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ETHANOL EFFECTS ON THE OLIVOCEREBELLAR SYSTEM

By

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ABSTRACT

The detrimental effect of ethanol on coordinated movement and the similarity of this effect to that of pathological damage to the cerebellum or the inferior olive (IO) suggests that ethanol profoundly effects the olivocerebellar system. Each cerebellar Purkinje cell (PC) displays two distinct action potential responses: the simple spike (SS), evoked intrinsically and via the parallel fibres, and the complex spike (CS), evoked via a solitary climbing fibre (CF) which originates from the IO.

Ethanol (1.5 g/kg, i.v.) was found to have no significant effect on the CS activity evoked by direct electrical activation of CFs, while it significantly reduced, in parallel, that evoked by cerebral cortex stimulation and that occurring spontaneously. In addition, ethanol, applied both systemically and locally by micropressure, reduced the spontaneous activity of inferior olive (IO) neurones. These results strongly suggest an action of ethanol local to the IO nucleus, perhaps by a direct action on the IO neurones themselves.

Elimination of spontaneous CS activity by the electrolytic lesioning of the contralateral IO did not significantly alter spontaneous SS firing rate. However, subsequent administration of ethanol i.v. did produce a significant increase of SS rate, indicating that only part of ethanol-evoked SS rate increase is dependant upon the reduction of CS activity. Simple spike regularity was not significantly altered by either IO lesioning or i.v. ethanol.

Ethanol applied locally to PC's by micropressure was found to reduce the period of inhibition evoked by local stimulation of the cerebellar cortex in the majority of the cells tested. This result is in agreement with the previously reported effects of intravenous ethanol on this inhibition, thought to be mediated by gamma-aminobutyric acid (GABA). Both intravenously and locally applied ethanol antagonized the inhibition of PC's evoked by locally applied GABA. These results, while in agreement, are contrary to the reported effects of ethanol in other systems.

Intravenously administered ethanol did not significantly alter Golgi cell spontaneous firing, while it did significantly reduce the period of inhibition of Golgi cell firing evoked by IO stimulation.

John G. Sinclair, Ph.D.

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LIST OF ABBREVIATIONS

3-AP	- 3-acetylpyridine
AP	- anterioposterior
CF	- climbing fibre
CS	- complex spike
DAO	- dorsal accessory olive
DV	- dorsoventral
GABA	- gamma-aminobutyric acid
GABA-T	- GABA transamidase
IO	- inferior olive
i.p.	- intraperitoneal
ISI	- interspike interval histogram
i.v.	- intravenous
LC	- locus coeruleus
LH	- latency histogram
MAO	- medial accessory olive
NA	- noradrenaline
mepps	- miniature endplate potentials
PAG	- periaqueductal grey
PC	- Purkinje cell
PF	- parallel fibre
PO	- principal nucleus of inferior olive
PSTH	- poststimulus time histogram
SEM	- standard error of the mean
SS	- simple spike
TTX	- tetrodotoxin

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I. INTRODUCTION

Acute alcohol intoxication results in disturbances in the modulatory control of proprioceptor and motor pathways; including symptoms of incoordination and nystagmus. These symptoms are similar to those observed after damage to the cerebellum or to the inferior olive (Dow and Morazzi, 1958; Murphy and O'Leary, 1971). In addition, chronic intoxication and fetal alcohol syndrome (Chernoff, 1977; Volk, 1977) have been reported to result in damage to the cerebellum. Although these studies do not preclude mediation of alcohol-induced ataxia by other mechanisms, they do suggest that the olivocerebellar system is involved.

Each cerebellar Purkinje cell displays two distinct action potentials. The first of these is the simple spike (SS) and is evoked both intrinsically and by parallel fibre activity. The second potential is called the complex spike (CS), and is evoked by activity on the single climbing fibre (CF) projecting to the PC from the inferior olive (IO).

Previous studies have reported that ethanol increased cerebellar Purkinje cell simple spike firing and regularity (Colin et al., 1980; Sinclair et al., 1980; Sinclair and Lo, 1981). Its effect on Purkinje cell complex spike activity is controversial: slow intravenous infusion reduced CS activity (Sinclair et al., 1980, 1982; Sinclair and Lo, 1981), while intraperitoneally injected ethanol increased CS-like bursting (Rogers et al., 1980).

The present study was directed at examining the sites of action of ethanol in the cerebellum and the associated inferior olivary nuclei. Three sets of experiments were conducted to this end. The first and second of these involved the activation of CS activity by stimulation of the cerebellar white matter and the cerebral cortex, respectively. The effect of intravenously administered ethanol on these activations was then studied. The last set of experiments were directed at examining the effect of intravenously and locally applied ethanol on the spontaneous firing of IO neurones by recording directly from IO neurones.

The action of ethanol on SS activity is not clear because of the apparent interaction between CS and SS activity outlined above. Therefore, this study examined the effect of ethanol on SS rate and regularity in the absence of CS activity. To this end, complex spike activity was eliminated in these experiments by the electrolytic lesioning of the IO.

In addition, since ethanol has been shown to modify gamma-aminobutyric acid (GABA) transmission at other sites in the central nervous system (Nestoros, 1979, 1980; Newlin et al., 1981), and because GABA is a ubiquitous neurotransmitter in the cerebellum, other sets of experiments were performed to study the effect of ethanol on this transmitter's actions in the cerebellum. These included experiments designed to study of the effect of ethanol on the inhibition of Purkinje cells evoked by local surface stimulation, which is thought to be mediated by GABA (Woodward et al., 1971), as well as inhibition evoked by exogenous GABA.

Finally, since Golgi cells are an integral part of the cerebellar circuitry and have been reported to be inhibited by IO stimulation, the effect of ethanol upon their spontaneous firing and upon IO stimulation-evoked inhibition was also studied.

II. BACKGROUND

II.A. Anatomy, Pharmacology, and Physiology

II.A.1. General Overview

The cerebellum is a relatively large part of the brain which is generally responsible for coordinating and modulating individual muscle movements into a smooth and integrated whole. It has been anatomically divided into three cellular layers: the molecular layer; the Purkinje cell layer; and the granular layer (cf. review by Eccles et al., 1967). The molecular layer contains mainly axonal fibres (the parallel fibres), dendrites of Purkinje, Golgi, basket, and stellate cells, and the sparsely spaced cell bodies of basket and stellate neurones. The thin Purkinje cell layer contains mostly Purkinje cell bodies and the vertical axons and dendrites of Golgi cells, whereas the granular layer is densely packed with granule and Golgi cell bodies.

There are two major inputs to the cerebellum, the mossy fibres and the climbing fibres (cf. Gould, 1980). The mossy fibres originate from a large variety of sources, such as the vestibular and reticular systems. In addition, the mossy input includes fibres travelling with the spinocerebellar tracts which carry joint-muscle location and movement information directly to the cerebellum. The mossy fibres end in clusters of excitatory synapses, called rosettes, with granule cell dendrites. The climbing fibres, on the other hand, prob-

ably originate solely from neurones in the inferior olive (Eccles et al., 1966) and each projects to several Purkinje cells and other cerebellar cortical cells via collaterals. Each Purkinje cell is thought to receive exactly one climbing fibre on its dendritic tree, and to send collaterals to Golgi and basket cells, other folia, the cerebellar nuclei and Deiters' nucleus (Scheibel and Scheibel, 1954).

A single mossy fibre may branch into several folia and eventually end at several hundred rosettes. There is evidence to suggest that the transmitter at these synapses is acetylcholine (Isreal and Whittaker, 1965; Phillis, 1968). Output from the granule cells is distributed via their axons which ascend to the molecular layer, bifurcate and run parallel to the folium in either direction. These are referred to as the parallel fibres and they make extensive excitatory contacts with the perpendicularly and closely packed dendritic arborizations of Purkinje, Golgi, stellate, and basket cells. Basket cells make inhibitory axosomatic synaptic contacts on Purkinje cells, while stellate cells make inhibitory axosomatic and axodendritic connections on Purkinje and Golgi cells. Golgi cells in turn make inhibitory connections with the granule cells at the rosettes. The transmitter at these all inhibitory synapses is believed to be gamma-aminobutyric acid (GABA, Bisti et al., 1971). However, the putative transmitter from stellate cells may be taurine (Okamoto et al., 1983b).

The only output from the cerebellar cortex is via the Purkinje cell axons, which innervate the central cerebellar and other brainstem nuclei.

Extensive anatomical studies have been made on the interconnections of the olivocerebellar system (see Brodal and Kawamura, 1980; Courville et al., 1980). Briefly, the caudal third of the inferior olive projects to the cerebellar cortex of the contralateral vermis, while the rostral two-thirds supplies afferents to the para-vermal and lateral regions of the contralateral hemisphere. In turn, the Purkinje cells of the vermis terminate in the contralateral fastigial and Dentate nuclei. Similarly, the para-vermal region projects to the interpositus nuclei, and the lateral regions project to the dentate nuclei.

II.A.2. The Purkinje Cell

II.A.2.a. Anatomy

Purkinje cells are the largest cells of the cerebellar cortex and their axons provide its only efferent pathway (Eccles et al., 1967; Scheibel, 1981). Each Purkinje cell has an extensive dendritic arborization extending into the granular layer. This tree is flattened perpendicularly to the axis of the parallel fibres (PF).

In terms of function, it has been proposed that the cerebellar cortex is divided into narrow parasagittal strips (Armstrong, 1974; Voogd and Bigare, 1980; and others). The pattern of cerebellar folding is such that the folia are at right angles to the direction of these strips. This functional subdivision was reflected in the distribution of olivocerebellar projection found in electrophysiological studies by Armstrong (1974). Each strip is supplied by a localized group of climbing fibres, each of which supplies collaterals to widely separated sections of the strip. In addition, the output of each strip is topologically distributed to localized neurones of the deep cerebellar nuclei. Extensive anatomical tracing studies of the olivocerebellar system (see Brodal and Kawamura, 1980; Courville et al., 1980) suggest that it is divided into twelve semi-independant functional modules, i.e. six symmetrically opposed modules on each side (Voogd and Bigare, 1980). Each module consists of: a subdivision of the inferior olive (IO); a parasagittal strip of the cortex; a central nucleus; and their corresponding interconnections. The modules and

their associated parasagittal strips have been designated A, B, C1, C2, C3, and D. Strips A and B receive fibres from the caudal medial accessory olive (MAO) and dorsal accessory olive (DAO) and project to the fastigial and Dieter's nuclei, respectively. Strips C1 and C3 both receive inputs from the rostral DAO and project to the anterior interpositus nucleus, while strip C2, which is interposed between strips C1 and C3, receives its inputs from the rostral MAO and projects to the posterior interpositus nucleus. Finally, strip D receives fibres from the principal nucleus of the olive (PO) and projects to the dentate nucleus.

The parallel fibres are the first of the two major excitatory afferent systems to Purkinje cells. These fibres make extensive "en passage" synaptic connections to the Purkinje cell at dendritic spines (Hamori and Szentogathai, 1966). The estimated number of connections exceeds 200,000 per PC (Eccles et al., 1966a, 1967; Fox and Bernard, 1967; Palkovits et al., 1972). The transmitter at these synapses is believed to be glutamate. Purkinje cell dendrites can be directly activated by glutamate (Chujo et al., 1975). Also, the molecular and granule cell layers contain high concentrations of glutamate (Nadi et al., 1977) which is reduced in granule cell deficient cerebella, such as those from genetically deficient (Roffler-Tarlov et al., 1978), virally infected (Young et al., 1974), or x-irradiated animals (Rohde et al., 1979).

The second major excitatory input to Purkinje cells is the climbing fibre. Immature Purkinje cells receive a transient innervation from more than one climbing fibre (Kawa-

guchi, 1975; Barragan and Delhaye-Bouchaud, 1980), but this innervation is reduced to a single climbing fibre in the adult (Eccles et al., 1966a; Puro and Woodward, 1977). The presence of climbing fibres and their continued functioning has been suggested to be essential to the embryological development and the continued maintenance of the Purkinje cell dendritic tree and its associated dendritic spines (Kornguth and Scott, 1972; Kawaguchi et al., 1975; Woodward et al., 1977), and the functioning of the Purkinje cell itself (Ito et al., 1978, 1979; Ito, 1981). These latter workers reported that the destruction of the IO with 3-acetylpyridine (3-AP) caused a rapid failure of inhibitory transmission at the Purkinje cell-lateral vestibulospinal tract cell synapse. They suggested that this may have be due to the interruption of axonal flow and the consequent loss of a neurohumour required for the continued functioning of the PC. However, this experiment was repeated by another group (Montarolo et al., 1981). However, the results of Ito's group were not confirmed and therefore more studies are required to clarify the situation. In addition, another study (Sotello et al., 1975) reported that Purkinje cell dendritic spines were not reduced after IO destruction with 3-AP, but rather increased in number. This suggests that, while spine development may require climbing fibre innervation, spine maintenance does not appear to be dependent upon it.

Each climbing fibre makes an intimate and robust connection with at least one Purkinje cell in a topological arrangement, as was mentioned above. This connection is in the form of a great many "en passage" synapses over the PC's entire

dendritic tree (Eccles et al., 1967). The putative transmitter at this synapse has been suggested to be aspartate. Electrophysiological experiments support this idea since, for example, microiontophoretic application of L-aspartate produced a dose-dependent increase in the spontaneous firing rate of all Purkinje cells tested (Krnjevic, 1982). Autoradiographical support includes the report that injection of tritiated D-aspartate into the rat cerebellar cortex or deep nuclei resulted in the labelling of IO cell bodies (Wiklund et al., 1982). These workers also reported that a calcium-dependent in vitro release of endogenous L-aspartate, caused by increased potassium concentration, was reduced significantly after destruction of the IO by 3-AP administration, while the potassium-induced release of endogenous L-glutamate, gamma-aminobutyric acid (GABA), and glycine was unchanged.

The major inhibitory input of the Purkinje cell is from the cerebellar interneurons: the basket and stellate cells. Basket cells make inhibitory connections on or near the Purkinje cell somata (Eccles et al., 1967). Autoradiographical and electrophysiological evidence suggests an involvement of gamma-aminobutyric acid (GABA) as a neurotransmitter at this synapse (for more detail see Section II.B).

In contrast, the outer stellate cells make inhibitory connections onto Purkinje cells dendrites (Eccles et al., 1966b, 1967). The transmitter at these synapses also may be GABA, but there is some evidence to suggest it might be taurine. For example, selective x-ray destruction of stellate cells reduced the cerebellar cortical content of taurine

(McBride et al., 1976). Also, Purkinje cells are reported to be inhibited by the local microiontophoretic application of taurine (Frederickson et al., 1978; Okamoto et al., 1983a,b,c).

In addition to the inputs discussed above, Purkinje cells also receive extrinsic input from other sources. A noradrenergic input has had the strongest experimental support. Anatomical studies employing biochemical, fluorescent, ultra-structural, and immunochemical methods had suggested noradrenaline to be a neurotransmitter in the cerebellum (Hokfelt and Fuxe, 1969; Alexander et al., 1975; Melamed et al., 1976; Amenta et al., 1981). That these studies may have reflected a noradrenergic innervation of Purkinje cells has been supported recently by Scheibel's description of noradrenergic-like fibres in apposition to Purkinje cells in her electronmicrographic study of the cerebellar cortex (Scheibel, 1981). Studies involving application of phencyclidine, 6-hydroxydopamine, and X-ray irradiation of neonates also support a noradrenergic innervation onto the Purkinje cell (Marwaha et al., 1980). Finally, electrophysiological studies have indicated a source for at least part of this innervation. A noradrenergic pathway from the locus coeruleus to the Purkinje cell has been well characterized by anatomical, physiological, and pharmacological studies (Hoffer et al., 1973). Activity in this afferent system slows spontaneous Purkinje cell activity (Hoffer et al., 1971), while preserving activity evoked by mossy and climbing fibres (Freedman et al., 1977) and microiontophoretic application of putative neurotransmitter amino acids (Moises et al., 1979).

The cerebellar cortex also is reported to receive an inhibitory innervation of serotonergic fibres from the raphe nuclei (Strahlendorf et al., 1979) and biochemical studies have demonstrated the presence of histamine receptors in the cerebellum (Palacios et al., 1981). The response of Purkinje cells to the microiontophoretic and micropressure application of histamine agonists and antagonists suggest these receptors may reflect a histaminergic innervation of Purkinje cells (Marwaha et al., 1981).

The only output from the cerebellar cortex is through the axons of the Purkinje cells, which make inhibitory synaptic connections on the cerebellar nuclei and on brainstem sites such as Deiters' nucleus (cf. Jansen and Brodal, 1940). The transmitter of Purkinje cell axons onto Deiters' neurones has been well established to be GABA (Obata et al., 1967; Otsuka et al., 1971). Purkinje cell activity presumably modulates information from higher centres relayed by these nuclei to spinal cord sites. In addition, Purkinje cell axonal collaterals are reported to make inhibitory connections on neighbouring Purkinje cells, but the exact pattern of distribution may vary over different areas of the cerebellum (Bishop, 1982). Purkinje cell collaterals also innervate granule cells somata, presumably allowing Purkinje cells to modulate information entering the cerebellum via the mossy fibres in a manner similar to Golgi cells.

Purkinje cells are characterized by their generation of two distinctly different spikes in response to parallel and climbing fibre inputs. The first, called the simple spike

(SS), is evoked by intrinsic activity or by the excitatory parallel fibre input. It has a shape and time course similar to that of the action potentials of most other neurones. The spontaneous simple spike rate of a Purkinje cell is usually 30-50 Hz and is quite irregular reflecting the variability of the excitatory and inhibitory inputs to the cell.

The second spike response of the Purkinje cell is called the complex spike (CS). It is evoked by the powerful input from the climbing fibre. Purkinje cells have a spontaneous CS rate of about 1 Hz. Each CS is made up of a series of spikes: an initial large spike, with a normal shape and time course, followed by several smaller spikes or wavelets. Each CS is followed by an period of inhibition, which has been named the inactivation response (Granit and Phillips, 1956; Bloedel and Roberts, 1971).

Simple spike activity has been reported to be inversely related to CS activity (Ferin et al., 1971; Colin et al., 1980). For example, CS rates greater than about 8 Hz, evoked by stimulation or by the administration of harmaline, are associated with a complete cessation of SS activity (Colin et al., 1980; Rawson and Tilokschulchai, 1981; Montarolo et al., 1982). Colin et al. (1980) also demonstrated that cessation of climbing fibre activity, caused by the destruction of the inferior olive with 3-acetylpyridine (3-AP), was associated with an increased Purkinje cell SS rate and regularity. This study was recently confirmed by reversably halting CS activity by cooling the IO (Montarolo et al., 1982) and by local injection of lidocaine (Leonard and Simpson, 1982). Indeed,

this relationship has been reported by this laboratory: the reduction of CS activity caused by intravenous administration of ethanol was also associated with an increased SS activity and regularity (Sinclair et al., 1980; Sinclair and Lo, 1981). The increased SS rate does not appear to be due only to there being a reduced number of CS-associated inactivation responses after ethanol administration, but rather to some other factor. An explanation for this increased rate was suggested in a recent report by Ebner and Bloedel (1981). They found that the spontaneous firing rates of neighbouring Purkinje cells were correlated. Specifically, the firing rate of one was affected by a complex spike evocation in the other. This study suggests that not only is a PC under the direct inhibitory influence of one climbing fibre, but its overall activity is modulated by the activity of others, probably through the activation of cortical inhibitory interneurons (although see below). This would suggest that if the activity of the total climbing fibre population was reduced, for example by ethanol, then SS activity would be increased to a level above that which would be caused by only the lack of inactivation responses.

Many workers have reported that evoked CS activity is followed by a period of reduced SS activity which extends beyond the period of increased CS activity (Armstrong, 1974; Colin et al., 1980; Ito et al., 1981; Rawson and Tilokskulchai, 1981, 1982). This suppression of SS activity does not appear to be due to the activation of cerebellar cortical neurones by the activated climbing fibre because IO stimulation generally has little excitatory effect upon the discharges of Golgi,

basket and stellate cells (Ito et al., 1981; Rawson and Tilokskulchai, 1981). Similarly, it does not appear to be due to recurrent PC collaterals nor to actions at the soma since it has no effect on antidromic spike responses of the PC to cerebellar white matter stimulation (Rawson and Tilokskulchai, 1981). Rather, the SS suppression may be due to the shunting of PC dendritic activity by the prolonged depolarizations of PC dendrites which were recorded after complex spikes (Ekerot and Oscarsson, 1980), or to fluctuations in the extracellular concentrations of potassium and/or calcium ions which are seen after CS evocation (Stockle and ten Bruggengate, 1980). Both these observations could reflect a modification of the underlying membrane conductances responsible for the electrogenicity of the PC and its dendrites.

In contrast to the above studies, which found decreased SS rates during evoked CS activity, McDevitt and Bloedel (1982) reported SS activity to be increased in the period following the inactivation response after spontaneous CS or complex spikes evoked by natural stimulation such as wrist flexure. They suggested the difference between their results and those of the studies reported above reflects a difference between the effect of synchronous activation of many climbing fibres, as occurs during IO or juxtafastigial stimulation, and the asynchronous activity occurring naturally. The modulatory effects that climbing fibres have on PC firing described above could also be expected to cause differences in response to evoked versus spontaneous CS activity. In addition, the inhibitory action of IO stimulation on Golgi cells (Schulman and

Bloom, 1981) may provide an explanation for the increased SS rate after a CS seen in this experiment. However, Rawson and Tiloksekulschai (1981) reported some Golgi cells (3/12) to be activated by IO stimulation. This would be consistent with a CS being followed by a decreased SS rate.

Although some workers have assigned a physiological significance to climbing fibre activity, such as a control signal for cerebellar learning (Marr, 1969; Albus, 1971), its ultimate role in cerebellar function is unclear. Complex spikes can be evoked by intense stimuli applied to whole limbs (Thatch, 1967), but this response is not reliable and shows rapid acclimatization. Similarly, Kolb and Rubia (1980) have reported that climbing fibre activity is related to complex limb position and movement parameters. However, these responses again show great variation over time and between neurones.

