SOME EFFECTS OF ETHANOL ON RAT BRAIN MONAMINE OXIDASE, l-GLUTAMIC ACID DECARBOXYLASE AND ACETYLCHOLINESTERASE

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ABSTRACT

A study has been made of monamine oxidase (MAO), l-glutamic acid decarboxylase (GAD) and acetylcholinesterase (AChE) activities in five regions of chronically ethanol fed and liquid control rat brain.

Isocaloric replacement of carbohydrate content of the diet of the rats by ethanol (36% of the total caloric intake) produced changes in the activities of MAO, GAD and AChE levels. Levels of MAO and GAD were markedly different in the five regions studied, however levels of AChE were not significantly different.

Prolonged chronic ethanol feeding even in conjunction with nutritiously adequate diet, resulted in a decrease of 59% in MAO activity in the thalamic region and an average increase of 65% in superior and inferior colliculi. GAD activity showed an average decrease of 62% in all the regions studied, with the thalamic and cortical regions undergoing the most decrease in activity. AChE activity showed an increase of 46% in olfactory lobes with no significant changes occurring in the other regions studied.

For most of the regions studied, the removal of ethanol from the diet for 24 hr caused the activities of MAO, GAD and AChE to approach the values observed in the rats given the liquid control diet.

The observations suggest that for most of the brain regions studied MAO, GAD and AChE inhibitions are involved in ethanol action.

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I. INTRODUCTION

Some centuries of scientific and other writing have described effects of alcoholic drinks on mental and social phenomena, and ethanol remains the substance manufactured on the largest scale for its central effects. The mixture of excitation and depression in outward behavior which it produces has also long attracted attention and has contributed to ideas on the heterogeneity and evolutionary organization of the brain.

Huglings Jackson in 1873 and on other subsequent occasions writes or quotes that ".... alcohol, the nervous system appears to be paralyzed in inverse order of its development, the highest centres going first, next the middle, and then the lowest". This is a sequence seen in the action of several general depressants but among them alcohol occupies an unusual position. It is in a real, though limited sense, both a food and a drug and needs to invoke many scientific disciplines from the physiochemical to the psychosocial.

Alcohol, when consumed in moderate quantities, seems to be a relatively innocuous nutrient that can be tolerated without apparent harm to the organism. When consumed in quantities that consistently approach the upper limit of the mechanisms responsible for its metabolic elimination, however it becomes a drug that may

produce very serious metabolic derangements.

It is very important, and recognized, that one of the first steps towards understanding the problems underlying the central nervous system's (CNS) tolerance to, and dependence on, alcohol should be the study of its basic mechanism of action on nerve cells. Ethanol is classified as a general depressant. Among other compounds in this category are the higher alcohols, the volatile general anesthetics and the inert gases. One property shared by these compounds, is that of being lipid soluble. The potencies of the different general depressants *in vivo* correlate well with their ability to dissolve in a lipid phase. Equal degrees of narcosis are obtained when equal concentrations of the general depressants are present in the lipid phase of a biological system. Equal degrees of narcosis occur when an equal volume fraction of the cell membrane is occupied by the depressants. Proteins have lipophilic cores in which the general depressants may also be solubilized, thus changing the molecular characteristics of the protein.

Small quantities of ethanol are present normally in animals. Blood levels in man are equivalent to about 0.03 mM ethanol (Lester, 1962). In the brain of the rat, an average value of 0.37 μmoles/g was found (McManus, Contag & Olson, 1966). Ethanol is a normal metabolite of the mammalian body, independent of its dietary intake.

After administration of ethanol, it quickly

becomes generally distributed in all the body water and converted largely to CO_2 within few hours. A small proportion of it remaining in the brain, as in other tissues. Administered ethanol takes part in a number of metabolic interchanges in the brain. The scheme of Fig. 1, summarizes the general metabolic routes of ethanol metabolism. This scheme may be regarded as applying also to the brain.

The demonstrated stages in the metabolism include aldehyde dehydrogenase (EC 1.1.1.1), identified as the nicotinamide-adenine dinucleotide oxidoreductase. When this enzyme is isolated from brain tissue it is found to have properties similar to those of the enzyme as isolated from liver tissue. Raskin and Sokoloff (1968) found that its activity rate is relatively low: some 2.4 µmoles/g brain/hr contrasting with 9 µmoles/g liver/hr in the same animals.

Studies by Battey, Heyman & Patterson Jr. (1953), in humans show that ethanol in concentrations of about 300 mg/100 ml of blood significantly reduces the brain O_2 consumption by about 30%.

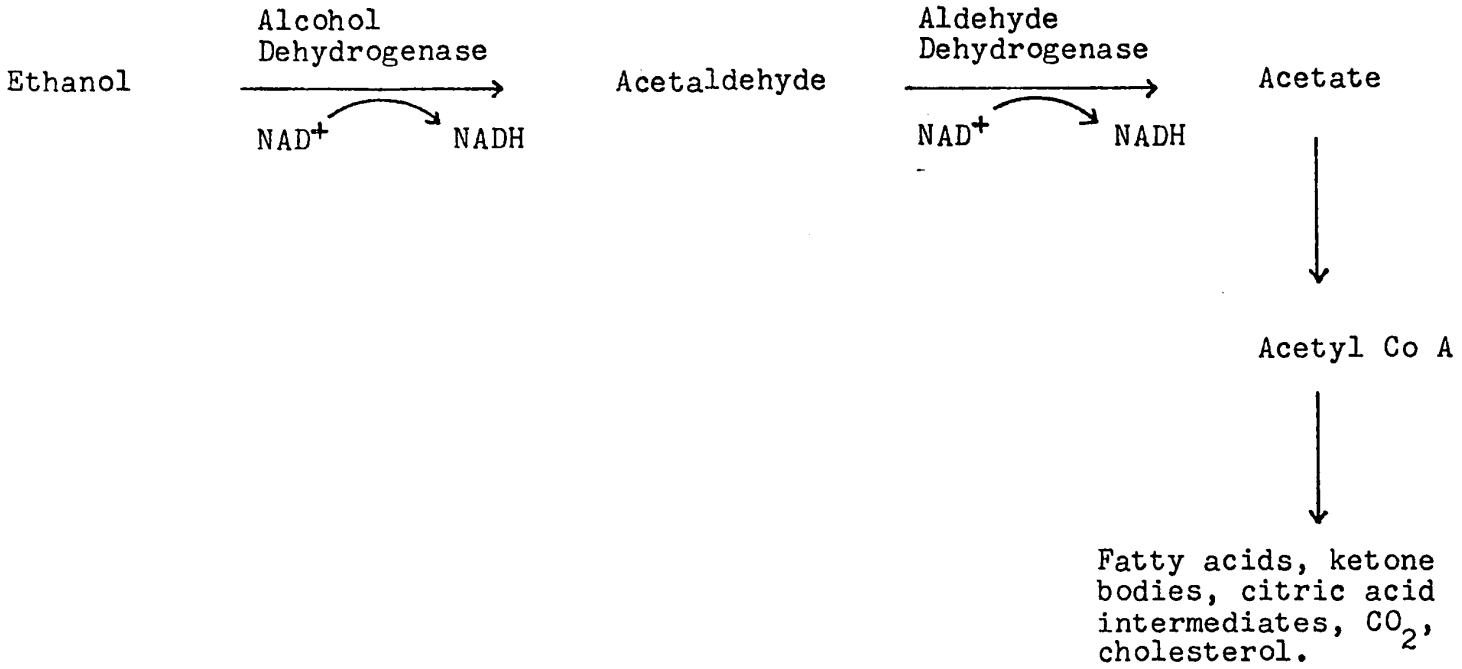
Studies by Machrowicz (1962, 1965) show that ethanol and the higher alcohols inhibit the utilization of glucose in KCl-stimulated brain cortex slices.

Studies by Roach (1970) show that ethanol in small doses (2.5 g/Kg) markedly inhibits the utilization of glucose in the brain of the hamster <u>in vivo</u>.

Ethanol inhibits the conversion of the carbon

Figure 1

Routes of Ethanol Metabolism



skeleton of glucose into glutamate, aspartate, glutamine and γ-aminobutyric acid, thus suggesting an inhibition of the Krebs cycle.

The processes leading to increased O_2 and glucose consumption in the stimulated nerve cell can be summarized as follows:

1. A certain degree of depolarization produces changes in the nerve cell membrane that lead to the production of an action potential: Na^+ ions enter the cell and K^+ ions leave it (Hodgkin, 1964; Huxley, 1964).

2. This is followed by increased active transport of Na^+ and K^+ with a concomitant hydrolysis of ATP (Cummins, 1961).

3. Thus, the availability of ADP to the mitochondria is increased and consequently;

(a) The O_2 consumption is increased.

It is also to be expected that the oxidation of reducing equivalents provided by glucose and the Krebs cycle intermediates may increase, thus;

(b) Increasing glucose utilization and Krebs cycle turnover.

At the same time the steady-state level of creatine phosphate, a compound in equilibrium with ATP is reduced (Atkinson, 1966; Wallgren, 1963).

$$CrP + ADP \longrightarrow ATP + Cr$$

The data accumulated with brain slices, isolated mitochondria, and experiments in vivo suggest that ethanol does not interfere with the synthesis of high-energy phosphates but that it inhibits their utilization. The effects of ethanol are thus probably exerted on the molecular processes that lead to the action potential or on the subsequent active transport of Na^+ and K^+.

Because ethanol is a CNS depressant, and it affects its function as reflected by euphoria, loss of motor control, unconsciousness, and severe psychological and physical dependence, it is reasonable to look for ethanol-induced effects on neurotransmitters and the enzymes intimately associated with their metabolism.

Monamine oxidase, l-glutamic acid decarboxylase and acetylcholinesterase are the enzymes studied in this project and they will be described in the following sections.

A. Monamine Oxidase (monamine: oxygen oxidoreductase (deaminating) EC 1.4.3.4)

1. Discovery, Occurrence and Metabolic Function of MAO

In 1928, Hare described an enzyme that catalyzed the oxidative deamination of tyramine. This is the first mention of the enzyme now known as monamine oxidase. Subsequently it became clear that this enzyme was widely distributed in animals. In 1937, Pugh and Quastel observed

an amine oxidase in brain. It had been shown that tyramine tryptamine and isoamylamine on being added to brain slices incubated in a physiological saline-glucose medium bring about a diminution of brain respiration. It was found that aliphatic amines are oxidized in brain to liberate NH_3 and dinitrophenylhydrazine-reacting substance, subsequently shown to be the corresponding aldehyde. Tyramine and tryptamine of the brain are also attacked by the amine oxidase, which attacks the aliphatic amines liberating NH_3 and the corresponding aldehyde. The brain amine oxidase was found to have similar properties to those of hepatic amine oxidase.

Amine oxidase has been found in all vertebrates tested, in mollusks, in echinoderms and in annelids. It also occurs in bacteria and plants but a typical monamine oxidase has been only found in animals.

Amine oxidase occurs in many vertebrate tissues. The liver is a good source, but all glands contain amine oxidase. The enzyme is also found in nervous tissue, in the gonads, in smooth muscle, and in smaller amounts in cardiac muscle and blood. It seems to be absent from skeletal muscle.

Amine oxidase or monamine oxidase is the term used for the enzyme which attacks the physiologically active monamines. Only the monamines containing a terminal amino group are attacked. Those with a substituent on the α-carbon atom are not attacked by the enzyme.

When monamines are oxidized by monamine oxidase the following transformations take place:

$$R.CH_2NH_2 + O_2 + H_2O \longrightarrow R.CHO + NH_3 + H_2O_2$$

$$2\ R.CHO + H_2O \longrightarrow R.CH_2OH + R.COOH$$

where (R = hydrogen, an alkyl or an aryl group)

The brain is equipped with an aldehyde dehydrogenase and also with a mutase or reducing system that brings about the formation of the corresponding alcohol. These aldehyde-removing systems have turned attention away from the possible implications of aldehyde formation from cerebral amines. Nevertheless, there are observations indicating that aldehydes formed from neuramines may influence various metabolic changes and neuronal function.

2. Chemical Properties and Substrates of MAO

Monamine oxidase is a relatively insoluble enzyme. A number of solubilizing agents have been tested and found to be useful in producing a moderate purification of the enzyme. Blaschko and Jacobson (1942) used lysolecithin to produce a clear solution. Barsky, Berman and Zeller (1953) have treated the enzyme with sonic

oscillations in the presence of deoxycholate to produce a clear solution. Cotzias, Serlin and Greenough (1954) have used the detergent "Cutscum" with isooctylphenoxypolyphenoxyethanol to solubilize monamine oxidase.

Because of the inhibition of the enzyme by sulfhydryl reagents, there is the likelihood that the enzyme contains a sulfhydryl group (Smith, 1960).

Alles and Heegaard in 1943, suggested that MAO is not one enzyme but a mixture of different enzymes. Hagan and Weiner (1959), and Hope and Smith (1960) have shown independently that enzymes from different organs differ in their action on different substrates.

Monamine oxidase has 3 groups of substrates:

(a) N-substitution

Primary and secondary amines are readily oxidized by MAO, but the latter only if the substituent is a methyl group. MAO attacks N-methylated amines in contrast to other amine oxidases that attack primary amines (Blaschko, 1960). Tertiary amines are oxidized very slowly.

(b) Aliphatic amines

The enzyme does not act on methylamine, but ethylamine is slowly oxidized. In the homologous series $CH_3(CH_2)_nNH_2$, maximal rates are attained with amylamine or hexylamine. The fall in the rate of oxidation with longer chains is probably due in part to the relative insolubility of the long-chain members.

(c) Other substrates

The most important naturally occurring substrates of MAO carry cyclic substituents. These are derivatives of β-phenylethylamine, of tryptamine and of histamine. The catechol compounds are dopamine, norepinephrine, epinephrine, metanephrine and normetanephrine. Of the tryptamine derivatives, 5-hydroxytryptamine is the most important naturally occurring substrate. Of the histamine derivatives, 1,4-methylhistamine is an important substrate of MAO.

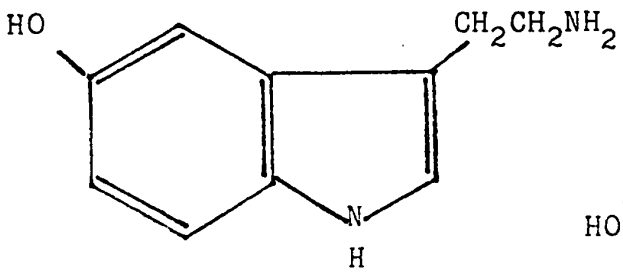
3. Detection and Estimation of Monamine Oxidase

Histochemical techniques for the detection of MAO in tissues make use of the fact that in the presence of tryptamine or 5-hydroxytryptamine (serotonin), the enzyme produces a melaninlike pigment, brown or black, firmly absorbed on the tissue slices, whose location gives an indication of its site in tissues. This pigment in vivo, is also oxidized to the corresponding carboxylic acid, which is excreted and can be estimated.

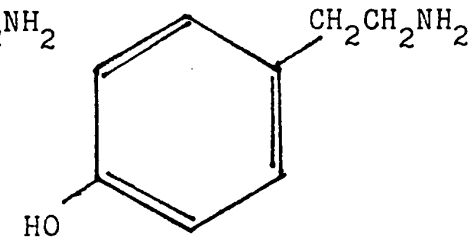
Another method for the detection of the enzyme is that the aldehyde formed from the amine reduces tetrazolium with the formation of formazan.

Arioka and Tanimukai (1953) developed a histochemical method using serotonin as substrate. Koelle and Valk (1954) developed another method using tryptamine as substrate.

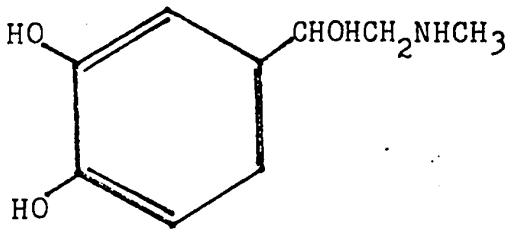
Figure 2
Some of the Substrates of Monamine Oxidase



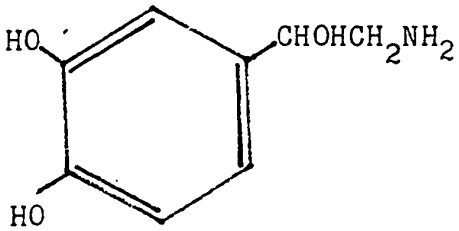
5-hydroxytryptamine



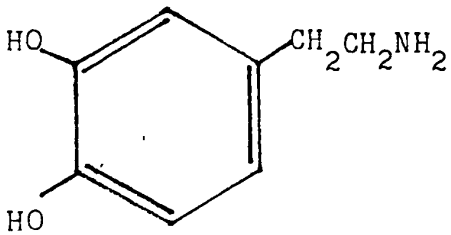
Tyramine



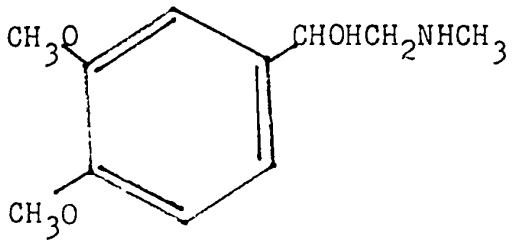
Epinephrine



Norepinephrine



Dopamine



Metanephrine

Pugh and Quastel (1937) described a colorimetric method for the estimation of MAO, based on the observation of the red color that turns yellow-orange. This color is formed in the solution in alkali of the dinitrophenylhydrazone of the aldehyde derived from the amine.

There are several spectrophotometric assays for MAO. The oldest is that of Tabor et al., (1954) employing benzylamine as substrate and following the rate of formation of benzaldehyde at 250 nm. A similar assay employs m-iodobenzylamine as substrate (Zeller, Ramachander and Zeller, 1965). These assays suffer the primary disadvantage of being carried out in the ultraviolet range where protein and other materials absorb appreciably.

Another method is that of Weissbach et al., (1960) and depends upon decrease in absorbance of kynuramine at 370 nm. This assay is carried out at a higher wavelength than previously reported assays. However, the substrate is unstable and relatively expensive. Deitrich and Erwin (1969),used p-dimethylaminoaldehyde as a substrate for the assay of MAO, since it has a large molar extinction coefficient at 355 nm ($\varepsilon = 2.77 \times 10^4$).

MAO activity is also estimated by measurement of the ammonia formed during incubation of the tissue with tyramine in a Conway diffusion chamber, the ammonia

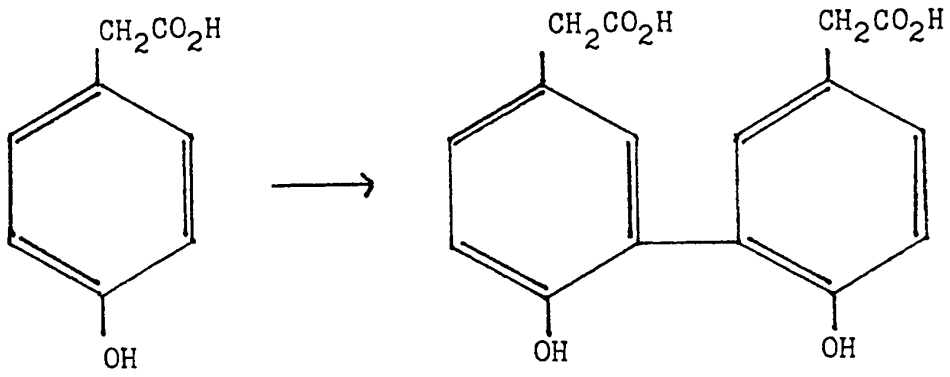
being absorbed by boric acid placed in the centre well of the chamber (Aprison & Takahashi, 1964).

Radiometric methods have been described for the assay of MAO utilizing labeled tryptamine by Otsuka and Kobayashi, 1964; Wurtman and Axelrod, 1963. Because of limitations in molar absorptivities or of changes in pH, most methods described previously for measuring components in enzyme reactions except radiometric methods are limited to reactions of reagents present at concentrations greater that 10^{-6} M. Because fluorimetric methods are generally several orders of magnitude more sensitive than chromogenic ones, a large increase in the sensitivity of measurement results. Thus, much lower concentrations of reactants would be needed and methods have been devised to measure substances 10^{-9} M concentrations or lower.

Guilbault et al., (1968) reported homovanillic acid and p-hydroxyphenylacetic acid to be ideal substances for the determination of MAO.

$$\text{substrate} + H_2O + O_2 \xrightarrow{\text{oxidase}} \text{aldehyde} + NH_3 + H_2O_2$$

$$H_2O_2 + \text{p-hydroxyphenyl acetic acid} \xrightarrow{\text{peroxidase}} \text{oxidized product (fluorescent)}$$



4. Cellular and Subcellular Distribution of MAO

Although MAO is found in all vertebrates tested, in some invertebrates, in plants and in bacteria, yet the typical MAO is only found in animals. The enzyme occurs in a variety of vertebrate tissues, particularly liver. It is present also in the nervous tissue.

