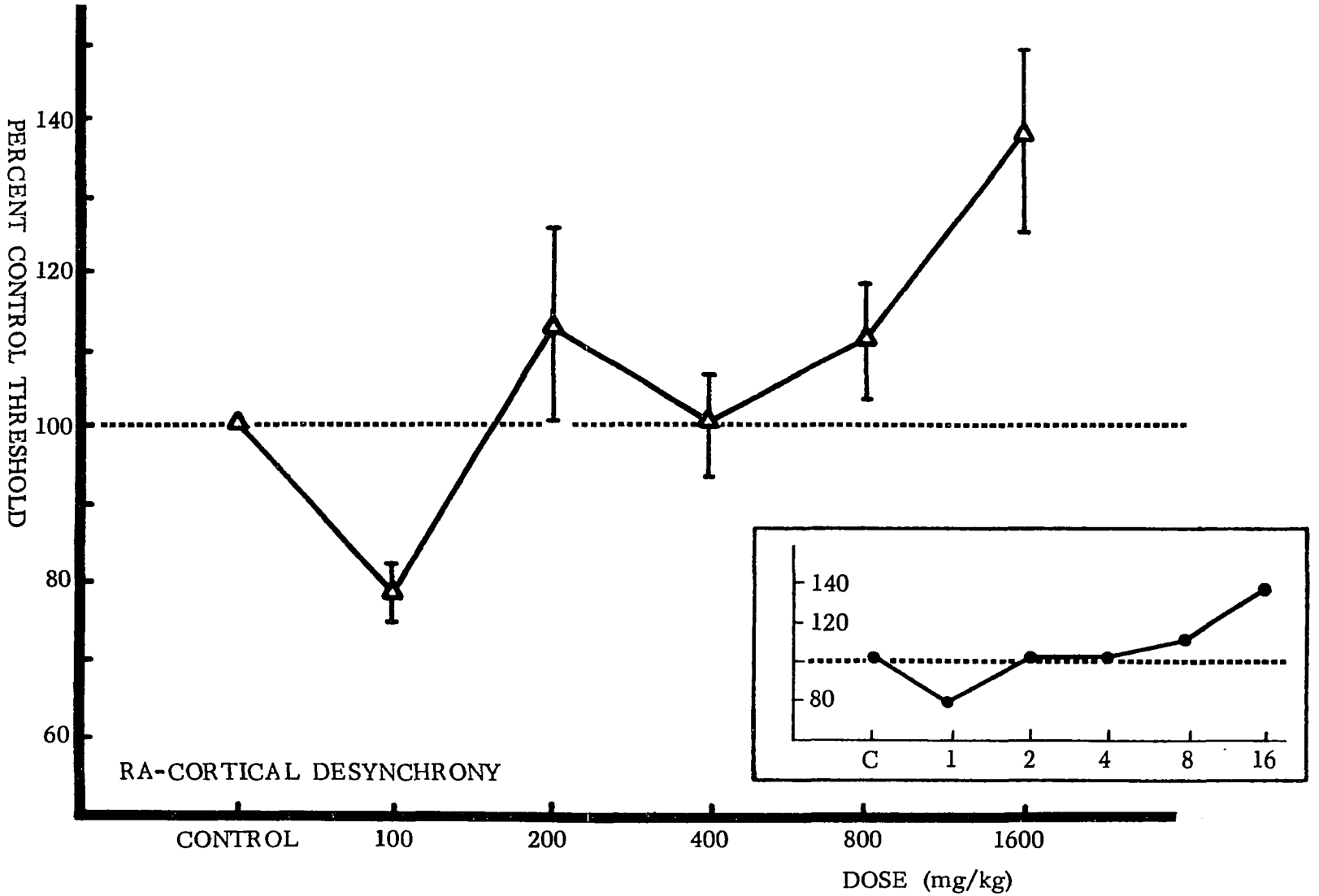
The inset in Figure 14 shows the results of analysis of differences between ethanol dose thresholds and control. Thresholds for cortical desynchrony response were significantly ($p<.01$) below control at 100 mg/kg and significantly above control at 1600 mg/kg ($p<.02$). Thresholds at the intervening doses (Table 3) were not reliably different than control although the overall ethanol effect on cortical desynchrony was significant (Friedman, $\underline{df} = 4$, $p<.001$).

An analysis of the significance of inter-dose threshold differences showed (Table 4) that thresholds at 100 mg/kg were lower than those at all other doses, thresholds at 1600 mg/kg were higher than at all other doses. No differences were found between thresholds at the intervening doses.

In general, the results show that thresholds for cortical desynchrony are sharply decreased at 100 mg/kg, return to and are maintained at control values over the 200, 400 and 800 mg/kg dosage levels and are elevated above control values at 1600 mg/kg.

Figure 14. Dose-Response Threshold Profile for Cortical Desynchrony.

This graph represents the mean dose-response threshold profile derived from 12 experiments.


PERCENT CONTROL THRESHOLD
140
120
100
80
60
RA-CORTICAL DESYNCHRONY
CONTROL
100
200
400
800
1600
DOSE (mg/kg)
140
120
80
C
1
2
4
8
16

## Augmenting Responses

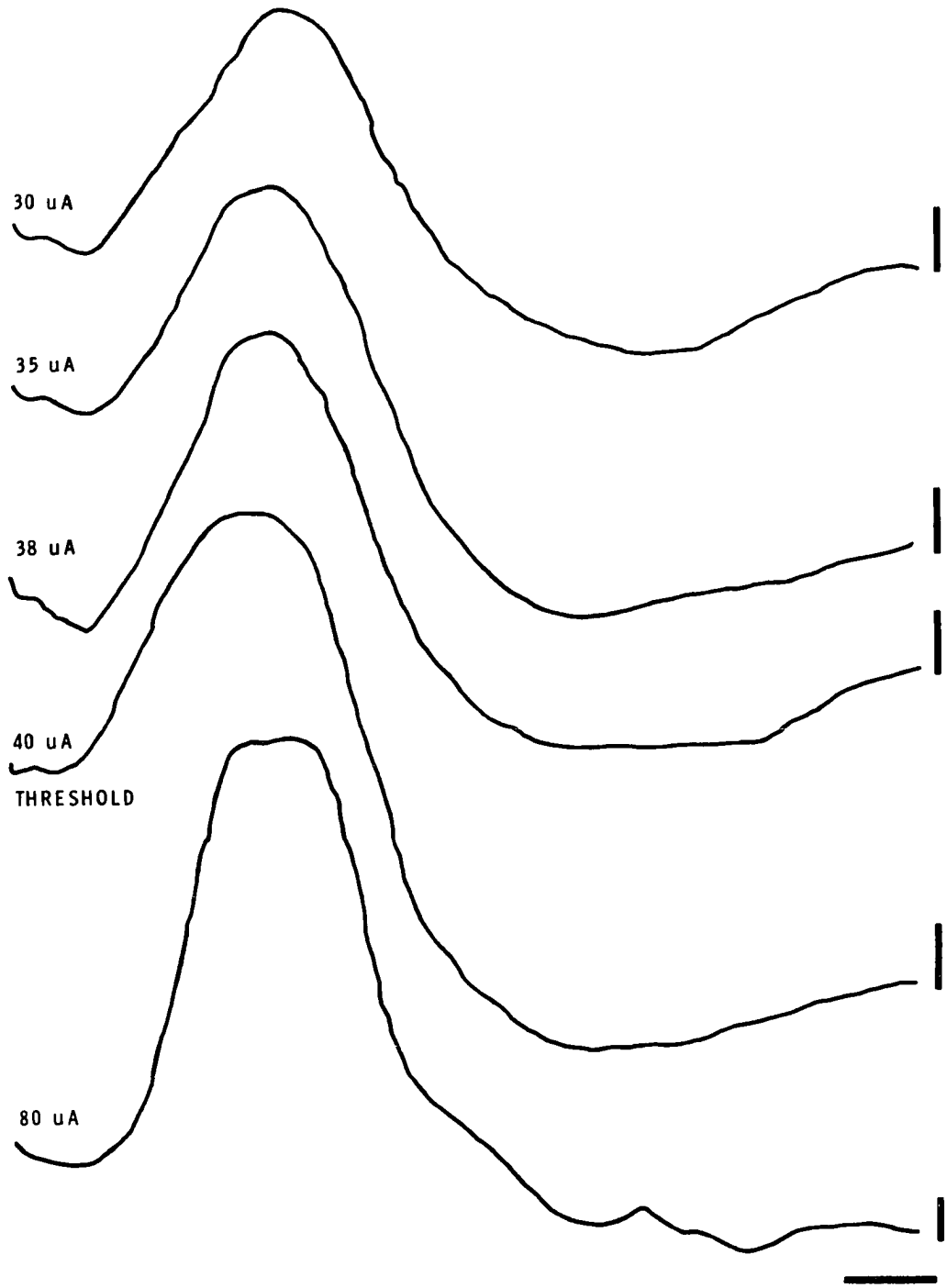
Control responses. Characteristics of suprathreshold augmenting responses conformed to earlier descriptions (Spencer and Brookhart, 1961). They were observed as short latency (peak latency 10-20 msec) surface positive-negative events with highest amplitude over sensorimotor cortex ipsilateral to the stimulated thalamic site (n. ventrolateralis thalami). In contrast to the slow development of the recruiting response to full amplitude, the augmenting responses achieved maximal amplitude (Figure 3) by the third to fifth stimulus and remained relatively constant over the remainder of the stimulus train.

Subthreshold augmenting responses (Figure 3) exhibited characteristics similar to subthreshold recruiting: with increasing current, periods of frequency specific activity increased in duration. In many experiments near-threshold stimulation elicited a "burst" of 5-10 responses which might be delayed from the onset of stimulation by several seconds. The range of current between an intensity eliciting no discriminable response and that eliciting a threshold response was usually quite small and often spanned no more than 3-5 μA. As with recruiting, subthreshold augmenting responses could be amplified by computer and exhibited waveform characteristics similar to those of threshold and suprathreshold responses (Figure 15).

The transition from subthreshold to suprathreshold augmenting response (Figure 3) was usually marked by the increased duration of periods of frequency specific activity, a rapid shift to the fully developed response for a variable period, then increased duration of

Figure 15. CAT Amplification of Augmenting Responses.

Sub-threshold responses could be observed by response summation on a computer of average transients (CAT). The illustration shows the waveform of augmenting responses elicited at various current intensities. The waveforms shown resulted from the summation of 80 responses. Augmenting threshold was obtained at 40 μA in this experiment. Calibration, 50 μV and 10 msec, cortical negativity upwards.




CAT AMPLIFICATION OF AUGMENTING RESPONSES

response repetition with higher currents. Mean control threshold current for the augmenting response was 52.1 μA (range 20 to 175 μA).