Purkinje cell responses to parallel fibre and related climbing fibre input, evoked by natural limb movement, were modified by the concurrent activation of climbing fibre input to the PC by electrical stimulation of the corresponding sensory cortical cortex (Mariani et al., 1982). The cortical stimulation resulted in the enhancement or reduction the responses, or caused a disruption of the established relationship between CS and SS activity.

II.A.2.b. Motor Learning

The site of motor learning is still unknown and is contraversial. Many believe that the learning and retention functions are performed by plasticity changes to the parallel

fibre-Purkinje cell dendrite synapse, and indeed, several theories have been published proposing mechanisms by which these changes may effect motor learning (Marr, 1969; Albus, 1971; Eccles, 1977). Marr (1969) proposed that a climbing fibre acts as a learning signal which results in the parallel fibre "context", of the selected PC's, being learned. More specifically, a CF activation of a PC dendritic tree is proposed to strengthen those PF-PC synapses which are active conjointly with the CF. Hence, over time, a PC would "learn" to respond to a given context. These ideas were reiterated more recently by Eccles (1977). Albus' theory was essentially identical except that it proposed that a CF input would decrease the effectiveness of the conjointly active PF-PC synapses. Marr's theory was modified by Gilbert (1974) to include the concept that Purkinje cells are arranged in units, with each unit projecting to a particular muscle and receiving similar inputs. He suggested that this would increase the capacity of the cerebellum to learn movements and also increase its robustness to damage by "spreading" a memory trace across many neurones. Obviously, the modular arrangement of the cerebellum suggested by Voogd and others appears to support this theory, at least superficially.

Although, there is some experimental support for these theories, some of which has been quoted above, they have been seriously questioned by some investigators. Some of these theories imply that once learning has taken place, the cerebellar cortex retains the learned responses in the absence of climbing fibre input. However, Llinas et al. (1975) found that

recovery from vestibular nerve lesions in rats was reversed when the IO was lesioned with 3-acetylpyridine (3AP). Further, cerebellar decortication, which spared the cerebellar nuclei and their connections to the brainstem, allowed the recovery to vestibular nerve lesioning to take place, indicating that this recovery was not dependent upon the cerebellar cortex. These findings were reiterated in a later paper (Llinas and Walton, 1979). They proposed that CF's were solely a selection mechanism, and that learning and retention took place at an extra-cerebellocortical site or sites. A mathematical model compatible with their views was developed based upon viewing the cerebellar cortex as a tensor system in which PC functioned to yield "lookahead" functions which are selected by CF and used to predict movement results (Pellionisz and Llinas, 1979).

These two opposing views of motor learning need not be incompatible since the experimental results may indicate only that plasticity occurs at many locations throughout the central nervous system. Also it should be realized that plasticity changes may occur in experimental conditions which are not physiologically important to motor learning.

A recent experiment has lent support to the learning theory of cerebellar function proposed by Marr (1969) and Gilbert (1974). Ito et al. (1981, 1982) have reported that conjunctive climbing fibre-parallel fibre activity caused a long-lasting depression of the effectiveness of the PF synapses on Purkinje cells. Simple spike activity evoked by stimulation of one vestibular nerve at 20 Hz was depressed by conjunctive IO stimulation at 4 Hz, but that evoked by stimulation of the

other nerve alone, which did not receive conjunctive stimulation, was not effected in PC's of decerebrate rabbits (Ito et al., 1981, 1982). This depression recovered about ten minutes after the IO stimulation was stopped, but was followed by a slow depression lasting for about an hour. Similar depressions of PC sensitivity to glutamate, the putative transmitter at the PF-PC synapse, were also tested. Responses to iontophoretically applied glutamate were depressed after IO stimulation, while those to aspartate were unaffected or reduced to a lesser extent. Furthermore, the depression of glutamate sensitivity had a identical time course to the depression of the parallel fibre responses. It is notable that not all PF synapses were effected since neither the spontaneous activity of PC's nor the activation of basket cells and the subsequent inhibition of Purkinje cells was changed. Additional studies involving conjunctive stimulation of parallel fibres and climbing fibres found confirmatory results (Ito and Kano, 1982). The authors concluded that the most likely site of action for the depressions was the PF-PC synapse.

I.A.2.c. Ionic Basis of Electrogenicity

Purkinje cells, as well as the classical voltage-dependent conductances responsible for somatic and axonal electrogenicity, have unusual somatic and dendritic conductances which mediate the unique responses of these neurones to climbing fibre and parallel fibre inputs. Llinas and Sugimori

(1980a,b) in a series of in vitro experiments, using a cerebellar tissue slice preparation from guinea pigs, have demonstrated the underlying conductances responsible for the electroresponsiveness of Purkinje cells.

The Purkinje cell soma had two main types of electroresponsiveness. The first was a classical fast sodium-dependent spike probably generated by inactivating voltage-dependent sodium and potassium conductances. This spike was blocked by the application of tetrodotoxin (TTX) to the bathing medium. The second was a non-inactivating sodium conductance, evident only after calcium conductance had been blocked with the perfusion of the chloride salts of cobalt, cadmium, or manganese or D-600. This conductance was capable of generating prolonged plateaus with amplitudes probably determined by an equilibrium between voltage-dependent non-inactivating sodium and potassium conductances.

Purkinje cell dendrites demonstrated a set of ionic conductances and electroresponsive behaviours distinct from those of the soma. Firstly, they had a calcium-dependent spiking which was TTX insensitive but was blocked by calcium blockers. The resulting spikes were slow rising, fast falling, and were followed by a prolonged after-hyperpolarization. The inactivation and after-hyperpolarization were probably due to calcium and voltage-dependent potassium conductances. Secondly, PC dendrites displayed a calcium-dependent plateau, similar to the sodium dependent plateau of the soma. This may be another manifestation of the calcium conductance which caused spiking or may reflect another distinct conductance.

It is possible that Purkinje cell spontaneous activity may be due solely to the calcium conductances present in the PC, and not require the presence of sodium since the calcium spiking behaviour was independent of sodium. Similarly, climbing fibre action may be explained by changes in the voltage-dependent calcium conductances in the dendritic tree. The long term modification of spike generation and neuronal excitability might be a reflection of the prolonged plateau-type responses causing modification of membrane resistance and potassium conductance. Therefore, given such a rich set of ionic conductances, many of the unique interactions and theories alluded to above may be explained in terms of the conductances elucidated in these studies (Llinas and Sugimori, 1980a,b).

II.A.3. The Inferior Olive

The inferior olive nucleus (IO) was so named because of the shape it gives to the ventral brainstem. Internally it can be divided into three subnuclei: the dorsal accessory olive (DAO); the medial accessory olive (MAO); and the principal olive (PO). However, recent studies have indicated that the IO is more complex than was previously thought as it can be divided into subdivisions which differ from one another in terms of structure and afferentation (Brodal and Kawamura, 1980). The nucleus receives inputs from a wide range of sources, such as the spinal cord, the raphe nuclei, the periaqueductal grey (PAG) and the cortex, and it projects to the cerebellar cortex and deep nuclei. The inferior olive appears to be implicated in motor learning and retention. Lesioning the IO has been reported to not only prevent motor learning but to also reverse the recuperation from labyrinthine lesions (Llinas et al., 1975).

II.A.3.a. Anatomy

Experiments involving retrograde and orthograde tracing techniques, autoradiography, and degeneration have been used to document the sources of input to the IO. Olivary neurones receive these inputs on the distal dendrites, sometimes at synaptic clusters. Afferents from the spinal cord terminate mainly on dendritic shafts in the caudal part of the MAO and the dorsolateral DAO (King, 1980; Nicholson and Gwyn, 1974; Martin et al., 1980). The deep cerebellar nuclei project

to the whole of the IO except parts of the MAO (King et al., 1976, 1978; Angaut and Cicirata, 1982; Haroian, 1982). Cortical afferents are limited to parts of the MAO (Martin et al., 1975; Sousa-Pintal and Brodal, 1969), whereas the whole IO receives inputs from the midbrain, including the tegmentum, red nucleus, and ventral PAG, and from the thalamus, specifically the nucleus subparafascicularis (Brown et al., 1977; King et al., 1978; Linauts and Martin, 1978; Saint-Cyr and Courville, 1978).

A catecholaminergic innervation of the IO has been reported (Fuxe, 1965; Hoffman et al., 1973; Chan-Palay, 1977; Wiklund et al., 1977) whose origin has been attributed to the caudal medulla (Chan-Palay, 1977), the periaqueductal grey (Garver and Sladek, 1975), and the nucleus of the optic tract (Hoffman et al., 1973). Terminals which degenerate after PAG lesions (King et al., 1978) and which may be monoaminergic are present throughout the IO but mainly in the PO. An intense substance-P-like immunoreactivity has been reported in the lateral tip of the DAO in the rat (Ljungdahl et al., 1978), but terminals containing substance-P have not been identified as yet in the IO. The presence of acetylcholinesterase in the neuropil (Marani et al., 1977) and alpha-bungarotoxin binding sites in the IO (Hunt and Schmidt, 1978) is suggestive that the nucleus receives a cholinergic input. Schulman (1981) has recently reported leu-enkephalin-like immunoreactivity heterogeneously throughout the IO. Additionally, local application of enkephalin by iontophoresis or micropressure depressed both spontaneous and firing evoked by iontophoretically applied

DL-homocysteic acid (Schulman, 1981).

The IO also has been reported to receive an indol-aminergic input in the cat, rat and monkey (Fuxe, 1965; Chan-Palay, 1977; and Wiklund et al., 1977). Sources of these fibres may be the raphe nuclei (Bobillier et al., 1976) or neurones within or surrounding the IO (Chan-Palay, 1977). Electrophysiological evidence also suggests serotonin (5-HT) activity in the IO. Harmaline causes rhythmic, synchronous firing in the IO after intravenous (de Montigny and Lamarre, 1973; Llinas and Volkind, 1973; Biscoe et al., 1973) or local (Headley et al., 1976) administration. Destruction of serotonergic innervation of the IO by specific neurotoxic drugs (5,6- or 5,7-dihydroxytryptamine) markedly reduced the effects of harmaline (Sjolund et al., 1977). Headley et al. (1976) suggested that harmaline acts by suppressing a tonic 5-HT input to olivary cells because 5-HT was the only substance tried that antagonized the firing induced by harmaline. It is known that iontophoretically applied harmaline causes a hyperpolarization of olivary cells (Llinas and Yarom, 1980) which may cause the oscillatory firing by unmasking the somatic calcium conductance (Llinas and Yarom, 1981a, see below). Consistent with this idea, 5-HT has been reported to cause a marked reduction in the late phase of olivary cell action potentials, although it only slightly modifies firing rate (Headley and Lodge, 1976).

The inferior olive is the main, if not sole, source for climbing fibres to the cerebellar cortex and deep nuclei (Desclin, 1974; Batini et al., 1976; Chan-Palay, 1977). The projection is topographically arranged, with the caudal third

of the nucleus projecting to the vermis and the rostral two-thirds projecting to the paravermal and lateral regions of the hemispheres, respectively (see Brodal and Kawamura, 1980).

Each cerebellar Purkinje cell appears to receive input from one and only one climbing fibre in the adult (Eccles et al., 1966; Puro and Woodward, 1977), as opposed to the situation in the immature animal where more than one climbing fibre may innervate a single PC (Kawaguchi et al., 1975; Barragan and Delhaye-Bouchaud, 1980). The presence of climbing fibres and their continued functioning has been suggested to be essential to the embryological development (Kornguth and Scott, 1972; Kawaguchi et al., 1975; Woodward et al., 1977) and the continued maintenance (Hamori, 1973) of the Purkinje cell dendritic tree, and the functioning of the Purkinje cell. However, contrary to these reports, Sotello et al. (1975) reported that Purkinje cell dendritic spines were not reduced after IO destruction with 3-AP, but rather increased in number. However, another study indicated a role for climbing fibre on Purkinje cell functioning: Ito et al. (1978, 1979) reported that destruction of the IO with 3-AP caused a rapid failure of inhibitory transmission at the Purkinje cell-lateral vestibulospinal tract cell synapse. They suggested that this may be due to the interruption of axonal flow. Unfortunately, a recent attempt designed to repeat this finding was unsuccessful (Montarolo et al., 1981). It remains to be seen, therefore, whether or not the Purkinje cell requires the IO to maintain its function.

In addition to innervation of Purkinje cells, climbing fibres are reported to have collaterals to the cerebellar nuclei (Chan-Palay, 1977; Desclin and Colin, 1980) and Golgi cells (Eccles et al., 1967; Desclin and Colin, 1980). They are reported not to project to molecular layer interneurons (Desclin and Colin, 1980). However, electrophysiological studies have reported electrical stimulation of the IO to inhibit molecular layer interneurons (Bloedel and Roberts, 1971).

The constituent neurones of the IO have been reported to have a firing rate of about 0.3-1.5 Hz (Armstrong and Harvey, 1966; Armstrong and Rawson, 1979) and to tend to fire in synchrony. Spontaneous firing is probably caused by the pacemaker activity of the underlying ionic mechanisms of electrogenicity (see below, Llinas and Yarom, 1981a,b). The synchronous firing between cells is likely due to the presence of gap junctions and dendrodendritic synapses which would allow the electrotonic spread of membrane depolarizations between neighbouring cells. Evidence for these has been published from both electron microscopic (King, 1980) and electrophysiological (Llinas et al., 1974; Llinas and Yarom, 1981a) studies. The latter experiments also indicated that olivary neurones receive an inhibitory input from recurrent collaterals, which was suggested to be the cause of the slow firing rates of these cells. However, Desclin and Colin (1980) found no axonal terminal degeneration in the IO or its periphery after destruction of the IO with 3-acteylpyridine (3-AP), a drug which preferentially destroys IO neurones. They concluded that this

ruled out the existence of recurrent olivary axons at these locations.

II.A.3.b. Ionic Basis of Electrogenicity

Extracellular recordings from olivary neurones demonstrate the atypical action potentials generated by these neurones. Initially, as the microelectrode approaches the cell, the potentials have a sharp monophasic deflection followed by a prolonged plateau extending to about 5 ms (Headley and Lodge, 1976). As the electrode moves closer to the cell, the initial monophasic deflection converts to a diphasic potential and the plateau is usually reduced in amplitude. As shall be seen below, these changes probably reflect a change in the electrode position from a dendritic to a somatic position.

Olivary action potentials recorded intracellularly have an appearance very similar to the dendritic extracellular recordings described above (Crill, 1970 and Llinas et al., 1974). In addition to the early fast-rise and the late depolarization components, there is also a late hyperpolarization which lasts for up to 250 ms. Tissue slice experiments have allowed the characterization of the ionic conductances underlying these components of the olivary action potentials and have also allowed the elucidation of the rhythmic character of olivary firing (Llinas and Yarom, 1980, 1981a,b). These experiments have demonstrated that the initial fast portion of the potential is due to the familiar voltage dependent increase in sodium conductance, since this conductance was blocked by tetrodotoxin (TTX). In contrast the late-depolarization and

late hyperpolarizations are not sodium dependent as they remain intact following treatment with TTX. However, these late spike components could in turn be blocked by applications of solutions of cadmium, cobalt, or magnesium chloride which are known to block calcium conductance. Llinas and Yarom concluded that these late polarizations were due to an inward calcium current which triggers a potassium conductance. The action potentials resulting from these conductances appear to originate in the dendrites of olivary neurones.

In addition to these dendritic conductances, the olivary neurone has another calcium conductance limited to the neuronal somata, which is only observable when the cell is hyperpolarized (Llinas and Yarom, 1980, 1981a). A small internally injected hyperpolarizing current will trigger calcium dependent spikes which do not display refractoriness. These ionic conductances: dendritic sodium, potassium, and calcium and somatic calcium; provide an ionic basis for the slow oscillatory behaviour of olivary cells. In progression, an externally produced depolarization causes a sodium spike followed by calcium and potassium mediated late depolarization and hyperpolarization, respectively. The late depolarization, in turn, causes the unmasking of the somatic calcium conductance and the subsequent calcium spike. This calcium spike closes the oscillatory loop by triggering another dendritic action potential. It is clear that external modifications, for example through synaptic inputs or the changes to the neuronal environment, to the cells membrane potential would result in a modulation of the neurone's period of oscillation. For example, the mecha-

nism of harmaline's excitatory action may be due the unmasking of the somatic calcium conductance and the subsequent oscillatory behaviour (Llinas and Yarom, 1981b).

II.A.4. The Golgi Cell

Golgi cells are relatively large neurones situated in the granular layer of the cerebellar cortex having firing rates of 4-31 Hz (Schulman and Bloom, 1981). Golgi cells have extensive dendritic trees and receive an excitatory input from a large number of parallel fibres and from mossy fibres (Hamori and Szentagothai, 1966). In addition, anatomical studies indicate that they probably also receive axosomatic synapses from collaterals of climbing fibres and Purkinje cell axons (Scheibel and Scheibel, 1954; Palay and Chan-Palay, 1974). Stimulation of the cerebellar cortex, which activates climbing fibre collaterals, was found to produce a significant increase in firing rate of Golgi cells (Rawson and Tilokskulchai, 1981). However, Schulman and Bloom (1980) failed to find excitation of Golgi cells during electrical stimulation of the IO, but rather found them to be inhibited.

Electrophysiological studies indicate Golgi cells have inhibitory actions both post-synaptically on granule cell dendrites and pre-synaptically on mossy fibre terminals as well as on Purkinje cells (Eccles et al., 1967). The putative neurotransmitter at these synapses has been suggested to be GABA (Bisti et al., 1971). Experimental evidence supporting this transmitter includes the antagonism of Golgi cell-induced depression of evoked potentials, produced by the antidromic activation of PC's and the orthodromic activation of granule cells, by relatively large doses of picrotoxin and bicuculline, but not strychnine (Bisti et al., 1971). Golgi cells are also

reported to take up GABA (Hokfelt and Ljungdahl, 1972).

In terms of classical control theory the granule cell-Golgi cell inhibitory loop would tend to keep the granule cell activity at a constant level. One can speculate that this level is the one at which the cerebellar circuitry works most efficiently.

II.B. Gamma-aminobutyric acid

Gamma-aminobutyric acid (GABA) is well established as a neurotransmitter (see Roberts et al., 1976; Olsen, 1982). Exogenously applied GABA produces a conductance change which causes a hyperpolarization identical to that produced by the endogenous inhibitory transmitter in the cerebral cortex (Krnjevic and Schwartz, 1967). Biochemical and pharmacological studies have shown the GABA receptor to be specific (Curtis et al., 1971) and its synthesis and deactivation have been well studied. It is increasingly being suggested to mediate many of the sedative, anxiolytic, anticonvulsive, and muscle relaxant actions of the benzodiazepines, and also the sedative-hypnotic and anticonvulsant actions of barbituates, as well as the convulsant actions of picrotoxin and related substances (Haefely et al., 1978; Olsen, 1981; Simmonds, 1980).

The major synthetic route for the manufacture of GABA is decarboxylation of L-glutamic acid derived from the tricarboxylic acid cycle. This is the final and rate limiting step of the reaction and is catalyzed by L-glutamic acid decarboxylase (GAD, Roberts et al., 1979). A secondary route of GABA synthesis may be from ornithine (Yoneda and Roberts, 1981). Although sodium dependent GABA binding has been shown to occur in synaptosomes and GABA may be stored in vesicles in some terminals, it is likely that non-vesicle release also occurs. However, GABA release, similarly to that of other putative transmitters, is calcium-dependant. Deactivation of GABA is via reuptake from the synaptic space by a highly specific

transport system into both neuronal and non-neuronal cells (Snyder et al., 1973; Balcar and Johnston, 1973). These two systems appear to be differentiable since they can be preferentially inhibited by different blockers (Schousboe et al., 1981). Subsequently, the major enzymatic deactivation of GABA is via GABA-transaminase (GABA-T, Metcalf, 1979), which is located in the inner mitochondrial membrane (Schousboe et al., 1977).

Biochemical studies have revealed the GABA receptor to be a complex of interrelated, but independent, subunits. The agonists GABA and muscimol both bind at the same site, as well as the antagonist bicuculline (Enna and Snyder, 1977). However, differences in biological and pharmacological characteristics indicate two conformational states of the receptor: one which binds agonists, another which binds antagonists (Mohler and Okada, 1978).

It has been quite well established that an interaction exists between the benzodiazepines and GABA. Indirect evidence of behavioural correlations between GABA agonists and antagonists and the benzodiazepines had suggested this for many years (Haefely et al., 1978; Olsen, 1981; Simmonds, 1980). Recent studies have supported this interaction at the receptor level. In addition to the two subunits mentioned above, there appears to be a modulator subunit which reduces the affinity of the GABA receptor site (Enna and Snyder, 1977). This modulator is reported to be displaced by the binding of benzodiazepines (Costa, 1979). Conversely, GABA binding has been shown to increase the affinity of these benzodiazepine receptor sites

(Tallman et al., 1978). However, the presence of the subunit appears to be limited to a portion of the total number of GABA receptors, resulting in at least two receptor subtypes of different affinities and distributions (Costa, 1979). Of the two, only the low affinity sites show binding to the benzodiazepines (Braestrup et al., 1980).

Another group of agents, the barbituates, may interact with the receptor complex by binding to the ionophore portion of the GABA receptor (Olsen, 1981). Similarly, the GABA antagonist picrotoxin is thought to block the opening of the ionophore by also binding to this site (Ticku et al., 1978) since dihydropicrotoxin has been reported to label the chloride ionophore (Olsen, 1981).

The major postsynaptic event of the interaction of GABA and the receptor-ionophore complex is the rapid increase in the membrane conductance to chloride ion (McBurney and Barker, 1978; Nistri et al., 1980). This may result in either depolarization or hyperpolarization depending upon the transmembrane gradient of chloride ions. In the case of the spinal cord, GABA administration has been reported to cause membrane depolarization of spinal cord primary afferents, which has been suggested to reduce the amount of transmitter released by the nerve terminal (Eccles et al., 1963). This has been referred to as primary afferent depolarization or presynaptic inhibition. This effect is thought to be due to chloride ion efflux, because the chloride concentration intracellularly has been suggested to be greater than that extracellularly due to a inward facing chloride pump (Nishi et al., 1974). It may be

also due in part to a build up of potassium ion in the extracellular space due to the discharge of neighbouring afferents (Curtis et al., 1971). Recently, a more direct mechanism for pre-synaptic inhibition by GABA has been proposed by Dunlap and Fischbach (1978). They found GABA was able to block a slow-calcium mediated component of dorsal-root ganglion cell action potentials without any significant depolarization. Because the antagonism was insensitive to bicuculline and other GABA antagonists the antagonism does not appear to involve the post-synaptic GABA receptor. It was proposed, therefore, that the GABA interfered directly with inward calcium current. Postsynaptic inhibition by GABA involves the hyperpolarization of the post-synaptic membrane due to chloride ion influx since the extracellular chloride ion concentration generally is believed to be higher than intracellularly (Krnjevic, 1982).