Using the pigment-histochemical test, Arioka & Tanimukai (1957) found that the dark-brown coloration indicative of MAO is greater in the gray matter than in the white matter, and that coloration in the hypothalamus is very intense. The pigment granules are seen in the cytoplasm, especially the perinuclear region and absent in the nuclei of nerve cells of the paraventricular structures. No pigment formation is seen in the nerve fibres or in glial cells.

Usually, relatively high amounts of MAO are found in structures richly endowed with sympathetic nerves

as well as the nerves themselves. The brain differs in that it does not have a homogeneous distribution of the enzyme, the greatest activity being localized in the midbrain (Nachmias, 1960).

MAO activity is found in the neurohypophysis, oculomotor and optic nerves, the myelin sheaths of rat sciatic and optic nerves, the midbrain of the mouse, and the rat and the pons and mesencephalon of the squirrel monkey.

The greater part of liver MAO is found to be in mitochondria (Jarrott & Iverson, 1968).

Ladwon and Belpaire (1968), found that the mitochondria of adrenal medulla contain the greater part of its MAO.

Subcellular fractionation of whole rat brain shows that 70% of MAO activity is in the mitochondrial fraction, 17.5% in the nuclear fraction and 12.5% in the microsomal fraction (Davidson, 1958).

There is a close parallelism between the distribution of MAO and succinic dehydrogenase in rat brain (de Lores Arnaiz & de Robertis, 1962). These workers have shown that the activities of both enzymes are very low in cholinergic nerve endings, are high in the non-cholinergic nerve endings and they are highest in the mitochondria.

5. Inhibition of Monamine Oxidase

(a) Readily reversible inhibitors

In all substrates of MAO, the terminal carbon atom is unsubstituted. Amines that have one of the hydrogen atoms at C-1 replaced by methyl group are inhibitors of MAO. These compounds are competitive inhibitors whose action is readily reversible, e.g., ephedrine, harmaline and related compounds.

(b) Less readily reversible inhibitors

Amidines carry the amidino group -C-(:NH)NH_2 and are very potent inhibitors of MAO, but there are great differences in sensitivity among the enzymes of different species. The length of the polymethylene chain separating the two amidino groups affects inhibitory activity and the optimal chain length differs from species to species. In one and the same homologous series, different degrees of reversibility have been encountered.

Tedeschi at al., (1959) discovered that cyclopropylamines are potent inhibitors of MAO.

(c) Hydrazine derivatives

Zeller et al., (1959) discovered that isopropyl derivative of isonicotinic acid hydrazide was a potent inhibitor of MAO. Both the acidhydrazides of the structure R'.C(:O).NH.NHR" and alkylhydrazines have potent inhibitory properties.

(d) Other inhibitors

Methylene blue and certain aliphatic alcohols are active inhibitors of MAO.

Resenfeld (1960) measured serotonin metabolism and showed that there seemed to be an appreciable decrease in the formation of 5-hydroxyindolacetic acid (5HIAA) after sublethal doses of alcohol. He suggested that the oxidation of alcohol, competitively inhibited the oxidative metabolism of serotonin and perhaps in particular the oxidation of the aldehyde derived from it.

Feldstein et al., (1964) were able to demonstrate that in vivo, alcohol blocks the metabolism of serotonin to 5HIAA. They claim that it was hard to detect which enzyme was altered by alcohol, MAO or aldehyde dehydrogenase.

Maynard et al., (1962) showed that ethanol did inhibit MAO in mouse liver but not in brain.

In 1964, Towne demonstrated that acetaldehyde (0.12 M) inhibited both liver and brain MAO in vitro, by 26% and 36% respectively.

Ethanol by way of its proximate metabolite acetaldehyde seemed to be a nonspecific inhibitor of MAO. This is because of the high chemical reactivity of this 2-carbon aldehyde that could conceivably inactivate the enzyme. Acetaldehyde condenses with various amines to form alkaloids. The deamination rate varies markedly with the substrate concentration used, so that at any given substrate concentration when acetaldehyde is present it may

prevent the biogenic amine from being deaminated by condensing with it and forming the respective cyclic alkaloid derivative. This is due to actual inhibition of MAO as well as substrate limitation, resulting from condensation of the amine substrate with acetaldehyde.

The effect of acetaldehyde is apparent and indirect rather than non-specific direct action on the enzyme. This effect may not be of pharmacological significance, because of the high levels of acetaldehyde required to produce it in vitro. However, the endogenous systhesis of alkaloids that are effective MAO inhibitors with greater intrinsic activity than acetaldehyde alone, after alcohol ingestion could still make this effect of alcohol an important one and requires further research.

B. l-Glutamic Acid Decarboxylase (l-Glutamic l-Carboxyl-lyase, EC 4.1.1.15)

1. Occurrence and Metabolic Function of GAD

Among vertebrates the enzyme, l-glutamic acid decarboxylase (GAD) and the reaction product γ-aminobutyric acid (GABA) have been found solely in the central nervous system. The enzyme requires pyridoxal phosphate (PyP) as a coenzyme. Very minute amounts of GAD apoenzyme have been detected in the cerebrospinal fluid. Regional levels of GAD apoenzyme reflect, quite accurately, the regional

levels of GABA. The close correlation between the level of apoenzyme and product, in almost all of the brain areas, suggest that GAD is the only significant enzymatic pathway leading to GABA in nerve tissues.

The GAD enzyme, together with the enzyme γ-amino butyric-α-ketoglutaric transaminase and succinic semi-aldehyde dehydrogenase is responsible for the metabolic pathway: glutamate $\longrightarrow$ γ-aminobutyrate $\longrightarrow$ succinic semi-aldehyde $\longrightarrow$ succinate. Therefore, in the central nervous system, but not in other vertebrate tissues, there exists a metabolic shunt around the α-ketoglutarate oxidase system of the tricarboxylic acid cycle. It has been estimated that this shunt may account for as much as 40% of the oxidative metabolism of the brain (McKhann et al., 1960).

$$\underset{\text{(glutamic acid)}}{HOOC\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH} \xrightarrow[PyP]{GAD} CO_2 + \underset{\text{(GABA)}}{HOOC\text{-}CH_2\text{-}CH_2\text{-}CH_2NH_2}$$

2. General Properties of GAD

GAD extracted from mammalian tissues in crude form is quite unstable, and purification requires protection of the labile reactive sites on the apoenzyme.

Susz, Haber and Roberts (1966) were able to purify the enzyme by working with buffer solutions containing pyridoxal phosphate (PyP) at 1 x 10^{-4} M, and amino-ethylisothiuronium bromide at 1 x 10^{-3} M (a reagent that protects sulfhydryl and possibly other reactive groups) and by protecting the enzyme from exposure to light.

Roberts and Simonsen (1963) found that the pH optimum varies from 6.4 for crude GAD to 7.2 for the most highly purified enzyme with K_m values changing from 3 x 10^{-3} to 8 x 10^{-3} M. Shifts in pH and K_m suggest that there are configurational changes in the enzyme protein during purification. Below 40° the purified GAD is reasonably stable. Above 40° and below 60°, the enzyme is stable only when attached to calcium phosphate gel.

3. Significance of GABA

In the crustacean nervous system there are distinctly separate excitatory and inhibitory nerve fibres, and many findings support the hypothesis that GABA is a specific inhibitory transmitter at the neuromuscular junction.

Graham et al., (1967) found evidence showing that GABA has highest concentrations in the dorsal gray matter and very low concentrations in the white matter and spinal roots. This is compatible with the notion that GABA functions as a postsynaptic inhibitory transmitter.

In contrast to low levels of acetylcholine found in CNS of vertebrates there are large quantities of GABA. For this reason it is unlikely to suggest that GABA is associated with chemical transmitter mechanisms in the synaptic regions. However, it has been suggested that GABA exerts inhibitory effects in the neuronal environment.

GABA may have the role of a stabilizing agent. Wood (1967) proposed the homeostatic function of GABA under hypoxic conditions.

Baxter (1968) showed that GABA participates in the osmotic regulation of brain tissue in toad (Bufo boreas).

GABA is known to be incorporated into proteins of nervous tissue.

Many workers have claimed that derivatives of GABA are biologically active in the nervous system and that they might be important in neuronal physiology.

4. Assay Methods of l-Glutamic Acid Decarboxylase

The activity of GAD is measured either from the volume of CO_2 released or from the amount of GABA formed from glutamate.

CO_2 liberated at acidic pH values is usually measured by conventional manometric techniques in the Warburg apparatus.

Roberts (1951) was the first to describe a

manometric technique for the determination of GAD activity in nervous tissue, based upon the CO_2 evolved by the decarboxylase reaction. By using (^{14}C)-labeled glutamic acid substrate and measuring the $^{14}CO_2$ evolved, the sensitivity of the method is greatly enhanced and offers the possibility to do analyses with very small quantities of tissue. Kravitz (1962) and Lupien, Hinse & Berlinguet (1968) described simple isotopic methods for the measurement of GAD activity. They compared their results with those obtained manometrically by Davidson (1956) and found them to be similar.

When GAD activity is measured by product formation the level of GABA in a system must be determined before and after incubation.

Chromatographic and electrophotometric techniques have been adapted for use with brain tissue extracts and separate many amino acids of which GABA is one.

Biological detectors have also been used to assay GABA. The main shortcoming of these techniques for GABA assay is their lack of specificity.

GABA can be measured quantitatively by enzymatic assay. Jacobsy and Scott (1959) and then Cozzani (1970) described spectrophotometric techniques which were proved very rapid, accurate and sensitive to measure GAD activity. GABA formed during the decarboxylation reaction was measured by adding "Gabase system" (GABA transaminase coupled to succinic semialdehyde dehydrogenase).

Lowe et al., (1957) described the fluorimetric technique that permits determination of activity with as little as 3 µg dry weight of brain tissue. This technique is based upon the conversion of GABA under alkaline conditions to a highly fluorescent GABA-ninhydrin complex.

5. Cellular and Subcellular Distribution of GAD

The uniquely high activity of GAD in brain has been reported by Roberts (1950). Roberts in 1956, using pooled tissues, has shown that there is a five- to six-fold greater activity of GAD in gray matter than in white matter of cat brain. Lowe, Robins & Eyerman (1957) measured the activity of the enzyme in various areas of monkey and rabbit brain as well as in the whole brain of mouse and rat. They showed that the white matter is extremely poor in GAD. This suggests that each of the components of white matter contains little or none of the enzyme, and that GAD is not quantitatively important in their metabolism.

There has been considerable disagreement about the subcellular distribution of GAD. Albers (1960) and Shatunova & Sytinski (1964) found it in fractions operationally defined as mitochondrial. However, Løvtrup's (1961) mitochondrial fraction was the least active of all the fractions prepared. Weinstein et al., (1963) localized GAD in the densest of their synaptosome fractions and suggested that it was present within the mitochondria of

the synaptosomes. Salganicoff & de Robertis (1965) found GAD in their "non-cholinergic" synaptosome fraction and advanced evidence that it was linked to the synaptic vesicles by Ca^{++}. Balazs, Dahl & Harwood (1966) found that GAD was localized in the synaptosome fraction. Fonnum (1967) found that GAD was localized in an osmotically sensitive particle containing cytoplasm, whose sedimentation characteristics are identical with those of synaptosomes and also that the enzyme binds to membrane fractions by Ca^{++}.

6. Inhibition of l-Glutamic Acid Decarboxylase

(a) General inhibitors of GAD

Mammalian GAD is inhibited competitively by halogen anions at 0.05 M concentration. The inhibition by Cl^- decreases with elevated pH levels. Since glutamate is a potential excitatory agent and GABA is an inhibitory agent, it has been suggested that the regulation of GAD by Cl^- concentrations might have physiological significance by controlling the ratio of glutamate to GABA at specific nerve endings.

Tashian (1961) found that <u>in vitro</u>, mammalian GAD is inactivated by a variety of sulfhydryl reagents and by oxidation. These effects can be prevented by adding glutathione or other SH group protective agents. The enzyme is also inhibited by estrogens, salicylate and biologically important phenolic acids.

The pyridoxal phosphate cofactor of GAD is only loosely linked to the apoenzyme. Dietary B_6 deficiency in vivo, as well as a large number of carboxyl trapping agents inhibit the enzyme.

(b) Ethanol, inhibitor of GAD

The effect of ethanol has been studied on levels of GABA in the brain rather than on levels of GAD.

GABA is regarded as a potential inhibitory transmitter, but its position as an important intermediate in the energy metabolism of the nerve cells obscures its physiological role in the mammalian CNS.

The proposed inhibitory function of GABA was demonstrated by the findings of Häkkinen and Kulonen (1959), when they showed the 34% increase in GABA content in the brain of ethanol-intoxicated rats. However, subsequent work, mainly with rats has given contradictory results. Häkkinen and Kulonen (1961) reported that ethanol increased cerebral GABA, wheras Ferrari and Arnold (1961) and Higgens (1962) found insignificant decrease or no change.

Sutherland and Rikimuru (1964) measured regional changes in concentrations of GABA and the only change that they found was a decrease in the GABA content of the cerebellum and the medulla. By means of the freeze-stop technique, Gordon (1967) found a decrease in the GABA content of the cerebellum, wheras Mouton et al., (1967)

demonstrated an increase in the GABA content of mouse brain. Häkkinen and Kulonen (1963) could reproduce their prior finding in fasted rats but found no change in the GABA content of fed animals given ethanol. Flock et al., (1969) could show that ethanol had no effect on the concentration of GABA in either cerebrum or hindbrain. Roach (1970) found that ethanol produces no significant effects on brain GABA levels.

As demonstrated above, changes in concentrations of GABA induced by ethanol are somewhat controversial.

Some of the experimental observations indicate that GABA is not an important primary factor in the intoxication process. Some indicate that the changes in GABA content are a reflection rather than a cause of the action of ethanol and some others indicate that the increase in GABA content and decrease in glutamine content fit in with the evidence that ethanol slows reaction rates in the citric acid cycle.

C. Acetylcholinesterase (Acetylcholine Hydrolase, EC 3.1.1.7)

1. Occurrence and Significance of AChE

Acetylcholinesterase has been found in the innervated tissues of all vertebrates and invertebrates which have been studied. The enzyme activity is maximal

in the synaptic membranes of cholinergic synapses (Wilson, 1960). The specialized junctional tissues of electric organs are a good source of the enzyme.

The significance of AChE at postsynaptic membranes is demonstrated by the prolonged duration of AChE effects seen after esterase inhibitors. Many major insecticide and chemical warfare agents belong to the group of irreversible inhibitors of AChE. It seems likely that AChE in cholinergic nerves does hydrolyze some of the AChE which is synthesized throughout the same fibres, although the physiological merit of this section is unknown.

The presence of AChE in nonneuronal membranes raises questions about the functions of the enzyme. In the placenta there are generous quantities of ACh and AChE. It has been suggested that ACh could function in some way to permit antibody transfer through membranes (Hebb and Ratkovic, 1962). In red blood cells, the presence of AChE is not universal and there is little reason to believe that significant ACh metabolism occurs in them (Hebb, 1957).

2. Physical and Chemical Properties of AChE

Acetylcholinesterase is difficult to purify and relatively pure samples have not been prepared. Consequently, the molecular properties have not been extensively studied, and only the molecular and equiv-

alent weights have been investigated. During purification and storage processes of AChE, high dilution and many organic solvents bring about its denaturation. Kremzner and Wilson (1964) characterized the enzyme and found the molecular weight of AChE of the eel to be 230,000. By gel filtration the weight appeared to be 250,000 and no evidence of larger or smaller units was obtained.

It appears that AChE has four active sites per molecule and its isoelectric point to be at pH 3.1. There is excellent evidence for at least one anionic site on AChE which attracts the positively charged quaternary nitrogen of ACh, and evidence for one esteratic site at which hydrolysis occurs (Wilson, 1960; Cohen and Ooserbaan, 1963).

Available evidence supports the view that AChE is acetylated by ACh and then reacts with water to free acetic acid.

$$\underset{\text{(Acetylcholine)}}{(CH_3)_3N^+CH_2CH_2OCOCH_3} + H_2O \longrightarrow \underset{\text{(Choline)}}{(CH_3)N^+CH_2CH_2OH} + CH_3COOH$$

Attraction of ACh to the anionic site of AChE is believed to be due to coulombic and Van der Waals forces, primarily the former. The attractive force be-

tween ACh and the esteratic site of AChE appears to be due to a weak covalent bond between the carbonyl carbon atom of the acetyl group and at least one basic group on the enzyme itself.

3. Methods for the Assay of AChE

Measurement of the activity of a relatively pure AChE preparation is an easy matter for which there are a large number of effective and convenient methods. However, measurements involving tissue samples from most sources are complicated by the need to distinguish the separate activities of mixed amounts of AChE, ChE and simple esterases. The only suitable way to do so is with the use of relatively specific substrates and inhibitors.

AChE hydrolyzes several substrates at different rates (ACh $>$ acetyl-β-methylcholine $\gg$ butylcholine), but not benzoylcholine, and is readily inhibited by bis-quaternary compounds, like ambenonium.

ACh is the preferable substrate for AChE, because it is the natural one and its analogues are split at a different rate.

Hestrin (1949) used a procedure which measures the amount of unreacted ester by the ferric hydroxamate method. This procedure does not have any restriction to any substrate, pH medium and temperature. Ellman et al., (1964) used another method in which the free thiol of hydrolyzed ACh is measured with a sulfhydryl reagent.

Although this method is limited to thiocholine esters as substrates, the method is otherwise highly adaptable and sensitive. Most other methods for AChE assay, measure the amount of acetate produced by CO_2 production from bicarbonate buffer, by titration, pH change, electrometric or indicator method or by assay of radioactive acetate (Augustinsson, 1963).

4. Cellular and Subcellular Distribution of AChE

The factors which regulate the amount of AChE in nervous tissues are unknown. The total activity of the enzyme generally increases with growth, both during embryological life (Koelle, 1963) and in proximal regenerating tips of cut nerves (Hebb and Krnjević, 1962).

Three techniques have helped to define the localization of AChE: classical subcellular fractionation of tissues, microanalysis of single cells and histochemistry.

Kremzner and Wilson (1964) found during partial characterization of the enzyme that almost all of it remains associated with membrane fragments in sucrose solution, and its solubilization requires high concentrations of salts, organic solvents or detergents. This is the best evidence that AChE is localized in membranes <u>in vivo</u>. Early fractionation studies of brain tissue showed that AChE was associated with many membrane fragments, particularly microsomes (Whittaker, 1965). Subsequent studies

showed that much of the enzyme is associated with isolated nerve endings which have attached postsynaptic membranes (Potter, 1968; de Lorez Arnaiz et al.,1967).

Within the limits of dissection, the ultramicroassays of AChE by Giacobini (1959) and others, using Cartesian divers as small as 0.005 µliters, have greatly extended our knowledge of the quantitative amounts of enzyme in cells and parts of cells. These studies show that the amount of enzyme in end plates is at least 50 times that in extrajunctional muscle; that the enzyme is in high concentrations in cholinergic cells like horn cells; that there is a wide variation of enzyme levels between different juxtaposed cells.

Koelle, Couteaux and their coworkers (1963) have developed histochemical methods for the demonstration of AChE. These methods have shown that AChE is widely distributed in nerves, particularly known cholinergic ones, and it is present in dendrites, cell bodies, axons and at the surface of nerve terminals. It is maximal at muscle end plates and is seen in red blood cells.

5. Inhibition of Acetylcholinesterase

Alkyl phosphates such as tetraethyl pyrophosphate and diisopropyl fluorophosphate are potent inhibitors. These compounds are among the most toxic substances known, some are nerve gases and others are widely

used as insecticides.

All these inhibitors contain an easily hydrolyzed phosphorous bond, i.e. they are phosphorylating agents, and they act by phosphorylating the enzyme.

Any ammonium ion could be a reversible competitive inhibitor of AChE. 3-hydroxyphenyltrimethyl ammonium ion, phenyltrimethyl ammonium ion and dimethyl ammonium ion are potent inhibitors.

Some investigators have assumed that ethanol inhibits AChE (Lowe et al., 1962). According to Fujita (1954) AChE activity is unchanged in the brain of acutely intoxicated rabbits, and Kinard and Hay (1960) have found that both acute and repeated ethanol intoxication causes a functionally insignificant reduction of cerebral AChE activity in rats. Kalant et al., (1967) have reported that ethanol does not affect the AChE activity of brain tissue <u>in vitro</u>. Thus AChE inhibition is not involved in the action of ethanol.