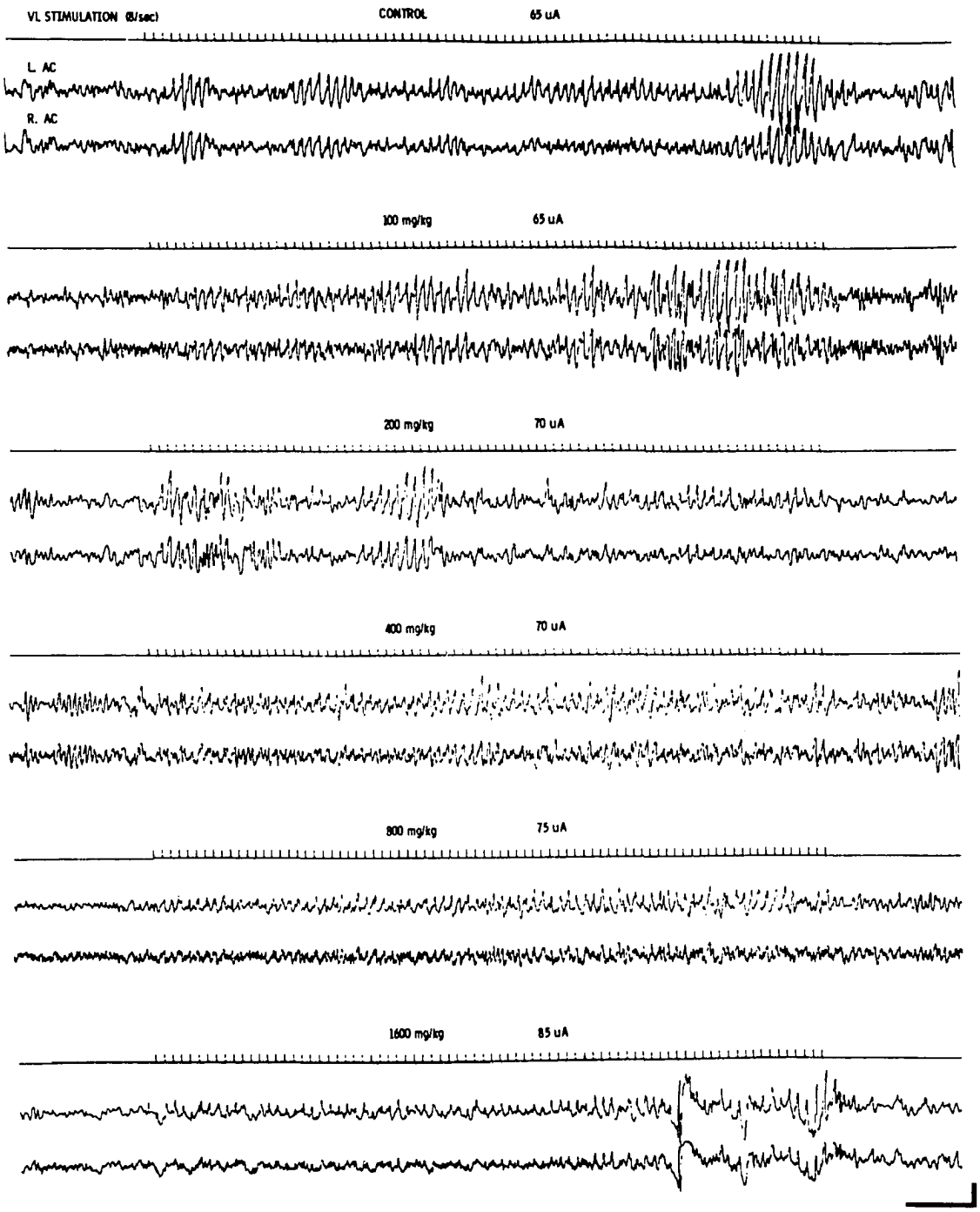
Effects of ethanol on augmenting response thresholds. Individual dose-response threshold profiles were generally elevated at 100 or 200 mg/kg and remained fairly constant at this increased level through all higher doses. In some experiments thresholds were noted to decline slightly from a low dose peak at intermediate doses; many profiles showed some increase over the initial elevation at higher doses. Generally, the effects of ethanol were seen as a low dose elevation of augmenting response thresholds with almost invariant continuation of this initial elevation at higher doses. Figure 16 shows records taken at threshold under control and five ethanol conditions in a typical experiment.

The mean dose-response threshold profile (Figure 17) shows these results quite clearly. Mean threshold is increased to 118.5% at the lowest dose, is decreased from this value only a few percent at the 200, 400 and 800 mg/kg doses and shows only a slight additional increase to 122.7% at 1600 mg/kg. These data are summarized in Table 3; the standard error tabulated there demonstrates that a much smaller degree of variability was associated with augmenting thresholds in comparison to other responses.

The results of Wilcoxen test analysis of differences between thresholds at the ethanol doses and control levels are shown in Table 3 and are graphically illustrated in the inset to Figure 17. Results of these tests showed that the initial increase above control at 100 mg/kg was significant and augmenting response thresholds were

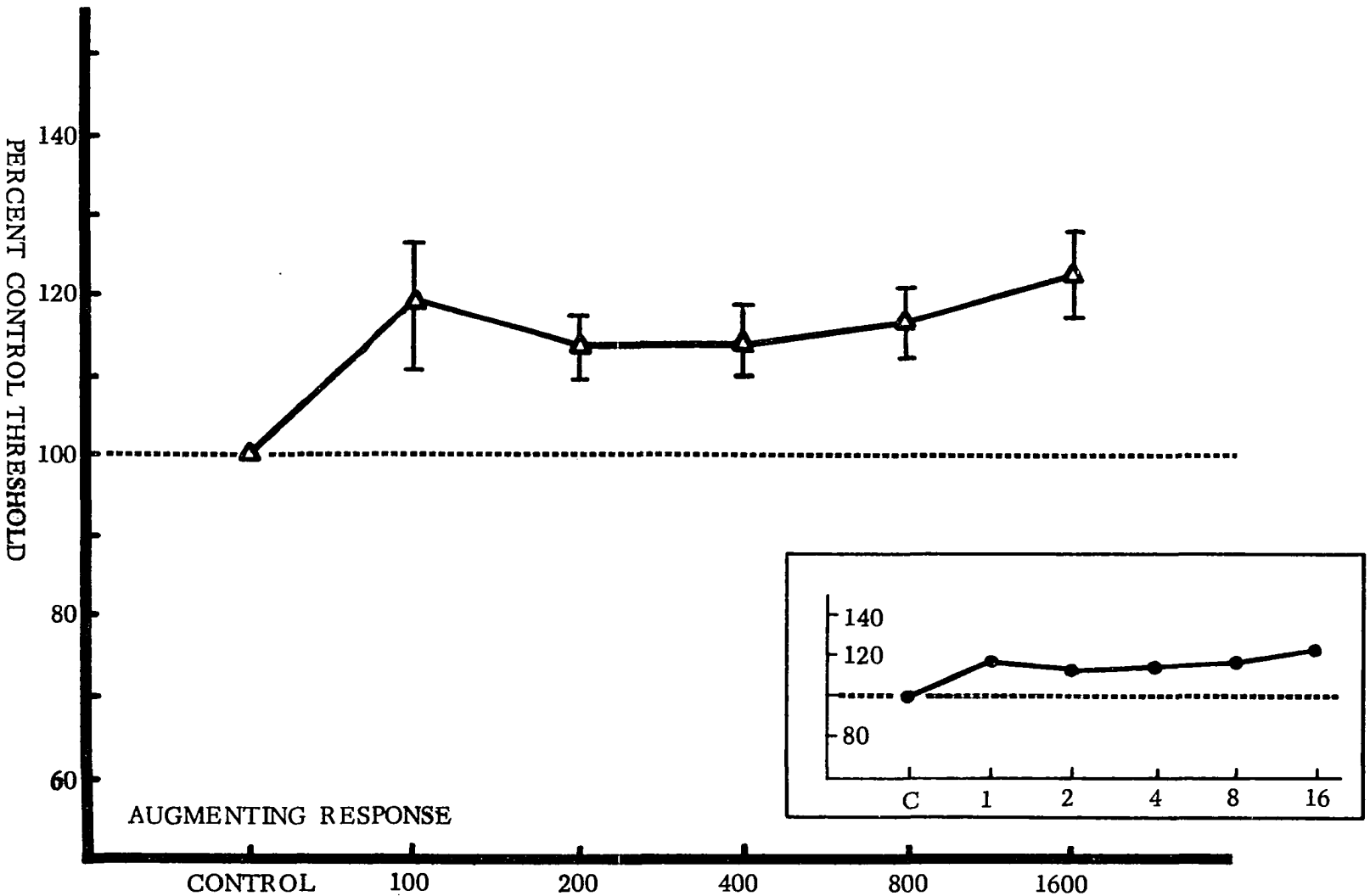
Figure 16. Effects of Ethanol on Augmenting Response Threshold.

In this experiment, augmenting response threshold elicited by stimulation (8/sec, 0.5 msec duration) of n. ventrolateralis (VL) of the specific afferent thalamus was measured as 65 μA. This threshold was maintained at 100 mg/kg, increased to 70 μA at the 200 and 400 mg/kg dose levels, then to 75 and 80 μA at the highest doses. Response amplitude was greatest on specific cortex ipsilateral (LAC) to the thalamic stimulation site. Calibration, 100 μV and 1 second.




EFFECTS OF ETHANOL ON AUGMENTING RESPONSE THRESHOLD

Figure 17. Dose-Response Threshold Profile for Augmenting Response. The profile represents mean response threshold data derived from 10 experiments.


PERCENT CONTROL THRESHOLD
140
120
100
80
60
AUGMENTING RESPONSE
CONTROL
100
200
400
800
1600
140
120
80
C
1
2
4
8
16

significantly increased over control values at all higher doses. As may be expected from the lack of large differences between threshold means across the doses, overall drug effect was not significant (Friedman, df = 4, $p<.20$). Analysis of inter-dose threshold differences also reflects the constancy of augmenting threshold over all dose levels. No differences were found between any of the thresholds (Table 4).

The overall effect of ethanol was to raise the threshold for augmenting responses at low doses. This increase remained fairly constant over higher doses. Mean thresholds ranged from 113.5 to 122.7% of control over all doses, and mean threshold at the highest dose was only 4% greater than at the lowest dose.

### Reticular Activation: Cardiovascular Pressor Responses

Control responses. Suprathreshold stimulation of the mesencephalic reticular formation resulted in a phasic pressor response with a latency of 3-5 seconds from the onset of stimulation (Figure 4). The response continued through the end of the stimulus train but peaked within the 10-second stimulation period. Increases of more than 100 mm Hg could be elicited in blood pressure with current intensities several times threshold.

The transition from subthreshold to suprathreshold response was characterized by an increased response amplitude in proportion to stimulation current intensity. Threshold was set at an observed rise of 10 mm Hg in systolic pressure above the baseline pressure established in the 10 seconds preceding the stimulation period. Although this

criterion was somewhat arbitrary, it was found to be sufficiently high in pilot experiments to prevent scoring of "false positives" due to spontaneous changes in pressure.

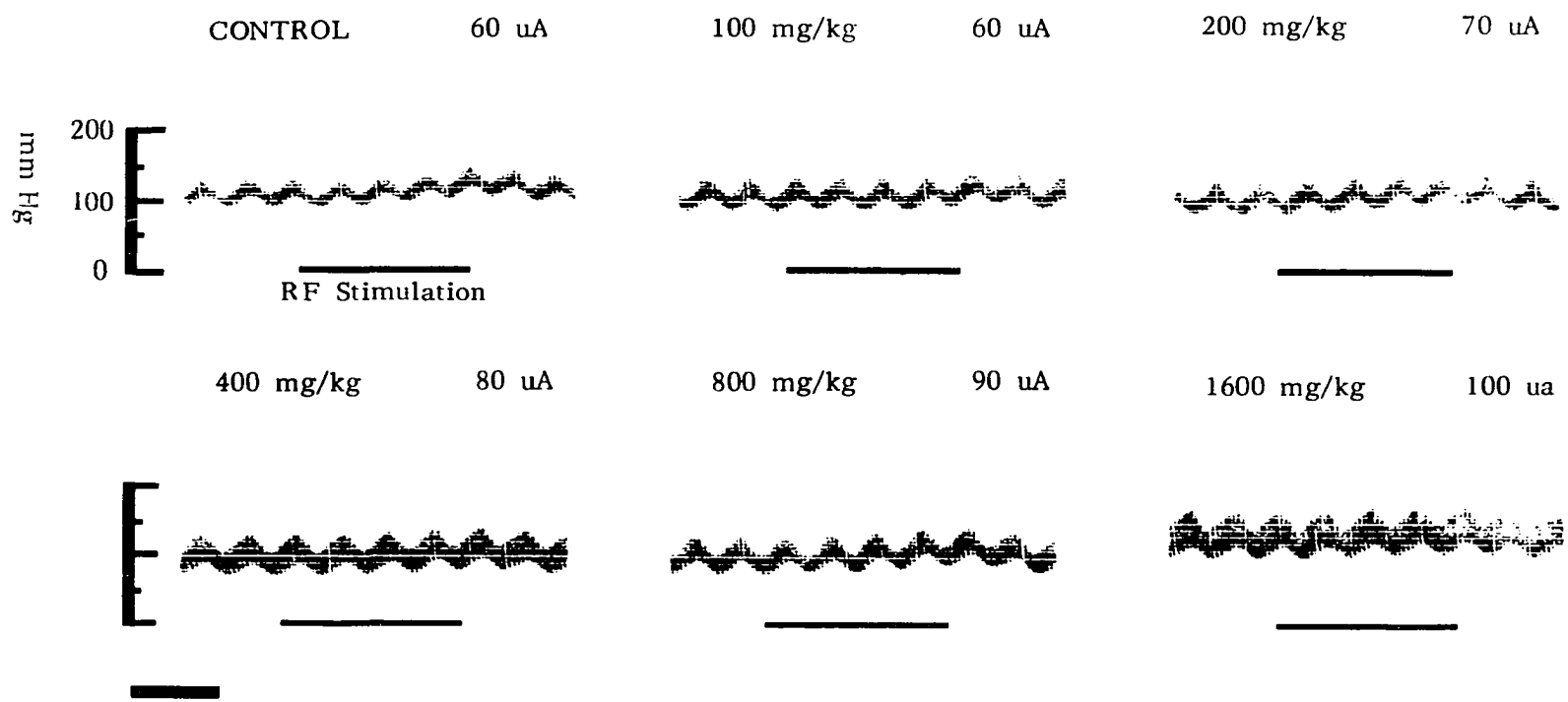
With increasing current intensity the pressor response was seen to emerge from the normal cyclic variation in blood pressure associated with respiration (Figure 4). Mean absolute threshold current was 60.1 μA (range 30 to 90 μA).