Gamma-aminobutyric acid has been quite well characterized as an inhibitory transmitter in the cerebellum. It may be the inhibitory transmitter used by Purkinje, stellate, basket, and Golgi cells. The Purkinje cell-Deiters' neurone synapse has been most extensively studied. Exogenous GABA has been shown to mimic the action of synaptically released transmitter evoked by stimulation of the cerebellar cortex (Obata et al., 1967; ten Bruggencate and Engberg, 1971). For example, inhibition of Deiters' neurones by exogenous and synaptically released GABA has been shown to be blocked by the GABA antagonists picrotoxin and bicuculline (Curtis, Duggan, and Felix, 1970; Bruggencate and Engberg, 1971). Obata and Takeda (1969) found GABA was released into the fourth ventricle after stimu-

lation of the cerebellar cortex. Also, the GABA content of individual Deiters' nucleus neurones was reduced by removal of the cerebellar vermis (Otsuka et al., 1971) and GABA has been shown to associated in high concentrations with the neuronal membranes of Deiters' neurones. These data again suggest GABA is the transmitter at this synapse.

Although the evidence is not as good as that from Deiters' nucleus, GABA also may be the transmitter of the cerebellar cortical inhibitory interneurones. It appears to mediate stellate and basket cell inhibition of Purkinje cells and Golgi cell inhibition of granule cells. GABA antagonists have been shown to block the inhibition of Purkinje cells produced by synaptically released GABA evoked by stimulation of parallel fibres (LOC stimulation), which is due to the activation of the stellate and basket cells and their subsequent inhibition of Purkinje cells. GABA antagonists have also blocked the inhibition produced by iontophoresed GABA (Bisti et al., 1971; Curtis and Felix, 1971; Woodward et al., 1971). In addition, histological studies have demonstrated accumulation of ^3H -GABA at stellate, basket, and Purkinje cells and also at nerve endings terminating on Purkinje cells (Hokfelt and Ljungdahl, 1972).

There is also some evidence to suggest that the action of other transmitters in the cerebellum may be mediated by modification of GABA transmission. For example, a recent study by Moises et al. (1979) demonstrated that inhibition of Purkinje cell by synaptically released GABA is enhanced by both electrical stimulation of the locus coeruleus and iontophoretic

noradrenaline. Further, Namima et al. (1983) found taurine to modulate the depolarization produced, calcium dependant release of 3H-GABA from guinea pig cerebellar slices. This modulation was blocked by bicuculline. They suggested that taurine may be acting upon GABA autoreceptors or other bicuculline sensitive receptors, or, tentatively, may be blocking the influx of calcium and hence GABA release.

II.C. Ethanol

It is unlikely that the molecular mechanism of action of ethanol on neuronal firing and synaptic transmission is mediated by a receptor since, other than alcohol dehydrogenase, no macromolecule is known which reacts with ethanol to any degree of specificity. Many theories have been postulated to explain ethanol's mechanism of action. It is conceivable that more than one mechanism may be important as they are not mutually exclusive, and it is probable that different mechanisms may be important in different neuronal systems. Unfortunately, even some of ethanol's basic physical effects are still controversial.

One of the first observations which suggested a mechanism for ethanol action was made by Overton and Meyer at the beginning of this century. They found the potency of an anaesthetic or similar compound, including ethanol, to be directly proportional to its lipid solubility (Meyer, 1937). This correlation led to the formation of the Overton-Meyer concept which holds that anaesthetic action is due to the attainment of a critical concentration of anaesthetic in cell lipids. This concept was extended by Mullins (1954) to include the molecular volume: bulkier molecules are more effective than small molecules. Seeman (1974) demonstrated that ethanol lowers the density of synaptosomes and further that the decrease in biological membranes was greater than that for model membranes containing no protein, strongly suggesting that conformational changes in the membrane proteins contributed to

the membrane expansion. In addition, the effects of anaesthetics can be reversed by the application of high pressures (Trudell et al., 1973; Kendig et al., 1975). These findings suggest that ethanol acts primarily at a hydrophobic site, such as the cell membrane, and may act by expanding biomembranes and disrupting their structure.

Although ethanol causes membrane fluidization, the local concentrations required to produce such non-specific effects as membrane leakage appear to be far in excess of those required to produce physiological effects, such as inebriation. However, it is probable that fluidization is an important mechanism at more sensitive and specialized sites within the membrane. More recently, the methods of electron para-magnetic resonance and fluorescence spectroscopy of membrane marker groups has allowed a quantitative measurement of membrane disruption. For example, Chin and Goldstein (1977) have reported that ethanol, at concentrations as low as 10 mM, caused fluidization of synaptosomal membranes. This concentration of ethanol in vivo would produce only mild to moderate intoxication. In addition, fluidization and changes to spatial arrangements of the lipids surrounding membrane bound enzymes is known to be critical to their functioning (Barondes et al., 1979). This class of proteins includes ionophores, ionic pumps, gates, and receptor complexes. For example, ethanol has been reported to produce a concentration-dependent inhibition of sodium-potassium-ATPase activity (Schwartz et al., 1975). This inhibition was shown to be caused by allosteric effects which were probably due to alterations of the enzyme-phospho-

lipid interaction.

As would be predicted from the above, low concentrations of ethanol have been found to reduce the amplitude of the electrical stimuli required to activate a compound action potential in an isolated nerve or to activate the isolated squid giant axon (Armstrong and Binstock, 1964; Moore et al., 1964). This result is due to ethanol producing a slight depolarization of the membrane by decreasing potassium conductance. Higher concentrations (>100 mM) cause a further depolarization, decreased sodium conductance, and the inactivation of sodium channels, resulting in a slower propagation velocity and a lower action potential amplitude (Seeman, 1972). Small unmyelinated fibres are affected more than larger myelinated fibres (Quevedo et al., 1978).

Although ethanol has been reported to alter calcium content and binding in a variety of tissues, the data are contradictory. Ross and coworkers found ethanol to cause the displacement of calcium from synaptosomes both in vivo and in vitro (Ross, 1977; Ross et al., 1979). Other workers have found ethanol to increase in vitro calcium binding (Seeman et al., 1971; Michaelis and Myers, 1979). Still other groups have reported ethanol to have no effect on in vivo membranes (Hood and Harris, 1979; Ferko and Bobjock, 1980). Kalant and Woo (1981), in their recent review, suggest that these results are a reflection of differences in membrane preparation and definition of calcium uptake. In addition to affecting calcium binding, anaesthetics and ethanol may cause calcium-dependent permeability systems, such as potassium, to be more sensitive

to intracellular ionized calcium, without necessarily affecting the concentration of calcium (Baker et al., 1980).

Modification of calcium binding or content would be predicted to modify neurotransmitter release. Ethanol has been reported to increase the frequency of miniature endplate potentials in the isolated nerve-muscle junction (Gage, 1965; Okada, 1967), but to inhibit release of acetylcholine from the cerebral cortex (Phillis, 1968; Sinclair and Lo, 1978; Phillis et al., 1980; Sunahara and Kalant, 1980). The release of other transmitters from the cerebral cortex was reported to also be reduced. These transmitters included serotonin, dopamine, noradrenaline, glutamate, and GABA, with GABA being least effected (Carmichael and Israel, 1975). However, Seeman and Lee (1974) found dopamine release to be increased by ethanol.

In addition to affecting transmitter release, ethanol also has an effect on post-synaptic events. It has been reported to antagonize the contracture of guinea-pig ileum induced by acetylcholine or potassium (Hurwitz et al., 1962, 1967; Mayer et al., 1980a). This effect could be reversed by increased calcium ion concentration (Mayer et al., 1980b). Similarly, in the neuromuscular junction ethanol has been reported to increase calcium-independent spontaneous and calcium-dependent depolarization-evoked miniature end-plate potential (mepp) number (Quastel et al., 1971), to prolong mepps (Carlen and Corrigan, 1980), and to increase the affinity of the acetylcholine receptor (Bradley et al., 1980). However, contrary to this latter result, acetylcholine binding, and that of dopamine and glutamate, was increased, decreased, or not

affected unpredictably by ethanol in preparations from different parts of the brain (Ciofalo, 1980). In another report, glutamate binding to brain synaptosomes was decreased by in vitro ethanol (Michaelis et al., 1980). A recent report by Carlen et al. (1982), which involved intracellular recording of CA1 cells from hippocampal slices, found that ethanol appears to augment calcium-mediated mechanisms both pre- and post-synaptically, possibly by increasing intracellular calcium concentration or effectiveness.

Ethanol induced alterations to transmitter release and binding would be expected to be reflected in the effects of ethanol on single unit spontaneous and evoked firing. For example, an early report found that a slowly infused intravenous dose of ethanol depressed cells presumed to be Purkinje cells, while tending to excite interneurons, in decerebrate cats (Eidelberg et al., 1971). Conversely, cerebellar explants have been found to have increased spontaneous firing and regularity, even in the absence of synaptic input (Seil et al., 1977). Similar results have been reported in vivo: ethanol at low to moderate doses caused an increased simple spike firing rate of Purkinje cells recorded in situ from intact rat cerebelli (Rogers et al., 1980; Sinclair et al., 1980; Sinclair and Lo, 1981). These latter workers also reported a concurrent increase in the regularity of SS firing. These effects may have been due to the decreased complex spike activity reported after ethanol. When ethanol was given more rapidly or in higher doses SS activity was depressed and complex spike-like burst activity was observed (Rogers et al., 1980).

A variety of neuronal responses to ethanol have been reported for other areas of the nervous system. For example, the spontaneous discharge of spinal dorsal horn interneurons was depressed by slow intravenous infusion of ethanol (Eidelberg and Wooley, 1970). Conversely, dorsal hippocampal neuronal firing was excited by low doses of intravenously infused ethanol and inhibited by higher doses (Grupp, 1980). A later study showed that this inhibition of firing could be antagonized by dopaminergic and cholinergic agonists and unaffected or enhanced by GABA-ergic and adrenergic antagonists (Grupp, 1981). Most cells in the locus coeruleus were found to be inhibited by intraperitoneally injected ethanol (Pohorecky and Brick, 1977).

Ethanol applied locally onto Purkinje cells by micro-pressure depressed their firing in a non-specific, local anaesthetic-like manner (Siggins and French, 1979). Similar application of ethanol onto hippocampal pyramidal cells caused about half of them to be excited, while the other half were inhibited (Siggins and Bloom, 1980). Carlen et al. (1982) reported that ethanol, either perfused or applied focally by microelectrode, caused a decreased firing in most of the neurons recorded intracellularly in hippocampal slices.

II.D. Ethanol Effects on GABA

Although not much direct evidence has been published showing an effect of ethanol on GABA-ergic function, there has been quite a lot of indirect evidence suggesting this. Behavioural studies have shown a similarity between the effects of ethanol and that of other substances, such as the benzodiazepines and barbituates, whose effects have been attributed to modification of GABA pathways. In addition, a few behavioural studies have been directed at GABA's role in ethanol intoxication. For example, Hakkinen and Kulonen (1976) reported that the GABA antagonist bicuculline reduced ethanol intoxication in rats, as measured by the tilting-plane test. Conversely, an inhibitor of GABA catabolism, amino-oxyacetic acid (AOAA), increased the level of intoxication.

Ethanol is reported to modify spinal cord presynaptic inhibition, which is generally agreed to be mediated by GABA (cf. Levy, 1977). Miyahara, Esplin, and Zablocka (1966) reported that ethanol as well as other anaesthetic agents enhanced presynaptic inhibition at a dose which produced sedation or light anaesthesia. Conversely, Eidenberg and Wooley (1969) reported that, in a similar system, ethanol did not produce any potentiation of dorsal root potential. However, Davidoff (1973) also reported that ethanol (150-450 mg/100 ml) enhanced primary afferent depolarization, but in a perfused amphibian spinal cord preparation. Therefore, it appears that ethanol enhances the spinal presynaptic inhibition produced by GABA.

Unfortunately, the studies on the effect of ethanol on GABA's actions on single neurones above the level of the spinal cord are often contradictory. Lake et al. (1973) reported that electro-osmotically applied ethanol antagonized the actions produced by iontophoretic acetylcholine, noradrenaline, and serotonin, but not that of GABA. On the other hand, Nestoros (1980a,b) reported ethanol, administered microosmotically or intravenously, potentiated inhibition of single feline cortical cells produced by microiontophoretically applied GABA or electrical stimulation of the surface of the cerebral cortex. It is notable that these effects were seen at remarkably low doses of ethanol (0.2-2 mg/Kg). Recent experiments by Newlin, Mancillas-Trevino, and Bloom (1981) have shown that ethanol modifies a hippocampal neuronal inhibition thought to be mediated by GABA. In these experiments, ethanol (3 g/kg i.p.) significantly increased the duration of rat hippocampal CA3 neuronal inhibition produced by stimulation of the dentate mossy fibre or commissural pathways. Conversely, Carlen et al. (1982) reported that ethanol, at low doses, did not augment GABA-induced CA1 pyramidal cell responses to GABA applied focally by micropressure ejection (0.1-10mM). Other workers have reported ethanol to antagonize the effects of GABA. For instance, Backstrom (1977) found that ethanol (11-45 mM) inhibited conventional GABA-ergic synapses in isolated frog retina, while not affecting the ribbon synapses. Siggins and French (1979) reported that direct application of ethanol antagonized the inhibition of Purkinje cells by GABA. However, they regarded this antagonism to be a non-specific effect since it was

often accompanied by reductions of spike amplitude and lengthening of spike duration. Finally, Sinclair and Lo (1981) found that ethanol (1.5 g/kg i.v.) reduced the period of inhibition of rat Purkinje cells produced by local stimulation of the cerebellar cortical surface. As this inhibition is thought to be mediated by GABA, the results were interpreted as an antagonism by ethanol of GABA inhibition.

III. METHODS

III.A. Animal Preparation and Surgery.

The animals used in these experiments were male albino Wistar rats weighing between 175 and 250 g obtained from either the University of British Columbia Animal Care Facility or Canadian Breeding Laboratories, La Prairie, Quebec. In each animal, anaesthesia was induced by intraperitoneal injection of urethane (1.4 g/kg, Sigma) administered as a 20% solution by weight in distilled water. Rectal temperature was monitored by an electronic thermometer and maintained within physiological limits automatically by a feedback controlled direct current heating pad. To allow subsequent intravenous injections to be made, the right femoral vein was cannulated with No. 10 polyethylene tubing (Clay Adams) prefilled with normal saline. In those experiments involving the electrolytic lesioning of the inferior olive, a tracheal cannulation was performed and the animal respired using a small animal respirator (Harvard Instrument 680).

The animal's head was then positioned in a stereotaxic frame (Narashige) according to the method of Pelligrino et al. (1979). The skin over the skull was retracted and burrholes (about 0.3 cm in diameter) were drilled with a Moto-tool (Dremel Manufacturing Company) to allow the placement of recording and stimulating electrodes into the appropriate brain locations. The dura was then reflected and the pia matter gently ripped with two pairs of forceps. Exposed tissue was covered with

normal saline or agar (4% in saline) to prevent drying.

III.B. Electrode Preparation, Placement, and Methods

Single barrel recording microelectrodes were prepared in a conventional manner from 1.5 mm diameter glass micropipettes (Glass Company of America, Omega Dot) containing a single strand of fibreglass to facilitate their filling by capillary action. The pipettes were pulled conventionally on a commercial electrode puller (Narashige) and the tips broken against a glass rod under a microscope to about 1 μ m. They were filled with a Pontamine Sky Blue dye (ICN Pharmaceuticals, Inc., 6BX) in 0.5 M sodium acetate, allowing both recording and later histological marking of recording sites by iontophoretic ejection of the dye. The electrodes had DC impedances of 15-25 megohms.

Multibarrel recording microelectrodes were constructed by glueing a number of short hockey stick shaped pipette blanks around a central full-length pipette with five minute epoxy (Lepage). The former were so shaped to allow their connection to the micropressure or iontophoresis equipment and to prevent interaction between barrels due to inter-barrel leakage. After curing, the resulting multibarrel assemblage was inserted into the electrode puller, heated, and given a half turn to bond the individual pipettes together. After a period for cooling, the electrode was pulled conventionally and the tip broken. For a three barreled electrode the resulting tip size was 3-4 μ m. The central barrel, filled as above, served as the recording barrel, while the surrounding barrels, filled with drug solutions,

served as drug application barrels.

The drugs administered intravenously were: ethanol (up to 1.5 g/kg, 20% w/v, given either in divided doses of 0.25, 0.25, 0.5, and 0.5 g/kg over 22 minutes or as one dose over 10 min) and harmaline (up to 5 mg/kg, given as a 10 mg/ml solution). The iontophoretically administered drugs were: ethanol (1 M in 145 mM NaCl) and gamma-aminobutyric acid (GABA, 2 M, pH 4). Two addition barrels of the same electrode were filled with saline: the first for control applications of saline to test for current effects; and the second as a current balancing channel to minimize current effects (Palmer, 1982). Ejection currents were in the range of 0-40 nA. The drugs administered by micro-pressure were ethanol (750 mM in saline) and sucrose (670 mM in saline). These concentrations were chosen to obtain equiosmolarity (Sorensen et al., 1981). Sucrose was used as a control to test for non-specific pressure effects. Ejection pressures were in the range of 0-20 psi.

Olivary and juxtafastigial stimulation was evoked by a bipolar concentric electrode (David Kopf Inst., NE-100). The cerebral cortex was stimulated by a comb-electrode constructed from four bipolar electrodes glued together in an planar array on 1.0 mm centers, and connected in parallel electrically.

Recording and stimulating electrodes were positioned in the brain by stereotaxic techniques. The coordinates of the target site were obtained from a stereotaxic atlas (Pelligrino et al., 1979) and used to position the electrode relative to its calibrated zero point. To avoid damage to the cranial circulation and because of the spatial requirements of the electrode

carriers, non-vertical approaches to brain sites were sometimes used.

Recording sites of interest were marked by passing a direct negative current (6 uA for 10 min) through the recording barrel. This procedure left a spot of the blue dye at the recording site. These spots were subsequently located after the brain had been sectioned. Stimulation sites were marked by lesioning the tissue at the electrode tip with direct current (0.3-0.9 mA, 30 s) passed through the electrode. At the end of the experiment the animal's chest was opened and a needle, attached to a 50 ml syringe filled with normal saline, was introduced into the left ventricle of the heart. The right ventricle was then slit and the animal perfused with the saline (30 ml over 2 min). The animal was then decapitated with a guillotine, the brain removed and stored in 10% formaldehyde solution for at least three days. The preserved brain was then sliced into 40 um sections with a freezing microtome (Damon/IEC Division) at -20 degrees Centigrade and mounted onto glass slides using an ethanol-gelatin solution (2 g gelatin in 2 l of 40% ethanol). Sections were stained with cresyl violet using conventional procedures. Micrographs were taken with a light microscope (Zeiss Photomicroscope III, 2.5x objective, ASA 6.3), black and white variable contrast film (Kodak, Technical Pan 2415), Kohler illumination, a green filter, and at a magnification of 32x.

III.C. Equipment

Single unit activity was recorded through a unity or 10x gain voltage follower. This signal was then amplified, filtered, and displayed on an oscilloscope (Tektronix, D11 or R5030). The filtering used to record Purkinje and Golgi cells consisted of a high pass filter with a 3 dB frequency cutoff of 3 kHz and a low pass filter with a 3 dB frequency cutoff of 1 kHz. This was optimal for neurones with action potentials durations of about 1 ms. However, the inferior olive neuronal action potential has a duration of about 5 ms and required the low pass filter's cutoff frequency to be adjusted at 1 to 10 Hz when recording from these neurones.

The filtered and amplified signal was then input to other equipment, as required. Typically, this would include a tape recorder (Sony TC-399, Teac A-3440, or Hewlett-Packard 3960), a window discriminator (constructed by the author), and a bucket brigade delay line (constructed by the author). The output of this latter device was input to the second channel of the oscilloscope and allowed the signal of interest to be displayed in the centre of the screen.

The window discriminator selected an action potential based upon its peak amplitude lying within an amplitude window and its width lying within a time window. These windows could be monitored visually. Selected action potentials were converted to standard pulses which were input to a spike integrator (constructed by the author, see Harris, 1979) or to a microcomputer system. The integrated spike rate could be recorded on a

polygraph (Gould 2200S or Narco Physiograph), if required.

Extensive use was made of the microcomputer system for the integration and display of neuronal firing rate and the construction of peri-stimulus (PSTH), interspike interval (ISI) and latency histograms (LH) and subsequent statistical analysis (see software). In addition, it controlled the equipment which administered drugs locally to neurones by iontophoresis (Dagan 6400) or micropressure ejection (Medical Systems Corporation PPM-2).

The microcomputer system hardware consisted of the following:

- Apple II plus with 48 kilobytes of random access memory;
- 16 kilobyte random access memory extension board (Orange Computers Inc. 16K Card);
- two 5 1/4 inch floppy disk drives (Apple Disk II);
- monitor (Electrohome ESM-914);
- dot matrix printer (Integral Data Systems 460) interfaced via a parallel interface board (Apple Parallel Card);
- and another parallel interface card (constructed by the author).

A laboratory stimulator (Grass, SD9) supplied the pulsed voltages for electrical stimulation and the currents used for electrolytic lesioning and the marking of recording sites. The "prepulse sync" output was recorded when appropriate on one channel of the tape recorder and provided a trigger signal for the oscilloscope and microcomputer system.

III.D. Software.