Ethanol augments the release of ACh from the synaptic vesicles at cholinergic membrane. It is not apparent whether this effect is on the combination of ACh with receptors or on subsequent changes in membrane components which govern the ionic movements involved in spike generation.

There is little evidence for effects of ethanol on other aspects of cholinergic transmission.

II. RESEARCH OBJECTIVE

The present investigation was designed to study some of the effects of chronic ethanol feeding on metabolism of neurotransmitters, by determining the apparent activity of enzymes intimately associated with them.

Monamine oxidase (MAO) is believed to be an enzyme that is involved in the catabolism of the most important naturally occurring neuramines, e.g. serotonin, epinephrine, etc.

l-Glutamic acid decarboxylase (GAD) is part of the only significant pathway leading to γ-aminobutyric acid (GABA) in nervous system. GABA is believed to be a negative feedback transmitter in the central nervous system, as well as an intermediate in the normal metabolic pathways of the brain.

Acetylcholinesterase (AChE) is believed to be the enzyme which hydrolyzes acetylcholine (ACh) at postsysnaptic membranes. ACh is an important neurotransmitter of impulses in nerves and muscles.

Since the three enzymes seem to be associated with the metabolism of neurotransmitters, therefore, it is probable that changes in their levels induced by various inhibitors correspond to functional changes at the synaptic levels as well as, to general metabolic

changes.

Many workers have reported discrepancies in the results for the distribution of these three enzymes in the whole brain of chronically ethanol fed rats. These results could reflect major differences in the distribution of the enzymes at regional and cellular levels. Therefore, it seemed worthwile to examine simultaneously their distribution in five regions of the brain, both in rats given the ethanol diet and rats given the liquid control diet.

III. MATERIALS AND METHODS

A. Preparation of the Enzyme from Rat Brain

1) Method

Rats weighing 100 to 120 g were sacrificed by cervical fracture, the brain was removed and kept cold on ice. The brain was homogenized in 1.5 to 2.5 ml of ice-cold isotonic solution of NaCl (0.9% by weight), in a motor driven glass homogenizer. The different regions dissected off the brain to be studied were:

a. Olfactory lobe

b. Superior colliculus

c. Inferior colliculus

d. Thalamus

e. Occipital lobe of cerebral cortex

The homogenates were kept in the freezer until used, so that the cell membranes would break to liberate the different enzymes. Aliquots of these homogenates were taken for enzyme assay.

2) Materials

The rats were obtained from Dr. E.R.Gordon at University Medical Centre, McGill University, Montreal 109, Quebec.

The rats used were male albino Sprague-Dawley strain. They were divided into three groups. Group 1 and 2 were used as experimental animals and group 3 as con-

trols. During the experimental period, animals in groups 1 and 2 were maintained on the normal liquid diet for 10 days, then ethanol was added at 20% of the total caloric intake. After 10 days, the ethanol concentration was increased to 36% of the total caloric intake. After 24 days at this ethanol concentration, animals of groups 1 and 2 were killed with the control animals. The animals of group 2 were given the normal liquid diet for one day before being killed. Group 3 maintained on the liquid diet, as described by Lieber, Jones, Mendelson & de Carli (1963) and Lieber & Spitz (1966), except that the source of fat was corn oil. All animals had excess of water and those on the liquid diet were given fresh diet daily in Richter tubes. The rats were not starved before being killed but in each experiment, they were killed at the same time of day. At the time of death, the rat was stunned by a blow on the head, the abdomen was quickly opened to obtain the liver for other experiments, after which the skull was dissected to obtain the brain. Minimal brain damage was noted at autopsy.

TABLE I

Composition of Liquid Diet of the Animals

	Group 1	Group 2	Group 3
Protein	19.4	19.4	19.4
Carbohydrate	4.8	4.8	40.8
Fat	39.8	39.8	39.8
Ethanol	36.0	36.0	0.0
Residue	0.0	0.0	0.0

B. Fluorimetric Measurement of Monamine Oxidase

The enzymatic oxidation of amines is accompanied by the uptake of oxygen and the production of ammonia, hydrogen peroxide and the corresponding oxidation products of the amine. All of these parameters, as well as the disappearance of substrate have been used as the basis for assaying oxidative enzymes.

Sydner & Hendley (1968), followed MAO activity using a technique based on the principle that hydrogen peroxide formed in the oxidase reaction can be measured when coupled with homovanillic acid to form a fluorophore in the presence of peroxidase.

Guilbault, Brignac & Juneau (1968a), showed that p-hydroxyphenylacetic acid could be used in place of homovanillic acid. This substrate had the advantages over homovanillic acid of low cost and a higher fluorescence coefficient.

Guilbault, Kuan & Brignac (1969), employed the better substrate p-hydroxyphenylacetic acid, instead of homovanillic acid to measure MAO activity in beef blood.

The method of Sydner & Hendley (1968), was followed to measure MAO activity with only one variation, p-hydroxyphenylacetic acid was used instead of homovanillic acid. This method is based on the formation of an intensely fluorescent product from p-hydroxyphenylacetic acid and hydrogen peroxide released during oxidative deamination of added substrate. Tyramine hydrochloride was

the substrate used.

For practical assay, triplicate samples for incubation were made-up to contain in a final volume of 3 ml:

(2.40 ± 0.02) ml of 0.1 N Na-K phosphate buffer pH 7.8

(0.2000 ± 0.0004) ml of (1.5 ± 0.1) mg/ml horse radish peroxidase solution.

(0.250 ± 0.002) ml of (2.43 ± 0.02) x 10^{-2} M p-hydroxyphenylacetic acid.

(0.1000 ± 0.0002) ml of brain homogenate.

(0.0500 ± 0.0002) ml Of (8.9 ± 0.1) x 10^{-3} M tyramine hydrochloride solution.

Before the addition of p-hydroxyphenylacetic acid and substrate, the tubes were preincubated for 10 min. with shaking at 37° in a Dubnoff metabolic incubator, to remove endogenous substrates of H_2O_2-producing enzymes. After preincubation, p-hydroxyphenylacetic acid and substrate were added and the tubes were incubated for 1 hr. at 37°, with shaking. The reaction was stopped by chilling the tubes to 4° and their fluorescence intensity was measured on a Baird-Atomic "Fluorispec" Model SF-1, at an excitation wavelength of 317 nm and fluorescence wavelength of 414 nm.

Blanks containing tissue enzymes but no added substrate were subtracted for calculation of enzyme activity.

Calibration of the instrument was accomplished by adding increasing amounts of freshly prepared standard hydrogen peroxide solution $(3.26 \pm 0.04) \times 10^{-6}$ M, to the cuvette containing in 3 ml:

(2.50 ± 0.02) ml of 0.1 N Na-K phosphate buffer, pH 7.8

(0.2000 ± 0.0004) ml of (1.5 ± 0.1) mg/ml horse radish peroxidase solution.

(0.250 ± 0.002) ml of $(2.43 \pm 0.02) \times 10^{-2}$ M p-hydroxyphenylacetic acid.

The experimental data for the calibration will be reported in "Appendix B".

The resultant fluorescence readings were used to calculate equivalent moles of H_2O_2 formed per hour per gram wet brain tissue.

One unit of MAO activity was defined as that amount which transformed 0.6 nanomoles of tyramine hydrochloride at 10^{-4} M concentration/min. at 37°.

C. Fluorimetric Measurement of l-Glutamic Acid Decarboxylase

The method of Lowe, Robins and Eyerman (1957) was used to measure the activity of GAD in various areas of rat brain. This method is based on the principle that certain amino acids and indole derivatives when allowed to react with ninhydrin at an alkaline pH, form highly

fluorescent products. γ-aminobutyric acid (GABA) has given the greatest amount of fluorescence among many substances tested.

For practical assay, triplicate samples were run.

Before the enzyme assay, the complete buffer-substrate-coenzyme mixture was prepared by combining:

(1.000 ± 0.002) ml of 0.4 M potassium phosphate buffer, pH 6.4

(1.000 ± 0.002) ml of (0.10 ± 0.01) M glutamic acid solution, pH 6.61

(0.0400 ± 0.0002) ml of (4.99 ± 0.01) x 10^{-2} M pyridoxal phosphate solution.

For the assay, 0.1 ml of brain homogenate was placed in a small test tube in an ice-bath and 0.1 ml of complete buffer-substrate-coenzyme solution was added. The tubes were parafilm-capped and incubated at 38° in a constant-temperature water bath, for exactly 60 min. After the incubation period, the tubes were returned to the ice-bath and 0.2 ml of 10% trichloroacetic acid (TCA) solution was added to each of them. The tubes were centrifuged at 3000 rev/min for 10 min. and 0.2 ml aliquot of the supernatant fluid was mixed with 0.4 ml of ninhydrin regent in a test tube. The tubes were capped with tightly-fitting stoppers and heated at 60° in a Dubnoff metabolic incubator for exactly 30 min. After the incubation period, 10 ml of copper tartrate reagent was

added. After 15 min., at R.T., the solution in each tube was transferred into a 4 ml fluorescence cuvette and read on the "Fluorispec" Model SF-1, at excitation wavelength of 375 nm and fluorescence wavelength of 485 nm.

Blanks were run by adding TCA directly to the same amount of homogenate before adding the buffer-substrate-coenzyme mixture and incubating in the water bath at 37°. The blank readings were subtracted for calculation of enzyme activity.

Calibration of the instrument was accomplished by adding increasing amounts of GABA solution (3.99 ± 0.02) x 10^{-3} M and following the same procedure.

The experimental data for the calibration will be reported in "Appendix B".

The resultant fluorescence readings were used to calculate moles GABA formed per hour per gram wet brain tissue.

D. Spectrophotometric Measurement of Acetylcholinesterase

The principle of the method used to measure the activity of AChE is that of Lowry, Roberts, Wu, Hixon and Crawford (1954) with some modifications.

Acetylcholine splits in a solution of 1.69 mM sodium barbital containing phenol red. The acid dissociation constants of phenol red and barbital are within 0.1 pH unit of each other. Therefore, as the liberated

acetic acid converts a stoichiometric amount of sodium barbital into free diethyl barbituric acid, a much smaller but almost proportionate amount of alkaline phenol red is converted into the acid form. A spectrophotometric reading at 561 nm, in which only the alkaline form is measured, becomes therefore a direct measure of the enzyme action. Phenol red in sodium barbital at the concentration used is not inhibitory.

The stock buffer consisted of 0.06% phenol red in 0.03 M sodium barbital. This was diluted exactly 1:20 on the day of use with 1 mM $MgCl_2.6H_2O$ in 0.1 N NaCl. This was a modification since this gave more a stable buffer solution and higher activity than using CO_2-free glass distilled water. The substrate was 0.6% (0.025) M acetylcholine bromide in the electrolyte solution. This solution was stored in small quantities (5 ml) to avoid repeated thawing which results in hydrolysis. In a room low in CO_2 (where people have not been working), 20 µl of the dilute phenol red buffer was added to 10 µl of brain homogenate in the test tube. The tube was then tapped to dispense the homogenate. After 15 min. of fore-period , 5 µl of substrate was added to the tube at zero time and the sample was buzzed.

Buzzing means mixing by holding a microtube to a small eccentric rotating at high speed. Tubes with a 1.5 to 3.0 mm bore were used. Mixing in these tubes would be difficult otherwise.

The tube was then put in the "Beckman DB Spectrophotometer" at a constant wavelength of 561 nm. The recorder was turned on and the rate of the reaction was followed for about 5 min. This procedure was standardized by reading few tubes after adding a trace of 0.099 N sodium hydroxide solution to the dilute phenol red buffer (R_1) and a slight excess of 0.111 N acetic acid (R_2). The difference in these two readings $\Delta R_{std.}$, is equivalent to: $20 \times 10^{-6} \times 1.69 \times 10^{-3} = 33.8 \times 10^{-9}$ moles of acetic acid liberation. In any tube, a color change corrected for blank of 32% of $\Delta R_{std.}$, indicates the liberation of 11.3×10^{-9} moles of acetic acid. The time (t) in minutes to reach 32% of $\Delta R_{std.}$ was calculated by interpolation.

The rate of enzyme activity is:

$$11.3 \times \frac{60}{t} \times \frac{10^{-9}}{\text{g wet brain}}$$ moles per hour per gram wet brain tissue

Few determinations were done using the purified acetylcholinesterase from electric eel.

E. Materials and Apparatus used for Assay of the Enzymes

Dubnoff Metabolic Shaking Incubator. Manufactured by P/S Precision Scientific Co., Chicago, U.S.A.

Beckman Expandomatic pH meter. Range 0-14 pH

or any 2 pH span. Accuracy: Standard 14 pH scale ± 0.05 pH. Expanded 2 pH scale ± 0.01 pH.

Fluorispec, Fluorescence Spectrophotometer Model SF-1, Baird Atomic, Inc., Cambridge, Massachusetts. Accuracy: Excitation or Fluorescence wavelength ± 2 nm. Uncertainty ± 1 nm. Front panel meter, uncertainty ± 0.4 μamp.

Spectrosil A cell-fluorescence. 10 mm path-length and 4 ml capacity.

Mosley 7035A X-Y Recorder, Hewlett-Packard. X-axis, excitation or fluorescence wavelength, full chart length. Uncertainty ± 1 nm. Y-axis, concentration units. Uncertainty ± ½ of smallest division.

Constant Temperature Water Bath.

International Clinical Centrifuge, Model CL. Manufactured by International Equipment Co., Needham Hts. Massachusetts.

Beckman DB Spectrophotometer. Analytical wavelength (approximately 200-800 nm). Uncertainty ± 0.5 nm.

Heathkit Servo Recorder by Daystrom. Model EUW-

20A. Full scale sensitivity 100 mv. Chart drive speeds:

Seconds per inch	30	120	600
Minutes per inch	0.5	2	10

1 inch of chart = 5 divisions. Uncertainty ± 0.5 division. Full scale sensitivity percentage transmission (0-100). Uncertainty ± 0.2 percent.

Peroxidase (horse radish) liophilized, B Grade, 62.7 units/mg.

Purchased from Calbiochem., San Diego, Calif. 92112, lot no. 20040.

Activity = 3120 o-dianisidine units/mcg, RZ 0.726, 1% 280, 11.6/cm.

(1.5 ± 0.1) mg/ml solution in glass distilled water.

p-Hydroxyphenylacetic acid, anhydrous, M.Wt. 152.2

Purchased from Sigma Chemical Company, lot no. 70C-3490.

0.1850 g in 50 ml glass distilled water.

Molarity = $(2.43 \pm 0.02) \times 10^{-2}$ M.

Tyramine hydrochloride, anhydrous, M.Wt. 173.6

Purchased from Sigma Chemical Company, lot no. 110C-0640.

0.0156 g in 10 ml glass distilled water.

Molarity = $(8.9 \pm 0.1) \times 10^{-3}$ M.

Phosphate and carbonate buffers were prepared according to the method of Gomori (1955).

γ-Aminobutyric acid, anhydrous, M.Wt. 103.1

Purchased from Sigma Chemical Company, lot no. 86B-2950.

0.0412 g in 100 ml glass distilled water.

Molarity = $(3.99 \pm 0.02) \times 10^{-3}$ M.

l-Glutamic acid, monosodium salt, anhydrous, M.Wt. 169.1

Purchased from Sigma Chemical Company, lot no. 118B-0750.

1.6910 g in 100 ml glass distilled water.

Molarity = (0.10 ± 0.01) M.

Trichloroacetic acid, certified A.C.S., M.Wt. 163.39

Purchased from Fisher Scientific Company, lot no. 780629.

10 g in 100 ml glass distilled water.

Molarity = $(61.2 \pm 0.1) \times 10^{-2}$ M.

Pyridoxal-5'-phosphate (codecarboxylase), anhydrous, M.Wt. 247.2

Purchased from Sigma Chemical Company, lot no. 21C-3030.

0.1235 g in 10 ml 0.4 M phosphate buffer, pH 6.4

Molarity = $(4.99 \pm 0.01) \times 10^{-2}$ M.

Ninhydrin (triketohydrindene hydrate), M.Wt. 178.14

Purchased from Fisher Scientific Company.

0.1247 g in 50 ml 0.5 M sodium carbonate buffer, pH 9.9

Molarity = $(14.00 \pm 0.02) \times 10^{-3}$ M.

Sodium carbonate, cupric sulfate and tartaric acid were all certified A.C.S. grade reagents, purchased from Fisher Scientific Company.

Acetylcholinesterase from electric eel, Type VI, lyophilized salt free powder.

Purchased from Sigma Chemical Company, lot no. 11C-8080.

325 μMolar units per mg solid.

Definition: One unit will hydrolyze 1.0 μMole of acetylcholine per min. at pH 8.0, 37°.

Acetylcholine bromide, anhydrous, M.Wt. 226.1, crystalline approx. 99%.

Purchased from Sigma Chemical Company, lot no. 106B-1980.

0.0564 g in 5 ml electrolyte solution.

Molarity = $(2.49 \pm 0.02) \times 10^{-2}$ M.

Phenol red, certified A.C.S. grade, M.Wt. 354.37

Purchased from Fisher Scientific Company. Visual transition interval pH 6.8 (yellow) to pH 8.2 (red).

0.06 g in 100 ml $(29.90 \pm 0.02) \times 10^{-3}$ M sodium barbital buffer.

Molarity = $(1.69 \pm 0.01) \times 10^{-3}$ M.

Sodium barbital, M.Wt. 206.18

0.6185 g in 100 ml glass distilled water.

Molarity = $(29.90 \pm 0.02) \times 10^{-3}$ M.

The electrolyte solution was prepared by useing certified A.C.S. grade reagents.

IV. RESULTS

The results are presented in Tables III to XI and in Figurs 3 through 5.

The activities of monamine oxidase, l-glutamic acid decarboxylase and acetylcholinesterase were measured in olfactory lobes, superior and inferior colliculi, thalamus and occipital cortex.

To do the measurements four ethanol induced rats, five ethanol induced rats that had been off ethanol diet for one day and five liquid control rats were used.

The rats used were kept in five different groups. They were sacrificed in groups on five different days. Each group consisted of one liquid control rat and one or two experimental rats (Table II).

Tables III through VII show the effect of ethanol on enzyme activities of MAO, GAD and AChE in different regions of brain obtained from five different groups of rats.

On the other hand, Tables VIII to X demonstrate the average enzymatic activities of MAO, GAD and AChE in different regions of brain obtained from ethanol induced, ethanol induced one day off and liquid control rats.

Table XI summarizes all the results and shows the effect of ethanol treatment on enzyme activities of MAO, GAD and AChE in different regions of rat brain.

TABLE II

Composition of Animal Groups

Group	Date Sacrificed	Ethanol induced	Ethanol induced one day off	Control
1	7-6-72	1	1	1
2	12-6-72	0	2	1
3	14-6-72	0	2	1
4	20-6-72	2	0	1
5	22-6-72	1	0	1

It also shows that some of the results obtained from ethanol induced rats are statistically significant with respect to the results obtained from liquid control rats. These data were analyzed statistically by using Student's t-test.

Figures 3 to 5 demonstrate comparative mean activities of rat brain MAO, GAD and AChE in five regions for all three treatments.

There are obvious differences in activities of the three enzymes under investigation:

- in the five regions studied.
- among animals of different treatments.
- between animals of same treatment but of different groups.
- within animals of same treatment and group.

A. Effect of Ethanol on Monamine Oxidase of Rat Brain

To study and compare the distribution of this enzyme in the brain, MAO was measured in larger areas of rat brain. MAO was found to be most active in the thalamic region and less active in olfactory lobes, superior and inferior colliculi.

Intermediate activities were observed in the occipital cortex region.

Statistically it was shown that:

- Within one group of animals, the differences in MAO activity were significant in the five regions studied.

- Within one group of animals, the differences in MAO activity were insignificant among the animals of the different groups.

- For each treatment, the differences in MAO activity were insignificant between animals of the different groups.

- The only significant interaction between groups and regions studied was for the liquid control animals.

- The only insignificant interaction between the treatments applied and regions studied, was for animals of group 2.

B. Effect of Ethanol on L-Glutamic Acid Decarboxylase of Rat Brain

To study and compare the distribution of this enzyme in the brain, GAD was measured in larger areas of rat brain. GAD was found to be most active in the thalamic region and less active in olfactory lobes, superior and inferior colliculi. Intermediate activities were observed in the occipital cortex region.

Statistically it was shown that:

- Within one group of animals, the differences in GAD activity were significant in the five regions studied.

- The differences in GAD activity were significant among the animals of different treatments for groups 1 and 5, and were insignificant for groups 2, 3 and 4.

- For each treatment, the differences in GAD activity were insignificant between animals of the different groups.

- The only insignificant interaction between groups of animals and regions studied, was for ethanol induced animals.

- The only insignificant interaction between the treatments and regions studied, was for animals of groups 1 and 2.