Effects of ethanol on pressor response thresholds. Individual dose-response threshold profiles were generally characterized by increases in pressor response threshold in proportion to the infused dose. Few profiles showed any decrease in threshold below control values with any dose. This is demonstrated in Figure 18, which shows record samples taken at threshold during control and five ethanol conditions from a typical experiment.

The mean dose-response threshold profile (Figure 19) shows that ethanol had little effect at the lowest dose wherein pressor thresholds remained relatively constant at control values. Mean threshold increases to 113.2% of control at 200 mg/kg and further increases to 121.5, 135.2 and 145.3% with succeeding doses (Table 3). Overall ethanol effect on pressor thresholds, shown clearly in the mean threshold profile as a monotonic increasing function of ethanol dosage, was significant (Friedman, $df = 4$, $p<.001$). Analysis of the difference between threshold means at each dose and those observed under control conditions showed that thresholds at 200 mg/kg and all higher doses were significantly higher than control values (Table 3).

Figure 18. Effects of Ethanol on Cardiovascular Pressor Response Threshold.

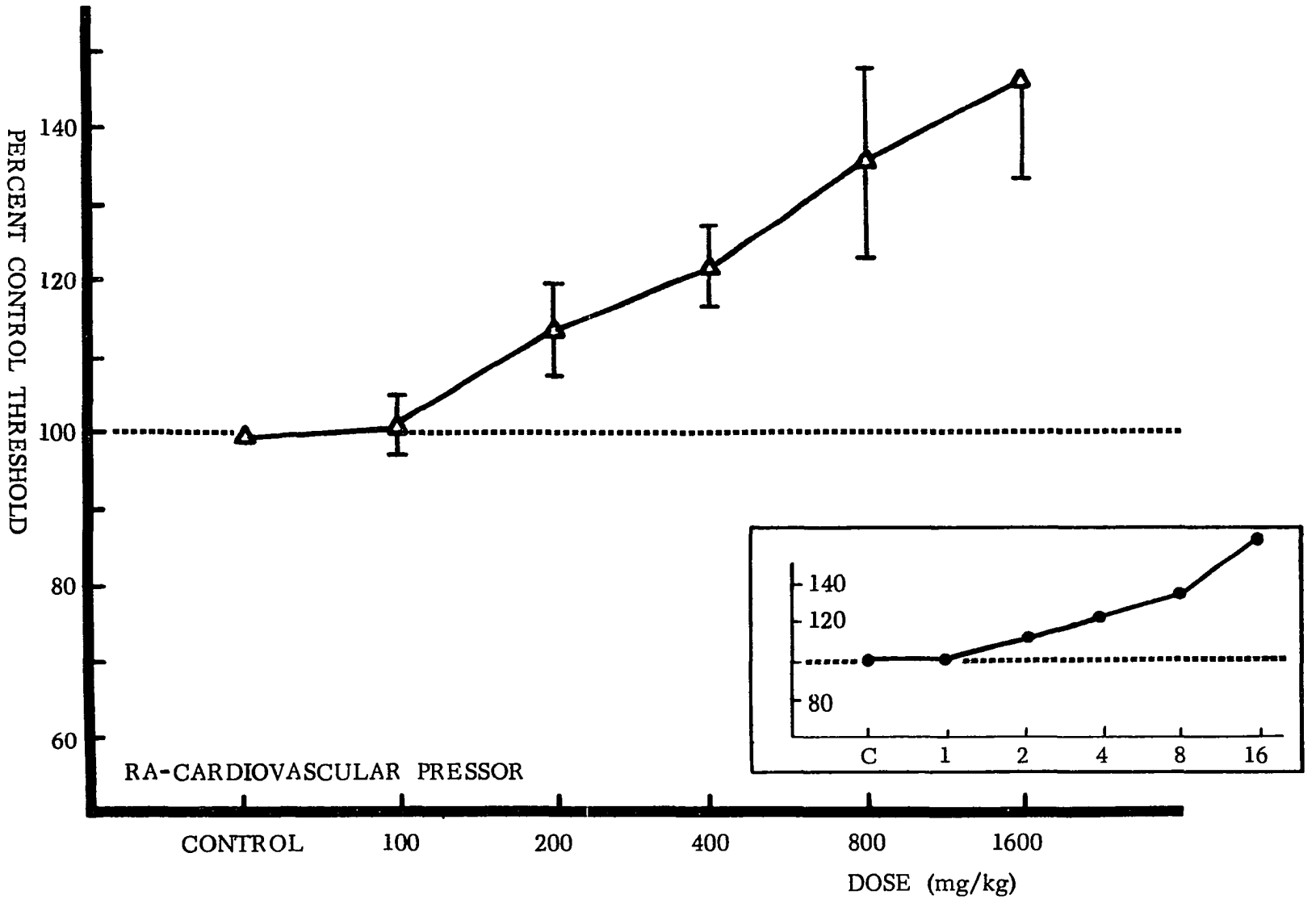
In the experiment illustrated, a criterion pressor response, elicited by stimulation (200/sec, 0.05 msec duration) of midbrain reticular formation was obtained at 60 μA. This threshold was maintained at the 100 mg/kg dose level and thereafter rose in proportion to the increasing dosage. Time calibration, 5 seconds.




EFFECTS OF ETHANOL ON CARDIOVASCULAR PRESSOR RESPONSE THRESHOLD

Figure 19. Dose-Response Threshold Profile for Pressor Response.

This graph represents the mean dose-response threshold profile derived from 11 experiments.


PERCENT CONTROL THRESHOLD
140
120
100
80
60
RA-CARDIOVASCULAR PRESSOR
CONTROL
100
200
400
800
1600
DOSE (mg/kg)
140
120
80
C
1
2
4
8
16

Analysis of inter-dose threshold differences (Table 4) showed that contiguous doses were not significantly different (e.g., thresholds at 400 mg/kg were significantly higher than at 100 mg/kg but they could not be differentiated from those at 200 mg/kg).

Overall, the effects of ethanol on the pressor response were observed as an increase in threshold proportional to dose; thresholds were not significantly higher than control values until the 200 mg/kg dose. Other changes in the response as a function of ethanol dosage were observed but not analyzed. These included a more rapid return to baseline pressure as dosage increased associated with an increase in the latency of the response from the onset of stimulation.

Reticular Activation: Skin Resistance Responses

Control responses. Skin resistance responses to high frequenty stimulation of the reticular formation were observed as phasic decreases in basal skin resistance; response amplitude was proportional to stimulation current and with sufficient intensity changes of several hundred ohms could be elicited. The first discriminable decrease in resistance could usually be observed within 2-3 seconds of stimulus onset; response peak latencies were usually within the range of 4-6 seconds and decreased with increasing current intensity. The transition from subthreshold to suprathreshold response (Figure 5) was similar to that observed with the pressor response: it emerged from a relatively flat baseline basal resistance and amplitude increased in proportion to the applied current. Threshold was set at a phasic decrease of 50 ohms, a value sufficiently high to prevent scoring of spontaneous

and artifactual changes yet sensitive to small changes due to reticular stimulation. Mean control threshold current intensity was 62.4 μuA with a range of 30 to 100 μA.

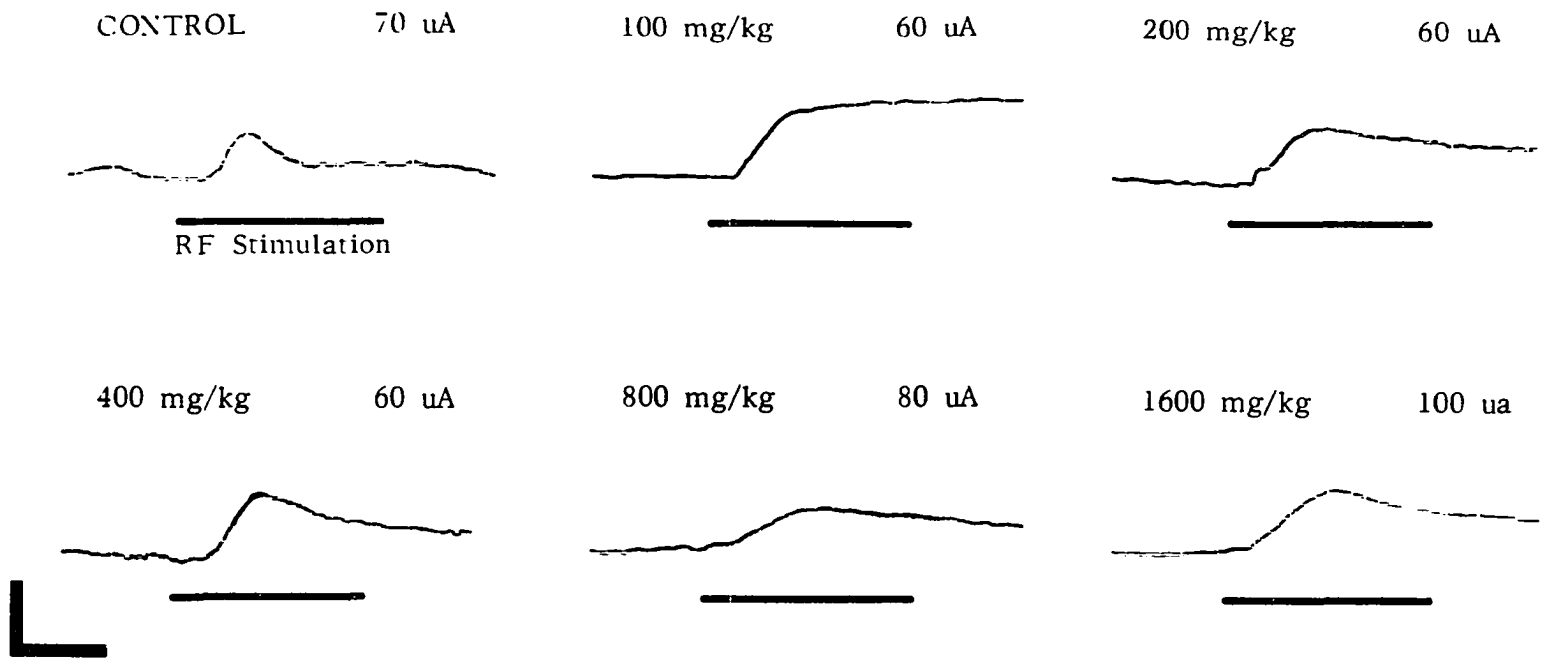
Effects of ethanol on skin resistance response thresholds. Individual thresholds were considerably more variable than those for other responses. Thresholds were generally decreased below control values through the lower doses with some tendency to increase to and above these values at the higher doses. Standard errors associated with the mean response at each threshold (Table 2) were not exceedingly large compared to those calculated for other response thresholds, but they do not reflect the fact that in one experiment skin resistance thresholds dropped to 40% of control threshold at one dose and in others, threshold changes were somewhat erratic. Figure 20 shows records derived from an experiment which reasonably approximated the mean dose-response threshold profile (Figure 21).