Extensive software was written by the author to allow the microcomputer system to be used for data collection, analysis and the real time control of equipment. It was written either in BASIC, for the display, control and analysis programs, or in 6502 assembly language for the data collection and timing routines.

Various versions of post-stimulus (PSTH), interspike interval (ISI), and latency histogram (LTH) programs were written to allow analysis of the neuronal responses to different treatments. For example, one of the programs allowed the simultaneous construction of a SS to SS ISI histogram, a CS to CS ISI histogram, and a latency histogram from each CS to the first SS. The programs used to collect data from Purkinje cells, as in this example, distinguished between complex and simple spikes on the basis of interspike interval. Specifically, an interspike interval of less than 4 ms indicated the beginning of a complex spike, while a following interval exceeding 7 ms indicated the completion the complex spike. As a consequence of this distinction, the SS interspike time histograms, for example, were constructed from only those SS to SS intervals which did not contain a CS. In most cases the histograms consisted of 250 bins with a bin width of 1 ms. However, the bin width could be increased to allow the measurement of longer time periods. Data collected by these programs was then stored onto floppy diskettes.

Companion programs accessed the stored data to redisplay and calculate the required statistical measures from it. Mean interspike intervals, together with their standard error of the mean (SEM), were calculated using standard formulae. Inhibitory periods were determined by constructing a PSTH of the inhibition and smoothing the resulting histogram by using a moving average. The period of inhibition was then found by determining the time at which the firing rate returned to 50% of the control rate (the average of the last 25 bins of the histogram).

Other programs were written to control the application of drugs by micropressure or iontophoresis while simultaneously recording and displaying the neuronal firing rate visually, both as a time versus rate display and numerically. Again, the collected data was stored on floppy diskette for recall by other programs for further display and analysis. These programs allowed a number of drug application trials to be averaged together. Inhibition by GABA would be determined from this averaged response as either the absolute or relative (percentage) decrease in firing rate. Absolute decrease was calculated from the averaged response by subtracting the mean rate during GABA application from the mean rate during the control period. Relative change was calculated similarly, but expressed as a percentage of the control firing rate.

Statistical significance was calculated using Student's paired t-test with a two-tailed critical value of $p < 0.05$. The data from each experiment trial were normalized by subtracting the average of the control values from each of the

test points. Then these differences were averaged over all the experiments at each time point and used to calculate the t-statistic. This procedure calculated SEM's for the control values and thus measured the variance of the control data as well as that of the test data.

Graph's were plotted on a digital plotter (Houston Instruments, DMP-4) controlled by another Apple II microcomputer system. The plotting software was written by Mr. Ahmad Fawzi.

III.E. Experimental Protocols

III.E.1. Ethanol on Complex Spikes

III.E.1.a. Climbing Fibre-Purkinje Cell Synapse

To determine whether ethanol decreases CS activity though an action at the climbing fibre-Purkinje cell synapse, we examined the effect of ethanol on the CS activity evoked by juxtafastigial stimulation. A bipolar stimulating electrode was positioned juxtafastigially (-4.5 AP, -2.5 DV, 1.8 lateral) to allow the subsequent evocation of complex spikes. Purkinje cell single unit activity was recorded ipsilaterally to the stimulating electrode in lobes IV and V of the cerebellum. After a Purkinje cell had been located by its characteristic firing rate and pattern, and a stable recording had been obtained, the minimum voltage required to consistently evoke complex spike activity was measured. This was defined as the voltage of stimulation required to evoke a complex spike in all of 10 consecutive stimulations. This measurement was made at regular intervals before and after ethanol administration (1.5 g/kg,

i.v. in a single dose over 10 min or in divided doses over 22 min). The neuronal spike activity was recorded on an FM tape recorder for later analysis.

The data was analyzed by counting the numbers of simple and total spikes per one minute epochs over the course of the experiment. In each experiment the voltage required to evoke complex spike activity were compared to the pre-control voltage at those times when the spontaneous complex spike rate was reduced significantly ($p < 0.001$) with a paired Student's t-test ($p < 0.05$).

III.E.1.b. Cerebral Stimulation

To study the effect of ethanol on CS activity further, inferior olive neurones were activated indirectly by stimulation of the cerebral cortex. An array of bipolar stimulating electrodes was positioned 1 mm below the surface of the cerebral cortex according to the method of Freedman et al. (1977). After a Purkinje cell had been located, complex spikes were evoked at regular intervals (80 stimuli, 0.4 ms duration, 1Hz, every 5 min). The stimulation intensity was adjusted to a submaximum level which evoked complex spike activity consistently. Neuronal spontaneous CS activity was recorded during the periods between stimuli.

The data were analyzed by constructing latency time histograms (LTH, in addition to SS and CS PSTH's) of the first CS after each stimuli. The number of evoked CS per 80 stimulations was taken from each LTH. The resulting data was pointwise averaged at each time period over all experimental trials. From

this averaged data, significant difference from the average of the pre-ethanol control counts (two time points) was determined by a Student's paired t-test ($p < 0.05$).

III.E.1.c. The Inferior Olive

Single or multibarreled glass micropipettes were used to record extracellular inferior olive neuronal activity. The effect of ethanol on firing rate was tested by administering the drug intravenously or locally by micropressure. The micropipettes were positioned stereotaxically (-4.5 AP, -7.5 DV, 0.6 lateral) using characteristic field potentials evoked by stimulation of the cerebellar white matter (-4.5 AP, -2.5 DV, 1.8 lateral) as an aid (Headley and Lodge, 1976). After a stable recording had been achieved, neuronal firing was collected and displayed by the microcomputer. Ethanol (1.5 g/kg) was intravenously infused over 10 min, while locally administered ethanol or sucrose (used as a control) was applied for one minute at three minute intervals under microcomputer control. At the end of an experiment, harmaline (up to 5mg, i.v.) was administered to verify the identity of the neurone.

The effect of ethanol on inferior olive firing was analysed by counting cell firing during six second epochs. The data from all of the intravenous ethanol experiments were point-wise averaged at each time point and a paired t-test was used to determine the significance of changes to the firing rate from the average of the pre-ethanol controls. In the local administration experiments, trials at similar ejection pressures were averaged together to obtain mean percentage change in firing

rate from pre-drug control rate. A paired Student's t-test was used to determine if there was a significant ($p < 0.05$) difference between the change in firing rates during the ethanol and glucose applications in each trial.

III.E.2. Ethanol on Simple Spikes

To examine the effect of ethanol on SS activity in the absence of indirect effects of CS activity, the inferior olive was lesioned to eliminate spontaneous CS. These experiments involved the positioning of a bipolar stimulating electrode in the inferior olive stereotaxically (-4.5 AP, -7.5 DV, 0.6 lateral). Purkinje cell single unit activity was recorded contralateral to the stimulating electrode in lobules IV and V of the cerebellum. After a Purkinje cell had been located which could be activated by stimulation of the inferior olive, the inferior olive was lesioned (0.1-0.6 mA over 2-5 min), resulting in the complete cessation of CS activity in the Purkinje cell. After acquisition of control data ethanol (1.5 g/kg) was infused over 10 min.

The effect of ethanol on SS firing rate was analyzed by constructing ISI histograms of SS to SS and CS to CS before and after the lesioning and the administration of ethanol. The mean interspike interval was calculated at each time point in each experiment. The data was then pointwise averaged at each time point over all experiments and significant differences from the averaged post-lesion, pre-ethanol means (two time points) were determined using Student's paired t-test ($p < 0.05$).

The effect of ethanol on SS regularity was analyzed by comparing the variance of the mean interspike intervals measured in these experiments. A measure of regularity was calculated at each time point in each experiment, by dividing the variance of the interspike interval (from the ISI histogram) by its mean and scaling by 1000. Again, the data from all experimental trials was pointwise averaged at each time point. Significant differences from the averaged post-lesion, pre-ethanol time points were determined using Student's paired t-test ($p < 0.05$).

III.E.3. Ethanol on Local Inhibition

These experiments were conducted to test the effect of locally applied ethanol on the period of inhibition evoked by local surface stimulation (LOC). Purkinje cell single unit activity was recorded from lobules IV and V of the cerebellar cortex with three barreled glass microelectrodes whose two drug barrels contained ethanol and sucrose. After the electrode had been positioned just below the surface, the local (LOC) stimulating electrode (a 0.3-0.4 mm diameter silver ball electrode insulated with glass) was positioned about 1.0 mm from the microelectrode along the longitudinal axis of the folium. A suitable Purkinje cell was then located and the effect of locally administered ethanol and sucrose on local inhibition tested.

Inhibition of firing was evoked by stimulating with the LOC electrode (1Hz, 0.5 ms duration) with the stimulus intensity adjusted to achieve a 10-30 ms inhibitory period. Post-stimulus histograms were constructed at regular intervals

with and without the local administration of ethanol or sucrose by micropressure over a range of pressures. The period of inhibition at each time point was measured by smoothing the PSTH and finding the time at which firing had returned to 50% of control. For each trial, significant differences in the period of inhibition during ethanol and sucrose, administered at the same pressure were determined by Student's paired t-test ($p < 0.05$).

III.E.4. Ethanol on Local GABA Inhibition

To test the effect of ethanol on the inhibition produced by local application of GABA, Purkinje cell single unit activity was recorded from lobules IV and V of the cerebellar cortex. When ethanol was infused intravenously GABA was applied by iontophoresis. After a Purkinje cell had been located, GABA was applied under microcomputer control for 20 s at 1 min intervals. After a series of control responses, ethanol was infused (1.5 g/kg, i.v. over 10 min). When ethanol was given locally, both GABA (10 s at 30 s intervals) and ethanol (over 10 GABA trials, i.e. 5 min) were applied by micropressure. In both sets of experiments, the application of GABA was adjusted to give submaximal depressions of cell firing.

Each group of five consecutive responses to GABA were averaged to calculate one inhibition value. This was calculated as absolute or percent decrease in firing rate from pre-GABA control. In the case of intravenously administered ethanol, the pointwise average of the data at each time point from all the experiments were averaged. The data from the local application

of ethanol were grouped as pre-ethanol, during ethanol, and recovery values. Significant differences in the level of inhibition from the averaged pre-ethanol controls was determined by Student's paired t-test ($p < 0.05$).

III.E.5. Ethanol on Golgi Cells

III.E.5.a. Spontaneous Firing Rate.

The effect of intravenous ethanol on Golgi cell firing rate was measured between the periods of stimulation in the protocol below (section III.E.4.b.). The data from all experimental trials were pointwise averaged at each time point. Significant difference from the average of the two pre-ethanol control rates was determined by Student's paired t-test ($p < 0.05$).

III.E.5.b. Inhibition by Inferior Olive Stimulation

The effect of ethanol on the inhibition of Golgi cells evoked by inferior olive stimulation was examined by recording Golgi cell single unit activity from lobules IV and V of the cerebellar cortex using single barrel electrodes. A bipolar stimulating electrode was positioned stereotaxically in the inferior olive (-4.5 AP, -2.5 DV, 1.8 lateral). A suitable Golgi cell was located using the criteria of a large amplitude action potential and a regular firing rate of 12-20 Hz (Schulman and Bloom, 1981). To test the effect of the ethanol on the inhibition evoked by stimulation of the inferior olive (1-2 Hz, single pulse, 0.5 ms), poststimulus histograms of the neurone's

response to the stimulation were constructed before and after ethanol administration.

The inhibitory period was measured by smoothing the PSTH and finding the time at which firing returned to 50% of control. The pointwise average at each time point from all experimental trials was then calculated. From this average significant differences from the average pre-ethanol control inhibitory period was determined by Student's paired t-test ($p < 0.05$).

IV. RESULTS

IV.A. Ethanol on Complex Spikes.

IV.A.1. Climbing Fibre-Purkinje Cell Synapse

Complex spikes (CS), are caused by afferent input from climbing fibres, which originate exclusively from the inferior olive (IO). Previously, intravenously administered ethanol has been found to reduce CS activity, however the locality of its action was not determined. This experiment was designed to test the hypothesis that this reduction in spontaneous CS activity caused by ethanol is due to climbing fibre activity failing to cross the climbing fibre-Purkinje cell synapse. To do this, climbing fibres were activated by electrical stimulation of the cerebellar white matter (juxtastagially) and the voltage required to activate CS activity measured at regular intervals. The effect of ethanol (1.5 g/kg, i.v., in divided doses over 22 min) upon this threshold was then studied.

An example of a Purkinje cell's response to this protocol is illustrated in Figure 1. Before ethanol administration, this Purkinje cell had a spontaneous simple spike rate of 58 Hz and a complex spike rate of 0.8 Hz. Administration of ethanol, 1.5 g/kg intravenously in divided doses over 22 min, resulted in the SS rate increasing to over 100 Hz and the almost complete cessation of CS activity (significant at $p < 0.001$, Student's t-Test). However, the voltage required to consistently evoke CS activity only rose from 10.9 to 11.2 V, a nonsigni-

Figure 1: An example of the effect of intravenous ethanol on the voltage for evoking a complex spike by juxtafastigial stimulation. (A,B, and C) Time histograms representing a Purkinje cell's (A) total and (B) complex spike activities, and (C) the voltage required to evoke a complex spike (C). Thresholds were measured during the time periods represented by the short bars. Cellular activity was not displayed during these periods because of contamination by the stimulation artifact. Ethanol administration was started and discontinued at the up and down arrows, respectively.

A **B** **C**

ficant change ($p > 0.2$).

This example was typical of the results from the other six experiments performed. In all cases, the voltages were unchanged ($p > 0.20$, Student's *t*-test) at those times when the spontaneous CS activity was reduced significantly ($p < 0.001$).

The action potential configurations of the complex spikes were highly variable. However, no discernable changes to their configurations were observed after the administration of ethanol. Examples of simple spike and complex spike activity is illustrated in Figure 2. It includes examples of spontaneous SS and CS, and examples of spontaneous SS and evoked CS activity before and after ethanol.

IV.A.2. Cerebral Stimulation

It may be hypothesized that the reduction of Purkinje cell CS activity by ethanol represents an action of ethanol on the source of the climbing fibres: the inferior olive. This hypothesis was investigated by two methods. In the first, the effect of intravenously administered ethanol on IO neuronal excitability was tested indirectly, by the activation of IO neurones by stimulation of the cerebral cortex.

Intravenous administration of ethanol was found to decrease the ability of cerebral cortical stimulation to evoke CS activity and to cause a parallel reduction of Purkinje cell spontaneous CS activity. An example illustrating these effects is shown in Figure 3, the total spike activity of a sample Purkinje cell, before and after ethanol, in the form of two post-stimulus histograms (PSTH). Note that the initial peak of

Figure 2: Examples of spontaneous and evoked extracellular Purkinje cell activity. (A) An oscilloscope trace showing typical spontaneous simple and complex spike activity. (B,C) Oscilloscope traces showing complex spikes evoked by juxtafastigial stimulation (B) before and (C) after ethanol (1.5 g/Kg in divided doses over 22 min). Stimulation occurred at the arrows.

A

B

C

10 ms

Figure 3: An example of the effect of intravenous ethanol on the evocation of complex spikes in a Purkinje cell by stimulation of the cerebral cortex. (Top) Peristimulus time histogram of the total pre-ethanol spike activity. (Bottom) Response of cell after ethanol (1.5 g/kg, over 10 min). Cerebral stimulation (1 Hz, 0.4 ms duration, 2-4 V) occurred at the arrow heads. Each histogram was constructed from 80 trials.

activity present in the first PSTH is absent from the second.

This effect can be seen more clearly in Figure 4, where neuronal activity has been divided into separate SS and CS activities, each represented by its own PSTH. In addition, a latency histogram was constructed that included only the first CS occurring after each stimulus. The majority of these CSs reflect activity evoked by the cerebral stimulation with only a very minor contribution from spontaneous CS activity. From this figure it is clear that the initial peak seen in Figure 3 represents evoked CS activity and that this was greatly reduced by the ethanol. This reduction is illustrated in both the post-ethanol complex spike PSTH and latency histogram. The CS activity recorded in the latency histograms, after 80 stimulations of the cerebral cortex, was reduced from 62 to 7 counts after ethanol administration. On the other hand, SS firing rate was increased from 36.7 to 51.6 Hz. Only a minor part of this increase can be attributed to the reduction of CS-induced inhibition of SS activity visible in the pre-ethanol SS histogram.

Similar responses were seen from the other cells tested and the averaged data from all of these is graphed in Figure 5. This figure illustrates that the reduction of evoked CS activity after ethanol administration is accompanied by a parallel reduction of spontaneous CS activity. Spontaneous CS activity was reduced from a pre-ethanol level of 40.2 Hz to a low level of 19.9 Hz. It was reduced significantly from 6.5 to 16.5 min after the start of ethanol administration. Similarly, evoked CS activity was reduced from 37.1 to a low of 13.3 spikes per 80 cerebral stimuli 9 min after the beginning of the ethanol

Figure 4: An example of the effect of intravenous ethanol on the evocation of complex spikes in a Purkinje cell by stimulation of the cerebral cortex. (A) Three pre-ethanol post-stimulus time histograms showing from top to bottom: simple spike activity; complex spike activity; and latency to the first complex spike after each stimuli. (B) Same as (A), except after ethanol administration (1.5 g/kg, over 10 min). The number above each set of histograms is the averaged total spike rate during the period of histogram construction. The numbers above the latency histograms are the number of evoked CS and their mean average latency and SEM (in brackets). Electrical stimulation (1 Hz, 0.4 ms duration, 2-4 V) of the cortex occurred at time zero. Each histogram was constructed from 80 trials.

A **B**

Figure 5: The effect of intravenous ethanol on spontaneous complex spike activity, and that evoked by cerebral electrical stimulation. The solid line represents the effect of ethanol on the averaged rate of spontaneous complex spike activity from nine neurones. The broken line represents the effect of ethanol on the number of complex spike evoked over 80 stimulations of the cerebral cortex (1 Hz, 0.4 ms duration, 2-4 V) in the same neurones. Each point represents the averaged data from nine Purkinje cells, and is plotted as mean and SEM. Significant difference from the pre-ethanol controls is indicated by the asterisks ($p < 0.05$, Student's paired t-Test). Ethanol (1.5 g/kg, over 10 min) was given over the period indicated by the solid bar.

administration. This reduction was significant at 4 through 22 min after ethanol administration. The mean CS latency from the stimulation was 22.6 ± 1.4 ms.

IV.A.3. The Inferior Olive

The second method used to test whether ethanol has an action on inferior olive (IO) neurones involved recording spontaneous unit activity directly from IO neurones. The effect of ethanol on this activity was tested by administering ethanol either intravenously or locally by micropressure ejection. The neurones typically had slow spontaneous firing rates of 0.4 to 3.5 Hz. Their action potential (AP) configurations were characteristic of IO neurones. An example of action potentials recorded from an IO neurone is illustrated in Figure 6. They display a long plateau phase after the initial sharp onset typically seen upon approaching the cell. Occasionally, this configuration would convert to a larger triphasic configuration if the electrode approached very close to the cell. A micrograph illustrating a recording site from one of these experiments is shown in Figure 7.

An example of an inferior olive cell's response to ethanol and to harmaline, both administered intravenously, is shown in Figure 8. The rate of discharge of this cell decreased after the ethanol and gradually recovered toward the pre-ethanol rate over the next 30 minutes. The subsequent massive discharge immediately after harmaline administration is characteristic of IO neurones.

Figure 6: Three superimposed oscilloscope traces showing typical action potentials from an inferior olive cell. Note the long plateau phase.

Figure 7: Micrograph showing a transverse section of the caudal inferior olive. The arrow points to the dye mark ejected by the recording barrel.

Figure 8: An example of an inferior olive cell's response to intravenous ethanol and harmaline. This figure represents a continuous extracellular rate recording. Ethanol was given over the period indicated by the long bar (1.5 g/kg, i.v., over 10 min) and harmaline at the arrowhead (5 mg/kg, i.v.). The vertical and horizontal calibration bars indicate 1 Hz and 3 min, respectively.

Over all, intravenous ethanol administration resulted in a major reduction of mean inferior olive neuronal firing rate. In no case was an increased firing rate observed after ethanol administration. The averaged results from these experiments are illustrated in Figure 9. As can be seen, the mean neuronal firing rate was reduced from a pre-ethanol control rate of 1.7 Hz to a low of 1.0 Hz at 9-12 min after the start of ethanol infusion. Firing rate was significantly ($p < .05$, paired Student's t-Test) different from control at all times after 6 min. Similar injections of saline did not cause any significant changes to firing rate in another four cells.

In addition to systemically administered ethanol, ethanol was also applied by micropressure ejection directly onto IO neurones and its effect on their firing rate studied. An example of an inferior olivary neurone's firing rate response to ethanol administered by micropressure is shown in Figure 10. This cell was inhibited by 8, 22, 33, and 77% by application of ethanol at pressures of 3, 7, 10, and 15 psi, respectively, while it was inhibited by only 5% when sucrose was applied at 19 psi.

Table I is a summary of the responses of the fourteen neurones which were successfully tested. The spontaneous firing of nine of the fourteen cells was decreased by ethanol. Seven of these were inhibited to a significantly ($p < 0.05$, Student's t-Test) greater extent by ethanol than by sucrose administered at an equal pressure. Two other neurones were inhibited significantly by ethanol but had no sucrose controls, allowing the possibility that these inhibitions were due solely to pressure

Figure 9: The effect of intravenous ethanol on inferior olive neuronal firing rate. Each point represents the averaged data from twelve neurones, and is plotted as mean and SEM. Ethanol was administered during the period indicated by the solid bar (1.5 g/kg, over 10 min). Significant difference ($p < 0.05$, Student's paired t-Test) from the two pre-ethanol controls is indicated by an asterisk.

(N=12)

Figure 10: An example of the effect of local ethanol on inferior olive neuronal firing. Each histogram represents the cell's firing over three minutes. Those in the rightmost column are the pointwise averages of the histograms to their immediate left. The numbers to right indicate the percent decrease in firing rate during drug application (indicated by the bars) from the corresponding control period immediately before. The number and letter to the left of each row indicate the pressure of application (psi) and the drug applied, respectively. Drug application occurred during the periods indicated by the solid bars. The vertical and horizontal calibration bars are 1 Hz and 1 min, respectively. C - control, i.e. no drug application; S - sucrose control; E - ethanol.