C. Effect of Ethanol on Acetylcholinesterase of Rat Brain

To study and compare the distribution of this enzyme in the brain, AChE was measured in larger areas of rat brain. AChE was found to be not appreciably different in the five regions studied. This might be due to complete rupture of cell membranes during the freezing process of the original homogenates.

Statistically it was shown that:

- Within one group of animals, the differences in AChE activity were significant in the five regions studied except for animals of group 5.

- Within one group of animals, the differences in AChE activity were insignificant among the animals of different treatments.

- For each treatment, the differences in AChE activity were insignificant between the animals of different groups.

- The only significant interaction between groups of animals and regions studied, was for liquid control animals.

- The only significant interaction between treatments applied and regions studied, was for animals of group 5.

TABLE III

Effect of Ethanol on Enzyme Activities in Different Regions of Brain Obtained from Rats of Group 1

Animal & Brain Region	MAO Moles/g*/hr x 10^{-8}	GAD Moles/g*/hr x 10^{-5}	AChE Moles/g*/hr x 10^{-5}
Ia	**	7.3 ± 1.4	6.1 ± 0.2
IIa	0.3 ± 0.1	3.1 ± 0.5	19.2 ± 0.4
IIIa	**	11.5 ± 2.6	7.6 ± 0.0
Ib	**	5.2 ± 0.5	4.9 ± 0.0
IIb	**	2.9 ± 0.1	16.7 ± 0.0
IIIb	**	3.9 ± 0.5	—
Ic	0.6 ± 0.3	6.9 ± 0.2	5.1 ± 1.2
IIc	0.5 ± 0.3	4.2 ± 0.7	10.6 ± 1.6
IIIc	**	12.3 ± 1.5	7.8 ± 0.0
Id	32.8 ± 0.7	28.1 ± 5.6	7.8 ± 2.0
IId	20.9 ± 0.4	10.8 ± 0.0	18.6 ± 9.5
IIId	36.6 ± 2.4	42.2 ± 2.1	8.3 ± 1.4
Ie	7.0 ± 4.9	13.0 ± 1.6	1.9 ± 2.1
IIe	7.6 ± 3.3	7.5 ± 0.6	14.2 ± 1.6
IIIe	13.4 ± 0.0	13.5 ± 1.3	11.3 ± 2.3

* g of wet brain tissue.

** Enzyme activity is less than 0.1 moles H_2O_2/g*/hr x 10^{-8}.

The values are expressed as means ± std. dev. of the mean.

TABLE IV

Effect of Ethanol on Enzyme Activities in Different Regions of Brain Obtained from Rats of Group 2

Animal & Brain Region	MAO Moles/g*/hr x 10^{-8}	GAD Moles/g*/hr x 10^{-5}	AChE Moles/g*/hr x 10^{-5}
IVa	1.2 ± 0.4	5.5 ± 1.2	15.0 ± 1.6
Va	**	2.2 ± 0.3	15.4 ±11.4
VIa	**	6.5 ± 0.7	7.9 ± 1.6
IVb	1.2 ± 0.5	8.7 ± 0.5	9.8 ± 0.0
Vb	4.4 ± 0.5	7.1 ± 0.6	10.9 ± 0.9
VIb	**	6.7 ± 0.7	1.1 ± 0.4
IVc	**	5.4 ± 1.1	6.7 ± 2.1
Vc	2.5 ± 1.4	4.7 ± 1.0	8.7 ± 0.0
VIc	**	7.4 ± 0.0	6.7 ± 0.5
IVd	22.1 ± 0.0	23.8 ± 1.1	12.5 ± 8.2
Vd	33.6 ± 8.9	39.5 ±10.2	11.1 ± 0.0
VId	46.9 ±14.3	41.4 ± 4.4	5.5 ± 0.0
IVe	5.5 ± 0.1	20.1 ± 1.7	6.7 ± 0.0
Ve	7.6 ± 0.7	27.5 ± 2.3	5.8 ± 1.3
VIe	15.6 ± 7.6	19.8 ± 4.6	14.7 ± 3.8

* g of wet brain tissue.
** Enzyme activity is less than 0.1 moles H_2O_2/g*/hr x 10^{-8}.
The values are expressed as means ± std.dev. of the mean.

TABLE V

Effect of Ethanol on Enzyme Activities in Different Regions of Brain Obtained from Rats of Group 3

Animal & Brain Region	MAO Moles/g*/hr x 10^{-8}	GAD Moles/g*/hr x 10^{-5}	AChE Moles/g*/hr x 10^{-5}
VIIa	**	3.0 ± 0.7	9.9 ± 2.8
VIIIa	2.8 ± 1.6	4.0 ± 0.2	10.8 ± 2.6
IXa	2.5 ± 0.0	11.6 ± 2.1	5.5 ± 0.0
VIIb	**	4.5 ± 0.4	9.9 ± 0.0
VIIIb	**	3.2 ± 1.0	15.9 ± 1.5
IXb	1.6 ± 0.0	4.6 ± 1.2	8.3 ± 1.6
VIIc	**	2.4 ± 0.3	9.2 ± 0.9
VIIIc	**	2.2 ± 0.5	14.6 ± 0.5
IXc	**	7.5 ± 0.5	10.1 ± 0.5
VIId	22.2 ± 1.9	32.4 ± 0.2	8.2 ± 0.0
VIIId	27.3 ± 0.4	28.7 ± 0.1	19.2 ± 1.3
IXd	45.1 ± 6.6	48.1 ± 0.4	10.4 ± 0.0
VIIe	5.6 ± 0.0	26.7 ± 6.9	8.9 ± 0.0
VIIIe	7.1 ± 0.5	26.5 ± 6.8	8.3 ± 0.0
IXe	15.5 ± 3.5	38.8 ± 1.8	10.2 ± 2.1

* g of wet brain tissue.
** Enzyme activity is less than 0.1 moles H_2O_2/g*/hr x 10^{-8}.
The values are expressed as means ± std.dev. of the mean.

TABLE VI

Effect of Ethanol on Enzyme Activities in Different Regions of Brain Obtained from Rats of Group 4

Animal & Brain Region	MAO Moles/g*/hr x 10^{-8}	GAD Moles/g*/hr x 10^{-5}	AChE Moles/g*/hr x 10^{-5}
Xa	1.0 ± 0.5	3.4 ± 0.7	16.4 ± 4.2
XIa	3.0 ± 1.7	3.6 ± 0.7	2.8 ± 1.6
XIIa	1.4 ± 0.3	3.7 ± 0.4	10.1 ± 0.5
Xb	5.6 ± 0.0	8.5 ± 0.6	
XIb	**	2.0 ± 0.3	7.0 ± 0.7
XIIb	**	12.7 ± 0.4	11.9 ± 0.9
Xc	**	6.5 ± 1.1	11.5 ± 0.7
XIc	1.0 ± 0.0	4.9 ± 0.2	11.2 ± 3.1
XIIc	**	13.0 ± 0.9	10.7 ± 0.6
Xd	18.5 ± 1.2	14.2 ± 1.7	7.8 ± 0.0
XId	15.8 ± 0.3	7.6 ± 1.2	11.9 ± 2.1
XIId	48.0 ± 1.5	28.1 ± 0.9	11.5 ± 0.3
Xe	9.4 ± 0.0	1.7 ± 0.0	8.4 ± 0.7
XIe	11.8 ± 1.6	6.2 ± 1.0	10.2 ± 0.0
XIIe	5.4 ± 2.7	17.3 ± 1.4	4.2 ± 3.1

* g of wet brain tissue.
** Enzyme activity is less than 0.1 moles H_2O_2/g*/hr x 10^{-8}.
The values are expressed as means ± std.dev. of the mean.

TABLE VII

Effect of Ethanol on Enzyme Activities in Different Regions of Brain Obtained from Rats of Group 5

Animal & Brain Region	MAO Moles/g*/hr x 10^{-8}	GAD Moles/g*/hr x 10^{-5}	AChE Moles/g*/hr x 10^{-5}
XIIIa	0.5 ± 0.0	3.5 ± 0.9	8.8 ± 0.0
XIVa	1.8 ± 0.6	5.4 ± 0.5	0.8 ± 0.0
XIIIb	0.7 ± 0.0	3.0 ± 0.6	7.8 ± 0.5
XIVb	**	9.8 ± 0.6	24.1 ± 4.5
XIIIc	**	1.6 ± 0.1	8.3 ± 0.7
XIVc	1.2 ± 0.4	6.9 ± 1.5	6.3 ± 0.2
XIIId	13.9 ± 0.3	2.1 ± 0.1	8.2 ± 0.7
XIVd	35.0 ± 1.5	25.6 ± 0.5	17.1 ± 6.0
XIIIe	8.8 ± 2.0	6.6 ± 0.7	10.3 ± 0.5
XIVe	7.1 ± 1.2	23.5 ± 1.6	8.7 ± 4.4

* g of wet brain tissue.
** Enzyme activity is less than 0.1 moles H_2O_2/g*/hr x 10^{-8}.
The values are expressed as means ± std.dev. of the mean.

TABLE VIII

Average Enzymatic Activities of MAO, GAD and AChE in Different Regions of Ethanol-Induced Rat Brain

Animal & Brain Region	MAO Moles/g*/hr x 10^{-8}	GAD Moles/g*/hr x 10^{-5}	AChE Moles/g*/hr x 10^{-5}
IIa	0.3 ± 0.1	3.1 ± 0.5	19.2 ± 0.4
Xa	1.0 ± 0.5	3.4 ± 0.7	16.4 ± 4.2
XIa	3.0 ± 1.7	3.6 ± 0.7	2.8 ± 1.6
XIIIa	0.5 ± 0.0	3.5 ± 0.9	8.8 ± 0.0
IIb	**	2.9 ± 0.1	16.7 ± 0.0
Xb	5.6 ± 0.0	8.5 ± 0.6	
XIb	**	2.0 ± 0.3	7.0 ± 0.7
XIIIb	0.7 ± 0.0	3.0 ± 0.6	7.8 ± 0.5
IIc	0.5 ± 0.3	4.2 ± 0.7	10.6 ± 1.6
Xc	**	6.5 ± 1.1	11.5 ± 0.7
XIc	1.0 ± 0.0	4.9 ± 0.2	11.2 ± 3.1
XIIIc	**	1.6 ± 0.1	8.3 ± 0.7
IId	20.9 ± 0.4	10.8 ± 0.0	18.6 ± 9.5
Xd	12.5 ± 1.2	14.2 ± 1.7	7.8 ± 0.0
XId	15.8 ± 0.3	7.6 ± 1.2	11.9 ± 2.1
XIIId	13.9 ± 0.3	2.1 ± 0.1	8.2 ± 0.7
IIe	7.6 ± 3.3	7.5 ± 0.6	14.2 ± 1.6
Xe	9.4 ± 0.0	1.7 ± 0.0	8.4 ± 0.7
XIe	11.8 ± 1.6	6.2 ± 1.0	10.2 ± 0.0
XIIIe	8.8 ± 2.0	6.6 ± 0.7	10.3 ± 0.5

* g of wet brain tissue.
** Enzyme activity is less than 0.1 moles H_2O_2/g*/hr x 10^{-8}.
The values are expressed as means ± std.dev. of the mean.

TABLE IX

Average Enzymatic Activities of MAO, GAD and AChE in Different Regions of Ethanol-Induced one day off Rat Brain

Animal & Brain Region	MAO Moles/g*/hr x 10^{-8}	GAD Moles/g*/hr x 10^{-5}	AChE Moles/g*/hr x 10^{-5}
Ia	**	7.3 ± 1.4	6.1 ± 0.2
IVa	1.2 ± 0.4	5.5 ± 1.2	15.0 ± 1.6
Va	**	2.2 ± 0.3	15.4 ±11.4
VIIa	**	3.0 ± 0.7	9.9 ± 2.8
VIIIa	2.8 ± 1.6	4.0 ± 0.2	10.8 ± 2.6
Ib	**	5.2 ± 0.5	4.9 ± 0.0
IVb	1.2 ± 0.5	8.7 ± 0.5	9.8 ± 0.0
Vb	4.4 ± 0.5	7.1 ± 0.6	10.9 ± 0.9
VIIb	**	4.5 ± 0.4	9.9 ± 0.0
VIIIb	**	3.2 ± 1.0	15.9 ± 1.5
Ic	0.6 ± 0.3	6.9 ± 0.2	5.1 ± 1.2
IVc	**	5.4 ± 0.2	6.7 ± 2.1
Vc	2.5 ± 1.4	4.7 ± 1.0	8.7 ± 0.0
VIIc	**	2.4 ± 0.3	9.2 ± 0.9
VIIIc	**	2.2 ± 0.5	14.6 ± 0.5
Id	32.8 ± 0.7	28.1 ± 5.6	7.8 ± 2.0
IVd	22.1 ± 0.0	23.8 ± 1.1	12.5 ± 8.2
Vd	33.6 ± 8.9	39.5 ±10.2	11.1 ± 0.0
VIId	22.2 ± 1.9	32.4 ± 0.2	8.2 ± 0.0
VIIId	27.3 ± 0.4	28.7 ± 0.1	19.2 ± 1.3
Ie	7.0 ± 4.9	18.0 ± 1.6	1.9 ± 2.1
IVe	5.5 ± 0.1	20.1 ± 1.7	6.7 ± 0.0
Ve	7.6 ± 0.7	27.5 ± 2.3	5.8 ± 1.3
VIIe	5.6 ± 0.0	26.7 ± 6.9	8.9 ± 0.0
VIIIe	7.1 ± 0.5	26.5 ± 6.8	8.3 ± 0.0

* g of wet brain tissue.
** Enzyme activity is less than 0.1 moles H_2O_2/g*/hr x 10^{-8}.
The values are expressed as means ± std.dev. of the mean.

TABLE X

Average Enzymatic Activities of MAO, GAD and AChE in Different Regions of Control Rat Brain

Animal & Brain Region	MAO Moles/g*/hr x 10^{-8}	GAD Moles/g*/hr x 10^{-5}	AChE Moles/g*/hr x 10^{-5}
IIIa	**	11.5 ± 2.6	7.6 ± 0.0
VIa	**	6.5 ± 0.7	7.9 ± 1.6
IXa	2.5 ± 0.0	11.6 ± 2.1	5.5 ± 0.0
XIIa	1.4 ± 0.3	3.7 ± 0.4	10.1 ± 0.5
XIVa	1.8 ± 0.6	5.4 ± 0.5	0.8 ± 0.0
IIIb	**	3.9 ± 0.5	
VIb	**	6.7 ± 0.7	1.1 ± 0.4
IXb	1.6 ± 0.0	4.6 ± 1.2	8.3 ± 1.6
XIIb	**	12.7 ± 0.4	11.9 ± 0.9
XIVb	**	9.8 ± 0.6	24.1 ± 4.5
IIIc	**	12.3 ± 1.5	7.8 ± 0.0
VIc	**	7.4 ± 0.0	6.7 ± 0.5
IXc	**	7.5 ± 0.5	10.1 ± 0.5
XIIc	**	13.0 ± 0.9	10.7 ± 0.6
XIVc	1.2 ± 0.4	6.9 ± 1.5	6.3 ± 0.2
IIId	36.6 ± 2.4	42.2 ± 2.1	8.3 ± 1.4
VId	46.9 ±14.3	41.4 ± 4.4	5.5 ± 0.0
IXd	45.1 ± 6.6	48.1 ± 0.4	10.4 ± 0.0
XIId	48.0 ± 1.5	28.1 ± 0.9	11.5 ± 0.3
XIVd	35.0 ± 1.5	25.6 ± 0.5	17.1 ± 6.0
IIIe	13.4 ± 0.0	18.5 ± 1.3	11.3 ± 2.3
VIe	15.6 ± 7.6	19.8 ± 4.6	14.7 ± 3.8
IXe	15.5 ± 3.5	38.8 ± 1.8	10.2 ± 2.1
XIIe	5.4 ± 2.7	17.3 ± 1.4	4.2 ± 3.1
XIVe	7.1 ± 1.2	23.5 ± 1.6	8.7 ± 4.4

* g of wet brain tissue.
** Enzyme activity is less than 0.1 moles H_2O_2/g*/hr x 10^{-8}.
The values are expressed as means ± std.dev. of the mean.

TABLE XI

Effect of Ethanol Treatment on Enzyme Activities in Different Regions of Rat Brain

Treatment	No. of Animals	Brain Region	MAO Moles/g*/hr x 10^{-8}	GAD Moles/g*/hr x 10^{-5}	AChE Moles/g*/hr x 10^{-5}
Ethanol	4	a	1.2 ± 0.6	3.4 ± 0.7	11.8 ± 1.6
Ethanol 1 day off	5	a	0.8 ± 0.4	4.4 ± 0.8"	11.4 ± 3.7"
Control	5	a	1.1 ± 0.2	7.7 ± 1.3	6.4 ± 0.4
Ethanol	4	b	1.6 ± 0.0"	4.1 ± 0.4"	10.5 ± 0.4
Ethanol 1 day off	5	b	1.1 ± 0.2"	5.7 ± 0.6	10.3 ± 0.5"
Control	5	b	0.3 ± 0.0	7.5 ± 0.7	11.4 ± 1.9
Ethanol	4	c	0.4 ± 0.1"	4.3 ± 0.5	10.4 ± 1.5
Ethanol 1 day off	5	c	0.6 ± 0.3"	4.3 ± 0.4"	8.9 ± 0.9
Control	5	c	0.2 ± 0.1	9.4 ± 0.9	8.3 ± 0.4
Ethanol	4	d	17.3 ± 0.6"	8.7 ± 0.8"	11.6 ± 3.1
Ethanol 1 day off	5	d	27.6 ± 2.4"	30.5 ± 3.4"	11.8 ± 2.3
Control	5	d	42.3 ± 4.9	37.1 ± 1.6	10.6 ± 1.5
Ethanol	4	e	9.4 ± 1.7	5.5 ± 0.6	10.8 ± 0.7"
Ethanol 1 day off	5	e	6.6 ± 1.2	23.8 ± 3.9	6.3 ± 0.7"
Control	5	e	11.4 ± 3.0	23.6 ± 2.1	9.8 ± 3.1

* g of wet brain tissue.
" Significant with respect to control treatment ($P < 0.05$).
The values are expressed as means ± std.dev. of the mean.

Figure 3

Comparative Mean Activities of Rat Brain Monamine Oxidase in Five Regions for all Three Treatments
Ethanol-Induced Treatment is Represented by Diagonal Bar,
Ethanol-Induced one day off Treatment is Blank and Control Treatment is Represented by Stippled Bar.

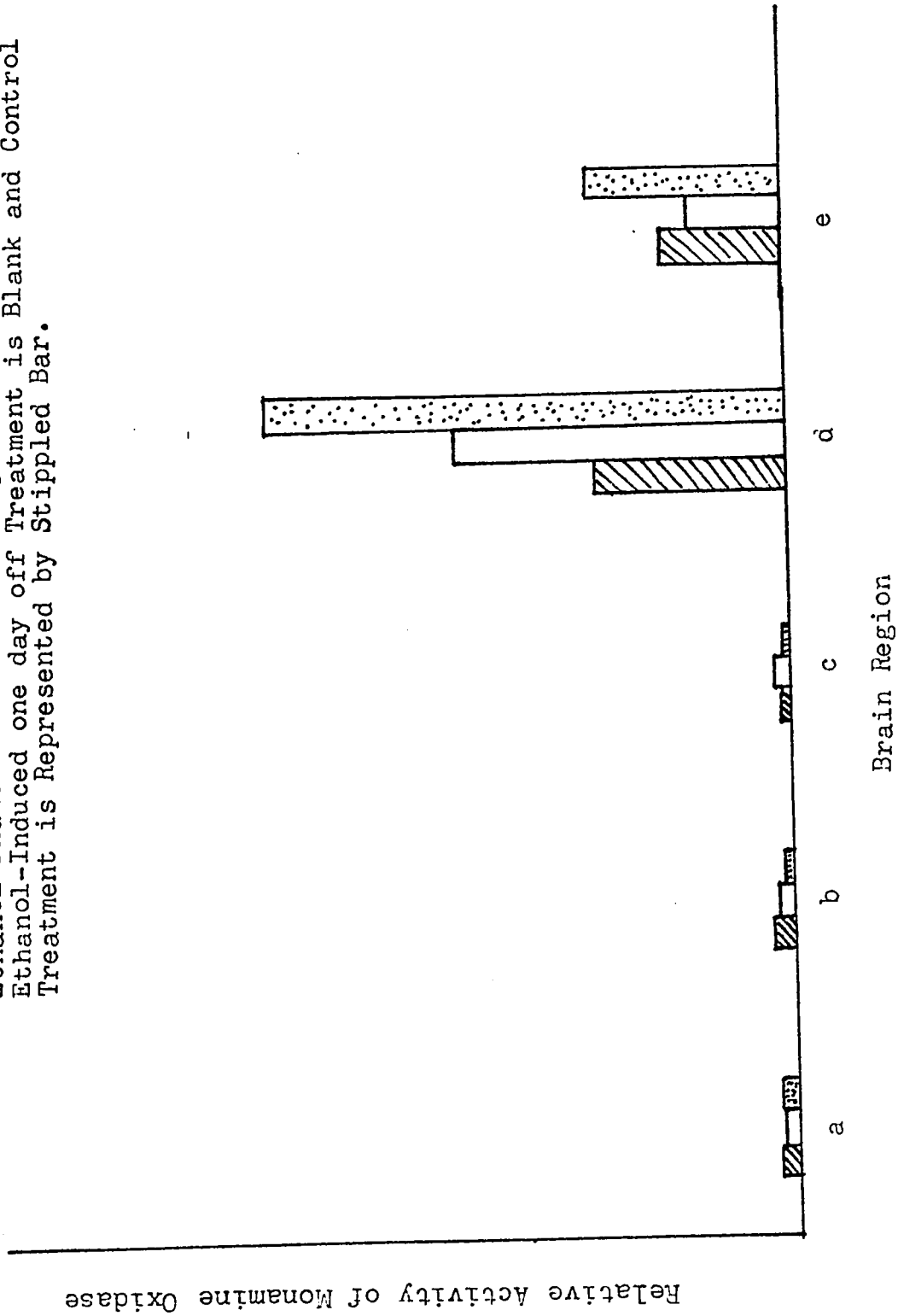


Figure 4

Comparative Mean Activities of Rat Brain L-Glutamic Acid Decarboxylase in Five Regions for all Three Treatments Ethanol-Induced Treatment is Represented by Diagonal Bar, Ethanol-Induced one day off Treatment is Blank and Control Treatment is Represented by Stippled Bar.