Although mean threshold values declined to 93% at 100 and 200 mg/kg and increased above control values at the higher doses, none of these changes were significant. The results of inter-dose difference tests (Table 4) showed, however, that thresholds at 1600 mg/kg were significantly higher than those at all other doses except at 800 mg/kg. The only other significant difference found was between thresholds at 200 and 800 mg/kg.

Increasing concentrations of ethanol led to a decrease in the duration of the skin resistance response and usually increased the latency of the response after the onset of reticular stimulation (Figure 20). Basal resistance was also sensitive to the effects of

Figure 20. Effects of Ethanol on Skin Resistance Response Threshold.

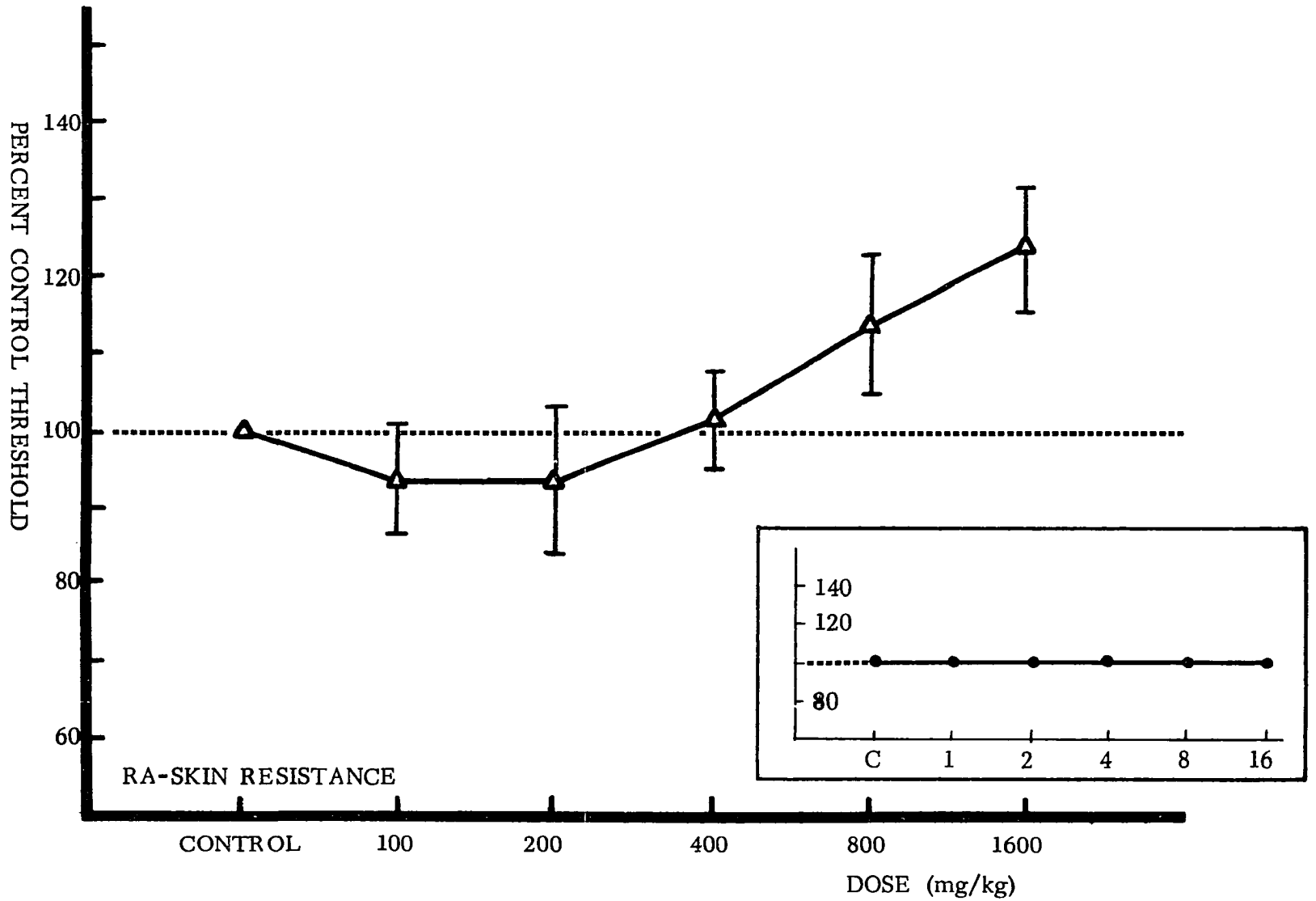
Under control conditions, a criterion threshold skin resistance response, elicited by stimulation (200/sec, 0.05 msec duration) of the mesencephalic reticular formation, was obtained at 70 μA in this experiment. Threshold was decreased below control at the 100, 200 and 400 mg/kg dose levels then increased above control values at 800 and 1600 mg/kg. The response at 100 mg/kg demonstrates one of the measurement problems associated with this response. Skin resistance showed a marked phasic decrease in response to stimulation. There apparently was a slight change in basal resistance during the response to a level approximating the peak decrease of the phasic response and, hence, resistance did not return to the previous baseline. Time calibration, 5 seconds.




EFFECT OF ETHANOL ON SKIN RESISTANCE RESPONSE THRESHOLD

Figure 21. Dose-Response Threshold Profile for Skin Resistance.

Means data for this profile of skin resistance thresholds were derived from 11 experiments.


PERCENT CONTROL THRESHOLD
140
120
100
80
60
RA-SKIN RESISTANCE
CONTROL
100
200
400
800
1600
DOSE (mg/kg)
140
120
80
C
1
2
4
8
16

higher doses; in many cases the infusion of 800 and 1600 mg/kg doses was accompanied by a 100-150 ohm decrease in basal resistance although pre-infusion values were usually recovered by the beginning of threshold measurements 10 minutes after the end of infusion.

In general, the effects of ethanol on skin resistance response thresholds were variable but were observed to be most frequently a small decrease in threshold below control values at lower doses and a rise above control threshold at the higher doses. Neither of these changes were significant. Increased variability was not obviously seen in basal resistance.

## Interaction of Changes in Response Thresholds

Figure 22 shows a combined plot of all mean dose-response threshold profiles. This figure shows that all mean response thresholds rise monotonically above 400 mg/kg. Three responses, recruiting, cortical desynchrony and skin resistance, have mean thresholds approximating control values at 400 mg/kg. Below 400 mg/kg the pattern becomes more complex although there is an obvious difference between recruiting and cortical desynchrony thresholds at 100 mg/kg. Statistical analysis of differences between response thresholds at each dose were made (Table 5) and for clarity each dose will be discussed separately.

### 100 mg/kg

The most striking effect at this dose is the significant difference between cortical desynchrony and recruiting. Skin resistance and pressor responses could not be differentiated, but both of

Figure 22. Combined Dose-Response Threshold Profiles for all Responses.

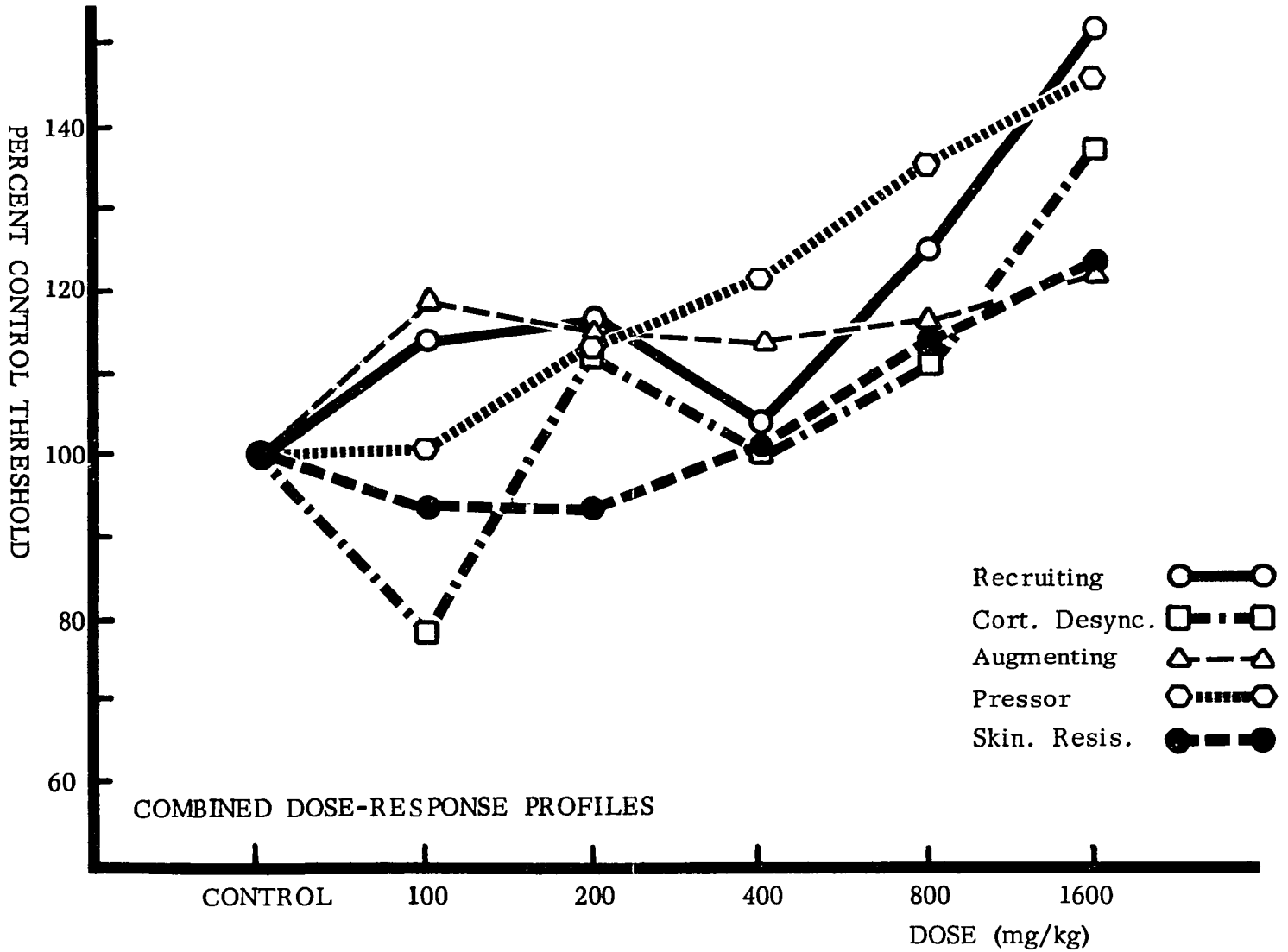

PERCENT CONTROL THRESHOLD
140
120
100
80
60
COMBINED DOSE-RESPONSE PROFILES
CONTROL
100
200
400
800
1600
DOSE (mg/kg)
Recruiting
Cort. Desync.
Augmenting
Pressor
Skin. Resis.

Table 5

RESULTS OF WILCOXEN TEST ANALYSIS OF RESPONSE THRESHOLD DIFFERENCES BETWEEN RESPONSES

| Dose | Response | Response CDS | AUG | CV | SR |
|---|---|---|---|---|---|
| 100 mg/kg | RR | .01[a] | -- | .05 | .02 |
| | CDS | | .01 | .01 | -- |
| | AUG | | | -- | .05 |
| | CV | | | | -- |
| 200 mg/kg | RR | -- | -- | -- | -- |
| | CDS | | -- | -- | -- |
| | AUG | | | -- | -- |
| | CV | | | | .05 |
| 400 mg/kg | RR | -- | -- | .05 | -- |
| | CDS | | -- | .02 | -- |
| | AUG | | | -- | -- |
| | CV | | | | .02 |
| 800 mg/kg | RR | -- | -- | -- | -- |
| | CDS | | -- | .02 | -- |
| | AUG | | | -- | -- |
| | CV | | | | .02 |
| 1600 mg/kg | RR | -- | .01 | -- | .05 |
| | CDS | | -- | -- | -- |
| | AUG | | | -- | -- |
| | CV | | | | -- |

[a]Probability of test result less than stated value.