 TABLE I: Summary of the effect of local ethanol on the spontaneous firing rate of inferior olive neurones.

Response	Number of neurones
-----	-----
Decreased Firing Rate	
Significantly More than Sucrose	7
Decreased Firing Rate	
but No Sucrose Controls	2
Increased Firing Rate	0
No Change	5

Total Number of Neurones	14
-----	-----

effects. In the remaining five neurones, ethanol did not have a significantly different effect than did sucrose at equal pressures of application. In three neurones, ethanol produced a reduction of spike amplitude at higher application pressures, possibly indicating a nonspecific effect. However, in no case did ethanol increase the firing rate above that of its sucrose control.

IV.B. Ethanol on Simple Spikes

There appears to be an inverse correlation between simple and complex spike activities. For example, stimuli which increase CS activity, such as harmaline or electrical stimulation, result in a reduction of SS activity. Conversely, treatments which decrease CS activity, such as ethanol or 3-acetylpyridine, result in increased SS activity. Therefore, the increase of SS activity seen after ethanol administration may be due to a direct action of the ethanol on the neurone. Another possibility is that it is due to an indirect action such as ethanol's reduction of CS activity. Similarly, ethanol has been reported to reduce the variability of SS firing (Sinclair et al., 1980). Again, this may be due to a direct or indirect action of ethanol.

Therefore, the following experiments were designed to remove the influence of CS by eliminating their activity via the electrolytic lesioning of the inferior olive. Figure 11 illustrates an example of a unilateral inferior olive lesion. Although each lesion halted the CS activity in the PC under study, histological examination found that only three of the

Figure 11: A micrograph of a transverse section through the inferior olive showing a unilateral electrolytic lesion.

lesions appeared to completely destroy the IO nucleus. Therefore, climbing fibre input to the cerebellar cortex in most trials was probably not completely eliminated. However, this treatment did allow the effects of ethanol on SS rate and regularity to be investigated in the absence of CS activity.

An example of the response of a Purkinje cell to IO lesioning and the subsequent intravenous administration of ethanol is shown in Figure 12 as several interspike interval histograms (ISI). Initially, this neurone had a mean spontaneous SS and CS rates of 14 and 1.3 Hz, respectively. These rates are reflected in Figure 12 as SS-SS and CS-CS interspike intervals of 57.1 and 775 ms, respectively. Lesioning of the inferior olive caused the complete cessation of CS activity, and increased the SS firing rate to 33.2 Hz, which corresponded to an ISI of 30.2 ms. Intravenous ethanol further increased SS firing to 42.3 Hz and reduced the ISI to 23.65 ms.

This result was repeated in all but two of the other ten Purkinje cells tested. Figure 13 shows the averaged SS firing rate over time from all eleven Purkinje cells, and the effect of inferior olive lesioning and ethanol administration. The decreased ISI after IO lesioning was not significantly ($p > 0.05$, paired Student's t-Test) different from the pre-lesion values. However, the further reduction after ethanol administration was significantly ($p < 0.05$) different from the average of the two post-lesion pre-ethanol points.

In addition to measuring the effects of IO lesioning and ethanol on SS rate, an attempt was made to measure the effect of these two treatments on the regularity of SS firing in

Figure 12: The response of a Purkinje cell to electrolytic lesioning of the inferior olive and to the intravenous administration of ethanol. (A) Two interspike interval histograms of (top) simple and (bottom) complex spikes before inferior olive lesioning and ethanol administration. (B and C) Simple spike interspike interval histograms: (B) immediately after inferior olive lesioning and (C) 18 minutes after the start of ethanol administration. The numbers above each histogram represent the mean interval between two successive spike of the same type and SEM (in brackets), and the number (N) of intervals used to calculate them. Means were calculated with intervals up to 250 ms but were plotted to 200 ms only. Counts at greater than 200 ms were added into the bin at 200 ms.

Figure 13: The effect of inferior olive lesioning and intravenous ethanol administration on the mean interspike interval between Purkinje cell simple spikes. Each point represents the averaged data from eleven Purkinje cells, and is plotted as mean and SEM. The inferior olive was lesioned at the time denoted by the arrow head (at time -4 min), and ethanol was administered during the period indicated by the bar (1.5 g/kg, over 10 min). The interspike intervals were normalized around the two lesioned controls (i.e. times -2.5 and zero min).

IV ETHANOL ON PC'S AFTER IO LESIONING
(o) (N=11)

these same Purkinje cells. To do this, the scaled variance of the mean interspike interval from all cells was averaged at each time point and represents a measure of SS irregularity. The resulting curve is displayed in Figure 14. Although SS irregularity was reduced after IO lesioning, from 9.2 to 2.2, the reduction was not significantly different from pre-lesion values ($p < 0.05$, paired Student's t-Test). Similarly, intravenous ethanol also had no significant effect upon SS irregularity.

IV.C. Ethanol on Local Inhibition.

This laboratory has reported that intravenous ethanol antagonizes the inhibition evoked by the local surface stimulation of the cerebellar cortex. The following experiments were designed to further investigate the effect of ethanol on this inhibition by applying ethanol locally by micropressure.

An example of a Purkinje cell's response to local stimulation (LOC), and the effect of locally applied ethanol on the resulting inhibition, is shown in Figure 15. In this case, ethanol shortened the inhibitory period, from 34.0 to 13.3 ms, at a dose at which sucrose had little effect (35.5 ms).

This example was representative of the majority of cells tested in this manner, and Table II summarizes the results of these experiments. Eight of the twelve cells were found to have shortened inhibitory periods after ethanol. Seven of these were shortened significantly ($p < 0.05$). In the eighth, significance could not be calculated, but its inhibitory period was reduced 55% by application of ethanol at 12 psi, while sucrose at the same pressure increased firing by only 4%. Another

Figure 14: The effect of inferior olive lesioning and intravenous ethanol on Purkinje cell simple spike regularity over time. Each point represents the averaged data from 11 cells, and is plotted as mean and SEM. The inferior olive was lesioned at the time denoted by arrow head (-4 min), and ethanol was administered during the period indicated by the solid bar (1.5 g/kg, over 10 min). The ordinate represents the variance of the interspike interval divided by its mean and multiplied by 1000. The points were normalized to the average of the two lesioned controls (i.e. time points -2.5 and zero).

Effect of i.v. Ethanol on SS Regularity
(o) After IO Lesioning (n=11)

Figure 15: The effect of local ethanol on the inhibition of a Purkinje cell evoked by local stimulation. The three poststimulus histograms represent responses (A) before drug application, (B) during sucrose application (12 psi), and (C) during ethanol application (12 psi). Local stimulation occurred at time zero. The resulting histograms were smoothed by a running average (25 ms width) and the time to 50% of the rate during the last 25 ms measured (I-50%). This time is indicated by the arrow and quantitated by the number above each histogram. Electrical stimulation (1 Hz, 0.5 ms duration, 5.5 V) of the cerebral cortex occurred at time zero. Each histogram was constructed from 50 trials.

 TABLE II: Summary of the effect of local ethanol on the period
 of inhibition evoked by local stimulation of the cerebellar
 cortex.

Response	Number of neurones responding
Decreased Inhibitory Period	8
Increased Inhibitory Period	1
No Change	3

Total Number of Neurones	12

cell's inhibitory period was lengthened significantly over its sucrose control, while the inhibitory periods of the three remaining cells were not significantly changed.

IV.D. Ethanol on Local GABA Inhibition.

The putative transmitter responsible for the inhibition evoked by surface stimulation is thought to be gamma-aminobutyric acid (GABA, Woodward et al., 1971). As was reported above, this inhibition is antagonized by ethanol. Therefore, the effect of ethanol on the inhibition of PC induced by locally applied GABA was studied. The following experiments included protocols in which ethanol was administered either intravenously or locally by micropressure.

An example of the effect of intravenously administered ethanol on the inhibition of a Purkinje cell's SS firing by locally applied GABA is illustrated in Figure 16. In this example the ethanol appears to have antagonized the inhibitory action of GABA since the amount of inhibition evoked by GABA was reduced after the administration of the ethanol. Initially, each application of GABA reduced cell firing by about 82%. Six to ten minutes after the start of ethanol administration, this inhibition was reduced to 65% but had returned to control levels (83%) by about 23 min.

Figure 17 summarizes the results of this set of experiments. It shows the averaged response of 12 Purkinje cells to locally applied GABA and intravenously administered ethanol (1.5 g/kg, i.v. over 10 min). Before ethanol administration, the mean reduction of cell firing produced by each GABA application

Figure 16: An example of the effect of intravenous ethanol on the inhibition of Purkinje cells induced by GABA applied locally by micropressure. The main block of 35 histograms represents a continuous record of the cell's firing rate. Each of the histograms in the rightmost column is the pointwise average of the five histograms to its immediate left. The number to its right is the percent inhibition produced by the application of GABA (for 20 s during the periods indicated by the bars) from the control period (the 20 s immediately preceding the bar). The long bars indicate the period of application of ethanol (1.5 g/kg, i.v. over 10 min). The vertical and horizontal calibration bars represent 25 Hz and 20 sec, respectively.

Figure 17: The effect of intravenous ethanol on the inhibition of Purkinje cells induced by local GABA. The solid line represents the spontaneous firing rate during the periods when GABA was not being applied. The broken line represents the amount of inhibition evoked by application of GABA (percent decrease of firing rate from the immediately preceding 20 seconds). Each point represents the averaged data from twelve neurones, and is plotted as the mean and SEM. Ethanol was administered during the period indicated by the solid bar (1.5 g/kg, i.v. over 10 min). Significant difference from the averaged pre-ethanol controls is indicated by asterisks ($p < 0.05$, Student's paired t-Test).

Effect of i.v. Ethanol on

(x) PC Firing Rate (n=12)

(o) up-GABA Inhibition of PC (n=12)

was 65.8%. Six to ten minutes after the start of ethanol administration this value had fallen to 54.0%. By 25 min it had returned to a value of 58.1%. The observed reduction of the inhibition evoked by GABA was significantly ($p < 0.05$, paired Student's t-Test) reduced at the time point between 6 and 20 minutes.

An example of the effect of locally applied ethanol on the inhibition of PC firing by exogenous GABA is shown in Figure 18. In this cell application of ethanol by micropressure reduced the GABA evoked inhibition from a pre-ethanol value of a 76% reduction in firing rate to a 63% reduction at 2.5 to 7.5 minutes after the beginning of ethanol application. By thirteen minutes the inhibition had returned to pre-ethanol levels (76%).

Figure 19 summaries the effect locally applied ethanol had on absolute inhibition of Purkinje cell firing produced by GABA. It illustrates the averaged results from nine Purkinje cells. As indicated by the asterisks, the inhibition by GABA was only significantly antagonized from 2.5 to 5 minutes after the start of ethanol application. GABA reduced the mean firing rate of the cells by 15.3 Hz before ethanol was applied, but only reduced it by 12.7 Hz at 2.5 to 5 minutes. However, the ethanol application also caused a significant fall in PC spontaneous firing rate, from 27.8 Hz before ethanol to 23.8 Hz at 2.5 to 5 minutes after the start of application. Therefore, the reduction of GABA induced inhibition, when expressed as a percent change from control, was not significantly changed by the ethanol application (Figure 20).

Figure 18: An example of the effect of local ethanol on the inhibition of a Purkinje cell induced by local GABA. The block of 30 histograms represents a continuous rate recording of the neurone. Each histogram in the rightmost column is the point-wise average of the five histograms to its immediate left. The associated number represents the percent inhibition produced by the application of GABA (for 10 s during the periods indicated by the bars) from the control period (the 10 s preceding the bar). The long and short horizontal bars indicate the period of application by micropressure of ethanol and GABA, respectively. The vertical and horizontal calibration bars represent 25 Hz and 10 s, respectively.

76
68
63
63
66
76

Figure 19: The effect of locally applied ethanol on the absolute inhibition of Purkinje cells induced by locally applied GABA. The leftmost bar of each pair represents spontaneous firing rate of the neurones during those 20 s periods immediately before GABA application, while the rightmost represents the averaged absolute inhibition of firing rate evoked by the 20 s application of GABA. Each bar represents the data from nine neurones over 2.5 min. The bars represent mean and SEM. Ethanol was applied during the collection of the central bars. The left pair of bars are the control rate and inhibition, while the right pair is the recovery values at 5-7.5 after the application of ethanol. Bars significantly different from control are indicated by asterisks ($p < 0.05$, paired Student's t-Test).

Figure 20: The effect of local ethanol on the relative inhibition of Purkinje cells induced by locally applied GABA. The data was calculated as the percent decrease of simple spike firing during 20 s of application of GABA by micropressure from the immediately preceding control 20 s. Each bar represents the averaged data from nine neurones and over 2.5 min, and is plotted as the mean and SEM. Ethanol was applied by micropressure during collection of the data making up the middle pair of bars. None of the bars are significantly different from their controls ($p > 0.05$, paired two-tailed Student's t-Test).

(N=9)

The results from this series of experiments are summarized in Table III. The GABA-induced absolute decrease of firing rate was reduced in the majority of Purkinje cells (6/9) but unchanged in the remaining third of the cells. However, the inhibition, when expressed as percent decrease from control, was reduced in only four of the nine neurones, while in two other cells it was unchanged and in the remaining three it was enhanced.

IV.E. Ethanol on Golgi Cells.

Golgi cells are an integral part of the cerebellar circuitry and their responses to ethanol have not been previously studied. Therefore, these experiments were designed to study the effect of ethanol on the spontaneous firing rate of Golgi cells and its effect upon the inhibition of these cells by inferior olive stimulation.

Figure 21 summarizes the effect of ethanol on Golgi cell spontaneous discharge. It shows the averaged response over time of five Golgi cells to intravenously administered ethanol. The cells' averaged firing rate was not significantly different from the average of the pre-ethanol rates ($p > 0.05$, paired Student's t-Test) at any time.

An example of the effect of intravenously administered ethanol (1.5 g/kg, over 10 min) on the period of inhibition of a Golgi cell evoked by stimulation of the inferior olive is illustrated in Figure 22. The long lasting period of inhibition in this cell was reduced by ethanol, from 774 to 600 ms, at 5 minutes after the start of ethanol administration. By 21 min-

TABLE III: Summary of the effect of local ethanol on the inhibition of Purkinje cells evoked by locally administered gamma-aminobutyric acid.

Response	Number of neurones	
	Absolute Inhibition	Relative Inhibition
Decreased Inhibition (>10% change)	6	4
Increased Inhibition (>10% change)	0	2
No Change (<10% change)	3	3
Total Neurones	9	9

Figure 21: The effect of intravenous ethanol on the spontaneous firing rate of Golgi cells. Each point represents the averaged data from five cells, and is plotted as the mean and SEM. Ethanol was administered during the period indicated by the solid bar (1.5 g/kg, over 10 min). None of the points are significantly different from the average of the two pre-ethanol controls ($p > 0.05$, paired Student's t-Test).

EFFECT OF IV ETOH ON
(o) GOLGI CELL FIRING RATE (N=5)

Figure 22: An example of the effect of intravenous ethanol on the inhibition of a Golgi cell evoked by stimulation of the inferior olive. The three post-stimulus histograms were constructed (A) pre-ethanol and (B) 5 min and (C) 21 min after start of ethanol. In each of the histograms the period of inhibition was measured by smoothing the histogram with a moving average (250 ms width) and determining the I-50%, the time at which the firing rate returned to 50% of the control period (the rightmost 25 points). This time is indicated by the arrow and quantified by the value above each histogram. The inferior olive was electrically stimulated (single pulse, 1-2 Hz, 0.5 ms) at time zero. Ethanol was administered intravenously over 10 min (1.5 g/Kg).

utes it had returned to 747 ms, although in this case the firing rate had decreased.

The graph of the averaged data from these experiments is shown in Figure 23. The average latency to the start of the inhibitory period was about 70 ms (range 53-80 ms). The period of inhibition evoked by IO stimulation was significantly ($p < 0.05$, paired Student's t-Test) reduced after the intravenous administration of ethanol at 10, 15, and 20 min, reducing the inhibitory period from 520 to 412, 412, and 436 ms, respectively. By forty minutes it had almost returned (503 ms) to the pre-ethanol value.

Figure 23: The effect of intravenous ethanol on the inhibition of Golgi cells evoked by stimulation of the inferior olive. Each point represents the averaged data from five cells, plotted as the mean and SEM. Ethanol (1.5 g/kg) was administered during the period indicated by the solid bar. The inferior olive was electrically stimulated (single pulse, 1-2 Hz, 0.5 ms) at time zero. The asterisks indicate those time points at which the mean period of inhibition differed significantly from the pre-ethanol control ($p < 0.05$, paired Student's t-Test).

EFFECT OF IV ETOH ON
(o) IO STIM INHIBITION OF GOLGI (N=5)

V. DISCUSSION

V.A. Ethanol on Complex Spikes

Spontaneous complex spike activity has been reported to be reduced after intravenous ethanol administration. We hypothesized that this action of ethanol was due to a failure of the climbing fibre-Purkinje cell synapse to transmit the activity arriving on the climbing fibre. To test this hypothesis, complex spike activity was evoked by juxtafastigial electrical stimulation, and the effect of ethanol upon this evoked activity studied. The hypothesis predicts that spontaneous and stimulation-evoked complex spike activity would be reduced in parallel.

In fact, stimulation-evoked CS activity was not effected by ethanol infusion, even at those times when spontaneous CS activity was reduced significantly ($p < 0.001$). This result implies that ethanol does not reduce the security of the climbing fibre-Purkinje cell synapse. This is not unexpected as the CF innervation of the Purkinje cell is extensive, covering the dendritic tree and forming multiple synapses. This results in a high degree of synaptic security. Indeed, when Purkinje cells are damaged, even to such an extent as to cause complete cessation of SS activity, CS activity often continues for an extended time. It is, therefore, not surprizing that ethanol was ineffective in decreasing the safety of this synapse. In addition, the stability of the voltage required for CS activation implies that ethanol also did not effect the ability of climbing fibres to be activated by the electrical stimulation.

Complex spike activity has also been reported to be evoked by stimulation of the cerebral cortex (Freedman et al., 1977; Hoffer et al., 1978). In a set of experiments similar to the above, complex spike activity was evoked from the cerebral cortex, and the effect of intravenous ethanol measured. Intravenous ethanol decreased spontaneous CS activity significantly, confirming previous reports (Sinclair et al., 1980; Sinclair and Lo, 1981). Electrical stimulation of the cerebral cortex evoked CS activity with an average latency of 22.6 ± 1.4 ms, again confirming previous reports (for example Freedman et al., 1977). Ethanol was found to reduce significantly the ability of the cortical stimulation to evoke CS activity. Together with the finding above, this result implies ethanol's site of action is intermediate between the site of stimulation and the source of the climbing fibres, the inferior olive.

The reduction of evoked CS activity could reflect a reduced recruitment of cortical fibres by the stimulation, an inhibition of synapses interposed between the site of stimulation and the IO, or a direct action on IO neurones. The former two actions would presumably reduce the excitatory input to the inferior olive and consequently decrease CS rate. An action on interposed synapses is the more likely of the two possibilities since, while ethanol has been reported to increase the sensitivity of isolated nerves to stimulation (Armstrong and Binstock, 1964), polysynaptic pathways are quite sensitive to ethanol (cf. Kalant and Woo, 1981). However, since cortical projections account for only a portion of the total input to the IO, both of these mechanisms would be unlikely to produce the parallel

reduction of spontaneous and evoked CS activity seen in this study. Therefore it can be postulated that the ethanol was depressing either the total input to the IO, for example by inhibiting the synapses terminating on its neurones, or the inferior olive neurones themselves.

This proposal is supported by the results of the experiments where the effect of locally applied ethanol by micropressure on IO neuronal firing rate was studied. Again, the reduction of spontaneous activity observed in these experiments may have been due to a direct action of ethanol upon the inferior olive neurones, or may have reflected a modification of afferent input to these neurones, either on nearby neurones or upon the synapses impinging on the IO neurones. However, it is clear that ethanol's action must be in the immediate area of recording. The reduction of spontaneous activity by local ethanol may have reflected a non-specific inhibition of the neurones since local application may have produced concentrations at the cellular membrane high enough to cause non-specific depression of the neurone. However, although action potential configuration changes were noted in a few cells, the correspondence between the systemic and local results implies that ethanol has depressant effects on IO neuronal activity at levels which result in mild to moderate intoxication.

In any event, in none of the trials, with either systemically or locally administered ethanol, was a significant increase in IO neuronal firing rate observed. This is in direct conflict with the data reported by Rogers et al. (1980, 1982). Specifically, they have reported an increased PC activity after

intraperitoneal injection of ethanol. They reported that intraperitoneal ethanol increases both CS rate, recorded from single unit recordings from Purkinje cells (1-4 g/kg, Roger et al., 1980), and single and multiple unit activity and field potentials recorded from the inferior olive (2 g/kg, i.p., Roger et al., 1982).

In a series of experiments which repeated Rogers' et al.'s experimental parameters, including halothane anaesthesia, Sinclair et al. (1982) found that ethanol (4 g/kg, i.p.), in respired rats, failed to increase CS activity, but rather decreased it, thus confirming their previous findings (Sinclair et al., 1980; Sinclair and Lo, 1981). In addition, between 7 and 18 minutes after the ethanol injection most of the cells (6/8) exhibited a bursting behavior. However, the configuration of the spike bursts did not resemble those of typical complex spikes. Also they did not appear to be due to the anoxia because reducing or halting artificial respiration also halted the bursting behaviour. The majority of non-respired animals died within 15 minutes of injection. It was concluded that the intraperitoneal injections of ethanol, which resulted in very high blood ethanol levels (about 500 mg/100 ml at 9 min after injection), caused bursting behaviour in the Purkinje cells. Indeed, non-uniform blood ethanol levels have been reported to result from intraperitoneal administration by Rogers laboratory (Bloom et al., 1982) and other reseachers have expressed reservations about this route of administration (Grupp, 1980). Roth (1980) has reported that anaesthetic agents, including ethanol, can cause bursting behaviour in an isolated crayfish

stretch receptor at concentrations (520 mM) just below those which cause complete depression. Purkinje cell bursting behaviour has been described by Llinas and Sugimori (1980a,b) in cerebellar tissue slices. They reported that this behaviour was based upon dendritic calcium conductances. Therefore, it can be speculated that the bursting behaviour reported by Sinclair and Lo (1982) was due to high concentrations of ethanol and altered Purkinje cell calcium conductances, and was not due to an increased CF activity.