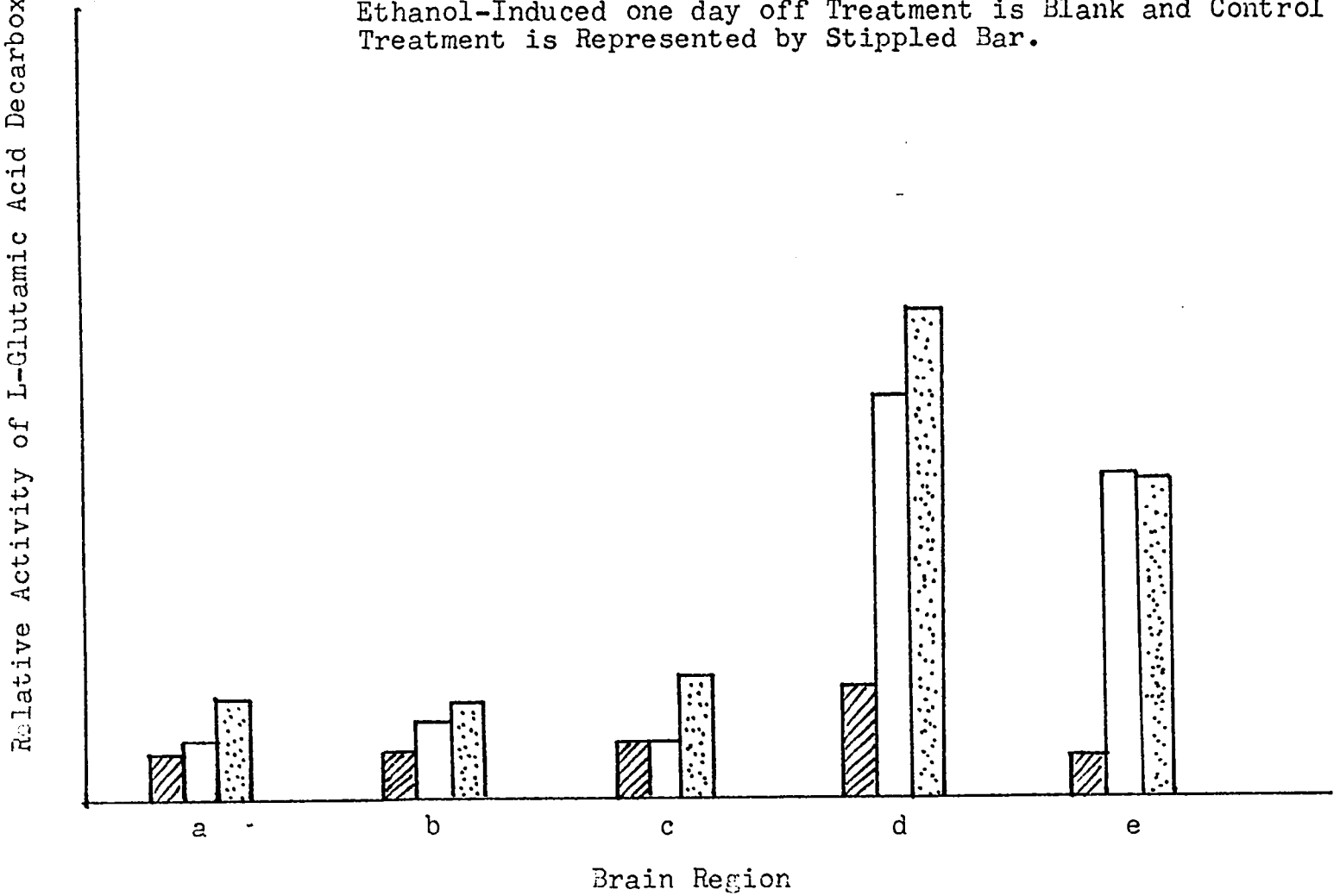
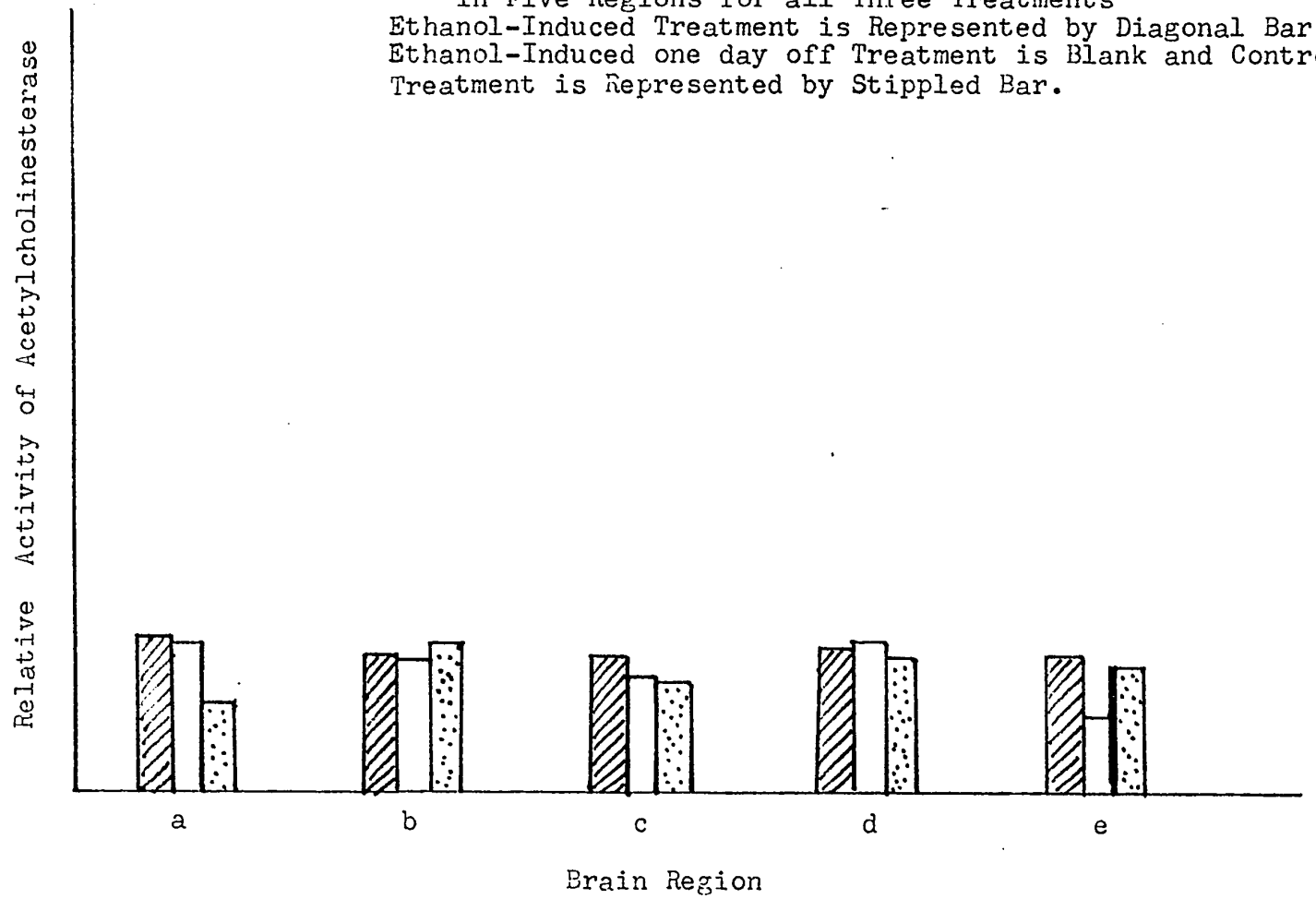


Figure 5

Comparative Mean Activities of Rat Brain Acetylcholinesterase in Five Regions for all Three Treatments
Ethanol-Induced Treatment is Represented by Diagonal Bar,
Ethanol-Induced one day off Treatment is Blank and Control Treatment is Represented by Stippled Bar.



V. DISCUSSION AND CONCLUSION

Even if it is believed that ethanol acts primarily on the functioning of excitable membranes, its effect may be mediated by changes in the metabolism of special compounds. These compounds would have specific functions in the nervous system, such as central synaptic transmitter substances.

Because biogenic amines, γ-aminobutyric acid and acetylcholine are all believed to be neurotransmitters, we chose to study the evidence for interaction between ethanol and those enzymes that have intimate association with these neurotransmitters.

An outline of the composition and possible function of the five regions of brain under investigation would be helpful in explanation of the results (Ranson & Clark, 1956).

a) Olfactory Lobes: These are formed mainly of synapses of the axons of the olfactory nerve fibres with the mitral cells. The rhinencephalon or the olfactory lobes of the cerebral hemispheres are highly developed in rodents because of their dependence to a great extent on the sense of smell in their search for food.

b) Superior Colliculi: Or superior quadrigeminal bodies are composed of laminated gray matter. Each consists of four superimposed dorsally convex

layers. The majority of the afferent fibres of the superior colliculi come from the optic tract. They contain important reflex centres and are dominated by visual impulses.

c) Inferior Colliculi: These contain laminated gray matter of the tectum and a large mass known as the nucleus of the inferior colliculus. Many of the fibres of the central auditory path terminate in these portions of brain. They contain important reflex centres and are concerned with auditory impulses.

d) Thalamus: This is a large avoid mass consisting chiefly of gray matter placed obliquely across the rostral end of the cerebral peduncle. The thalamus contains many nuclei. Certain of these nuclei serve as relay stations on pathways to the cerebral cortex. Some nuclei relay auditory, visual and somatic sensory impulses. Some others are relays on the somatesthetic sensory pathway or receive impulses from other thalamic nuclei, correlate them and then pass them on to cortex lying outside the sensory areas. It has been suggested that the thalamic diffuse projection system is related to the state of wakefulness and sleep and so perhaps to consciousness. This concept is significant in the light of the possible regulatory effects of the thalamic projection on the cortex (Ranson & Clark, 1956).

e) Occipital Lobes of Cerebral Cortex: These are well defined areas of the cortex. They form a small

part of the dorsolateral surface of the hemisphere. These are triangular in area at the occipital extremity, bounded rostrally by a line joining the parieto-occipital fissure and the reoccipital notch. These lobes are a structural and functional entity, probably all concerned directly or indirectly with the visual process.

Monamine oxidase was most highly concentrated in the thalamic region for rat brain, and less concentrated in olfactory lobes, superior and inferior colliculi. Intermediate activities of the enzyme were observed in the occipital lobes of the cerebral cortex (Tables III to VII). This pattern of distribution is essantially the same as that observed for different sections of dog and cow brain (Weiner, 1960).

Monamine oxidase is a particulate enzyme, believed to be localized in the outer membrane of mitochondria.

Chronic ethanol feeding resulted in significant increase in MAO activity in superior colliculi (81%), significant increase in inferior colliculi (50%) and significant decrease in MAO activity in thalamic region (59%), Table XI.

By contrast there were no significant changes in the activity of MAO in the other regions of rat brain that were studied.

For most of the brain regions under investigation, removal of ethanol from the diet for 24 hr,

caused the activities of MAO to approach the values observed in rats given the liquid control diet (Tables VIII to X). It can be assumed that removal of ethanol from the diet for several days would result in changes in MAO activities and eventually the values observed would return to values similar to those observed in animals given the liquid control diet (Walker & Gordon, 1970). This indicates a specific effect of ethanol on brain metabolism and it appears that ethanol or one of its metabolites may markedly influence monamine metabolism.

In humans, ethanol ingestion diverts the metabolism of glycol aldehyde, an intermediate of catecholamines derived from the action of MAO, from the normal oxidative route to a reductive pathway (Davis et al. 1970). It has been suggested that this alteration could be mediated by the elevated levels of reduced NAD brought about by ethanol metabolism and/or by competitive inhibition of alcohol dehydrogenase (ADH) with acetaldehyde. In 1970, Walsh et al. showed that the mechanism of alteration of catecholamine metabolism in humans, is most probably due to a competitive interaction between acetaldehyhe and the aldehyde intermediate of the neuramine for the active site on ADH.

Surely, the phenomenon of reducing ethanol induction in rats by 24 hr or longer and the resulting changes in MAO activities to return towards the values observed in animals given the liquid control diet, sup-

ports the hypothesis of alteration of monamine metabolism by competitive inhibition of ADH with acetaldehyde. However, the effects of ethanol in humans, after stopping from heavy or long term drinking does not indicate that the alteration in monamine metabolism is just due to a simple competitive inhibition where the degree of inhibition depends on the ratio of the concentration of inhibitor to the concentration of substrate.

Although the results suggest that chronic ethanol feeding alters MAO activity, yet they show clearly that the alteration in monamine metabolism is different in the various sections of the rat brain.

Raskin & Sokoloff (1972), have shown that the levels of ADH in different parts of the brain are lower than those of liver, but probably sufficient to perform local functions in the metabolism of ethanol or other endogenous substrates.

Variance in concentrations of ADH might thus account for the differences in MAO activities in response to ethanol treatment in various regions of the rat brain.

It is highly probable that the metabolism of any biogenic amine which is a substrate of MAO would be subject to an ethanol-evoked blockade at the intermediate aldehyde level. Condensation of the intermediate aldehyde with the parent amine results in the formation of an alkaloid. This alkaloid is a probable requisite intermediate in the biosynthesis of a variety of complex

alkaloids (morphine like compounds). This endogenous generation of alkaloids might well contribute to some of the diverse pharmacological effects of chronic ethanol ingestion.

The distribution of l-glutamic acid decarboxylase was found to parallel the distribution of MAO in rat brain.

It was found that GAD was most highly concentrated in the thalamic region for rat brain and least concentrated in olfactory lobes, superior and inferior colliculi. Intermediate activities of the enzyme were observed in the occipital lobes of the cerebral cortex (Tables III to VII). This pattern of distribution is essentially the same as that observed for different sections of mouse, rabbit, rat and monkey brain (Lowe et al., 1957). These findings are in the expected direction since GAD was found to be richer in gray matter than in white matter of the brain.

l-Glutamic acid decarboxylase is believed to be localized in the synaptosomes of nerve cells.

Chronic ethanol feeding resulted a general decrease in GAD activity of the rat brain. The range of decrease in GAD levels was (45-77)%. Different regions of brain showed different amounts of changes in enzymatic levels due to ethanol ingestion (Table XI).

In ethanol treated animals, GAD of superior colliculi and thalamus was significantly lower ($P < 0.05$)

than that in liquid control animals.

In 1967, Gordon reported a decrease in GABA after an intoxicating dose of ethanol administered in the blood of rats. However, her study indicated that it was necessary to immerse the brain immediately in liquid N_2 to avoid postmortem changes in GABA.

Although both observations show decrease in GAD levels, it is difficult to compare the data of Gordon (1967) with the results obtained presently, since her investigation utilized whole cerebrum and cerebellum after 3 or 5 hrs of ethanol intoxication. However, it is interesting that she also found a decrease in the levels of GAD.

For most of the regions under investigation, removal of ethanol from the diet for 24 hr caused the activities of GAD to approach the values observed in rats given the liquid control diet (Table VIII to X). However, GAD content of olfactory lobes, inferior colliculi and thalamus were significantly greater than that of ethanol treated animals and significantly lower ($P < 0.01$) than that of liquid control animals.

This indicates that possibly ethanol or one of its metabolites markedly influence the metabolism of glutamic acid and inhibit it in reversible fashion.

Changes in the concentration of GABA is of interest metabolically although it does not directly explain the mechanism of ethanol action. Since GABA is

believed to have profound inhibitory effects on transmission in the central nervous system, this suggests that perhaps the distribution of GAD may parallel the concentration of inhibitory synapses in the different regions of the nervous system.

Another tentative explanation of the results could be depression of the reaction rates of the intermediate steps in the GABA shunt. It is possible that a balance of GAD and α-ketoglutaric acid transaminase is necessary to maintain a given level of GABA. This suggests that for better interpretation of ethanol action in a specific site we would require a knowledge of the α-ketoglutaric acid transaminase and GAD levels at the same site.

Acetylcholinesterase appears to be distributed evenly in the different regions of rat brain under investigation. The results are not striking, because AChE is a membrane-bound enzyme and its activity tends to vary more with age and strain of animals.

Chronic ethanol feeding resulted in significant increase ($P < 0.05$) in AChE activity in cerebral cortex as compared to liquid control animals.

By contrast there were no significant changes in the activity of AChE in all the other brain regions that were studied.

Removal of ethanol from the diet for 24 hr resulted in significant changes ($P < 0.05$) in AChE ac-

tivity in olfactory lobes, superior colliculi and cerebral cortex with respect to liquid control animals.

Removal of ethanol from the diet for 24 hr caused the activities of AChE in some of the brain regions studied to approach the values observed in rats given the liquid control diet. Removal of ethanol from the diet for longer time would probably cause significant changes in almost all brain regions and eventually the values would return to values similar to those observed in liquid control animals.

These results augment the findings of other workers (Kinard and Hay, 1960; Lowe et al., 1962). These workers have reported that ethanol inhibits AChE activity of brain tissue in vitro.

In conclusion, this study suggests that chronic ethanol feeding was sufficient to produce MAO and GAD induction and also it resulted in significant biochemical changes for AChE in most of the brain regions studied in rats.

Regardless of the mechanism, the data obtained indicate that AChE may respond equally to chronic ethanol feeding in different regions of rat brain, while MAO and GAD may not respond in the same way.

The differences between the responses of MAO, GAD and AChE activities after withdrawal of ethanol from the diet emphasize the fact that the metabolic pathways

in brain have a differential response to ethanol and also that effect of ethanol on brain metabolism is possibly of reversible nature.

The data obtained indicate the relationship between ethanol induction and some aspects of cholinergic and non-cholinergic transmission of impulses but do not indicate the extent of this relationship.

VI. SUGGESTIONS FOR FUTURE WORK

Many experiments and projects can be suggested which arise from this thesis that might lead to an understanding of the processes underlying addiction and tolerance to alcohol.

1. A detailed study is needed in the area of the formation and biological activity of aberrant biological amine metabolites, to determine the precise role of these compounds in the disease of alcoholism.

2. For better interpretation of ethanol action on GABA in a specific site, it is necessary to study simultaneously the activities of the enzymes involved in GABA shunt. These enzymes are GAD, α-ketoglutaric acid transaminase and succinic semialdehyde dehydrogenase.

3. To determine whether the effect of ethanol on GAD is a reflection of changes in general metabolism rate in the mixed tissue or a reflection of preliminary changes at the synaptic levels, it is essential to isolate inhibitory synapses and cells and analyze them chemically.

4. Further studies are required to prove ethanol action on ACh liberation in central nervous system. The effects of ethanol on ACh liberation in neuromuscular transmission seems to be a specific one.

5. Removal of ethanol from the diet for 24 hr

caused the activities of the enzymes to approach the values observed in the rats given the liquid control diet. Further studies in this area are required to understand the exact chemical nature of the inhibition of the enzymes and also long term effects of ethanol.