RR = Recruiting Response
CDS = Cortical Desynchrony Response
AUG = Augmenting Response
CV = Pressor Response
SR = Skin Resistance Response

these responses were significantly different than recruiting and desynchrony. Augmenting thresholds were not different than recruiting thresholds. Hence, the five responses may be roughly divided into three classes at this dose: (1) thalamic response thresholds increased, (2) autonomic activation thresholds remained unchanged, and (3) cortical activation thresholds decreased at this dose.

200 mg/kg

At this dose statistical separation between the five response thresholds failed and showed only that pressor response thresholds are higher than skin resistance thresholds. All response thresholds are essentially equivalent.

400 mg/kg

At this dose recruiting, cortical desynchrony and skin resistance are not different than control, while augmenting and pressor response thresholds are significantly higher than control. Statistically, only the pressor threshold can be separated from the remaining responses. Augmenting, recruiting, desynchrony and skin resistance thresholds are essentially equivalent at this dose.

800 mg/kg

The pattern of threshold differences at 800 mg/kg is about the same as that at the next lower dose. The pressor response threshold can be differentiated from all other responses but the remaining thresholds cannot be separated.

1600 mg/kg

At the highest dose recruiting is significantly different than augmenting and skin resistance thresholds. The pressor threshold is not different than recruiting. Comparison of all differences at this dose suggested that a rough dichotomy can be made between more and less sensitive thresholds with recruiting, cortical desynchrony and pressor thresholds forming a group more sensitive to ethanol and augmenting, and skin resistance thresholds forming a less sensitive group.

Overall, this analysis demonstrated a large difference between cortical desynchrony and recruiting at the 100 mg/kg dose. It also showed that pressor thresholds were increased to a greater extent at lower doses than any other response and this greater increase continued through higher doses. Recruiting and augmenting thresholds were not significantly different at any dose despite the obvious differences in mean dose-response threshold profiles.

## Control Experiments

Two sets of control experiments were deemed necessary to show that observed changes in response thresholds were a function of ethanol dosage and not of extraneous uncontrolled factors. The first series examined the stability of response thresholds over time. Four animals were observed for a maximum of six hours beyond the start of normal control threshold recording. Since a total of three hours was allowed for recovery from general anesthesia, this meant that these control animals were observed for up to nine hours after the completion of

surgery. No significant changes were found in any response threshold over the time usually used for recording in ethanol experiments. Thresholds were found to remain relatively constant during the latter half of the six hour observation period as well, although there was some suggestion of slight increases in cortical desynchrony thresholds.

The second series of experiments examined the effects of high volume infusions of the ethanol diluent on response thresholds. This control was thought necessary because the 7% ethanol solution used required a total infusion of 22.85 ml/kg of Ringer's Solution to reach the highest dose of 1600 mg/kg. Raising the dose from 800 to 1600 mg/kg required a final infusion of 11.4 ml/kg or half the total infused volume. This final infusion represented an increase of 20-25% in the animal's blood volume within the space of 4-7 minutes depending upon its weight. Most of this excess volume was, of course, quickly reduced by absorption from the blood and diuresis.

In four experiments infusions were made with ethanol-free Ringer's Solution in the same volume as that required to reach 800 and 1600 mg/kg. No significant deviations were found in response thresholds although there were slight changes in other parameters. At the 1600 mg/kg level, hematocrit, derived from the first post-infusion blood sample, was usually 2-3% lower than when measured prior to infusion. It was found to be within the normal range in the second post-infusion measurement. Also, blood pressure declined 10-20 mm Hg during the high dose sham infusion but recovered to previous baseline values before the onset of the ordinarily scheduled threshold measurement period. No significant changes were found in blood gas values with either of the sham doses.

## Verification of Electrode Placements

Figures 23 and 24 show the results of histological verification of typical subcortical electrode placements. In all but two experiments used for analysis, electrodes with n. ventrolateralis as the specific thalamic target fell well within its borders (Figure 23). The remaining two fell approximately on the atlas-defined (Jasper and Ajmone-Marsan, 1954) border of this nucleus. Differences between augmenting responses as a function of electrode placement were not observed. Electrodes directed at non-specific thalamus were well placed in either n. centre median or n. reuniens depending upon the intended locus. Recruiting responses recorded at the cortex could not be used to differentiate between the two nuclei but recruiting-like responses recorded in the hippocampus were only associated with stimulation of n. centre median. Electrodes directed to the mesencephalic reticular formation (Figure 24) fell within a relatively small locus. Activation responses were observed to occur with placements anywhere within the general vicinity of the target locus used in these experiments (A2, L3, H3). Hippocampal placements were considerably more varied than with other targets. Several experiments apparently had electrodes placed more medially than the classic borders of the hippocampus. Although it might be expected that similar activity should be recorded in this medial area, the best records of hippocampal activation were derived from electrodes placed more laterally.

Figure 23. Histological Verification of Electrode Placements in Specific and Non-Specific Thalamus.

This illustration shows histological verification of typical electrode placements in n. ventrolateralis (VL, squares) of the specific afferent thalamus and n. centre median (NCM, triangles) and n. reuniens (RE, circles) of the non-specific thalamus. Verification was made by comparison of unstained frozen section slides taken from the point of deepest electrode penetration with appropriate stereotaxic atlas plates. (Adapted from Jasper and Ajmone Marsan, 1954.)

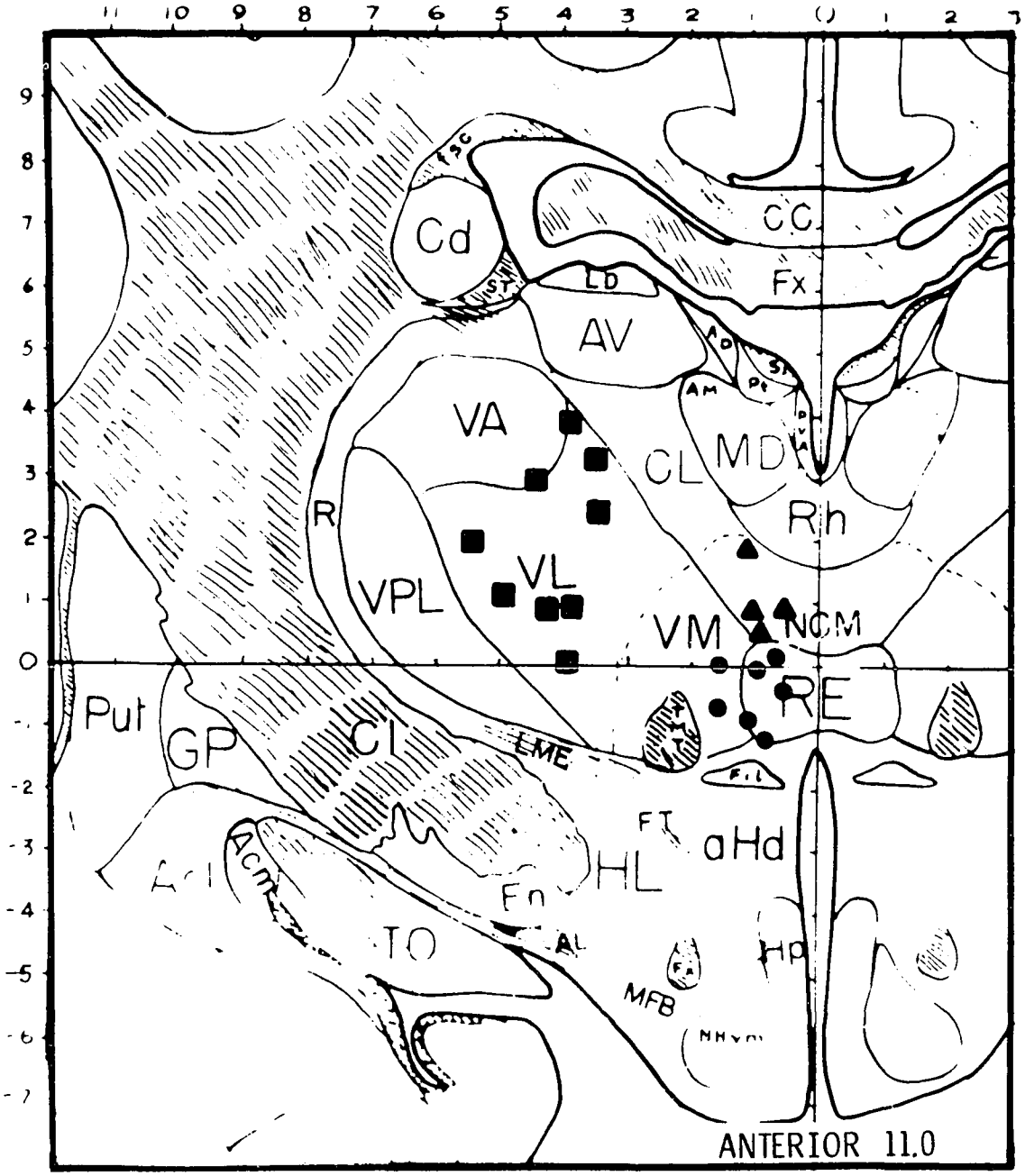
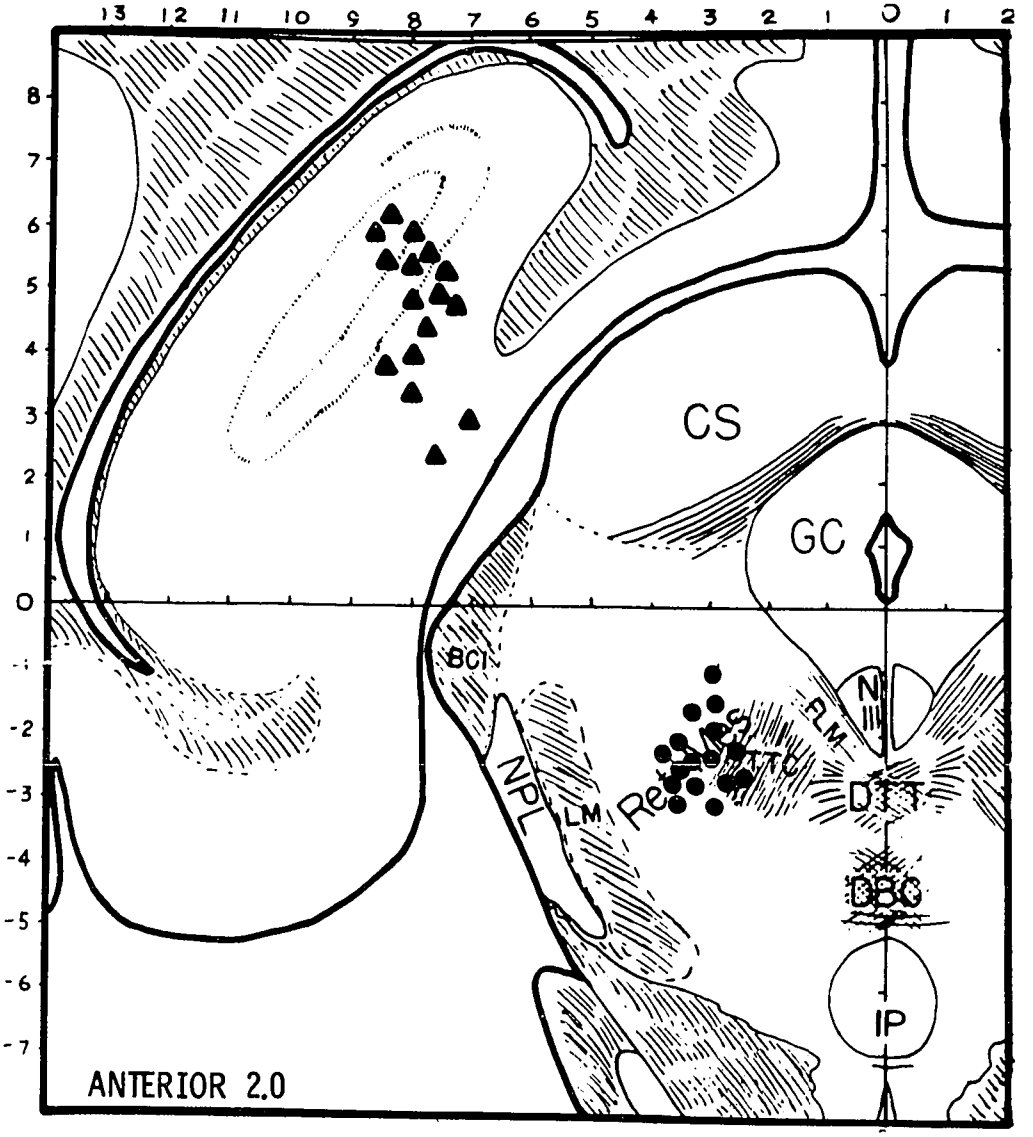

Cd
CC
Fx
LD
AV
VA
CL
MD
Rh
R
VPL
VL
VM
NCM
RE
Put
GP
CI
LME
aHd
HL
Fn
TO
AL
HP
MFB
ANTERIOR 11.0

Figure 24. Histological Verification of Electrode Placements in Hippocampus and Mesencephalic Reticular Formation.