The disparity between the effect of ethanol on inferior olive spontaneous activity found in this study and that found by Rogers et al. (1982) may also reflect ethanol level differences, although the dose used in these latter experiments was relatively low (2 g/kg, i.p.). Alternately, they may represent a dissimilarity in the cell populations studied. Rogers et al. (1982) verified their neurones histologically and/or physiologically, by stimulation of the contralateral hindpaw. Similarly, we are confident that the population of cells in this study are IO neurones since the neuronal firing and action potential characteristics, the effect of harmaline, and the histological examinations all indentified the cells as inferior olive neurones. Unfortunately, Roger et al. (1982) did not include details of the characterization of the neurones that were studied. Resolution of these discrepancies will have to wait further studies.

V.B. Ethanol on Simple Spikes

There have been several reports that ethanol causes increased simple spike (SS) rate and regularity in Purkinje cells (PC), both in vitro (Seil et al., 1977) and in vivo (Sinclair et al., 1980; Sinclair and Lo, 1981), and decreased CS activity in vivo (Sinclair et al., 1980; Sinclair and Lo, 1981). Contrary to these reports some workers have found ethanol to reduce PC total spike activity (Eidelberg et al., 1971) or increase CS activity (Rogers et al., 1980). However, evoked complex spike (CS) activity has been reported to also reduce SS activity and regularity (Ferin et al., 1971; Colin et al., 1980). Therefore, it was not clear whether the observed increases in SS rate and regularity induced by ethanol were due to a direct effect of this drug or to an indirect effect such as via its depression of CS activity.

To study this question, the effects of CS activity upon SS rate and regularity were eliminated by the electrolytic lesioning of the inferior olive. Although lesioning caused an increased firing rate, the SS interspike interval (ISI) was not significantly different from the pre-lesion interval. The lack of significance reflects the large variance in pre-lesion spontaneous CS rate. However, ethanol was still able to produce a significant decrease in ISI from the post-lesion. This suggests that the action of ethanol upon a neurone's SS rate is not wholly due to its effects upon that neurone's CS activity, but rather to a direct action of ethanol on the Purkinje cell or an indirect effect upon its other afferent input. However, recent

studies have indicated that each Purkinje cell is under the (diffuse) inhibitory influence of many climbing fibres (Ebner and Bloedel, 1981; Leonard and Simpson, 1982; Montarolo et al., 1982). Ebner and Bloedel (1981) reported that neighbouring PC activities were correlated such that the two Purkinje cells had coincident reductions of SS activity. Montarolo et al. (1982) reported that the increase in rate observed during IO cooling was dependent upon a active population of IO neurones. Purkinje cells from rats with 3-acetylpyridine lesioned IO nuclei did not increase their firing rates significantly upon IO cooling. Therefore, incomplete lesions may explain the results in the present study since any depression of activity by ethanol in those olivary neurones remaining could have produced the increased rate seen after ethanol administration. As the electrolytic lesions did not completely destroy the contralateral IO, it is possible that this is indeed the explanation. Complete lesioning of the olivary nuclei, with 3-AP for example, will be necessary to better evaluate the effect of ethanol on Purkinje cells in the absence of CS activity.

No significant difference from control was found in the measure of regularity calculated in these studies. This may simply reflect the inadequacy of the measure of variability used and may not reflect the true situation. Other workers have observed increased regularity associated with reduced CS activity induced by 3-AP (Colin et al., 1980) or ethanol (Sinclair et al., 1980; Sinclair and Lo, 1981). However, these observations were not quantitatively analyzed. The variance of a ISI is automatically more constrained at higher SS frequencies due to

the shorter ISI. Therefore, the increase in regularity observed by these workers may have been an artifactual consequence of the increased firing rate, the reduced numbers of inactivation responses, and the reduced asynchronous input to Purkinje cells as a consequence of the reduced CS activity. However, any SS regularity increase beyond these effects, if it occurred, may be explained by the inhibitory action exerted by the IO on Golgi cells (see Section V.E).

V.C. Ethanol on Local Inhibition

Purkinje cells are known to be inhibited by local stimulation (LOC) of the cerebellar cortex. This inhibition is thought to be mediated by the transmitter GABA. Ethanol has been previously found to modify GABA function and transmission (Nestor^o, 1980a,b; Newlin et al., 1981). LOC inhibition has been reported to be antagonized by ethanol since intravenously administered ethanol reduced the period of LOC inhibition (Sinclair and Lo, 1981). This latter finding was confirmed, and extended, in this study: locally applied ethanol also significantly reduced LOC inhibition in the majority of the neurones.

The lack of antagonism seen in three of the neurones may have been due to a mismatch between the position of the electrode tip and that of the nerve terminals mediating the inhibition. Such a displacement could be expected to result in ethanol not reaching an effective concentration at its site of action, since ethanol concentration would have fallen exponentially with distance from the electrode. This problem would have been exacerbated with somatic recording if the site of nerve

termination is the dendritic tree, such as is the case with stellate cell innervation of Purkinje cells (Eccles et al., 1967). Obviously, systemic administration results in a more uniform distribution of ethanol and does not pose this problem to the investigator.

In one experimental trial, LOC inhibition was prolonged by the application of local ethanol. As this neurone had no superficial differences from the other neurones tested, this may just represent an anomalous finding.

Previous studies have reported ethanol to variously antagonize (Backstrom, 1977; Sinclair and Lo, 1981), have no effect (Lake et al., 1973), or enhance (Nestor^os, 1980a,b; Newlin et al., 1981) putative GABA transmission or exogenous GABA-evoked inhibition in nonspinal systems. In addition, presynaptic inhibition appears to be enhanced by ethanol, at least in the spinal cord (Miyahara et al., 1966; Davidoff, 1973), although Eidelberg and Wooley (1969) found ethanol not to have a significant effect. Nestor^os concluded that in his system (cerebral cortical cells) ethanol was acting post-synaptically.

There is some experimental evidence to suggest that GABA receptors may differ over the brain. GABA receptors have been divided into two subtypes on the basis of their affinities and distributions (Costa, 1979). Only one of the subtypes shows benzodiazepine binding (Braestrup et al., 1980). Therefore, differences of ethanol-GABA receptor interactions between various areas of the brain may account for the differences between studies.

Alternatively, one may postulate that the ethanol induced antagonism of LOC inhibition seen in this study is due to ethanol's modification of transmitters other than GABA. Since taurine is a proposed transmitter for stellate cells (Okamoto et al., 1983b) it is plausible that at least some of the LOC inhibition is mediated by this transmitter. It is therefore possible that ethanol is antagonizing LOC inhibition by modifying the activity of this transmitter, for example by decreasing its release or reducing the affinity of its receptor. Another possibility is that ethanol enhances the release of taurine which in turn inhibits the release of GABA, since taurine has been reported to decrease the depolarization-induced calcium-dependent release of GABA from guinea pig cerebellar slices (Nanima, 1983).

Similarly, locally applied noradrenaline (NA) has been reported to enhance the GABA-induced inhibition of Purkinje cells (Moises et al., 1979; Moises and Woodward, 1980) and LOC inhibition (Hoffer et al., 1978) at doses that do not significantly depress spontaneous firing. In addition, ethanol has been reported to inhibit NA release from the cerebral cortex (Carmichael and Isreal, 1975) and to antagonize NA-evoked inhibition of cortical cells (Nesteros, 1980a,b) and Purkinje cells (Sorensen et al., 1981). Therefore, ethanol may antagonize the LOC inhibition of Purkinje cells by reducing the NA-mediated enhancement of the inhibition by either reducing NA's release or antagonizing its post-synaptic actions.

V.D. Ethanol on Local GABA Inhibition

Ethanol, administered either systemically by slow intravenous infusion or locally by micropressure ejection, was found to antagonize the inhibition of firing caused by exogenous GABA applied locally by micropressure. This result supports the finding that locally applied ethanol antagonized LOC inhibition, since this inhibition is believed to be GABA-mediated (Woodward et al., 1971). In addition, it suggests that ethanol was antagonizing GABA's actions post-synaptically.

Other workers have reported that the ethanol enhanced (e.g. Nesteros, 1980a,b), had no effect (Lake et al., 1973), or antagonized (Backstrom, 1977) the actions of locally applied GABA. For example, Nesteros (1980a,b) reported that electro-osmotically released ethanol enhanced the inhibition of cerebral cortical cells by iontophoresis, and also enhanced that released neuronally by electrical stimulation. Similarly, ethanol (3 g/kg, i.p.) prolonged the inhibition of CA3 neurones of the hippocampus evoked by stimulation of the dentate mossy fibre or commissural pathways, which is thought to be mediated by GABA. The disagreement of these results with those of the present study may be due to different populations of GABA receptors mediating the observed effects. In addition, the antagonism of GABA-mediated inhibition by intravenous ethanol seen in the present study may reflect a modification of NA function, since, as well as the mechanisms outlined above in Section V.C., the following reports suggest a possible mechanism of action: (1) ethanol transiently inhibits locus coeruleus (LC) neurones

(Pohorecky and Brick, 1977); (2) the LC projects noradrenergic fibres to the cerebellar cortex (e.g. Hoffer et al., 1973); and (3) LC stimulation enhances Purkinje cell's inhibition by exogenous GABA (Moises and Woodward, 1980). It can therefore be postulated that intravenously administered ethanol depresses LC neurones and, as a consequence, reduces a tonic LC-mediated enhancement of GABA-induced inhibition of Purkinje cells.

Locally applied ethanol produced a significant decrease of Purkinje cell spontaneous firing rate. This complicated the interpretation of the data. Locally applied ethanol produced a significant absolute- but nonsignificant percent-decrease of GABA-evoked inhibition. Difficulties in interpretation in such situations has been expounded by Soja and Sinclair (1983). An antagonism of GABA-mediated inhibition by local ethanol could support a non-pre-synaptic site of action for this effect, by modification of the post-synaptic receptor, uptake, or other modulators of GABA action such as NA. On the other hand, a lack of antagonism would suggest a pre-synaptic site of action or a differential effectiveness of drug action due to a differential distribution of ethanol and GABA. These mechanisms could be differentiated by the use of available pharmacological tools and intracellular recordings.

V.E. Ethanol on Golgi Cells

Ethanol had no significant effect on the spontaneous firing rate of the Golgi cells in this study. However, ethanol caused a significant decrease in the period of inhibition evoked by stimulation of the inferior olive.

The inhibition of Golgi cells by IO stimulation confirmed the results of Schulman and Bloom (1981), and indicates that Golgi cells are under an inhibitory influence from the IO. Although the spontaneous firing rate of IO neurones is slow, the long duration of the inhibition seen in these experiments would suggest that Golgi cells are tonically inhibited by the IO. The inhibition does not represent a monosynaptic innervation of these cells by climbing fibres since the long latency (about 70 ms) rules this out. Also, such an innervation would be expected to be excitatory in analogy to the response of climbing fibres on Purkinje cells, unless climbing fibres release more than one transmitter. More probably the inhibition is the result of a multisynaptic pathway to the Golgi cells via cerebellar interneurons.

This inhibition could not mediate the inhibitory influence that climbing fibres appear to exert over Purkinje cells (see Section V.A.1.) as it would result in an increased parallel fibre input to the Purkinje cells, not the decreased input required. However, it may mediate the reduction of PC simple spike regularity that has been reported with ethanol (Sinclair et al., 1980) and the cessation of CS activity with 3-acetylpyridine (Colin et al., 1980), although not found in this study. Elimination or reduction of the total climbing fibre activity would tend to disinhibit the cerebellar Golgi cells such that the Purkinje cells' asynchronous input, via the granule cells and their parallel fibres, would be decreased. This would be expected to increase the regularity of the Purkinje cell SS firing as it would result in a reduction of the

asynchronous input to the cell. The results of this study may support such a hypothesis since intravenously administered ethanol may reduce a tonic IO-mediated inhibition of Golgi cells and hence reduce the asynchronous input to Purkinje cells via the granule cells and their parallel fibres.

VI. CONCLUSIONS

- 1.a. Infused ethanol (1.5 g/kg, i.v. over 22 min in divided doses) significantly reduced spontaneous CS activity ($p < 0.001$) but did not effect the ability of juxtafastigial stimulation to evoke CS activity. In addition, the activation of climbing fibres was not significantly effected by the ethanol ($p > 0.05$).
- b. Infused ethanol (1.5 g/kg, i.v. over 10 min) significantly reduced the CS activity evoked by electrical stimulation of the cerebral cortex in parallel to its significant reduction in spontaneous CS activity ($p < 0.05$).
- c. Ethanol, administered both intravenously and locally by micropressure, reduced the spontaneous firing of IO neurones.

These results strongly suggest that ethanol has an action local to the inferior olive to reduce the excitability of IO neurones. This action could be either on the input to the neurones or directly on the neurones themselves.

- 2.a. Electrolytic lesioning of the IO caused a decreased SS to SS interval and increased SS regularity, although neither were reduced significantly ($p > 0.05$).
- b. Subsequent perfusion of ethanol (1.5 g/kg, i.v. over 10 min) significantly reduced the interspike interval, but had no significant effect on SS regularity.

These results indicate that at least part of the increased spontaneous SS rate was due to a mechanism which was independent

from the reduction of CS activity by ethanol. However, this increase may still reflect a reduction by ethanol of the total CF activity.

- 3.a. Ethanol applied onto PCs locally by micropressure reduced the period of inhibition evoked by local electrical stimulation of the cerebellar cortex.
- b. Ethanol, administered both systemically and locally, antagonized the inhibition of PCs evoked by the local application of GABA by micropressure, in the majority of the cells tested.

These results show that ethanol can antagonize the actions of both endogenous and exogenous GABA, but may reflect pre- or post-synaptic actions of ethanol.

- 4.a. Infused ethanol (1.5 g/kg, i.v. over 10 min) had no significant effect on spontaneous Golgi neuronal firing.
- b. Infused ethanol significantly reduced the period of inhibition evoked by electrical stimulation of the IO.

These results suggest that some of the effects of ethanol on the olive-Purkinje cell axis may be mediated by ethanol's action on input to Golgi cells from the IO.

VII. BIBLIOGRAPHY

- ALBUS, J. S. (1971). A theory of cerebellar function. *Math. Biosci.* 10: 25-61.
- ALEXANDER, R. W., DAVIES, J. N. and LEFKOWITZ, R. J. (1975). Direct identification and characterization of beta-adrenergic receptors in rat brain. *Nature (Lond.)* 233: 437-440.
- AMENTA, F., CAVALLOTTI, C., DE ROSSI, M. and FERRANTE, F. (1981). Direct immunohistochemical detection of binding sites for beta-blocker within rat cerebellum. *Neurosci. Lett.* 24: 59-63.
- ANGAUT, P. and CICIRATA, F. (1982). Cerebello-olivary projections in the rat. *Brain Behav. Evol.* 21: 24-33.
- ARMSTRONG, D. M. (1974). Functional significance of connections of the inferior olive. *Physiol. Rev.* 54: 358-417.
- ARMSTRONG, D. W. and BINSTOCK, L. (1974). The effects of several alcohols on the properties of the squid giant axon. *J. gen. Physiol.* 48: 265-277.
- ARMSTRONG, D. M. and HARVEY, R. J. (1966). Responses in the inferior olive to stimulation of the cerebellar and cerebral cortices in the cat. *J. Physiol. (Lond.)* 187: 553-574.
- ARMSTRONG, D. M. and RAWSON, J. A. (1979). Activity patterns of cerebellar cortical neurones and climbing fibre afferents in the awake cat. *J. Physiol. (Lond.)* 289: 425-448.
- BACKSTROM, A. -C. (1977). Effects of ethanol on ganglion cell receptive field properties and sensitivity in the frog retina. In: *Advances in Experimental Medicine and Biology*, Vol. 85B. GROSS, M. M. (ed). Plenum Press, New York and London, pp. 187-208.
- BRAESTRUP, C., NIELSEN, M., KROGSGAARD-LARSEN, P. and FALCH, E. (1980). Two or more conformations of benzodiazepine receptors depending on GABA receptors and other variables. In: *Receptors for Neurotransmitters and Peptide Hormones*, PAPEU, G., KUCHAR, M. J., and ENNA, S. J. (eds). Raven Press, New York, pp. 301-312.
- BAKER, P. F. and SCHAPINA, A. H. V. (1980). Anaesthetics increase light emission from aequorin at constant ionized calcium. *Nature* 284: 168-169.
- BALCAR, V. J. and JOHNSTON, G. A. R. (1973). High affinity uptake of transmitters: studies on the uptake of L-aspartate, GABA, L-glutamate and glycine in cat spinal cord. *J. Neurochem.* 20: 529-539.

- BARONDES, S. H., TRAYNOR, M. E., SCHLAPFER, W. T. and WOODSON, P. B. J. (1979). Rapid adaptation to neuronal membrane effects of ethanol and low temperature: Some speculations on mechanism. *Drug Alcohol Dependence* 4: 155-166.
- BARRAGAN, L. A. and DELHAYE-BOUCHAUD, N. (1980). Harmaline-induced activation of the olivo-cerebellar system in young rabbits: further evidence for a transient multiinnervation of Purkinje cells by climbing fibres. *Neuropharmacol.* 19: 305-310.
- BATINI, C., CORVISIER, J., DESTOMBES, J., GIONANNI, H. and EVERETT, J. (1976). The climbing fibres in the cerebellar cortex, their origin and pathway in the cat. *Exp. Brain Res.* 26: 407-422.
- BISCOE, T. J., DUGGAN, A. W., HEADLEY, P. M. and LODGE, D. (1973). Rythmical field potentials induced in the inferior olivary complex by iontophoretically applied harmaline and other unrelated alkaloids. *Br. J. Pharmac.* 49: 174-175.
- BISHOP, G. (1982). The pattern of distribution of the local axonal collaterals of Purkinje cells in the intermediate cortex of the anterior lobe and paramedian lobule of the cerebellum. *J. Comp. Neurol.* 210: 1-9.
- BISTI, S., IOSIF, G., MARCHESI, G. F. and STRADA, P. (1971). Pharmacological properties of inhibitions in the cerebellar cortex. *Exp. Brain Res.* 14: 24-37.
- BLOEDEL, J. R. and ROBERTS, W. J. (1971). Actions of climbing fibres in cerebellar cortex of the cat. *J. Neurophysiol.* 34: 17-31.
- BLOOM, F., PUSHKARAJ, L., PITTMAN, Q. and ROGERS, J. (1982). blood ethanol levels in rats: non-uniform yields from intraperitoneal doses based on body weight. *Br. J. Pharmac.* 75: 251-254.
- BOBILLIER, P., SEQUIN, S., PETITJEAN, F., SALVERT, D., TOURET, M. and JUVET, M. (1976). The raphe nuclei of the cat brain stem: A topological atlas of their efferent projections as revealed by autoradiography. *Brain Res.* 113: 449-486.
- BRADLEY, P. M. and BERRY, M. (1976). Quantitative effects of climbing fibre deafferentation of the adult Purkinje cell dendritic tree. *Brain Res.* 122: 133-140.
- BRODAL, A. and KAWAMURA, K. (1980). Olivocerebellar projection: a review. *Adv. Anat. Embriol. Cell Biol.* 64: 1-140.

- BROWN, J. T., CHAN-PALAY, V. and PALAY, S. L. (1977). A study of of the afferent input to the inferior olivary complex in the rat by the retrograde axonal transport of horseradish peroxidase. *J. Comp. Neurol.* 176: 1-22.
- BRUGGENCATE, G. TEN and ENGBERG, I. (1971). Iontophoretic studies in Deiters' nucleus of the inhibitory actions of GABA and related amino acids and the interactions of strychnine and picrotoxin. *Brain Res.* 25: 431-448.
- CARLEN, P. L. and CORRIGAL, W. A. (1980). Ethanol tolerance measured electrophysiologically in hippocampal slices and not in neuromuscular junctions from chronically ethanol-fed rats. *Neurosci. Lett.* 17: 95-100.
- CARLEN, P. L., GUREVICH, N. and DURAND, D. (1982). Ethanol in low doses augments calcium-mediated mechanisms measured intracellularly in hippocampal neurones. *Science* 215: 306-309.
- CARMICHAEL, F. J. and ISRAEL, Y. (1975). Effects of ethanol on neurotransmitter release by rat brain cortical slices. *J. Pharmac. exp. Ther.* 193: 824-834.
- CHAN-PALAY, V. (1977). *Cerebellar Dendate Nucleus: Organization, Cytology, and Transmitters*. Springer-Verlag, Berlin.
- CHERNOFF, G. F. (1977). The fetal alcohol syndrome in mice: An animal model. *Teratology* 15: 223-230.
- CHIN, J. H. and GOLDSTEIN, D. B. (1977). Electron paramagnetic resonance studies of ethanol on membrane fluidity. In: *Advances in Experimental Medicine and Biology*, Vol. 85B. GROSS, M. M. (ed). Plenum Press, New York and London, pp. 111-122.
- CHUJO, T., YAMADA, Y. and YATAMOTO, C. (1975). Sensitivity of Purkinje cells to glutamic acid. *Exp. Brain Res.* 23: 293-300.
- CIOFALO, F. R. (1980). Ethanol, neuroreceptors and postsynaptic membrane function. *Proc. West. Pharmac. Soc.* 23: 441-448.
- COLIN, F., MANIL, J. and DESCLIN, J. C. (1980). The olivocerebellar system. I. Delayed and slow inhibitory effects: an overlooked salient feature of cerebellar climbing fibres. *Brain Res.* 187: 3-27.
- COSTA, E. (1979). The role of gamma-aminobutyric acid in the action of 1,4-benzodiazepines. *Trends Pharmacol. Sci.* 1: 41-44.
- COURVILLE, J., DE MONTIGNY, C. and LAMARRE, Y. (1980). *The Inferior Olivary Nucleus*. Raven Press, New York.