6. A detailed study is needed of the basic properties of the cell membrane and of the primary interaction between ethanol and the membrane. Valuable tools are artificial lipid membranes and improved techniques for the study of macromolecular conformation.

VII. APPENDIX A

Preparation of Solutions

a. Preparation of brain enzymes

Male albino rats weighing 100 to 120 g were sacrificed by cervical fracture and the brain was removed and kept cold on ice. The brain was dissected and the different regions were homogenized in 1.5 to 2.5 ml of ice-cold isotonic (0.9%) NaCl solution in a motor driven homogenizer. Aliquots of whole homogenate were taken for enzyme assay.

b. Preparation of solutions for Monamine oxidase assay

1. The buffer solution: 0.1 N Na-K phosphate buffer at pH 7.8 was prepared by adding (50.00 ± 0.04) ml 0.2 M KH_2PO_4 to (44.50 ± 0.04) ml 0.1 N NaOH in a 100 ml volumetric flask and making it up to the volume with glass distilled water. This solution was stored at 4°, and 100 ml of it was prepared for each series of enzyme assays.

2. The horse radish peroxidase solution was prepared as a (1.5 ± 0.1) mg/ml solution in glass distilled water. This solution was stored at 4°, and 10 ml of it was prepared for each series of enzyme assays.

3. The p-hydroxyphenylacetic acid solution was prepared as a $(2.43 \pm 0.02) \times 10^{-2}$ M solution in glass distilled water. This solution was stored at 4°, and 50 ml of it was prepared for each series of enzyme assays.

4. The tyramine hydrochloride solution was

prepared as a $(8.9 \pm 0.1) \times 10^{-3}$ M solution in glass distilled water. This solution was stored at 4^{o}, and 10 ml of it was prepared for each series of enzyme assays.

5. The hydrogen peroxide (H_2O_2) solution was prepared in glass distilled water. The exact molarity of the stock solution was determined by titration against standardized potassium permanganate solution. The solution was unstable, therefore 10 ml of the working solution was prepared before each series of runs.

I. Stock solution: (0.1000 ± 0.0002) ml of (0.99 ± 0.01) M H_2O_2 solution was diluted to 100 ml.

0.1 ml transfer pipette and 100 ml volumetric flask were used.

$$\frac{(0.1000 \pm 0.0002) \text{ ml} \times (0.99 \pm 0.01) \text{ M}}{(100.00 \pm 0.04) \text{ ml}}$$

$$= (0.99 \pm 0.01) \times 10^{-3} \text{ M}$$

II. Working solution: (33.00 ± 0.02) µl of the stock solution was diluted to 10 ml.

50 µl syringe and 10 ml volumetric flask were used.

$$\frac{(0.03300 \pm 0.00002) \text{ ml} \times (0.99 \pm 0.01) \times 10^{-3} \text{ M}}{(10.00 \pm 0.01) \text{ ml}}$$

$= (3.26 \pm 0.04) \times 10^{-6}$ M

Note: (10.00 ± 0.02) ml burette was used to measure the buffer solution.

(0.2000 ± 0.0004) ml transfer pipette was used to measure horse radish peroxidase solution.

(1.000 ± 0.002) ml syringe was used to measure p-hydroxyphenylacetic acid solution.

(0.1000 ± 0.0002) ml transfer pipette was used to measure tyramine hydrochloride solution.

(50.0 ± 0.2) µl syringe was used to measure H_2O_2 solution.

The three ml reaction volume was made up of:

(2.50 ± 0.02) ml of 0.1 N Na-K phosphate buffer, pH 7.8

(0.2000 ± 0.0004) ml of horse radish peroxidase solution.

(0.250 ± 0.002) ml of $(2.43 \pm 0.02) \times 10^{-2}$ M p-hydroxyphenylacetic acid solution.

(1.0 to 25.0 ± 0.2) µl of $(3.26 \pm 0.04) \times 10^{-6}$ M H_2O_2 solution.

or

(2.40 ± 0.02) ml of 0.1 N Na-K phosphate buffer,

pH 7.8

(0.2000 ± 0.0004) ml of horse radish peroxidase solution.

(0.250 ± 0.002) ml of (2.43 ± 0.02) x 10^{-2} M p-hydroxyphenylacetic acid solution.

(0.050 ± 0.002) ml of (8.9 ± 0.1) x 10^{-3} M tyramine hydrochloride solution.

(0.1000 ± 0.0002) ml brain homogenate.

c. Preparation of solutions for l-Glutamic acid decarboxylase assay

1. The sodium bicarbonate buffer was prepared as 0.5 M solution at pH 9.9, ionic strength 0.0432. The buffer was prepared by adding (50.00 ± 0.04) ml 1 M $NaHCO_3$ to (9.10 ± 0.02) ml 0.1 N NaOH in a 100 ml volumetric flask and making it up to the volume with glass distilled water.

2. The ninhydrin (triketohydrindene hydrate) solution was prepared as a (14.00 ± 0.02) x 10^{-3} M solution in 0.5 M sodium bicarbonate buffer, pH 9.9. This solution was stored at -20°. The solution is stable at least for two months.

3. The potassium phosphate buffer was prepared as a 0.4 M solution at pH 6.4. To prepare the buffer (46.9511 ± 0.0002) g KH_2PO_4 and (9.5821 ± 0.0002) g K_2HPO_4 were dissolved in glass distilled water and

made up to one liter. The solution was stored at 4°. The solution is stable at least for four months.

4. The pyridoxal phosphate solution was prepared as a $(4.99 \pm 0.01) \times 10^{-2}$ M solution in 0.4 M potassium phosphate buffer pH 6.4. This solution was stored at 4°, and 10 ml of it was prepared for each series of enzyme assays.

5. The glutamic acid,monosodium salt was prepared as a (0.10 ± 0.01) M solution in glass distilled water. This solution was stored at 4°. The solution is stable at least for four months.

6. The trichloroacetic acid solution (TCA) was prepared as a $(6.12 \pm 0.01) \times 10^{-2}$ M solution in glass distilled water. The solution was stored at 4°. The solution is stable at least for four months.

7. The copper tartrate reagent was prepared as follows: (1.60 ± 0.02) g Na_2CO_3, (0.329 ± 0.002) g tartaric acid and (0.30 ± 0.02) g $CuSO_4 \cdot 5H_2O$ were dissolved in one liter glass distilled water. In order to avoid precipitation, these three compounds were dissolved separately, added in the order mentioned to 900 ml of glass distilled water and made up to volume. This solution was stored at 20°, and one liter of it was prepared for each series of enzyme assays.

8. The standard solutions of γ-aminobutyric acid (GABA) were prepared in glass distilled water. A

stock (3.99 ± 0.02) x 10^{-3} M solution was prepared in 100 ml volumetric flask. This solution was stored at 4^{o}.

9. The complete buffer-substrate-coenzyme mixture was prepared just before each series of enzyme assays by combining (1.000 ± 0.002) ml of 0.4 M potassium phosphate buffer, pH 6.4, (1.000 ± 0.002) ml of (0.10 ± 0.01) M glutamic acid solution, pH 6.61 and (0.0400 ± 0.0002) ml of (4.99 ± 0.01) x 10^{-2} M pyridoxal phosphate solution.

Note: (1.000 ± 0.002) ml graduated pipette was used to measure potassium phosphate buffer solution.
(1.000 ± 0.002) ml graduated pipette was used to measure glutamic acid solution.
(50.0 ± 0.2) µl syringe was used to measure pyridoxal phosphate solution.
(0.2000 ± 0.0004) ml transfer pipette was used to measure buffer-substrate-coenzyme mixture.
(0.2000 ± 0.0004) ml transfer pipette was used to measure GABA solution or brain homogenate.
(0.2000 ± 0.0004) ml transfer pipette was used to measure buffer-substrate-coenzyme-GABA solution.
(0.2000 ± 0.0004) ml transfer pipette was used to measure TCA solution.
(0.2000 ± 0.0004) ml transfer pipette was used to measure buffer-substrate-coenzyme-GABA-TCA solution.

(1.000 ± 0.002) ml graduated pipette was used to measure ninhydrin solution.

(50.00 ± 0.04) ml burette was used to measure copper tartrate reagent.

The total 0.2 ml enzyme incubation mixture was made up of:

(0.1000 ± 0.0004) ml buffer-substrate-coenzyme mixture.

(0.1000 ± 0.0004) ml brain homogenate or GABA solution.

The total 10.6 ml final reaction volume was made up of:

(0.2000 ± 0.0004) ml buffer-substrate-coenzyme-TCA-brain homogenate solution or buffer-substrate-coenzyme-TCA-GABA solution.

(0.400 ± 0.002) ml of ninhydrin solution.

(10.00 ± 0.04) ml of copper tartrate regent.

d. Preparation of solutions for Acetylcholinesterase assay

1. The electrolyte solution was prepared by dissolving 1.0×10^{-3} M $MgCl_2.6H_2O$ in 0.1 N NaCl. This solution was stored at 20°.

2. The sodium barbital solution was prepared

as a $(2.99 \pm 0.02) \times 10^{-2}$ M solution in glass distilled water. This solution was stored at 4°, and 100 ml of it was prepared for each series of enzyme assays.

3. The stock buffer solution was prepared as a 0.06% phenol red $(1.69 \pm 0.01) \times 10^{-3}$ M solution in $(2.99 \pm 0.02) \times 10^{-2}$ M sodium barbital solution. This solution was stored at 4°. On the day of use, this solution was diluted 1:20 with the electrolyte solution.

4. The acetylcholine bromide solution was prepared as a $(2.49 \pm 0.02) \times 10^{-2}$ M solution in the electrolyte solution. This solution was stored at -20°, in small quantities to avoid repeated thawing.

5. The sodium hydroxide solution was prepared as a (0.099 ± 0.001) N solution in glass distilled water.

6. The acetic acid solution was prepared as a (0.111 ± 0.003) N solution in glass distilled water.

7. The acetylcholinesterase solutions were prepared in the electrolyte solution and stored at -20°.

I. Stock solution: 1 mg/100 ml using 100 ml volumetric flask.

$$\frac{(0.0010 \pm 0.0002) \text{ g} \times (325 \ \mu\text{M units})}{(100.00 \pm 0.04) \times 10^{-3} \text{ l}}$$

$$= (3.25 \pm 0.65) \ \mu\text{M units/ml}$$

II. Working solution: 5 ml stock solution was diluted to 10 ml.

5 ml transfer pipette and 10 ml volumetric flask were used.

$$\frac{(5.000 \pm 0.005)\ \text{ml} \times (3.25 \pm 0.65)\ \mu\text{M units/ml}}{(10.00 \pm 0.01)\ \text{ml}}$$

$$= (1.6 \pm 0.3)\ \mu\text{M units/ml}$$

Note: $(10.00 \pm 0.02)\ \mu$l syringe was used to measure brain homogenate.

$(10.00 \pm 0.02)\ \mu$l syringe was used to measure acetylcholinesterase solution.

$(10.00 \pm 0.02)\ \mu$l syringe was used to measure acetylcholine bromide solution.

$(50.0 \pm 0.2)\ \mu$l syringe was used to measure the diluted phenol red buffer solution.

The total 35 μl reaction volume was made up of:

$(10.00 \pm 0.02)\ \mu$l brain homogenate.

$(20.0 \pm 0.2)\ \mu$l diluted phenol red buffer solution.

$(5.00 \pm 0.02)\ \mu$l acetylcholine bromide solution.

or

$[(0.50 \rightarrow 10.00) \pm 0.02]\ \mu$l acetylcholinesterase

solution.

$[(20.0 \rightarrow 29.5) \pm 0.2]$ µl diluted phenol red buffer solution.

(5.00 ± 0.02) µl acetylcholine bromide solution.

VIII. APPENDIX B

Instrument Calibration

a. Calibration of fluorescence spectrophotometer with standardized H_2O_2

1. A standard sodium oxalate solution was prepared by dissolving (17.0000 ± 0.0002) g of sodium oxalate in 250 ml glass distilled water, N=(0.799 ± 0.001)

2. A standard potassium permanganate solution was prepared by dissolving (8.0000 ± 0.0002) g of potassium permanganate in 250 ml glass distilled water. This solution was titrated against 25 ml sodium oxalate and found to be (0.796 ± 0.002) N.

3. Approximatly 5 N sulphuric acid solution was prepared by dissolving (34.70 ± 0.04) ml concentrated sulphuric acid in 250 ml glass distilled water. 25 ml of this solution and 25 ml of sodium oxalate solution were used to titrate potassium permanganate solution.

4. Standard hydrogen peroxide (H_2O_2) solution was prepared by dissolving 0.25 ml of 30% (w/w) H_2O_2 in 25 ml glass distilled water and titrating against (0.796 ± 0.002) N potassium permanganate solution. The normality of H_2O_2 solution was found to be (1.99 ± 0.01) N and the molarity (0.99 ± 0.01) M.

5. The following H_2O_2 solutions were then prepared by dilution.

I. Stock solution: (0.1000 ± 0.0002) ml of (0.99 ± 0.01) M H_2O_2 solution was diluted to 100 ml in a volumetric flask. Molarity is equal to (0.99 ± 0.01)

x 10^{-3}.

II. Working solution: (0.03300 ± 0.00002) ml of the stock solution was diluted to 10 ml in a volumetric flask. Molarity is equal to (3.26 ± 0.04) x 10^{-6}.

6. The buffer solution : 0.1 N Na-K phosphate buffer, at pH 7.8

7. The horse radish peroxidase solution: (1.5 ± 0.1) mg/ml solution.

8. The p-hydroxyphenylacetic acid solution: (2.43 ± 0.02) x 10^{-2} M.

For instrument calibration different volumes of (3.26 ± 0.04) x 10^{-6} M H_2O_2 solution were added to 3 ml reaction volume that contained:

(0.250 ± 0.002) ml (2.43 ± 0.02) x 10^{-2} M p-hydroxyphenylacetic acid solution.

(0.2000 ± 0.0004) ml horse radish peroxidase solution.

(2.50 ± 0.02) ml 0.1 N Na-K phosphate buffer, pH 7.8

TABLE B.I

Concentration of H_2O_2 in the reaction volume in nanomolar units	
µl of $(3.26 \pm 0.04) \times 10^{-6}$ M H_2O_2 added	(Nanomoles ± m.p.e) H_2O_2 in the reaction volume
1.00 ± 0.02	$1.0^{9} \pm 0.0^{5}$
2.00 ± 0.02	$2.1^{7} \pm 0.0^{8}$
3.00 ± 0.02	$3.2^{6} \pm 0.1^{0}$
4.00 ± 0.02	$4.3^{4} \pm 0.2^{8}$
5.00 ± 0.02	$5.4^{3} \pm 0.1^{6}$
6.00 ± 0.02	$6.5^{2} \pm 0.1^{9}$
7.00 ± 0.02	$7.6^{0} \pm 0.2^{2}$
8.00 ± 0.02	$8.6^{9} \pm 0.2^{4}$
9.00 ± 0.02	$9.7^{7} \pm 0.2^{7}$
10.00 ± 0.02	$10.8^{6} \pm 0.3^{0}$
11.0 ± 0.2	11.9 ± 0.5
12.0 ± 0.2	13.0 ± 0.5
13.0 ± 0.2	14.1 ± 0.6
15.0 ± 0.2	16.3 ± 0.6
20.0 ± 0.2	21.7 ± 0.8
25.0 ± 0.2	27.2 ± 0.9

TABLE B.II

Experimental Data for the Calibration of Fluorescence Spectrophotometer with Standardized H_2O_2

Run No.	H_2O_2 (Nanomoles ± m.p.e)	ΔF div. ± 0.5	Dev. div.	Std.Dev. (s) div.	Rel. Std. Dev.
1	$(1.0^9 \pm 0.0^5)$	5.2	0.8		
2	1.1 ± 0.1	3.6	-0.8		
Av.		4.4	±0.8	$1.^1$	0.2^6
1	$(2.1^7 \pm 0.0^8)$	8.8	1.1		
2	2.2 ± 0.1	6.4	-1.3		
3		8.0	0.3		
Av.		7.7	±0.9	$1.^2$	0.1^6
1	$(3.2^6 \pm 0.1^0)$	13.0	1.0		
2	3.3 ± 0.1	12.0	0.0		
3		11.2	-0.8		
4		10.4	-1.6		
5		12.4	0.4		
6		12.8	0.8		
Av.		12.0	±0.8	$1.^0$	0.08^3
1	$(4.3^4 \pm 0.2^8)$	15.2	-0.5		
2	4.3 ± 0.3	16.0	0.3		
3		17.2	1.5		
4		16.0	0.3		
5		15.2	-0.5		
6		15.2	-0.5		
7		15.0	-0.7		
Av.		15.7	±0.6	$0.^8$	0.05^0
1	$(5.4^3 \pm 0.1^6)$	19.0	0.0		
2	5.4 ± 0.2	20.0	1.0		
3		20.0	1.0		
4		18.0	-1.0		
5		18.0	-1.0		
Av.		19.0	±0.8	$1.^2$	0.06^4
1	$(6.5^2 \pm 0.1^9)$	20.8	-0.7		
2	6.5 ± 0.2	21.6	0.1		
3		22.0	0.5		
Av.		21.5	±0.4	$0.^6$	0.02^8

TABLE B.II (con't)

Run No.	H_2O_2 (Nanomoles ± m.p.e)	ΔF div. ±0.5	Dev. div.	Std. Dev. (s) div.	Rel. Std. Dev.
1	$(7.6^0 \pm 0.2^2)$	23.6	-1.5		
2	7.6 ± 0.2	26.8	1.7		
3		24.8	-0.3		
Av.		25.1	±1.2	$1.^6$	0.06^4
1	$(8.6^9 \pm 0.2^4)$	29.6	0.5		
2	8.7 ± 0.2	29.6	0.5		
3		28.0	-1.1		
Av.		29.1	±0.7	0.9	0.03^2
1	$(9.7^7 \pm 0.2^7)$	36.8	-0.6		
2	9.8 ± 0.3	36.0	-1.4		
3		39.0	1.6		
4		39.0	1.6		
5		36.0	-1.4		
Av.		37.4	±1.3	$1.^5$	0.04^1
1	$(10.8^6 \pm 0.3^0)$	37	-4		
2	10.9 ± 0.3	43	2		
3		41	0		
4		44	3		
5		40	-1		
Av.		41	±2	$2.^7$	0.06^7
1	11.9 ± 0.5	44	0		
2		44	0		
3		44	0		
Av.		44	±0	$0.^0$	0.0
1	13.0 ± 0.5	50	0		
2		50	0		
3		49	-1		
Av.		50	±0	$1.^0$	0.02^0
1	14.1 ± 0.6	54	1		
2		53	0		
3		53	0		
4		53	0		
Av.		53	±0	$0.^5$	0.01^0
1	16.3 ± 0.6	59	-2		
2		59	-2		
3		61	0		
4		61	0		
5		63	2		

TABLE B.II (con't)

Run No.	H_2O_2 (Nanomoles ± m.p.e)	ΔF div. ±0.5	Dev. div.	Std. Dev. (s) div.	Rel. Std. Dev.
6		64	3		
7		65	4		
8		64	3		
9		64	3		
10		61	0		
11		54	-7		
Av.		61	±2	$3.^2$	0.05^2
1	21.7 ± 0.8	66	-4		
2		67	-3		
3		68	-2		
4		69	-1		
5		68	-2		
6		72	2		
7		74	4		
8		73	3		
9		73	3		
Av.		70	±3	$3.^0$	0.04^3
1	27.2 ± 0.9	103	5		
2		103	5		
3		97	-1		
4		96	-2		
5		96	-2		
6		96	-2		
7		96	-2		
8		97	-1		
9		102	4		
10		96	-2		
11		93	-5		
Av.		98	±3	$3.^4$	0.03^4

b. Calibration of fluorescence spectrophotometer with γ-aminobutyric acid (GABA)

1. The GABA solution was prepared as a $(3.99 \pm 0.02) \times 10^{-3}$ M solution in glass distilled water.

2. The following GABA solutions were prepared by dilution using:

10 ml standard burette	uncertainty ± 0.01 ml
10 ml volumetric flask	uncertainty ± 0.01 ml

The total 10.6 ml final reaction volume was made up of:

(0.2000 ± 0.0004) ml buffer-substrate-coenzyme-TCA-GABA solution.

(0.400 ± 0.002) ml ninhydrin solution.

(10.00 ± 0.04) ml copper tartrate regent.