This illustration shows verification of typical electrode placements in hippocampus (triangles) and midbrain reticular formation (Ret Mes, circles). (Adapted from Jasper and Ajmone-Marsan, 1954.)


13 12 11 10 9 8 7 6 5 4 3 2 1 0 1 2
8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7
CS
GC
BCI
NPL
LM
RLM
DBC
IP
ANTERIOR 2.0

# CHAPTER V

## DISCUSSION

These results show that ethanol exerts profound effects upon CNS response thresholds, but they also demonstrate that only at high doses are these effects simple functions of increasing ethanol concentration. Comparison of dose-response threshold profiles reveals the existence of three distinct phases of ethanol action on the CNS. The first phase is defined at low doses by a decrease in threshold for cortical desynchrony responses and a concomitant increase in the threshold for recruiting responses. A second phase is defined at intermediate doses by the return of cortical desynchrony and recruiting response thresholds to control values. The observed increase in all response thresholds at high doses may define a third phase in which ethanol produces a non-specific depression of all CNS responses.

One question must be answered prior to any detailed interpretation of these results: Given that changes in CNS response thresholds are demonstrated, what information about the CNS is conveyed by these changes? Threshold was defined as that amount of energy, calibrated in units of current intensity, required for the elicitation of a given response. Energy was added to the observed system incrementally until its characteristic response satisfied certain preset criteria; a threshold response was elicited when the sum of all excitation in the

system was sufficient for its production.

If the artificially introduced energy required for response elicitation is less under some circumstances than others than either the total energy required for the response has decreased or the level of endogenous (or naturally imposed) excitation has increased. Operationally, these alternatives are equivalent: if the threshold is lower, than it may be assumed that the level of endogenous excitation has increased. A change in threshold may then be taken as equivalent to a change in excitation in the system, at least on some arbitrary ordinal scale.

Note that if the animal is maintained in some arbitrary but constant state of CNS activation, as measured by certain common indices (e.g., gross cortical electrical activity or autonomic stability), than the endogenous excitation should be maintained at some fairly constant level. If a centrally-active compound, such as ethanol, is added, the changes which it produces in the system will be seen as an increase or decrease in excitation of the system. Change in threshold should then be proportional to the excitatory effect of the drug. It may then be assumed that the information conveyed by a change in threshold is a change in excitation within the observed system.

Note also that whether criterion threshold is taken as the first observation of the characteristic response in the gross electroencephalograph or some other arbitrary amplitude there is little difference in interpretation so long as constancy is maintained. Examination of any of the observed responses at higher amplification or with the aid of computer summation (Figures 10 and 15) demonstrates presence

of the response at current levels lower than that defined as threshold. Nevertheless, the use of threshold criteria, setting the threshold as near as possible to the "real threshold," if one exists, is desirable because of special properties this information may have. Namely, the real threshold may be extremely sensitive to changes in CNS excitation.

## Low Dose Effects of Ethanol

The low dose phase of ethanol action is clearly defined by the decreased cortical desynchrony response thresholds at 100 mg/kg. Decrease in this threshold suggests that ethanol produces either an increase in the relative excitability of the reticular activating system or a direct increase in cortical excitability. Two factors suggest that the latter may be the best alternative.

First, the cardiovascular pressor response is mediated via activation of adrenergic vasoconstrictor and cardioregulatory mechanisms centered in the medulla (Gunn, et al, 1968). It is assumed that this activation is derived through descending pathways from the mesencephalic reticular formation. Unless there was a dissociation between ascending and descending activation derived from the same stimulus, constancy of the pressor threshold indicates that midbrain reticular formation excitability remains relatively unchanged.

Secondly, at this dosage ethanol caused a decrease in the excitability of the non-specific thalamic system demonstrated by an increase in recruiting response thresholds. This change in non-specific thalamic responsivity suggests the following interpretation: The primary effect of ethanol in the low dose range is to decrease cortical inhibition derived from the non-specific thalamic system which results in

an observed increase in cortical excitability. At the lowest dose level, the effects of ethanol are undoubtedly depressive, but the induced depression is predominantly rostral to the midbrain reticular formation since there is no change in pressor thresholds mediated caudal to the reticular formation. And, these effects are exerted primarily upon inhibitory systems.

## Functional Significance of Decreased Thalamic Excitability

The definition of the non-specific thalamic system as the primary mediator of cortical inhibition is not a radical departure from accepted neurophysiology, but its use here to explain some of the effects of ethanol on the CNS requires some explanatory comment. Although Jasper (1954) suggested that the non-specific thalamic nuclei were a rostral extension of the mesencephalic reticular formation, there is now substantial evidence that these nuclei may be, instead, a source of inhibition exerting modulatory control over the cerebral cortex. The cortical synchronizing abilities of non-specific thalamus are well known. Considering the correlation of cortical slow wave activity with sleep, inattention and CNS depression, events known to be dependent upon the integrity of thalamocortical connections, the non-specific thalamic system may be cited as a source of "internal inhibition." Magoun (1963) identifies this system with internal inhibition and states that "the consequences of the action of this mechanism are the opposite of the ascending reticular activating system for internal activation."

Proposal of the non-specific thalamic system as an extension of the midbrain reticular formation was founded upon the ability of high frequency stimulation of non-specific nuclei to produce cortical activation similar to that produced by direct reticular stimulation (Hunter and Jasper, 1949). However, Schlag and Chaillet (1963) found that such activating effects were mediated only through descending connections to midbrain reticular formation. Reversible cryogenic blockade of ascending, cortically directed, non-specific outflow results, not in cortical depression, but in sensory evoked potential augmentation, abolition of recruiting responses and spontaneous spindle bursts and deficits in behavior highly suggestive of frontal brain lesions and loss of internal inhibition (Skinner and Lindsley, 1967).

Correlation of frontal lobe symptoms and non-specific thalamus blockade suggests the existence of functional relations between these areas. Anatomical evidence exists for well developed bidirectional connections between frontal cortex and non-specific nuclei. More importantly, lesions of orbital frontal cortex result in complete abolition of spontaneous spindle waves and recruiting (Velasco, et al, 1968). Blockade of non-specific thalamus functions by lesions of a site known to mediate powerful inhibitory functions (Sauerland, Nakamura and Clemente, 1967) implicates the non-specific thalamus as a part of a thalamo-orbito-cortical inhibitory system (Velasco, et al, 1968).

If recruiting is taken as the characteristic response of the non-specific thalamus then increased activation should reduce recruiting in some fashion. Velasco and Lindsley (1968a) found peripheral and direct midbrain reticular stimulation to be equivalent in reducing

recruiting amplitude. Similarly, Weinberger, et al (1968) found suppression of recruiting with presentation of a conditioned stimulus but not with an habituated non-conditioned stimulus. Conversely, lesions of the reticular formation, leading to decreased activation, lower the threshold for recruiting, hasten its development and increase its amplitude modulation (waxing and waning) (Velasco and Lindsley, 1968b).

Taken together, these studies indicate a much more intimate relationship between non-specific thalamus and frontal cortex than between thalamus and reticular formation. Anatomical connections between non-specific thalamus and reticular formation have been well described (Bowsher, 1966; Nauta and Kuypers, 1958), however. The functions of these pathways could be to mediate inhibition of non-specific thalamus by the midbrain reticular formation; hence, activation of the reticular formation would produce direct cortical activation as well as a decrease in non-specific thalamic influence on the cortex. Preliminary support for this hypothesis of reticular formation to non-specific thalamus inhibition is indicated by showing that the predominant effect of reticular formation stimulation on neurons in non-specific thalamus is inhibition (Dila, 1971; Mancia, et al, 1971).

While these studies suggest that the non-specific thalamus mediates some aspects of "internal inhibition," it may also control information processing to some extent through its cortical modulatory powers. When cortical sensory units are directly stimulated during surgery on humans, it is found that perception of this stimulation requires a minimum of 0.3-0.5 seconds of repetition (Libet, 1964; Libet, et al, 1965). Single stimuli, regardless of their intensity,

are not perceived. Observation of cortical after-discharge outlasting the normal cortical evoked potential to stimuli (Cobb and Dawson, 1960) suggests that normal perception requires repetition of afferency. On this basis, Andersen and Andersson (1968) hypothesize that the cortex requires a repetitive volley at 10-20 Hz to react to afferent impulses with some sign of perception. Such repetitive after-discharge is probably the result of recurrent thalamocortical inhibitory circuits. It may be assumed that such rhythmic after-discharge will raise the probability of perception of near threshold stimuli. Hence, the inhibitory functions of the non-specific thalamus serve to emphasize certain information received by the CNS, a function shared with the midbrain reticular formation but accomplished by entirely different means.

In this light, a decrease in non-specific thalamus excitability, demonstrated by the rise of recruiting response threshold, strongly suggests loss of inhibitory control over the rostral CNS, especially the cerebral cortex, and thus, a significant decrease in potential modulatory control of afferent channels. Further disintegration of potential information processing capabilities is suggested by increasing thresholds for the augmenting response. If the augmenting response is indicative of the integrity of integration and information processing in specific thalamocortical afferent channels than these results suggest an early, sustained decrease in information processing abilities in the low dose phase of ethanol action.

The 200 mg/kg dose is included in the low range phase for several reasons. Although this phase is best defined by the threshold changes observed at 100 mg/kg, changes observed at the 200 mg/kg dose

suggest a transition between the low and intermediate phases. This dose produces a range of ethanol concentrations at which variability in thresholds is the greatest. Standard errors for cortical desynchrony, recruiting and skin resistance response are larger at this dose than at any other. Although the threshold for cortical desynchrony cannot be differentiated from control at 200 mg/kg its dramatic rise from the lowest dose may demonstrate that direct depression of excitatory systems begins in this concentration range.

Skin resistance thresholds are not significantly different than control within the low dose range. Their decline with low doses does suggest a trend towards an initial activation with a maximum at an ethanol concentration greater than that required for maximal decreases in cortical desynchrony threshold. The skin resistance data were for the most part, much more variable than those for other responses. This may be primarily a function of poor measurement technique.

## Effects of Ethanol at Intermediate Doses

The intermediate phase of ethanol action is best defined by threshold changes at 400 mg/kg although the examination of individual dose-response threshold profiles suggested that this phase may continue through ethanol concentrations produced by 800 mg/kg. At 400 mg/kg both recruiting and cortical desynchrony thresholds cannot be differentiated from those observed under control conditions. However, no such trend is observed in pressor thresholds, which at this dosage are clearly increased.

Return of recruiting and cortical desynchrony thresholds to control levels may indicate that ethanol-induced depression of both inhibitory and excitatory systems has reached a point where a new balance is achieved, a balance which has a "set point" different than that which exists under control conditions. At this dose thresholds for recruiting and cortical desynchrony are not different than control operationally, but considering their history at lower doses, they may be different intrinsically.