- CRILL, W. E. (1979). Unitary multiple-spiked responses in the cat inferior olive nucleus. *J. Neurophysiol.* 33: 199-209.
- CURTIS, D. R. and FELIX, D. (1971). The effect of bicuculline upon synaptic inhibition in cerebral and cerebellar cortices of the cat. *Brain Res.* 34: 301-321.
- CURTIS, D. R., DUGGAN, A. W. and FELIX, D. (1970). GABA and inhibition of Deiters' neurones. *Brain Res.* 23: 117-120.
- CURTIS, D. R., DUGGAN, A. W., FELIX, D. and JOHNSTON, G. A. R. (1971). Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord. *Brain Res.* 32: 69-96.
- DAVIDOFF, R. A. (1973). Alcohol and presynaptic inhibition in an isolated spinal cord preparation. *Arch. Neurol.* 28: 60-63.
- DECLIN, J. C. (1974). Histological evidence supporting the inferior olive as the major source of cerebellar climbing fibers in the rat. *Brain Res.* 77: 365-384.
- DECLIN, J. C. and COLIN, F. (1980). The olivocerebellar system. II. Some ultrastructural correlates of inferior olive destruction. *Brain Res.* 187: 29-46.
- DOW, R. S. and MORUZZI, G. (1958). *The Physiology and Pathology of the Cerebellum*. The University of Minnesota Press, Minneapolis.
- DUNLAP, K. and FISCHBACH, G. D. (1978). Neurotransmitters decrease the calcium component of sensory nerve action potentials. *Nature* 276: 827-839.
- EBNER, T. J. and BLOEDEL, J. R. (1981). Correlation between activity of Purkinje cells and its modification by natural peripheral stimuli. *J. Neurophysiol.* 45: 948-960.
- ECCLES, J. C. (1977). An instructional theory of learning in the cerebellar cortex. *Brain Res.* 127: 327-352.
- ECCLES, J. C., SCHMIDT, R. F. and WILLIS, W. D. (1963). Pharmacological studies on presynaptic inhibition. *J. Physiol. (Lond.)* 168: 500-530.
- ECCLES, J. C., LLINAS, R. and SASAKI, K. (1966a). The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. *J. Physiol. (Lond.)* 182: 268-296.
- ECCLES, J. C., LLINAS, R. and SASAKI, K. (1966b). The inhibitory interneurons within the cerebellar cortex. *Exp. Brain Res.* 1: 1-16.

- ECCLES, J. C., ITO, M., and SZENTAGOTHAÏ, J. (1967). *The Cerebellum as a Neuronal Machine*. Springer, Berlin, Heidelberg, New York.
- EIDELBERG, E. and WOOLEY, D. F. (1970). Effects of ethyl alcohol upon spinal cord neurones. *Arch. int. Pharmacodyn.* 185: 388-396.
- EIDELBERG, E., BOND, M. L. and KELTER, A. (1971). Effects of alcohol on cerebellar and vestibular neurones. *Arch. int. Pharmacodyn.* 192: 213-219.
- EKEROT, C. -F. and OSCARSSON, O. (1980a). Prolonged dendritic depolarizations evoked in Purkinje cells by climbing fibre impulses. *Brain Res.* 192: 272-275.
- EKEROT, C. -F. and OSCARSSON, O. (1980b). Climbing fibre elicited prolonged depolarizations in Purkinje cell dendrites. In: *Adv. Physiol. Sci. Vol. 2. Regulatory Functions of the C.N.S. Subsystems*, SZENTAGOTHAÏ, J., HAMORI, J. and PALKOVITS, M. (eds). Pergamon Press, Akademiai Kiado, Hungary, pp. 117-131.
- ENNA, S. J. and SNYDER, S. H. (1977). influences of ions, enzymes, and detergents on gamma-aminobutyric acid binding in synaptic membranes of rat brain. *Mol. Pharmacol.* 13: 442-453.
- FERIN, M., GRIGORIAN, R. A. and STRADA, P. (1971). Mossy and climbing fiber activation in the cat cerebellum by stimulation of the labyrinth. *Exp. Brain Res.* 12: 1-17.
- FERKO, A. P. and BOBYOCK, E. (1980). A study on regional brain calcium concentrations following acute and prolonged administration of ethanol in rats. *Toxicol. appl. Pharmac.* 55: 179-187.
- FOX, F. A., HILLMAN, D. E., SUGESMUND, K. A. and DUTTA, C. R. (1969). The primate cerebellar cortex: a Golgi and electron microscope study. *Progr. Brain Res.* 25: 174-225.
- FREDERICKSON, R. C. A., NEUSS, M., MORZORATI, S. L. and MCBRIDE, W. J. (1978). A comparison of the inhibitory effects of taurine and GABA on identified Purkinje cells and other neurones in the cerebellar cortex of the rat. *Brain Res.* 145: 117-126.
- FREEDMAN, R., HOFFER, B. J., WOODWARD, D. J. and PURO, D. (1977). Interaction of norepinephrine with cerebellar activity evoked by mossy and climbing fibres. *Exp. Neurol.* 55: 269-288.

- GAGE, P. W. (1965). The effect of methyl, ethyl, and n-propyl alcohol on neuromuscular transmission in the rat. *J. Pharmac. exp. Ther.* 150: 236-243.
- GARVER, D. L. and SLADEK, F. R. (1975). Monoamine distribution in primate brain. I. Catecholamine-containing perikarya in the brain stem of *Macaca speciosa*. *J. Comp. Neurol.* 159: 289-304.
- GILBERT, P. F. C. (1974). A theory of memory that explains the function and structure of the cerebellum. *Brain Res.* 70: 1-18.
- GOULD, B. B. (1980). Organization of afferents from the brain stem nuclei to the cerebellar cortex in the cat. *Adv. Anat. Embriol. Cell Biol.* 62: 1-90.
- GRANIT, R. and PHILLIPS, C. G. (1956). Excitatory and inhibitory processes acting upon individual Purkinje cells of the cerebellum in cats. *J. Physiol. (Lond.)* 133: 520-547.
- GRUPP, L. A. (1980). Biphasic action of ethanol on single units of the dorsal hippocampus and the relationship to the cortical EEG. *Psychopharmacol.* 70: 95-103.
- GRUPP, L. A. (1981). the influence of some neurotransmitter agonists and antagonists on the response of hippocampal units and cortical EEG to ethanol in the awake rat. *Psychopharmacol.* 75: 327-334.
- HAEFELY, W., POLC, P., SCHAFFNER, R., KELLER, H. H., PIERI, L. and MOHLER, H. (1978). Facilitation of GABA-ergic transmission by drugs. In: *GABA-Neurotransmitters*, KROGSGAARD-LARSEN, P., SCHEEL-KRUGER, J. and KOFOD, H. (eds). Munksgaard, Copenhagen, pp.357-375.
- HAKKINEN, H. -M. AND KULONEN, E. (1976). Ethanol intoxication and gamma-aminobutyric acid. *J. Neurochem.* 27:631-633.
- HAMORI, J. (1973). The inductive role of presynaptic axons in the development of postsynaptic spike. *Brain Res.* 62: 337-344.
- HAMORI, J. and SZENTAGOTHAJ, J. (1966). Identification under electron microscope of climbing fibres and their contacts. *Exp. Brain Res.* 1: 65-81.
- HAROIAN, A. J. (1982). Cerebello-olivary projections in the rat: an autoradiographic study. *Brain Res.* 235: 125-130.
- HARRIS, D. P. (1980). Studies on Characterized Nucleus Gigantocellularis Neurones. M.Sc. Thesis, University of British Columbia, Vancouver.

- HEADLEY, P. M. and LODGE, D. (1976). Studies on field potentials and on single cells in the inferior olivary complex in the rat. *Brain Res.* 101: 445-459.
- HEADLEY, P. M., LODGE, D. and DUGGAN, A. W. (1976). Drug-induced rhythmical activity in the inferior olivary complex of the rat. *Brain Res.* 101: 461-478.
- HOFFER, B. J., SIGGINS, G. R., WOODWARD, D. J. and BLOOM, F. E. (1971). Spontaneous discharge of Purkinje neurons after destruction of catecholamine-containing afferents by 6-hydroxydopamine. *Brain Res.* 40: 425.
- HOFFER, B. J., SIGGINS, G. R., OLIVER, A. P. and BLOOM, F. E. (1973). Activation of a pathway from the locus coeruleus to rat cerebellar Purkinje neurons: Pharmacological evidence of noradrenergic central inhibition. *J. Pharmacol. exp. Ther.* 184: 553-569.
- HOFFER, B. J., FREEDMAN, R., WOODWARD, D. J., PURO, D. and MOISES, H. (1978). A functional role for adrenergic input to the cerebellar cortex: Interaction of noradrenaline with mossy and climbing fibre excitation and GABA-mediated inhibition. In: *Interactions between Putative Neurotransmitters in the Brain*, GARATINNI, S., PUJOL, J. F. and SAMANIN, R. (eds). Raven Press, New York, pp. 231-243.
- HOFFMAN, D. L. and SLADEK, J. R. (1973). The distribution of catecholamines within the inferior olivary complex of the gerbil and rabbit. *J. Comp. Neurol.* 151: 101-112.
- HOKFELT, T. and FUXE, K. (1969). Cerebellar monoamine nerve terminals, a new type of afferent fibre to the cortex cerebelli. *Exp. Brain Res.* 9: 63-72.
- HOKFELT, T. and LJUNGDAHL, A. (1972). Autoradiographical identification of cerebral and cerebellar cortical neurons accumulating labelled gamma-aminobutyric acid (3H-GABA). *Exp. Brain Res.* 14: 354-362.
- HOOD, W. F. and HARRIS, R. A. (1979). Effects of pentobarbital, ethanol, and morphine on subcellular localization of calcium and magnesium in brain. *Biochem. Pharmacol.* 28: 3075-3080.
- HUNT, S. and SCHMIDT, J. (1978). Some observations of the binding patterns of alpha-bungarotoxin in the central nervous system of the rat. *Brain Res.* 157: 213-232.
- HURWITZ, L., BATTLE, F. and WEISS, G. B. (1962). Action of calcium antagonists cocaine and ethanol on contraction and potassium efflux of smooth muscle. *J. gen. Physiol.* 46: 315-332.

- HURWITZ, L., VON HAGEN, S. and JOINER, P. D. (1967). Acetylcholine and calcium on membrane permeability and contraction of intestinal smooth muscle. *J. gen. Physiol.* 50: 1157-1172.
- ISRAEL, M. and WHITTAKER, V. P. (1965). The isolation of mossy fibre endings from the granular layer of the cerebellar cortex. *Experientia* 21: 325-327.
- ITO, M. (1981). Remote depressant effect of destruction of the inferior olive upon Purkinje cell inhibition in vestibular nuclei. In: *Problems in GABA Research from Brain to Bacteria*, OKADA, Y. and ROBERTS, E. (eds). *Excerpta Medica*, Amsterdam, Oxford, Princeton, pp. 80-86.
- ITO, M. and KANO, M. (1982). Long-lasting depression of parallel fibre-Purkinje cell transmission induced by conjunctive stimulation of parallel fibres and climbing fibres in the cerebellar cortex. *Neurosci. Lett.* 33: 253-258.
- ITO, M., ORLOV, I. and SHIMOYAMA, I. (1978). Reduction of the cerebellar stimulus effect on rat Deiters' neurons after chemical destruction of the inferior olive. *Exp. Brain Res.* 33, 143-145.
- ITO, M., NISIMARU, N. and SHIBUKI, K. (1979). Destruction of inferior olive induces rapid depression in synaptic action of cerebellar Purkinje cells. *Nature* 277: 568-569.
- ITO, M., SAKURAI, M. and TONGROACH, P. (1981). Experimental support for Marr's modifiable neuronal network of the cerebellum. *Neurosci. Lett., Supp.* 6: S28.
- ITO, M., SAKURAI, M. and TONGROACH, P. (1982). Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. *J. Physiol.* 324: 113-134.
- JANSEN, J. and BRODAL, A. (1940). Experimental studies on the intrinsic fibers of the cerebellum. II. Cortico-nuclear projection. *J. Comp. Neurol.* 73: 267-321.
- KALANT, H. and WOO, N. (1981). Electrophysiological effects of ethanol on the nervous system. *Pharmac. Ther.* 14: 431-457.
- KAWAGUCHI, S., YAMAMOTO, T., MIZUNO, N. and IWAHORI, N. (1975). The role of climbing fibres in the development of Purkinje cell dendrites. *Neurosci. Lett.* 1: 301-304.
- KENDIG, J. J., TRUDELL, J. R. and COHEN, E. N. (1975). effects of pressure and anaesthetics on conduction and synaptic transmission. *J. Pharmacol. exp. Ther.* 195: 216-224.

- KING, J. S. (1980). Synaptic organization of the inferior olivary complex. In: *The Inferior Olivary Complex: Anatomy and Physiology*, COURVILLE, J., DE MONTIGNE, C. and LAMARRE, Y. (eds). Raven Press, New York, pp. 1-33.
- KING, J. S., ANDREZIK, J. A., FALLS, W. M. and MARTIN, G. F. (1976). The synaptic organization of the cerebello-olivary circuit. *Exp. Brain Res.* 26: 159-170.
- KING, J. S., HAMOS, J. E. and MALEY, B. E. (1978). The synaptic terminations of certain midbrain-olivary fibres in the opossum. *J. Comp. Neurol.* 182: 185-200.
- KOLB, F. P. and RUBIA, F. J. (1980). Information about peripheral events conveyed to the cerebellum via the climbing fibre system in the decerebrate cat. *Exp. Brain Res.* 38: 363-373.
- KORNGUTH, S. E. and SCOTT, G. (1972). The role of climbing fibers in the formation of Purkinje cell dendrites. *J. Comp. Neurol.* 146: 61-82.
- KRNJEVIC, K. (1982). GABA and other transmitters in the cerebellum. In: *Exp. Brain Res. Supp. 6: The Cerebellum-New Vistas*, PALAY, S. L. and CHAN-PALAY, V. (eds). Springer-Verlag, New York, pp. 533-549.
- KRNJEVIC, K. and SCHWARTZ, S. (1967). The action of gamma-aminobutyric acid on cortical neurones. *Exp. Brain Res.* 3: 320-336.
- LAKE, N., YARBOROUGH, G. G. and PHILLIS, J. W. (1973). Effects of ethanol on cerebral cortical neurones: Interactions with some putative transmitters. *J. Pharm. Pharmac.* 25: 582-584.
- LEONARD, C. S. and SIMPSON, J. I. (1982). Effects of suspending climbing fiber activity on the discharge patterns of floccular Purkinje cells. *Soc. Neurosci. Abst.* 8: 830.
- LEVY, R. A. (1977). The role of GABA in primary afferent depolarization. *Prog. Neurobiol.* 9: 211-267.
- LINAUTS, M. and MARTIN, G. F. (1978). An autoradiographical study of midbrain-diencephalic projections to the inferior olivary nucleus in the opossum (*Didelphus virginiana*). *J. Comp. Neurol.* 179: 325-354.
- LJUNGDAHL, A., HOKFELT, T. and NILSSON, G. (1978). Distribution of substance P-like immunoreactivity in the central nervous system of the rat. I. Cell bodies and nerve terminals. *Neurosci.* 3: 861-944.

- LLINAS, R. and SUGIMORI M. (1980a). Electrophysiological properties of in vitro Purkinje cell somata in mammalian cerebellar slices. J. Physiol. 305: 171-195.
- LLINAS, R. and SUGIMORI M. (1980b). Electrophysiological properties of in vitro Purkinje cell dendrites in mammalian cerebellar slices. J. Physiol. 305: 197-213.
- LLINAS, R. and VOLKIND, R. A. (1973). The olivocerebellar system: functional properties as revealed by harmaline tremor. Exp. Brain Res. 18: 69-87.
- LLINAS, R. and WALTON, K. (1979). Place of cerebellum in motor learning. In: Brain Mechanisms in Memory and Learning: From the Single Neuron to Man, BRAZIER, M. A. B. (ed). Raven Press, New York, pp. 18-36.
- LLINAS, R. and YAROM, Y. (1981a). Electrophysiology of mammalian inferior olivary neurones in vitro. Different types of voltage-dependent ionic conductances. J. Physiol. 315: 549-567.
- LLINAS, R. and YAROM, Y. (1981b). Properties and distribution of ionic conductances generating electroresponsiveness of mammalian inferior olivary neurones in vitro. J. Physiol. 315: 569-584.
- LLINAS, R., BAKER, R. and SOTELO, C. (1974). Electrotonic coupling between neurons in cat inferior olive. J. Neurophysiol. 37: 560-571.
- LLINAS, R., WALTON, K., HILLMAN, D. E. and SOTELLO, C. (1975). Inferior olive: Its role in motor learning. Science 190: 1230-1231.
- MCBRIDE, W. J., NADI, N. S., ALTMAN, J. and APRISON, M. H. (1976). Effects of selective doses of x-irradiation on the levels of several amino acids in the cerebellum of the rat. Neurochem. Res. 1: 141-152.
- MCBURNEY, R. N. and BARKER, J. L. (1978). GABA-induced conductance fluctuation in cultured spinal neurones. Nature 274: 596-597.
- MCDEVITT, C. J., EBNER, T. J. and BLOEDEL, J. R. (1982). The changes in Purkinje cell simple spike activity following spontaneous climbing fiber inputs. Brain Res. 237: 484-491.
- MELAMED, E., LAHAV, M. and ATLAS, D. (1976). Direct localization of beta-adrenoceptor sites in rat cerebellum by a new fluorescent analogue of propranolol. Nature (Lond.) 261: 420-422.

- MARIANI, E., VOOGD, J. and BOEKU, A. (1977). Acetylcholinesterase staining in subdivisions of the cat's inferior olive. *J. Comp. Neurol.* 174: 209-226.
- MARIANI, E., RUBIA, F. J., KOLB, F. P. and BAUSWEIN, E. (1982). Cortical influence upon cerebellar Purkinje cells responding to natural, peripheral stimulation in the cat. *Neurosci. Lett.* 33: 55-59.
- MARR, D. (1969). A theory of cerebellar cortex. *J. Physiol. (Lond.)* 202: 437-470.
- MARTIN, G. F., DOM, R., KING, J. S., ROBARDS, M. and WATSON, C. R. R. (1975). The inferior olivary nucleus of the opossum (*Didelphia virginiana*): Its organization and connections. *J. Comp. Neurol.* 160: 507-534.
- MARTIN, G. F., CULBERSON, J., LAXSON, C., LINAUTS, M., PANNETON, M. and TSCHISMADIA, I. (1980). Afferent connections of the inferior olivary nucleus with preliminary notes on their development. In: *The Inferior Olivary Nucleus: Anatomy and Physiology*, COURVILLE, J., DE MONTIGNY, C. and LAMARRE, Y. (eds). Raven Press, New York, pp. 35-72.
- MARWAHA, J., PALMER, M. R., WOODWARD, D. J., HOFFER, B. J. and FREEDMAN, R. (1980). Electrophysiological evidence for pre-synaptic actions of phencyclidine on noradrenergic transmission in the rat. *J. Pharmacol. exp. Ther.* 215: 606-613.
- MARWAHA, J., HOFFER, B. J. and FREEDMAN, R. (1981). electrophysiological evidence for histamine receptors in rat cerebellum. *Exp. Neurol.* 74: 285-292.
- MAYER, J. M., KHANNA, J. M., KALANT, H. and SPERO, L. (1980a). Cross-tolerance between ethanol and morphine in guinea-pig ileum longitudinal-muscle / myenteric-plexus preparation. *Eur. J. Pharmacol.* 63: 223-227.
- MAYER, J. M., KHANNA, J. M., KALANT, H. and SPERO, L. (1980b). A role of calcium in the acute and chronic actions of ethanol in vitro. *Eur. J. Pharmacol.* 68: 223-227.
- METCALF, B. W. (1979). Inhibitors of GABA metabolism. *Biochem. Pharmacol.* 28: 1705-1712.
- MEYER, K. H. (1937). Contributions to the theory of narcosis. *Trans. Faraday Soc.* 33: 1062-1068.
- MICHAELIS, E. K. and MEYERS, S. L. (1979). Calcium binding to brain synaptosomes. Effects of chronic ethanol intake. *Biochem. Pharmacol.* 28: 2081-2087.

- MICHAELIS, E. K., MICHAELIS, M. L., and FREED, W. J. (1980). Chronic ethanol intake and synaptosomal glutamate binding activity. In: *Advances in Experimental Medicine and Biology*, Vol. 126, BEGLEITER, H. (ed). Plenum Press, New York, pp. 43-56.
- MIYAHARA, J. T., ESPLIN, D. W. and ZABLOCKA, B. (1966). Differential effects of depressant drugs on presynaptic inhibition. *J. Pharmacol. exp. Ther.* 154: 119-127.
- MOHLER, H. and OKADA, T. (1978). GABA receptor in the brain: demonstration of an antagonist binding site. In: *Amino Acids as Chemical Transmitters*, FONNUM, F. (ed). Plenum Press, New York, pp. 493-498.
- MOISES, H. C. and WOODWARD, D. (1980). Potentiation of GABA inhibitory action in cerebellum by locus coeruleus stimulation. *Brain Res.* 182: 327-344.
- MOISES, H. C., WOODWARD, D. J., HOFFER, B. J. and FREEDMAN, R. (1979). Interactions of norepinephrine with Purkinje cell responses to putative amino acid neurotransmitters applied by microiontophoresis. *Exp. Neurol.* 64: 493-515.
- MONTAROLO, P. G., RASCHI, F. and STRATA, P. (1981). Are climbing fibres essential for Purkinje cell inhibitory action? *Exp. Brain Res.* 42: 215-218.
- MONTAROLO, P. G., PALESTINI, M. and STRATA, P. (1982). The inhibitory effect of the olivocerebellar input on the cerebellar Purkinje cells in the rat. *J. Physiol. (Lond.)* 332: 187-202.
- MONTIGNY, C. DE and LAMARRE, Y. (1973). Rhythmical activity induced by harmaline in the olivo-cerebellar-bulbar system of the cat. *Brain Res.* 53: 81-95.
- MOORE, J. W., ULBRICHT, W. and TAKAGI, M. (1964). Effect of ethanol on the sodium and potassium conductances of the squid axon membrane. *J. gen. Physiol.* 48: 276-279.
- MULLENS, L. J. (1954). Some physical mechanisms in narcosis. *Chem. Rev.* 54: 289-323.
- MURPHY, M. G. and O'LEARY, J. L. (1971). Neurological defects in cats with lesions of the cerebellar system. *Arch. Neurol.* 24: 145-157.
- NADI, N. S., MCBRIDE, W. J. and APRISON, M. H. (1977). Distribution of several amino acids in regions of the cerebellum of the rat. *J. Neurochem.* 28: 435-455.