TABLE B.III

Concentration of GABA in the Reaction Volume

ml of $(3.99 \pm 0.02) \times 10^{-3}$M GABA added	Initial GABA Concentration μM $\pm$ m.p.e.	Final GABA Concentration μM $\pm$ m.p.e.
$0.6^{3} \pm 0.0^{2}$	0.25 ± 0.01	$1.1^{8} \pm 0.0^{6}$
$1.2^{5} \pm 0.0^{2}$	0.50 ± 0.01	$2.3^{6} \pm 0.0^{6}$
$1.8^{8} \pm 0.0^{2}$	0.71 ± 0.01	$3.3^{5} \pm 0.0^{7}$
$2.5^{0} \pm 0.0^{2}$	1.00 ± 0.01	$4.7^{2} \pm 0.0^{8}$
$3.7^{5} \pm 0.0^{2}$	1.50 ± 0.02	7.1 ± 0.1
$5.0^{0} \pm 0.0^{2}$	2.00 ± 0.02	9.4 ± 0.2
$6.2^{5} \pm 0.0^{2}$	2.49 ± 0.02	11.7 ± 0.2
$7.5^{0} \pm 0.0^{2}$	2.99 ± 0.02	14.1 ± 0.2
$8.7^{5} \pm 0.0^{2}$	3.49 ± 0.03	16.5 ± 0.3
$10.0^{0} \pm 0.0^{2}$	3.99 ± 0.01	18.8 ± 0.2

TABLE B.IV

Experimental Data for the Calibration of Fluorescence Spectrophotometer with GABA

Run No.	GABA μMoles ± m.p.e.	ΔF div. ± 0.5	Dev. div.	Std. Dev. (s) div.	Rel. Std. Dev.
1	$(1.1^{8} \pm 0.0^{6})$	10.0	0.4		
2	1.2 ± 0.1	9.0	-0.6		
3		10.0	0.4		
4		10.0	0.4		
5		10.0	0.4		
6		10.0	0.4		
7		9.0	-0.6		
8		9.0	-0.6		
9		9.0	-0.6		
10		10.0	0.4		
Av.		9.6	±0.5	0.5^{2}	0.05^{4}
1	$(2.3^{6} \pm 0.0^{6})$	19.0	0.0		
2	2.4 ± 0.1	21.0	2.0		
3		20.0	1.0		
4		21.0	2.0		
5		21.0	2.0		
6		18.5	-0.5		
7		16.5	-2.5		
8		21.0	2.0		
9		16.0	-3.0		
10		16.0	-3.0		
Av.		19.0	±2.1	2.3^{1}	0.12^{1}
1	$(3.3^{5} \pm 0.0^{7})$	28	0		
2	3.4 ± 0.1	28	0		
3		28	0		
4		28	0		
5		28	0		
Av.		28	±0	$0.^{0}$	0.0
1	$(4.7^{2} \pm 0.0^{8})$	34.0	-1.7		
2	4.7 ± 0.1	35.0	-0.7		
3		33.0	-2.7		
4		33.0	-2.7		
5		33.0	-2.7		
6		40.5	4.8		
7		28.5	-7.2		
8		30.0	-5.7		
9		37.5	1.8		

TABLE B.IV (con't)

Run No.	GABA µMoles ± m.p.e.	Δ F div. ± 0.5	Dev. div.	Std. Dev. (s) div.	Rel. Std. Dev.
10		34.0	-1.7		
11		40.0	4.3		
12		40.0	4.3		
13		41.0	5.3		
14		38.0	2.3		
15		39.0	3.3		
Av.		36.0	±3.4	3.9^{6}	0.11^{1}
1	7.1 ± 0.1	50.0	-5.3		
2		48.0	-7.3		
3		54.0	-1.3		
4		61.5	5.2		
5		59.0	4.7		
6		47.0	-8.3		
7		53.0	-2.3		
8		54.0	-1.3		
9		54.0	-1.3		
10		55.0	-0.3		
11		58.0	2.7		
12		57.0	1.7		
13		59.0	3.7		
14		61.0	5.7		
15		60.0	4.7		
Av.		55.3	±3.8	4.5^{6}	0.08^{2}
1	9.4 ± 0.2	81.5	9.8		
2		69.0	-2.7		
3		66.0	-5.7		
4		63.5	-8.2		
5		78.5	6.8		
Av.		71.7	±6.6	7.8^{9}	0.11^{0}
1	11.7 ± 0.2	89.5	-2.3		
2		90.0	-1.8		
3		92.0	0.2		
4		95.5	3.7		
5		92.0	0.2		
Av.		91.8	±1.6	2.3^{6}	0.02^{6}
1	14.1 ± 0.2	121.5	2.9		
2		119.0	0.4		
3		117.0	-1.6		
4		116.5	-2.1		
5		119.0	0.4		
Av.		118.6	±1.5	1.9^{8}	0.01^{7}

TABLE B.IV (con't)

Run No.	GABA μMoles ± m.p.e.	ΔF div. ± 0.5	Dev. div.	Std.Dev. (s)	Rel. Std..Dev.
1	16.5 ± 0.3	138.0	0.3		
2		134.0	-3.7		
3		138.5	0.8		
4		144.0	6.3		
5		134.0	-3.7		
Av.		137.7	±2.9	4.1^{2}	0.03^{0}
1	18.8 ± 0.2	160.0	2.9		
2		156.0	-1.1		
3		157.0	-0.1		
4		158.0	0.9		
5		154.5	-2.6		
Av.		157.1	±1.5	2.0^{7}	0.01^{3}

c. Calibration of DB spectrophotometer with standardized sodium hydroxide and standardized acetic acid

1. A primary standard solution of sodium carbonate was prepared by dissolving (1.35 ± 0.02) g of Na_2CO_3 in 250 ml glass distilled water.

2. Hydrochloric acid solution N = (0.048 ± 0.001), was prepared to be used as a secondary standard solution.

3. Sodium hydroxide solution was prepared and titrated against (0.048 ± 0.001) N HCl. The normality was found to be (0.099 ± 0.002) N.

4. Acetic acid solution was prepared from 17.6 N glacial acetic acid. This solution was titrated against (0.099 ± 0.002) N NaOH and found to be (0.111 ± 0.003) N.

5. The electrolyte solution was 1.0×10^{-3} $MgCl_2.6H_2O$ in 0.1 N NaCl.

6. The stock buffer solution was $(1.69 \pm 0.01) \times 10^{-3}$ M phenol red solution in $(2.99 \pm 0.02) \times 10^{-2}$ M sodium barbital solution.

The total 35 µl reaction volume was made up of:

(20.0 ± 0.2) µl diluted phenol red buffer solution.

(10.00 ± 0.02) µl brain homogenate.

(5.00 ± 0.02) µl acetylcholine bromide solution.

The procedure was standardized by reading few tubes after adding a trace of (0.099 ± 0.002) N NaOH (R_1) and a slight excess of (0.111 ± 0.003) N acetic acid (R_2).

$$\Delta R = (R_2) - (R_1)$$

$$\doteq (20.0 \pm 0.2) \times 10^{-6} \times (1.69 \pm 0.01) \times 10^{-3}$$

$$= (33.8 \pm 0.5) \times 10^{-9} \text{ moles of acetic acid liberation.}$$

In any tube a color change of 32% of $\Delta R_{std.}$ indicates the liberation of $(11.3 \pm 0.5) \times 10^{-9}$ moles of acetic acid. The time (t) in minutes to reach 32% of $\Delta R_{std.}$ is calculated by interpolation.

The rate of enzyme activity is:

$$(11.3 \pm 0.5) \times \frac{60}{t} \times 10^{-9}$$ moles of acetic acid formed per hour.

TABLE B.V

Calibration Data for Acetylcholinesterase Assay

Run No.	R_1 %T ± 0.2%	R_2 %T ± 0.2%	$\Delta R_{std.}$ %T ± 0.4%	1/3 $\Delta R_{std.}$ %T ± 0.4%
1	27.0	80.5	53.5	17.8
2	51.5	99.0	48.5	16.1
Av.	39.2	89.7	50.5	16.8

IX. APPENDIX C

Experimental Data for Monamine Oxidase Assay

TABLE C.I

Fluorimetric Measurement of Monamine Oxidase in Different Regions of Experimental and Control Rat Brain+

Date	Animal & Sample	Experimental Condition	ΔF div. ± 0.5	Dev. div.
7-6-72	Ial	Ethanol	0.5	0.0
	2	induced	0.5	0.0
	3	one day	0.5	0.0
	Av.	off	0.5	±0.0
	Ibl		0.5	0.0
	2		0.5	0.0
	3		0.5	0.0
	Av.		0.5	±0.0
	Icl		1.5	0.3
	2		1.0	-0.2
	3		1.0	-0.2
	Av.		1.2	±0.2
	Idl		45.5	0.9
	2		43.5	-1.1
	3		45.0	0.4
	Av.		44.6	±0.8
	Iel		18.5*	-
	2		8.0	2.5
	3		3.0	-2.5
	Av.		5.5	±2.5
7-6-72	IIal	Ethanol	1.5	0.3
	2	induced	1.0	-0.2
	3		1.0	-0.2
	Av.		1.2	±0.2
	IIbl		0.5	-0.2
	2		0.5	-0.2
	3		1.0	0.3
	Av.		0.7	±0.2

+ (I-XIV) denote animal no., (a-e) denote brain region no. and (1-3) denote enzyme assay no.
* Rejected by 95% confidence level.

TABLE C.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	IIc1		1.0	-0.3
	2		2.0	0.7
	3		1.0	-0.3
	Av.		1.3	±0.4
	IId1		22.0	0.0
	2		21.5	-0.5
	3		22.5	0.5
	Av.		22.0	±0.3
	IIe1		7.5	-6.0
	2		19.0	5.5
	3		14.0	0.5
	Av.		13.5	±4.0
7-6-72	IIIa1	Control	0.5	0.0
	2		0.5	0.0
	3		0.5	0.0
	Av.		0.5	±0.0
	IIIb1		1.0	0.3
	2		0.5	-0.2
	3		0.5	-0.2
	Av.		0.7	±0.2
	IIIc1		0.5	0.0
	2		0.5	0.0
	3		0.5	0.0
	Av.		0.5	±0.0
	IIId1		69.0	-2.8
	2		77.5	5.7
	3		69.0	-2.8
	Av.		71.8	±3.8
	IIIe1		27.0	0.0
	2		43.0*	-
	3		27.0	0.0
	Av.		27.0	±0.0
12-6-72	IVa1	Ethanol	2.0	0.0
	2	induced	2.5	0.5
	3	one day	1.5	-0.5
	Av.	off	2.0	±0.3

TABLE C.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	IVb1		1.5	-0.3
	2		2.5	0.7
	3		1.5	-0.3
	Av.		1.8	±0.4
	IVc1		1.5	0.2
	2		0.5	-0.8
	3		2.0	0.7
	Av.		1.3	±0.6
	IVd1		24.0	0.0
	2		24.0	0.0
	3		24.0	0.0
	Av.		24.0	±0.0
	IVe1		5.5	-0.1
	2		5.5	-0.1
	3		5.6	0.0
	Av.		5.6	±0.1
12-6-72	Va1	Ethanol induced one day off	1.5	0.3
	2		1.5	0.3
	3		0.5	-0.7
	Av.		1.2	±0.4
	Vb1		4.0	0.2
	2		3.5	-0.3
	3		0.5*	-
	Av.		3.8	±0.3
	Vc1		1.5	-0.5
	2		1.5	-0.5
	3		3.0	1.0
	Av.		2.0	±0.7
	Vd1		20.5	-7.5
	2		28.5	0.5
	3		35.0	7.0
	Av.		28.0	±5.0
	Ve1		4.5	0.2
	2		4.0	-0.3
	3		0.5*	-
	Av.		4.3	±0.3

TABLE C.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
12-6-72	VIa1	Control	0.5	0.0
	2		0.5	0.0
	3		0.5	0.0
	Av.		0.5	±0.0
	VIb1		0.5	0.0
	2		0.5	0.0
	3		0.5	0.0
	Av.		0.5	±0.0
	VIc1		1.5	0.3
	2		1.5	0.3
	3		0.5	-0.7
	Av.		1.2	±0.4
	VId1		33.5	-3.5
	2		49.5	12.5
	3		28.0	-9.0
	Av.		37.0	±8.3
	VIe1		16.0	0.5
	2		8.0	-7.5
	3		22.5	7.0
	Av.		15.5	±5.0
14-6-72	VIIa1	Ethanol induced one day off	1.5	0.5
	2		0.5	-0.5
	3		1.0	0.0
	Av.		1.0	±0.3
	VIIb1		0.5	0.0
	2		0.5	0.0
	3		0.5	0.0
	Av.		0.5	0.0
	VIIc1		0.5	-1.0
	2		1.5	0.0
	3		2.5	1.0
	Av.		1.5	±0.7
	VIId1		18.0	-0.6
	2		17.5	-1.1
	3		20.5	1.9
	Av.		18.6	±1.2

TABLE C.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	VIIe1		3.5	0.0
	2		3.5	0.0
	3		3.5	0.0
	Av.		3.5	±0.0
14-6-72	VIIIa1	Ethanol	3.5	1.2
	2	induced	2.0	-0.3
	3	one day	1.5	-0.8
	Av.	off	2.3	±0.8
	VIIIb1		1.0	0.2
	2		0.5	-0.3
	3		1.0	0.2
	Av.		0.8	±0.3
	VIIIc1		0.5	0.0
	2		0.5	0.0
	3		0.5	0.0
	Av.		0.5	±0.0
	VIIId1		20.5	-0.3
	2		21.0	0.2
	3		21.0	0.2
	Av.		20.8	±0.3
	VIIIe1		5.0	0.2
	2		4.5	-0.3
	3		5.0	0.2
	Av.		4.8	±0.3
14-6-72	IXa1	Control	2.0	0.0
	2		2.0	0.0
	3		2.0	0.0
	Av.		2.0	±0.0
	IXb1		1.5	0.0
	2		1.5	0.0
	3		1.5	0.0
	Av.		1.5	±0.0
	IXc1		0.5	0.0
	2		0.5	0.0
	3		0.5	0.0
	Av.		0.5	±0.0

TABLE C.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	IXdl		47.0	3.2
	2		36.5	-7.3
	3		48.0	4.2
	Av.		43.8	±4.8
	IXel		11.5	-1.6
	2		16.5	3.4
	3		11.5	-1.6
	Av.		13.1	±2.2
20-6-72	Xal	Ethanol	1.0	-0.5
	2	induced	2.0	0.5
	3		1.5	0.0
	Av.		1.5	±0.3
	Xbl		1.5	0.0
	2		1.5	0.0
	3		1.5	0.0
	Av.		1.5	±0.0
	Xcl		0.5	-0.5
	2		1.0	0.0
	3		1.5	0.5
	Av.		1.0	±0.3
	Xdl		15.5	-0.7
	2		5.0*	-
	3		17.0	0.8
	Av.		16.2	±0.8
	Xel		8.5	0.0
	2		8.5	0.0
	3		25.0*	-
	Av.		8.5	±0.0
20-6-72	XIal	Ethanol	3.5	1.2
	2	induced	2.0	-0.3
	3		1.5	-0.8
	Av.		2.3	±0.8
	XIbl		0.5	0.0
	2		0.5	0.0
	3		0.5	0.0
	Av.		0.5	±0.0

TABLE C.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	XIc1		1.0	0.0
	2		1.0	0.0
	3		1.0	0.0
	Av.		1.0	±0.0
	XId1		12.5	-0.3
	2		13.0	0.2
	3		13.0	0.2
	Av.		12.8	±0.2
	XIe1		10.5	1.2
	2		9.5	0.2
	3		8.0	-1.3
	Av.		9.3	±0.9
20-6-72	XIIa1	Control	1.5	-0.2
	2		1.5	-0.2
	3		2.0	0.3
	Av.		1.7	±0.2
	XIIb1		0.5	0.0
	2		0.5	0.0
	3		0.5	0.0
	Av.		0.5	±0.0
	XIIc1		0.5	-0.7
	2		1.5	0.3
	3		1.5	0.3
	Av.		1.2	±0.4
	XIId1		42.5	1.2
	2		40.0	-1.3
	3		41.5	0.2
	Av.		41.3	±0.9
	XIIe1		3.5	-1.2
	2		3.5	-1.2
	3		7.0	2.3
	Av.		4.7	±1.6
22-6-72	XIIIa1	Ethanol induced	1.0	0.0
	2		1.0	0.0
	3		1.0	0.0
	Av.		1.0	±0.0

TABLE C.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	XIIIb1		1.0	0.0
	2		1.0	0.0
	3		1.0	0.0
	Av.		1.0	±0.0
	XIIIc1		1.5	0.7
	2		0.5	-0.3
	3		0.5	-0.3
	Av.		0.8	±0.4
	XIIId1		12.0	0.2
	2		11.5	-0.3
	3		12.0	0.2
	Av.		11.8	±0.3
	XIIIe1		7.0	0.7
	2		7.5	1.2
	3		4.5	-1.8
	Av.		6.3	±1.2
22-6-72	XIVa1	Control	1.5	0.0
	2		2.0	0.5
	3		1.0	-0.5
	Av.		1.5	±0.3
	XIVb1		1.0	-0.1
	2		0.5	-0.6
	3		2.0	0.9
	Av.		1.1	±0.5
	XIVc1		1.5	0.2
	2		1.5	0.2
	3		1.0	-0.3
	Av.		1.3	±0.3
	XIVd1		13.5*	-
	2		30.5	-1.0
	3		32.5	1.0
	Av.		31.5	±1.0
	XIVe1		4.0	-0.8
	2		5.5	0.7
	3		5.0	0.2
	Av.		4.8	±0.6

TABLE C.II

Average Fluorimetric Measurements of Monamine Oxidase Activity in Different Regions of Experimental and Control Rat Brain

Animal & Brain Region	ΔF div. ±0.5	Dev. div. ±	Std. Dev. (s) div.	Rel. Std. Dev.
Ia	0.5	0.0	0.0	0.0^{0}
Ib	0.5	0.0	0.0	0.0^{0}
Ic	1.1	0.2	0.3^{0}	0.27^{2}
Id	44.6	0.8	1.0^{4}	0.02^{3}
Ie	5.5	2.5	$3.^{5}$	0.6^{4}
IIa	1.1	0.2	0.3^{0}	0.27^{2}
IIb	0.7	0.2	0.3^{0}	0.5^{0}
IIc	1.3	0.4	0.5^{7}	0.4^{3}
IId	22.0	0.3	0.5^{0}	0.02^{2}
IIe	13.5	4.0	$5.^{7}$	0.4^{2}
IIIa	0.5	0.0	0.0	0.0^{0}
IIIb	0.7	0.2	0.3^{0}	0.5^{0}
IIIc	0.5	0.0	0.0	0.0^{0}
IIId	71.8	3.8	$4.^{9}$	0.0^{6}
IIIe	27.0	0.0	0.0	0.0^{0}
IVa	2.0	0.3	0.5^{0}	0.25^{0}
IVb	1.8	0.4	0.5^{0}	0.25^{0}
IVc	1.3	0.6	0.7^{6}	0.58^{0}
IVd	24.0	0.0	0.0	0.0^{0}
IVe	5.6	0.1	0.3^{0}	0.05^{3}

TABLE C.II (con't)

Animal & Brain Region	ΔF div. ±0.5	Dev. div. ±	Std.Dev. (s) div.	Rel. Std. Dev.
Va	1.2	0.4	0.5^{7}	0.47^{5}
Vb	3.8	0.3	0.3^{6}	0.09^{5}
Vc	2.0	0.7	0.8^{6}	0.43^{0}
Vd	28.0	5.0	$7.^{2}$	0.2^{5}
Ve	4.3	0.3	0.3^{6}	0.08^{8}
VIa	0.5	0.0	0.0	0.0^{0}
VIb	0.5	0.0	0.0	0.0^{0}
VIc	1.2	0.4	0.5^{7}	0.47^{5}
VId	37.0	8.3	$11.^{2}$	0.3^{0}
VIe	15.5	5.0	$7.^{3}$	0.4^{6}
VIIa	1.0	0.3	0.5^{0}	0.5^{0}
VIIb	0.5	0.0	0.0	0.0^{0}
VIIc	1.5	0.7	$1.^{0}$	0.6^{6}
VIId	18.6	1.2	1.6^{1}	0.08^{6}
VIIe	3.5	0.0	0.0	0.0^{0}
VIIIa	2.3	0.8	1.0^{4}	0.45^{2}
VIIIb	0.8	0.3	$0.^{3}$	0.3^{6}
VIIIc	0.5	0.0	0.0	0.0^{0}
VIIId	20.8	0.3	0.2^{9}	0.01^{3}
VIIIe	4.8	0.3	$0.^{3}$	0.0^{6}
IXa	2.0	0.0	0.0	0.0^{0}
IXb	1.5	0.0	0.0	0.0^{0}

TABLE C.II (con't)