The implications of this re-established balance are not clear, but it may mean that the early loss of cortical inhibition observed in the low dose phase is, at this dose level, counterbalanced by direct depression of excitatory systems. In the low dose phase, constancy of pressor thresholds led to the conclusion that ethanol did not directly decrease the excitability of the reticular activating system. Increase of that threshold at intermediate doses may indicate that the reticular formation is directly depressed at this ethanol concentration and, hence, the unbalanced cortical activation, observed at low doses, is counteracted. If the intermediate phase of ethanol action is defined by depression of both inhibitory and excitatory influences, the operational reestablishment of a new balance between these systems may not truly reflect the state of the CNS.

Several factors suggest that this balance is illusory. Increased threshold for autonomic activation (pressor response) demonstrate that autonomic reactivity is decreased. Integration in specific thalamocortical channels continues to be depressed (augmenting response) and evoked potential amplitudes are decreased in this

concentration range (Nakai, 1964; Nakai, et al, 1966). This evidence leads to the conclusion that both CNS autonomic responsivity and information processing potential are decreased in this range. At the least, the intermediate phase of ethanol action represents a clear dissociation between central responsivity, measured by cortical desynchrony and recruiting, and peripheral preparatory responsivity, measured by autonomic thresholds.

## Effects of Ethanol at High Doses

Reference to dose-response threshold profiles (Figure 24) shows that, with the exception of augmenting, all response thresholds increase above 400 mg/kg in direct proportion to rising ethanol concentration. Control experiments demonstrate that these increases are not due to the passage of time or the infusion of large volumes of diluent. These results may be taken to define the third and final phase of ethanol action: a non-specific depression of CNS responsivity which will increase in depth until unconsciousness and finally death ensue.

The results show that recruiting response thresholds can be clearly differentiated from control at 800 mg/kg while neither cortical desynchrony nor skin resistance thresholds can be differentiated statistically until the final dose at 1600 mg/kg. The pressor and augmenting thresholds have already been shown to be significantly above control values before the final phase of ethanol action is entered. These differences provide some evidence of an overall difference in susceptibility to ethanol among the five systems observed. These differences

are most apparent at the 1600 mg/kg dose where statistical analysis suggests a rough division of the responses into two groups: augmenting and skin resistance thresholds are not different at this dose but both are substantially lower than the three remaining responses.

## Comparison with Earlier Investigations

The results of this investigation demonstrate that ethanol produces significant changes in CNS responsivity at a blood concentration range of 10-15 mg%, a range only 2-3 times the endogenous level (Mardones, 1963). While most activation effects observed by others occur at somewhat higher concentrations (Kalant, 1970), there is evidence to support the conclusion that blood concentrations just barely above endogenous levels are sufficient to cause changes in certain CNS responses.

In man, a concentration of 10 mg% is sufficient to significantly lower the rotation velocity required to produce fusion in the optokinetic drum (Blomberg and Wassen, 1962). A concentration of 26 mg% is sufficient to alter alpha power distribution to its fullest observed extent (Doctor, et al, 1966) and some observable decrease in the effect of orbital-frontal inhibition on somatic reflexes in cats is found at 25 mg% (Sauerland, et al, 1967). Also in cats, spontaneous cortical activation has been observed at less than 50 mg% (Horsey and Akert , 1953; Hadju-Dimo, et al, 1968) and this activation is correlated with a transient increase in cortical blood flow (Hadji-Dimo, et al, 1968).

Hence, there is considerable support for the conclusion that only a very small amount of ethanol is necessary to significantly alter

CNS functions. With the exception of the change in optokinetic fusion, all of these studies suggest that ethanol in low doses increases CNS excitation but, as discussed below, this change may also result from increased excitation.

Few studies provide any basis for comparison with results found at higher doses in this investigation. Story, et al (1961) examined the effects of ethanol on augmenting and recruiting (neither of which satisfied the accepted definitions of these responses since paired and not repetitive stimuli were used) and found complete blockade of recruiting and severe depression of augmenting responses at concentrations of 250-300 mg%. Blockade of these responses would not be expected on the basis of the results of the present study although examination of suprathreshold recruiting and augmenting might show that their appearance was delayed from the onset of the stimulus train in this concentration range. If only paired stimuli were used under these circumstances, responses would not be observed, leading to the conclusion that ethanol "blocked" these responses.

Kogi, et al (1960) examined thresholds for cortical activation to reticular stimulation and for recruiting but only within a range of 250-300 mg%. They found a 48% increase in recruiting thresholds, a result contrary to that found by Story, et al (1961), and an 83% increase in cortical desynchrony threshold. These changes further support the conclusion made in this study, that in the final phase of action ethanol exerts a non-specific depressive effect over the entire CNS.

When recruiting thresholds are examined under low doses of barbiturates a quite different pattern of action is found. King (1956)

found decreased recruiting thresholds and enhanced suprathreshold recruiting while cortical desynchrony thresholds were decreased. The doses used, 3-5 mg/kg, are probably roughly equivalent to the lower ethanol doses used here. At higher barbiturate doses recruiting thresholds decreased further while desynchrony responses were completely abolished. Alpha-chloralose, tested under the same conditions by King (1956), causes enhancement of recruiting at low doses and depression at higher doses; it does not alter thresholds for cortical desynchrony at low doses as ethanol did in this investigation.

Although other CNS-active drugs, including chlorapromazine, ether and mephenesin, have been tested (King, 1956) under similar conditions, not enough data are available to compile a dose-response relationship. Only urethan shows some similarity to ethanol (Takaori, et al, 1966; Gogolak, et al, 1969) and it should be important to test urethan under conditions similar to those used in this study.

Comparison of CNS evoked potentials with the present results shows only a few similarities. The results of Nakai (Takaori, et al, 1966; Nakai and Domino, 1969) demonstrate a monotonic decreasing evoked potential amplitude with increasing ethanol concentration. Biphasic changes were not observed. However, neither of these studies used a dose of 100 mg/kg comparable to the lowest dose used in this investigation. The latter study (Nakai and Domino, 1969) demonstrates relative resistance of the reticular formation to ethanol, compared to the cerebral cortex, a result in agreement with the present results.

Differences in results produced by the CNS evoked potential paradigm and response threshold data cannot be accounted for by different

preparations. The acute, paralyzed cat is common to both investigations. Hence, differences must be a function of the response measures themselves. It may be concluded that the standard evoked potential paradigm does not give access to the same information as threshold data. Evoked potentials are an entirely artificial production in the CNS, the result of single, massive stimuli having no significant duration. Therefore, any sensitive modulatory influences, which may alter cortical reception of afferent information, are not operative. Under most circumstances the evoked potential waveform is a measure of relay capabilities over the most favorable pathway to the cortex.

Potential CNS modulatory mechanisms may be invoked by repetitive stimulation. The neural connections and synaptic activities responsible for recruiting are mainly of a recurrent inhibitory nature (Andersen and Andersson, 1968). The response results when stimulation frequency approaches the natural frequency of the intrathalamic and thalamocortical circuitry. High frequency stimulation of the midbrain reticular formation also produces a modulation of afferent information--although biased towards excitation--which is not stimulus-bound or necessarily apparent until several hundred milleseconds after stimulus onset. It may be concluded that if systems are activated so that the resultant operations are a reasonable facsimile of their natural operations (e.g., modulation) than ethanol effects will be more apparent and more understandable.

The results suggest that the augmenting response may represent some middle ground between strict information transfer and modulation. Evoked potential studies (Takaori, et al, 1966; Nakai and Domino, 1969)

show that the first apparent decrease in amplitude occurs in the 200-400 mg/kg range. Augmenting thresholds are first significantly elevated above control at 200 mg/kg although there is no great change in thresholds at higher doses.

Nevertheless, both thresholds and evoked potentials were measured under relatively abnormal conditions--the acute, paralyzed cat. However, the acute preparation allows the non-traumatic infusion of large volumes of drugs as well as accurate measurement of the drug's blood concentration. Additionally, with full control over blood gases, most deviations from "normal" physiological tolerances resulting from drug side-effects can be adequately controlled and separated from pure CNS drug effects.

One aspect of ethanol action not accounted for in the acute experiment is the possible effects of adaptation to ethanol. Adaptation, though primarily a phenomenon of chronic alcoholism, may also be present on a fairly short term basis. It would be observed here as an increase in the dosage required to achieve effects found at lower doses after continued or repeated presence of ethanol in the system. If the animal is rapidly adapting to the presence of ethanol--if the response observed is "recovering" to some extent--the essential pattern of results would probably not be altered. Adaptation would require that CNS responsivity will be changed less with succeeding ethanol doses or that responsivity would partially recover if a constant concentration was maintained. If adaptation were occurring in these experiments observed changes in response thresholds would actually be found at a lower concentration. If infusion brought the animal directly to a given dose level the only

effect of adaptation on the present results would be to constrict the observed threshold changes over a more narrow dosage range.

## Metabolism of Ethanol

The results summarized in Figure 6 and Table 2 indicate that ethanol metabolism, as measured by the rate of clearance from the blood, is related to blood concentration by at least a first-order equation. Although these results are derived from only two measurements of concentration at each dose, the clearance rates at each of these doses are clearly different and are directly proportional to concentration. This relationship was verified by statistical correlation of the initial blood sample concentration with the calculated clearance rate for dose within every experiment for which complete data were available. The results of these calculations demonstrated significant correlations at 3 doses (100, 200 and 800 mg/kg). There is every reason to believe, therefore, that more precise, repeated examination of clearance rates in experiments designed for that purpose will give the same result.

Although Eggleton (1940b) found blood clearance rates in cats to be proportional to blood concentration, studies conducted on humans have found only a zero-order (rate independent of concentration) relationships (Jacobson, 1952; Lundquist and Wolthers, 1958; Sidell and Pless, 1971). If this is a species difference it suggests the presence of a more efficient alcohol dehydrogenase system in lower mammals. Clearance rates in the present study (ranging from 23 to 172 mg%/hr) are substantially faster than those found in humans (Lundquist and Wolthers, 1958, 21 mg%/hr; Edwards and Evans, 1967, 18.2 mg%/hr; Sidell and Pless, 1971, 18.9 mg%/hr).

Although methods of ethanol administration and measurement varied over these studies, one typical investigation (Sidell and Pless, 1971) found peak blood concentrations similar to those reported here. Doses of 400, 800 and 1600 mg/kg administered orally resulted in peak blood levels of 45, 70 and 200 mg%, respectively, compared to first measurement post-infusion mean concentrations of 49, 98 and 214 mg% in this study.

## Relationships of Threshold Changes to Behavioral Alterations

### Low Dose Phase

The primary effects of ethanol in the low dose phase appear to be a decrease in the efficiency of inhibitory control over the cerebral cortex. The source of that inhibition is probably the non-specific thalamus-frontal cortex system which provides modulation of channels afferent to the cortex as well as an upper limit for cortical activation. Partial loss of this inhibition will result in increased relative excitation of the cortex and wherever in the CNS this inhibitory system exerts some control.

The behavioral alterations associated with the low dose phase of ethanol will be a function of these changes in inhibitory control. Threshold changes observed in this phase suggest behavioral symptoms reminiscent of the "jag state" associated with sub-anesthetic doses of barbiturates and volatile anesthetics, a state marked by talkativeness, hyperexcitability, and inappropriate responses.

They further suggest that certain changes in psychophysical operations and information processing will be observed. Due to the

depression of modulatory mechanisms, there will be a reduction in the perceived contrast between spatially and temporally separated stimuli. This effect may be particularly evident in the perception of relatively weak stimuli and may explain why extremely low ethanol concentrations cause a significant decrease in the optokinetic drum velocity necessary for fusion (Blomberg and Wassen, 1962). At the same time, relatively strong stimuli may be enhanced since modulatory control is reduced. Thus, decreased reaction times will be observed for stimuli capable of eliciting an "orienting response" and those interpreted as "imperative" by the individual.

These alterations may provide an explanation for known effects on information processing. Processing of "important" information is shown to be increased at low doses but simultaneous perception of peripheral, and thus weak, stimuli is decreased (Hamilton and Copeman, 1970). Further, processing deteriorates in the presence of strong, irrelevant stimuli when presented in addition to an imperative central task (Forney, et al, 1961, 1964; Hughes and Forney, 1963).