- NANIMA, M., OKAMOTO, K. and SAKAI, Y. (1983). Modulatory action of taurine on the release of GABA in cerebellar slices of the guinea pig. *J. Neurochem.* 40: 1-9.
- NESTEROS, J. M. (1980a). Ethanol specifically potentiates GABA-mediated transmission in feline cerebral cortex. *Science* 209: 708-709.
- NESTEROS, J. M. (1980b). Ethanol selectively potentiates GABA-mediated inhibition in single feline cortical neurons. *Life Sci.* 26: 519-523.
- NEWLIN, S. A., MANCILLAS-TREVINO, J. and BLOOM, F. E. (1981). Ethanol causes increases in excitation and inhibition in area CA3 of the dorsal hippocampus. *Brain Res.* 209: 113-128.
- NICHOLSON, G. P. and GWYN, D. G. (1974). The spino-olivary projection in the rat. *Can. Fed. Biol. Soc.* 19: 77.
- NISHI, S., MINOTA, S. and KARCZMAR, A. G. (1974). Primary afferent neurones: the ionic mechanism of GABA-mediated depolarization. *Neuropharmacol.* 13: 215-219.
- NISTRI, A., CONSTANTII, A. and KRNJEVIC, K. (1980). Electrophysiological studies of the mode of action of GABA on vertebrate central neurons. *Adv. Biochem. Psychopharmacol.* 21: 81-90.
- OBATA, K. and TAKEDA, K. (1969). Release of gamma-aminobutyric acid into the fourth ventricle induced by stimulation of cat's cerebellum. *J. Neurochem.* 16: 1041-1047.
- OBATA, K., ITO, T., OCHI, R. and SATO, N. (1967). Pharmacological properties of the postsynaptic inhibition by the Purkinje cell and the action of gamma-aminobutyric acid on Deiters' neurones. *Exp. Brain Res.* 4:43-57.
- OKADA, K. (1967). Effects of alcohols and acetone on the neuromuscular junction of frog. *Jap. J. Physiol.* 17: 245-261.
- OKAMOTO, K., KIMURA, H. and SAKAI, Y. (1983a). Taurine-induced increase of the Cl-conductance of cerebellar Purkinje cell dendrites in vitro. *Brain Res.* 259: 319-323.
- OKAMOTO, K., KIMURA, H. and SAKAI, Y. (1983b). Effects of taurine and GABA on Ca spikes and Na spikes in cerebellar Purkinje cells in vitro: intrasomatic study. *Brain Res.* 260: 249-259.
- OKAMOTO, K., KIMURA, H. and SAKAI, Y. (1983c). Ionic mechanisms of the actions of taurine on cerebellar Purkinje cell dendrites in vitro: intradendritic study. *Brain Res.* 260: 261-269.

- OLSEN, R. W. (1981). GABA-benzodiazepin-barbiturate receptor interactions. *J. Neurochem.* 37: 1-13.
- OLSEN, R. W. (1982). Drug interactions at the GABA-receptor-ionophore complex. *Ann. Rev. Pharmacol. Toxicol.* 22: 245-277.
- OSCARSSON, O. (1980). Functional organization of olivary projection to cerebellar anterior lobe. In: *The Inferior Olivary Nucleus: Anatomy and Physiology*, COURVILLE, J., DE MONTIGNY, C., and LAMARRE, Y. (ed). Raven Press, New York, pp. 279-289.
- OTSUKA, M., OBATA, K., MIYATA, Y. and TANAKA, Y. (1971). Measurement of gamma-aminobutyric acid in isolated nerve cells of cat central nervous system. *J. Neurochem.* 18: 287-295.
- PALACIOS, J. M., WAMSLEY, J. K. and KULAR, M. J. (1981). The distribution of histamine H_1 receptors in the rat brain. An autoradiographic study. *Neurosci.* 6: 15-37.
- PALAY, S. and CHAN-PALAY, V. (1974). *The Cerebellar Cortex*. Springer-Verlag, New York.
- PALKOVITS, M., MAGNAR, P. and SZENTAGOTHAI, J. (1972). Quantitative histological analysis of the cerebellar cortex in the cat. IV. Mossy fibre-Purkinje cell numerical transfer. *Brain Res.* 45: 15-29.
- PALMER, M. R., SORENSEN, S. M., FREEDMAN, R., OLSEN, L., HOFFER, B. and SEIGER, A. (1982). Differential ethanol sensitivity of intraocular cerebellar grafts in long-sleep and short-sleep mice. *J. Pharmacol. Exp. Ther.* 222: 480-487.
- PELLIGRINO, L. J., PELLEGRINO, A. S. and CUSHMAN, A. J. (1979). *A Stereotaxic Atlas of the Rat Brain*. Plenum Press, New York and London.
- PELLIONISZ, A. and LLINAS, R. (1979). Brain modeling by tensor network theory and computer simulation. The cerebellum: distributed processor for predictive coordination. *Neurosci.* 4: 323-348.
- PHILLIS, J. W. (1968). Cholinesterase in cat cerebellar cortex, deep nuclei, and peduncles. *Experientia* 21: 266-268.
- PHILLIS, J. W. and JHAMANDAS, K. (1971). The effects of chlorpromazine and ethanol on the in vivo release of acetylcholine from the cerebral cortex. *Comp. Gen. Pharmacol.* 2: 306-310.
- PHILLIS, J. W., JIANG, Z. G. and CHELACK, B. J. (1980). Effects of ethanol on acetylcholine and adenosine efflux from the in vivo rat cerebral cortex. *J. Pharm. Pharmacol.* 32: 871-872.
- POHORECKY, L. A. and BRICK, J. (1977). Activity of neurons in the locus coeruleus of the rat: inhibition by ethanol. *Brain Res.* 131: 174-179.

- PURO, D. G. and WOODWARD, D. J. (1977). Maturation of evoked climbing fibre input to rat cerebellar Purkinje cells (I). *Exp. Brain Res.* 28: 85-100.
- QUASTEL, D. M. J., HACKETT, J. T. and COOKE, J. D. (1971). Calcium: Is it required for transmitter secretion? *Science* 172: 1034-1036.
- QUEVEDO, L., BALDEIG, J. and BENOIT, C. -I. (1978). Accomodation related to the action of ethanol on sciatic nerve. *Pharmacology* 17: 249.
- RAWSON, J. A. and TILOKSKULCHAI, K. (1981). Suppression of simple spike discharges of cerebellar Purkinje cells by impulses in climbing fibre afferents. *Neurosci. Lett.* 25: 125-130.
- RAWSON, J. A. and TILOKSKULCHAI, K. (1982). Climbing fibre modification of cerebellar Purkinje cell responses to parallel fibre inputs. *Brain Res.* 237: 492-497.
- ROBERTS, E., CHASE, T. N. and TOWER, D. B. (eds) (1976). *GABA in Nervous System Function*. Raven Press, New York.
- ROBERTS, E. (1979). New directions in GABA research I: Immunocytochemical studies of GABA neurons. In: *GABA-neurotransmitters. Pharmacochemical, Biochemical and Pharmacological Aspects*, KROGSGAARD-LARSEN, P., SCHEEL-KRUGER, J. and KOFOD, H. (eds). Academic Press, New York, pp. 28-45
- ROFFLER-TARLOV, S. and SIDMAN, R. L. (1978). Concentrations of glutamic acid in cerebellar in cerebellar and deep nuclei of normal mice and weaver, staggerer and nervous mutants. *Brain Res.* 12: 269-283.
- ROGERS, J., SIGGINS, G. R., SCHULMAN, J. A. and BLOOM, F. E. (1980). Physiological correlates of ethanol intoxication, tolerance, and dependence in rat cerebellar Purkinje Cells. *Brain Res.* 196: 183-198.
- ROGERS, J., SIGGINS, G. R., ASTON-JONES, G., KODA, L. Y. and BLOOM, F. E. (1982). Electrophysiological effects of acute ethanol on olivo-cerebellar neurotransmission. *Soc. Neurosci. Abst.* 8: 597.
- ROHDE, B. H., REA, M. A., SIMON, J. R. and MCBRIDE, W. J. (1979). Effects of X-irradiation induced loss of cerebellar granule cells on the synaptosomal levels and high affinity uptake of amino acids. *J. Neurochem.* 32: 1431-1435.
- ROSS, D. H. (1977). Adaptive changes in calcium-membrane interactions following chronic ethanol exposure. *Adv. Exp. Med. and Biol.* 85A: 459-471.

- ROSS, D. H., MUTCHLER, T. L. and GRADY, M. M. (1979). Calcium and glycoprotein metabolism as correlates for ethanol preference and sensitivity. *Alcoholism: Chem. Exp. Res.* 3: 64-69.
- ROTH, S. H. (1980). Differential effects of anaesthetics on the neuronal activity. *Prog. Anesthesiol.*, Vol. 2: *Molecular Mechanisms of Anesthesia*, FINK, B. R. (ed). Raven Press, New York, pp. 119-128.
- SAINT-CYR, J. A. and COURVILLE, J. (1933). Topology of descending projections to the inferior olive from the parafasciculus region, mesencephalon and superior colliculus in the cat. *Soc. Neurosci. Abst.* 4: 68.
- SCHEIBEL, M. E. and SCHEIBEL, A. (1955). The inferior olive: a Golgi study. *J. Comp. Neurol.* 102: 77-132.
- SCHEIBEL, A. B., PAUL, L. and FRIED, I. (1981). Scanning electron microscopy of the central nervous system. I. The cerebellum. *Brain Res. Rev.* 3: 207.
- SCHOUSBOE, I., BRO, B. and SCHOUSBOE, A. (1977). Intramitochondrial localization of 4-aminobutyrate-2-oxoglutarate transaminase from ox brain. *Biochem. J.* 162: 303.
- SCHOUSBOE, A., LARSSON, O. M., HERTZ, L. and KROGSGAARD-LARSEN, P. (1981). Heterocyclic GABA analogues as new selective inhibitors of astroglial GABA transport. *Drug Dev. Res.* 1: 115-127.
- SCHULMAN, J. A. (1981). Anatomical distribution and physiological effects of enkephalin in rat inferior olive. *Regulatory Peptides* 2: 125-137.
- SCHULMAN, J. A. and BLOOM, F. E. (1981). Golgi cells of the cerebellum are inhibited by inferior olive activity. *Brain Res.* 210: 350-355.
- SCHWARTZ, A., LINDENMAYER, G. E. and ALLEN, J. C. (1975). The sodium-potassium adenosine triphosphatase: pharmacological, physiological and biochemical aspects. *Pharmac. Rev.* 27: 3-134.
- SEEMAN, P. (1972). The membrane actions of anaesthetics and tranquilizers. *Pharmacol. Rev.* 24: 583-655.
- SEEMAN, P. (1974). The membrane expansion theory of anaesthesia: direct evidence using ethanol and a high-precision density meter. *Experientia* 30: 759-760.
- SEEMAN, P. and LEE, T. (1974). The dopamine-releasing actions of neuroleptics and ethanol. *J. Pharmacol. exp. Ther.* 190: 131-139.

- SEEMAN, P., CHAU, M., GOLDBERG, M., SAUKS, T. and SAX, L. (1971). The binding of Ca^{2+} to the cell membrane increased by volatile anaesthetics (alcohols, acetone, ether) which produce sensitization of nerve or muscle. *Biochem. Biophys. Acta* 225: 185-193.
- SEIL, F. J., LEIMAN, A. L., HERMAN, M. M. and FISK, F. A. (1977). Direct effects of ethanol on central nervous system cultures: An electrophysiological and morphological study. *Exp. Neurol.* 55: 390-404.
- SIGGINS, G. R. and BLOOM, F. E. (1980). Alcohol-related electrophysiology. *Drug Alc. Depend.* 6: 81-82.
- SIGGINS, G. R. and FRENCH, E. (1979). Central neurons are depressed by iontophoretic and micropressure application of ethanol and tetrahydropapaveroline. *Drug Alc. Depend.* 4: 239-243.
- SIMMONDS, M. A. (1980). A site for the potentiation of GABA-mediated responses by benzodiazepines. *Nature* 284: 558-560.
- SINCLAIR, J. G. and LO, G. F. (1978). Acute tolerance to ethanol on the release of acetylcholine from the cat cerebral cortex. *Can. J. Physiol. Pharmacol.* 56: 668-670.
- SINCLAIR, J. G. and LO, G. F. (1981). The effects of ethanol on cerebellar Purkinje cell discharge pattern and inhibition evoked by local surface stimulation. *Brain Res.* 204: 465-471.
- SINCLAIR, J. G. and LO, G. F. (1982). Ethanol effects on rat cerebellar Purkinje cells. *Gen. Pharmac.* 13: 449-451.
- SINCLAIR, J. G., LO, G. F., and TIEN, A. F. (1980). The effects of ethanol on the cerebellar Purkinje cells in naive and alcohol-dependent rats. *Can. J. Physiol. Pharmacol.* 58: 429-432.
- SINCLAIR, J. G., LO, G. F. and HARRIS, D. P. (1982). Ethanol effects on the olivocerebellar system. *Can. J. Physiol. Pharmacol.* 60: 610-614.
- SJOLUND, B. BJORKLUND, A. and WIKLUND, L. (1977). The indolaminergic innervation of the inferior olive. II. Relation to harmaline induced tremor. *Brain Res.* 131: 23-37.
- SNYDER, S. H., LOGAN, W. J., BENNETT, J. P. and ARREQUI, A. (1973). Amino acids as central nervous transmitters: biochemical studies. *Neurosci. Res.* 5: 131-157.
- SOJA, P. J. and SINCLAIR, J. G. (1983). Spinal versus supraspinal actions of morphine on cat spinal cord multireceptive neurons. *Brain Res.* (in press).

- SORENSEN, S., CARTER, D., MARWALA, J., BAKER, R. and FREEDMAN, R. (1981). Disinhibition of rat cerebellar Purkinje neurons from noradrenergic inhibition during rising blood ethanol. *J. stud. Alcohol* 42: 908-917.
- SOTELLO, C., HILLMAN, D. E., ZAMORA, A. J. and LLINAS, R. (1975). Climbing fiber deafferentation: its action on Purkinje cell dendritic spines. *Brain Res.* 98: 574-581.
- SOUSA-PINTO, A. and BRODAL, A. (1969). Demonstration of a somatotopical pattern in the cortico-olivary projection in the cat: An experimental anatomical study. *Exp. Brain Res.* 8: 364-386.
- STOCKLE, H. and TEN BRUGGENCATE, G. (1980). Fluctuation of extracellular potassium and calcium in the cerebellar cortex related to climbing fiber activity. *Neurosci.* 5: 893-901.
- STRAHLENDORF, J. C., STRAHLENDORF, H. K. and BARNES, C. (1979). Modulation of cerebellar neuronal activity by raphe stimulation. *Brain Res.* 169: 565-569.
- SUNAHARA, G. I. and KALANT, H. (1980). Effect of ethanol on potassium-stimulated and electrically stimulated acetylcholine release in vitro from rat cortical slices. *Can. J. Physiol. Pharmacol.* 58: 706-711.
- TALLMAN, J. R., THOMAS, J. W. and GALLAGER, D. W. (1978). GABA-ergic modulation of benzodiazepine binding site sensitivity. *Nature (Lond.)* 274: 383-385.
- THACH, W. T. (1967). Somatosensory receptive fields of single units in cat cerebellar cortex. *J. Neurophysiol.* 30: 675-696.
- TICKU, M. K. and OLSEN, R. W. (1978). Interaction of barbituates with dihydro-picrotoxin binding sites related to the GABA receptors in the membrane are identical. *FEBS Lett.* 126: 309-312.
- TRUDELL, J. R., HUBBEL, W. L. and COHEN, E. N. (1973). Pressure reversal of inhalation anaesthetic-induced disorder in spin-labeled phospholipid vesicles. *Biochem. Biophys. Acta* 291: 328-334.
- VOLK, B. (1977). Delayed cerebellar histogenesis in "embryofetal alcohol syndrome". *Acta Neuropath.* 39: 157-163.
- VOOGD, J. and BIGARE, F. (1980). Topographical distribution of olivary and corticonuclear fibers in the cerebellum. A review. In: *The Inferior Olivary Nucleus: Anatomy and Physiology*, COURVILLE, J., DE MONTIGNE, C. and LAMARRE, Y. (eds). Raven Press, New York, pp. 207-234.

- WIKLUND, L., BJORKLUND, A. and SJOLUND, B. (1977). The indol-aminergic innervation of inferior olive. I. Convergence with the direct spinal afferents in the areas projecting to the cerebellar anterior lobe. *Brain Res.* 131: 1-21
- WIKLUND, L., TOGGENBERGER, G. and CUENOD, M. (1982). Aspartate: Possible neurotransmitter in cerebellar climbing fibers. *Science* 216: 78-80.
- WOODWARD, D. J., HOFFER, B. J., SIGGINS, G. R. and OLIVER, A. P. (1971). Inhibition of Purkinje cells in the frog cerebellum. II. Evidence for GABA as the inhibitory transmitter. *Brain Res.* 33: 91-100.
- WOODWARD, D. J., SEIGER, A., OLSON, L. and HOFFER, B. J. (1977). Intrinsic and extrinsic determinations of dendritic development as revealed by Golgi studies of cerebellar and hippocampal transplants in Oculo. *Exp. Neurol.* 57: 984-998.
- YONEDA, Y. and ROBERTS, E. (1981). Synaptosomal biosynthesis of GABA from ornithine and its feedback inhibition by GABA. In: *Problems in GABA research from Brain And Bacteria*, OKADA, Y. and ROBERTS, E. (eds). Excerpta Medica, Amsterdam, Oxford, Princeton, pp. 55-65.
- YOUNG, A. B., OSTER-GRANITE, M. L., HERNDON, R. M. and SNYDER, S. H. (1974). Glutamic acid: selective depletion by viral induced granule cell loss in hamster cerebellum. *Brain Res.* 73: 1-13.

VII. APPENDIX

The following are brief descriptions of the programs developed in the course of the research reported in this thesis.

1) WAITER

WAITER is a machine language routine which counts the number of pulses within a specified number of seconds. It returns this count to the BASIC program which calls it. It makes use of the hardware timers present on the custom parallel interface board.

2) GABALC, GETGABALC and MULTILOT

GABALC uses WAITER and direct manipulation of the custom parallel interface board to control the local application of ethanol and GABA in a defined protocol as outlined in the Methods. Neuronal firing rate is displayed both as a time vs rate histogram on the monitor in high-resolution graphics and numerically. Data collected can be stored onto diskette and recalled by GETGABALC.

GETGABALC retrieves the data from diskette previously stored by GABALC. It allows the display of a selected series of GABA trials and calculates an average response from them. The amount of inhibition evoked by the applied drug is calculated and displayed automatically. The histograms and calculations can be printed on the dotmatrix printer.

MULTILOT retrieves the same data but plots all the trials, the averaged response from each group of five trials, and its associated inhibition value. For an example see Figure 16.

3) PSTH, PLOT.PSTH and PLOT.PSTH.INH

The BASIC program, PSTH, and its associated machine language routine, allows peri-stimulus histograms (PSTH) to be constructed. Each histogram is made up of 250 bins and is displayed on the monitor in high-resolution graphics along with the sweep number and total bin count. The machine language routine uses the interrupt capabilities of the custom interface board to intercept trigger and spike signals. Each trigger signal starts a new sweep through all the bins of the histogram. Each spike signal causes the value in the current bin to be incremented and the histogram display to be updated. PSTH handles user queries and saves the data onto diskette.

PLOT.PSTH retrieves data previously saved to diskette by PSTH and redisplayes it with calculated statistics.

PLOT.PSTH.INH is similar to PLOT.PSTH except it displays the raw data, smoothed data and calculated I-50 Values. For an example see Figure 15.

4) DUAL.PSTH and PLOT.DUAL.PSTH

These two programs are similar to PSTH and PLOT.PSTH except that they also construct a latency histogram concurrently with the construction of the PSTH. This latter histogram includes only the first spike signal to arrive after each trigger signal. PLOT.DUAL.PSTH replots the histograms and calculates the mean latency to the first spike response. A period of time after the trigger signal, in which spike signals are ignored, can be selected in order to avoid spurious counts due to stimulation artifacts.

5) TRI.PSTH and PLOT.TRI.PSTH

These two programs are similar to the above PSTH programs, except that they distinguish between simple (SS) and complex spike (CS) activity on the basis of interspike interval. They construct three histograms: a stimulus to SS PSTH; a stimulus to CS PSTH; and a stimulus to CS latency histogram. PLOT.TRI.PSTH replots the histograms and calculates the mean latency to the first CS. An example of this program's output is shown in Figure 4.

6) ISI and PLOT.ISI

These two programs construct and display an interspike interval histogram consisting of 250 bins. The machine language routine associated with the ISI program uses interrupts to intercept spike signals. Each spike signal causes the current bin to be incremented, the displayed plot to be updated, and a new sweep of the bins to be initiated. Mean interspike interval is calculated and displayed with the histogram by PLOT.ISI, see Figure 12.

7) TRI.ISI and PLOT.TRI.ISI

These two programs are similar to ISI and PLOT.ISI except they distinguish SS from CS activity. They concurrently construct and display three interspike histograms: SS to SS; CS to CS; and CS to SS. Mean interspike intervals are calculated by PLOT.TRI.ISI and are displayed with the histograms. Part of a TRI.ISI display is included in Figure 12.

8) Miscellaneous Programs

Various other programs were written. Some were variations of the above programs used for the preliminary analysis of the data or for specialized tasks. Other programs written included a screen to printer dump routine, various statistical routines, and various display routines.