The low phase of ethanol action will also be marked by increased autonomic lability. This feature is primarily suggested by observations in several experiments in which excessive variability in autonomic records accompanied by prolonged cortical activation prevented threshold measurement.

The net result of low dose phase action may be a narrowing of the perceptual field and a possible enhancement of stimuli at the center of attention accompanied by increased autonomic lability. This suggests that one of the rewards of intoxication is a state of dissociation

described by sharpened awareness of certain important features of the environment and an induced, decreased perception of weak and irrelevant stimuli.

### Intermediate Dose Phase

Electrophysiologically, the intermediate phase is marked by the apparent reestablishment of balance between CNS excitation and inhibition. This balance does not, however, include the cardiovascular system. Threshold changes associated with the intermediate phase suggest the possibility of a unique behavioral state in which thresholds for ascending activation are comparable to those in the non-intoxicated state but wherein there is increased threshold for autonomic activation. This dissociation between central, assumedly perceived, events and autonomic reactivity to them may provide the necessary physiological ingredients of "tranquilization."

In this phase, autonomic-central dissociation will delay adaptive somatic activation when it is required to meet changing demands of the environment. Depression of somatic activation will be observed as a general loss or slowing of the adaptive preparation, which includes increased blood flow to muscle, endocrine activation and a general increase in somatic tone, ordinarily occurring with the threat of acute stress. This will be especially true if the "balance state" of the intermediate phase is in fact an illusory balance at a decreased state of intrinsic activation. In this event, not only is the individual slowed in his somatic reaction to stress in emergency situations but he must begin that activation from an already depressed state.

Thus, loss of emotional reactivity (Kalant, 1970; Forney and Hughes, 1963), though perceived as a beneficial aspect of mild intoxication by the drinker, might rather suggest a dissociation between him and his environment that is both rewarding and dangerous.

## High Dose Phase

Above the 40-60 mg% concentration range produced by a 400 mg/kg dose, all thresholds rise in a monotonic fashion. It may be expected that there will be a parallel depression of not only modulatory mechanisms, whose failure in this phase will result in a flat perceptual field, but also of information transmission from the periphery. These changes in function will be accompanied by a deepening behavioral depression. Nevertheless, this investigation shows that all response thresholds were well within a measurable range at the highest blood concentrations produced within the protocol, suggesting that, if stimulus intensity is sufficiently great, the individual will still be able to react to his envirnoment.

This is probably unfortunate for him and his dependents. Although able to react to stress, it may be expected that his responses will be made through clouded perceptions, with great latencies, and with no guarantee of appropriateness. These results strongly emphasize that ethanol produces purely depressant effects at relatively low blood concentrations. The most stringent drunk-driving law considers 100 mg% prima facie evidence; even this concentration may be too high.

## The Motives for Intoxication

Being fully knowledgeable of the ultimate effects of continued ingestion of ethanol, individuals nevertheless drink and usually to excess. Several sources of motivation for the phenomenon of self-induced intoxication are apparent from this and other investigations. Ethanol induces a state of dissociation that is at least partially under control of the drinker. With a surprisingly small amount the drinker may voluntarily suppress many of the annoying aspects of his environment as well as enhance certain favorable ones. The induced state of euphoria (Ekman, et al, 1964) resulting in the low dose phase can probably be defined physiologically as a state of reduced inhibitory efficiency. If this state is perceived as such by the drinker it may be possible for reduced physiological inhibition to circularly reinforce reduced social inhibition.

In addition to low dose euphoria, the intermediate dose phase of physiological "balance" and behavioral tranquilization certainly has motivating properties. At higher concentrations tension reduction may be summed with the reduction of pain, whether real or imagined, and thus provide the most basic source of motivation.

Unfortunately, the more pleasant aspects of intoxication occur within a very compressed range of blood concentration. Sobriety is quickly replaced by euphoria, then tranquilization, and finally depression over a range produced by no more than less than 0.5 to 1.5 ounces of ethanol. One can only conclude that it takes a sober man to know when he has had too much to drink.

# CHAPTER VI

## SUMMARY

Electrophysiological and behavioral investigations have demonstrated the existence of a transient period of CNS excitation at ethanol blood concentrations ranging between 10 and 50 mg% (0.01-0.05%). These investigations have for the most part concluded that this low dose effect is a function of the differential susceptibility to ethanol of various parts of the CNS. However, a more parsimonious explanation is that ethanol, in low doses, primarily depresses those CNS mechanisms responsible for the modulation of excitation and inhibition. Extensive review of the effects of ethanol on CNS electrophysiology shows that ethanol is, in fact, depressive over all of its non-lethal concentration range. Nevertheless, behavioral studies show improvement on various tests of information processing and cognitive performance. This is true, however, only so long as the operations required are relatively simple and remain uncomplicated by extraneous, distracting stimuli presented in addition to the central task. These behavioral effects might be explained by ethanol's depression of CNS modulatory mechanisms which exert control over afferent information.

These factors suggested that direct examination of the effect of ethanol on CNS systems known to mediate certain aspects of modulatory

control would provide valuable insight into the changes in CNS function and behavior known to define the state of ethanol intoxication in man. Additionally, it was assumed that systematic examination of the excitation threshold required for eliciting the operation of these systems would provide a sensitive index of changes in the balance of CNS excitation and inhibition.

On the basis of these assumptions a series of pilot experiments was initiated to define threshold criteria for each of five CNS responses elicited by central stimulation. These responses were the recruiting and augmenting responses of the non-specific and specific thalamus, respectively, and the cortical desynchrony, cardiovascular pressor and skin resistance responses of the reticular activating system. These responses were selected because they were stereotypical to appropriate stimulation of the CNS, they were characteristic responses of the modulatory systems to be investigated, and they were well-defined in the literature.

The investigation consisted of determining the threshold current intensity required for the elicitation of each of the 5 responses under non-drug and 5 ethanol dose conditions. The responses were required to meet certain precise criteria for the assignment of threshold current intensity, and they had to be elicited during the presence of a "drowsy" pattern of spontaneous electrical activity; a pattern for which additional criteria were developed.

Subjects in the study were acute, paralyzed cats. Under brief general anesthesia (ether), endotracheal, femoral artery and femoral vein catheters were inserted and extensive local anesthesia secured.

To prevent the usual increasing physiological depression resulting from prolonged general anesthesia, the animals were allowed to partially recover before paralysis and artificial respiration was initiated; surgical procedures were then continued. After placement in a stereotaxic apparatus, the skull was exposed and epidural screw and bipolar subcortical electrodes were inserted. Monopolar recordings were made from anterior cruciate gyrus, bilaterally, and medial suprasylvian gyrus. Depth electrodes were placed in n. reuniens or n. centre median for stimulation (8 Hz, 0.5 msec) of the non-specific thalamic system, in n. ventrolateralis to elicit (8 Hz, 0.5 msec) augmenting responses and in the mesencephalic reticular formation to elicit (200 Hz, 0.05 msec) reticular activation. An additional recording electrode was placed in the intermediate hippocampus.

Throughout the surgical and experimental procedures Lead II electrocardiograph, systemic blood pressure, skin resistance and core temperature were continuously monitored. Additionally, periodic blood samples were taken and analyzed for blood gases (pH, $pO_2$, $pCO_2$) and hematocrit. During the three-hour ether blow-off period, respirator parameters were adjusted until the results of blood gas measurements were within normal values for the cat. Spontaneous electrical activity was recorded via a Grass Model VI polygraph and placed on FM tape; blood pressure and skin resistance (derived from the forepaw contralateral to reticular stimulation) were recorded on a Grass Model IV polygraph.

Ethanol infusion was made by calibrated pump (4.52 ml/min) and precisely timed to give weight calibrated doses. The 5 ethanol conditions

consisted of succeeding doses of ethanol in a 7% (w/v) solution of lactated Ringer's which produced total dose levels of 100, 200, 400, 800 and 1600 mg/kg. Ten minutes after the end of each infusion and after the completion of threshold measurements of each dose, arterial blood samples were taken for blood gas analysis and gas chromatographic measurement of serum ethanol concentrations.

The experimental protocol consisted of the measurement of the stimulus current intensity required for threshold responses at each of the 5 ethanol dose levels. Two threshold determinations were made under each condition, the resultant thresholds averaged, and the mean converted to percent of control threshold. The data consisted of a dose-response threshold profile for each response over 5 ethanol conditions. Non-parametric statistical analyses were performed on the resulting data to test for deviation of drug condition thresholds from those obtained under non-drug conditions, for differences in response thresholds between doses for each response and differences between response thresholds at each dose.

The results of these analyses demonstrated the existence of three phases of ethanol action on the CNS. At low doses, ethanol increased recruiting response thresholds (mean, 114% of control) and sharply decreased thresholds for the cortical desynchrony response (78%). Pressor and skin resistance response thresholds remained constant at the lowest dose but augmenting thresholds were immediately increased (mean, 118%) at the lowest dose and they remained elevated at a relatively constant value through all higher doses. At intermediate doses, recruiting

and cortical desynchrony thresholds returned to control levels (105 and 100%, respectively) while pressor thresholds increased in proportion to dose (113% at 200 mg/kg and 121% at 400 mg/kg). At high doses all thresholds were increased above control levels (except skin resistance which was not different than control at any dose). These results were interpreted to suggest three phases of ethanol action of which the first phase consists of a decrease in the modulating inhibition derived from the non-specific thalamus. This decrease results in a shift in the CNS excitation-inhibition balance towards a state of increased excitation and increased cortical excitability. The second phase of action consists of a recovery of CNS balance but at a "set point" associated with a lower than normal level of intrinsic excitation. This state of regained balance is accompanied by a reduction in autonomic responsivity. In the third phase, ethanol exerts a non-specific depressive effect on all CNS functions which would continue with increasing concentrations until unconsciousness and death ensued.

These data suggested that the primary effect of ethanol at low doses is to depress CNS modulatory functions. Such an effect explains many of the changes in CNS information processing known to define the early stages of ethanol intoxication. Additionally, the intermediate phase suggests that part of the definition of tranquilization induced by ethanol at these doses is a dissociation between depressed, but operationally adequate, central functions and autonomic responsivity. This dissociation might lead to a situation in which the individual is intellectually able to cope with his environment according to his own introspection, but autonomically unprepared for the demands of acute stress.

Results of the measurement of blood ethanol concentrations produced by the infused doses demonstrated a significant first-order relation between blood levels of ethanol and its rate of metabolism. Clearance rates were considerably faster than those observed in humans, and rose from a mean rate of 23 mg%/hr at the lowest dose to 174 mg%/hr at the highest dose. These results suggest that the cat possesses an ethanol dehydrogenase system much more extensive than that which is found in humans. Mean blood ethanol concentrations were 13, 20, 45, 91 and 190 mg%, respectively. Comparison of these data with observed changes in response thresholds shows that the final phase of non-specific depression may be attained with only minimal amounts of ethanol.

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## APPENDIX I

## THRESHOLD CRITERIA

Against a spontaneous background electrocorticogram consisting of low-voltage fast mixed with slow-wave activity with occasional spindling, the following characteristics are required for each response:

I. Recruiting response--In any or all cortical leads there must be greater than a two-second period in the ten seconds of stimulation that the electrocorticogram exhibits evoked activity following the frequency of the stimulus.

II. Augmenting response--In the left anterior cruciate lead (ipsilateral to the stimulus) there must be a greater than two-second period in the ten seconds of stimulation that the electrocorticogram exhibits evoked activity following the frequency of the stimulus.

III. Reticular activation--

(a) Cortical activation (cortical desynchrony response) consists of (1) at least a 50% reduction in mean amplitude, (2) loss of all waves less than 8/sec with no spindles or (3) the duration of the stimulation in at least one cortical channel.

(b) Cardiovascular activation (pressor response) consists of an increase of at least 10 mm Hg in systolic pressure which must begin within the period of stimulation.

(c) Skin resistance response consists of a phasic decrease in conductance of at least 50 ohms within the duration of the stimulus.