Animal & Brain Region	ΔF div. ±0.5	Dev. div. ±	Std. Dev. (s) div.	Rel. Std. Dev.
IXc	0.5	0.0	0.0	0.0^{0}
IXd	43.8	4.8	6.3^{7}	0.14^{0}
IXe	13.1	2.2	2.8^{8}	0.21^{0}
Xa	1.5	0.3	$1.^{6}$	0.0^{8}
Xb	1.5	0.0	0.0	0.0^{0}
Xc	1.0	0.3	0.5^{0}	0.5^{0}
Xd	16.2	0.8	1.0^{6}	0.06^{0}
Xe	8.5	0.0	0.0	0.0^{0}
XIa	2.3	0.8	1.0^{4}	0.45^{2}
XIb	0.5	0.0	0.0	0.0^{0}
XIc	1.0	0.0	0.0	0.0^{0}
XId	12.8	0.2	0.2^{9}	0.02^{2}
XIe	9.3	0.9	1.2^{5}	0.13^{0}
XIIa	1.7	0.2	$0.^{3}$	0.1^{8}
XIIb	0.5	0.0	0.0	0.0^{0}
XIIc	1.2	0.4	$0.^{5}$	0.5^{3}
XIId	41.3	0.9	1.2^{5}	0.03^{0}
XIIe	4.7	1.6	2.0^{2}	0.43^{9}
XIIIa	1.0	0.0	0.0	0.0^{0}
XIIIb	1.0	0.0	0.0	0.0^{0}
XIIIc	0.8	0.4	$0.^{5}$	0.7^{2}
XIIId	11.8	0.3	0.2^{9}	0.02^{4}

TABLE C.II (con't)

Animal & Brain Region	ΔF div. ±0.5	Dev. div. ±	Std. Dev. (s) div.	Rel. Std. Dev.
XIIIe	6.3	1.2	1.6^{0}	0.25^{0}
XIVa	1.5	0.3	$0.^{5}$	0.3^{3}
XIVb	1.1	0.5	$0.^{7}$	0.6^{9}
XIVc	1.3	0.3	0.2^{9}	0.22^{3}
XIVd	31.5	1.0	1.4^{1}	0.04^{4}
XIVe	4.8	0.6	0.7^{6}	0.15^{9}

TABLE C.III

Effect of Ethanol on the Activity of Monamine Oxidase in Different Regions of Rat Brain

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles H_2O_2/hr/ g wet brain x10^{-8}	Dev. ±	Std. Dev. (s)	Rel. Std. Dev.
Ia	34 ± 5	**			
Ib	19 ± 4	**			
Ic	29 ± 5	0.6	0.2	0.3^{0}	0.60^{0}
Id	38 ± 5	32.8	0.6	0.7^{8}	0.02^{4}
Ie	20 ± 4	7.0	3.5	$4.^{9}$	0.7^{0}
IIa	48 ± 6	0.3	0.1	$0.^{1}$	0.5^{7}
IIb	53 ± 7	**			
IIc	43 ± 6	0.5	0.3	$0.^{3}$	0.6^{9}
IId	29 ± 5	20.9	0.3	0.4^{1}	0.01^{9}
IIe	48 ± 6	7.6	2.3	$3.^{6}$	0.44^{2}
IIIa	30 ± 5	**			
IIIb	12 ± 4	**			
IIIc	36 ± 5	**			
IIId	55 ± 6	36.6	1.9	2.4^{8}	0.06^{8}
IIIe	56 ± 6	13.4	0.0	0.0	0.0^{0}
IVa	34 ± 5	1.2	0.3	$0.^{4}$	0.3^{3}
IVb	30 ± 4	1.2	0.4	$0.^{5}$	0.4^{8}
IVc	18 ± 4	**			
IVd	30 ± 5	22.1	0.0	0.0^{0}	0.0^{0}
IVe	26 ± 4	5.5	0.1	0.1^{0}	0.01^{8}

** Enzyme activity is less than 0.1 moles H_2O_2/hr/g wet brain x 10^{-8}

TABLE C.III (con't)

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles H_2O_2/hr/ g wet brain $x10^{-8}$	Dev. ±	Std. Dev. (s)	Rel. Std. Dev.
Va	24 ± 4	**			
Vb	21 ± 4	4.4	0.4	0.5^{0}	0.11^{4}
Vc	17 ± 3	2.5	1.1	1.4	0.5^{7}
Vd	23 ± 4	33.6	6.2	8.9^{2}	0.26^{5}
Ve	14 ± 3	7.6	0.6	0.7^{8}	0.10^{3}
VIa	31 ± 5	**			
VIb	18 ± 4	**			
VIc	19 ± 4	**			
VId	22 ± 4	46.9	10.7	14.3^{3}	0.30^{6}
VIe	27 ± 4	15.6	5.3	7.6^{1}	0.48^{8}
VIIa	27 ± 4	**			
VIIb	23 ± 4	**			
VIIc	19 ± 4	**			
VIId	23 ± 4	22.2	1.5	1.9^{9}	0.08^{9}
VIIe	15 ± 4	5.6	0.0	0.0	0.0^{0}
VIIIa	18 ± 4	2.8	1.2	$1.^{6}$	0.5^{8}
VIIIb	19 ± 4	**			
VIIIc	18 ± 4	**			
VIIId	21 ± 4	27.3	0.3	0.4^{1}	0.01^{5}
VIIIe	17 ± 4	7.1	0.4	0.5^{3}	0.07^{5}
IXa	17 ± 4	2.5	0.0	0.0	0.0^{0}
IXb	17 ± 4	1.6	0.0	0.0	0.0^{0}

TABLE C.III (con't)

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles H_2O_2/hr/ g wet brain $x10^{-8}$	Dev. ±	Std. Dev. (s)	Rel. Std. Dev.
IXc	18 ± 4	**			
IXd	27 ± 5	45.1	5.2	6.6^{4}	0.14^{7}
IXe	23 ± 4	15.5	2.7	3.5^{4}	0.22^{7}
Xa	28 ± 4	1.0	0.3	$0.^{5}$	0.5^{0}
Xb	5 ± 7	5.6	0.0	0.0	0.0^{0}
Xc	28 ± 4	**			
Xd	24 ± 4	18.5	0.9	1.2^{7}	0.06^{9}
Xe	24 ± 4	9.4	0.0	0.0	0.0^{0}
XIa	17 ± 4	3.0	1.3	$1.^{7}$	0.5^{8}
XIb	25 ± 4	**			
XIc	27 ± 4	1.0	0.0	0.0_{5}	0.0^{0}
XId	22 ± 4	15.8	0.3	0.3^{6}	0.02^{2}
XIe	21 ± 4	11.8	1.5	1.6^{6}	0.14^{1}
XIIa	22 ± 4	1.4	0.2	$0.^{3}$	0.2^{6}
XIIb	30 ± 4	**			
XIIc	29 ± 4	**			
XIId	24 ± 4	48.0	1.1	1.5^{1}_{2}	0.03^{1}_{4}
XIIe	21 ± 4	5.4	2.0	2.7^{2}	0.50^{4}
XIIIa	26 ± 4	0.5	0.0	0.0	0.0^{0}
XIIIb	20 ± 4	0.7	0.0	0.0	0.0^{0}
XIIIc	26 ± 4	**			
XIIId	23 ± 4	13.9	0.3	0.3^{5}	0.02^{5}
XIIIe	19 ± 4	8.8	1.6	2.0^{6}	0.23^{4}

TABLE C.III (con't)

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles H_2O_2/hr/ g wet brain 10^{-8}	Dev. ±	Std. Dev. (s)	Rel. Std. Dev.
XIVa	16 ± 4	1.8	0.6	0.6^{9}	0.38^{6}
XIVb	29 ± 4	**			
XIVc	19 ± 4	1.2	0.4	0.4^{6}_{5}	0.38^{6}_{4}
XIVd	25 ± 4	35.0	1.1	1.5^{8}	0.04^{1}
XIVe	17 ± 4	7.1	1.0	1.2^{8}	0.18^{1}

X. APPENDIX D

Experimental Data for L-Glutamic Acid Decarboxylase Assay

TABLE D.I

Fluorimetric Measurement of l-Glutamic Acid Decarboxylase Activity in Different Regions of Experimental and Control Rat Brain+

Date	Animal & Sample	Experimental Condition	ΔF div. ± 0.5	Dev. div.
7-6-72	Ial	Ethanol induced one day off	14.0	-4.5
	2		19.0	0.5
	3		22.5	4.0
	Av.		18.5	±3.0
	Ibl		6.5	0.7
	2		5.0	-0.8
	3		6.0	0.2
	Av.		5.8	±1.5
	Icl		15.0	0.5
	2		14.5	0.0
	3		14.0	-0.5
	Av.		14.5	±0.3
	Idl		107.0	19.8
	2		82.5	-4.7
	3		72.0	-15.2
	Av.		87.2	±13.2
	Iel		26.5	-1.2
	2		26.0	-1.8
	3		31.0	3.2
	Av.		27.8	±2.1
7-6-72	IIal	Ethanol induced	12.5	2.3
	2		10.0	-0.2
	3		8.0	-2.2
	Av.		10.2	±1.5
	IIbl		11.0	0.3
	2		10.0	-0.7
	3		11.0	0.3
	Av.		10.7	±0.4

+ (I-XIV) denote animal no., (a-e) denote brain region no. and (1-3) denote enzyme assay no.
* Rejected by 95% confidence level.

TABLE D.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	IIc1		10.0	-2.8
	2		14.0	1.2
	3		14.5	1.7
	Av.		12.8	±1.9
	IId1		24.0	0.0
	2		24.0	0.0
	3		9.5*	-
	Av.		24.0	±0.0
	IIe1		29.5	1.7
	2		26.0	-1.8
	3		9.5*	-
	Av.		27.8	±1.8
7-6-72	IIIa1	Control	31.5	4.8
	2		9.0*	-
	3		22.0	-4.7
	Av.		26.7	±4.8
	IIIb1		2.0	0.5
	2		1.0	-0.5
	3		1.5	0.0
	Av.		1.5	±0.3
	IIIc1		36.5	1.8
	2		38.0	3.3
	3		29.5	-5.2
	Av.		34.7	±3.4
	IIId1		191.7	-0.8
	2		194.0	1.5
	3		191.7	-0.8
	Av.		192.5	±1.0
	IIIe1		82.3	-2.3
	2		89.3	4.7
	3		82.3	-2.3
	Av.		84.6	±3.1
12-6-72	IVa1	Ethanol	16.5	3.2
	2	induced	9.5	-3.8
	3	one day	14.0	0.7
	Av.	off	13.3	±2.5

TABLE D.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	IVb1		19.0	-0.5
	2		21.0	1.5
	3		18.5	-1.0
	Av.		19.5	±1.0
	IVc1		6.0	0.3
	2		2.0*	-
	3		5.5	-0.2
	Av.		5.7	±0.2
	IVd1		32.0*	-
	2		55.5	-2.0
	3		59.5	2.0
	Av.		57.5	±2.0
	IVe1		43.5	2.0
	2		44.0	2.5
	3		37.0	-4.5
	Av.		41.5	±3.0
12-6-72	Va1	Ethanol induced one day off	6.0*	-
	2		2.5	0.5
	3		1.5	-0.5
	Av.		2.0	±0.5
	Vb1		11.0	0.8
	2		2.0*	-
	3		9.5	-0.7
	Av.		10.2	±0.8
	Vc1		3.5	-0.8
	2		3.5	-0.8
	3		6.0	1.7
	Av.		4.3	±1.1
	Vd1		88.0	14.0
	2		18.0*	-
	3		60.0	-14.0
	Av.		74.0	±14.0
	Ve1		29.5	-0.5
	2		33.0	3.0
	3		27.5	-2.5
	Av.		30.0	±2.0

TABLE D.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
12-6-72	VIa1	Control	13.0	-1.7
	2		17.0	2.3
	3		14.0	-0.7
	Av.		14.7	±1.5
	VIb1		7.0	-0.8
	2		8.5	0.7
	3		8.0	0.2
	Av.		7.8	±0.5
	VIc1		35.0*	-
	2		9.5	0.0
	3		9.5	0.0
	Av.		9.5	±0.0
	VId1		77.0	2.8
	2		80.5	6.3
	3		65.0	-9.2
	Av.		74.2	±6.1
	VIe1		50.5	8.0
	2		46.5	4.0
	3		30.5	-12.0
	Av.		42.5	±8.0
14-6-72	VIIa1	Ethanol induced one day off	5.0	0.5
	2		2.5	-2.0
	3		6.0	1.5
	Av.		4.5	±1.3
	VIIb1		5.5	-0.8
	2		7.0	0.7
	3		6.5	0.2
	Av.		6.3	±0.6
	VIIc1		1.5	0.0
	2		2.0	0.5
	3		1.0	-0.5
	Av.		1.5	±0.3
	VIId1		16.0*	-
	2		60.0	-0.2
	3		60.5	0.3
	Av.		60.2	±0.3

TABLE D.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	VIIel		39.5	8.2
	2		32.5	1.2
	3		22.0	-9.3
	Av.		31.3	±6.2
14-6-72	VIIIal	Ethanol	3.5	-0.2
	2	induced	1.0*	-
	3	one day	4.0	0.3
	Av.	off	3.7	±0.2
	VIIIbl		2.0	-0.7
	2		1.5	-1.2
	3		4.5	1.8
	Av.		2.7	±0.9
	VIIIcl		5.5*	-
	2		1.5	0.5
	3		0.5	-0.5
	Av.		1.0	±0.5
	VIIIdl		48.0	-0.2
	2		48.5	0.3
	3		48.0	-0.2
	Av.		48.2	±0.2
	VIIIel		27.5	-7.0
	2		32.5	-4.0
	3		46.5	11.0
	Av.		35.5	±7.3
14-6-72	IXal	Control	14.5	0.3
	2		17.0	2.8
	3		11.0	-3.2
	Av.		14.2	±2.1
	IXbl		6.0	1.8
	2		2.5	-1.7
	3		4.0	-0.2
	Av.		4.2	±1.2
	IXcl		8.5	-0.5
	2		10.0	1.0
	3		8.5	-0.5
	Av.		9.0	±0.6

TABLE D.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	IXdl		107.5	0.7
	2		107.5	0.7
	3		105.5	-1.3
	Av.		106.8	±0.9
	IXel		75.0	2.5
	2		52.5*	-
	3		70.0	-2.5
	Av.		72.5	±2.5
20-6-72	Xal	Ethanol induced	7.0	1.5
	2		6.0	0.5
	3		3.5	-2.0
	Av.		5.5	±1.3
	Xbl		1.0	-0.2
	2		1.5	0.3
	3		1.0	-0.2
	Av.		1.2	±0.2
	Xcl		14.5	1.5
	2		14.5	1.5
	3		10.0	-3.0
	Av.		13.0	±2.0
	Xdl		29.5	3.2
	2		27.0	0.7
	3		22.5	-3.8
	Av.		26.3	±2.5
	Xel		1.0	0.0
	2		1.0	0.0
	3		17.0*	-
	Av.		1.0	±0.0
20-6-72	XIal	Ethanol induced	2.5	-0.3
	2		4.0	1.2
	3		2.0	-0.8
	Av.		2.8	±0.7
	XIbl		1.5	-0.5
	2		2.5	0.5
	3		19.0*	-
	Av.		2.0	±0.5

TABLE D.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	XIcl		8.5	-0.5
	2		9.5	0.5
	3		9.0	0.0
	Av.		9.0	±0.3
	XIdl		14.0	1.2
	2		9.5	-2.3
	3		12.0	0.8
	Av.		11.8	±1.7
	XIel		9.5	0.8
	2		10.0	1.3
	3		6.5	-2.2
	Av.		8.7	±1.4
20-6-72	XIIal	Control	3.5	-1.0
	2		5.0	0.5
	3		5.0	0.5
	Av.		4.5	±0.6
	XIIbl		28.5	-1.2
	2		30.0	0.3
	3		30.5	0.8
	Av.		29.7	±0.7
	XIIcl		28.0	-1.3
	2		32.0	2.7
	3		28.0	-1.3
	Av.		29.3	±1.7
	XIIdl		53.5	-0.8
	2		56.5	2.2
	3		53.0	-1.3
	Av.		54.3	±1.4
	XIIel		25.5	-2.7
	2		28.5	0.3
	3		30.5	2.3
	Av.		28.2	±1.7
22-6-72	XIIIal	Ethanol induced	7.0	1.5
	2		3.0	-2.5
	3		6.5	1.0
	Av.		8.7	±1.6

TABLE D.I (con't)

Date	Animal & Sample	Experimental Condition	ΔF div. ±0.5	Dev. div.
	XIIIb1		1.5	-1.2
	2		3.0	0.3
	3		3.5	0.3
	Av.		2.7	±0.7
	XIIIc1		1.0	-0.2
	2		16.0*	-
	3		1.5	0.3
	Av.		1.2	±0.3
	XIIId1		1.5	-0.2
	2		1.5	-0.2
	3		2.0	0.3
	Av.		1.7	±0.2
	XIIIe1		1.5*	-
	2		7.5	-0.7
	3		9.0	0.8
	Av.		8.2	±0.8
22-6-72	XIVa1	Control	10.5*	-
	2		4.5	-0.5
	3		5.5	0.5
	Av.		5.0	±0.5
	XIVb1		20.5	1.2
	2		21.0	-0.7
	3		23.5	1.8
	Av.		21.7	±1.2
	XIVc1		11.5	2.8
	2		7.5	-1.2
	3		7.0	-1.7
	Av.		8.7	±1.9
	XIVd1		52.5	1.0
	2		50.5	-1.0
	3		51.5	0.0
	Av.		51.5	±0.7
	XIVe1		29.5	-1.7
	2		33.0	1.8
	3		45.5*	-
	Av.		31.2	±1.8

TABLE D.II

Average Fluorimetric Measurements of L-Glutamic Acid Decarboxylase Activity in Different Regions of Experimental and Control Rat Brain

Animal & Brain Region	ΔF div. ±0.5	Dev. div. ±	Std. Dev. (s) div.	Rel. Std. Dev.
Ia	18.5	3.0	4.2^{7}	0.23^{0}
Ib	5.8	1.5	0.7^{6}	0.13^{1}
Ic	14.5	0.3	0.5^{0}	0.03^{4}
Id	87.2	13.2	$17.^{9}$	0.2^{0}
Ie	27.8	2.1	2.7^{5}	0.09^{9}
IIa	10.2	1.5	2.2^{5}	0.22^{0}
IIb	10.7	0.4	0.5^{7}	0.05^{4}
IIc	12.8	1.9	2.4^{6}	0.19^{2}
IId	24.0	0.0	0.0	0.0^{0}
IIe	27.8	1.8	2.4^{8}	0.08^{9}
IIIa	26.7	4.8	6.7^{2}	0.25^{2}
IIIb	1.5	0.3	0.5^{0}	0.33^{3}
IIIc	34.7	3.4	4.5^{3}	0.13^{0}
IIId	192.5	1.0	1.3^{2}	0.00^{6}
IIIe	84.6	3.1	4.3^{5}	0.05^{1}
IVa	13.3	2.5	3.5^{4}	0.26^{6}
IVb	19.5	1.0	1.3^{2}	0.06^{7}
IVc	5.7	0.2	0.3^{6}	0.06^{3}
IVd	57.5	2.0	2.8^{2}	0.04^{9}
IVe	41.5	3.0	3.9^{0}	0.09^{4}

TABLE D.II (con't)

Animal & Brain Region	ΔF div. ±0.5	Dev. div. ±	Std.Dev. (s) div.	Rel. Std. Dev.
Va	2.0	0.5	0.7^{0}	0.35^{3}
Vb	10.2	0.8	1.0^{6}	0.10^{4}
Vc	4.3	1.1	1.4^{4}	0.33^{5}
Vd	74.0	14.0	$19.^{7}$	0.2^{6}
Ve	30.0	2.0	$2.^{7}$	0.0^{9}
VIa	14.7	1.5	2.0^{8}	0.14^{1}
VIb	7.8	0.5	0.7^{6}	0.09^{7}
VIc	9.5	0.0	0.0	0.0^{0}
VId	74.2	6.1	8.1^{2}	0.10^{9}
VIe	42.5	8.0	$10.^{5}$	0.2^{4}
VIIa	4.5	1.3	1.8^{0}	0.40^{0}
VIIb	6.3	0.6	0.7^{6}	0.12^{1}
VIIc	1.5	0.3	0.5^{0}	0.33^{3}
VIId	60.2	0.3	0.3^{6}	0.00^{6}
VIIe	31.3	6.2	8.8^{0}	0.28^{1}
VIIIa	3.7	0.2	0.3^{6}	0.09^{7}
VIIIb	2.7	0.9	1.6^{0}	0.59^{5}
VIIIc	1.0	0.5	0.7^{1}	0.70^{7}
VIIId	48.2	0.2	0.2^{9}	0.00^{6}
VIIIe	35.5	7.3	9.8^{4}	0.27^{7}
IXa	14.2	2.1	3.0^{1}	0.21^{2}
IXb	4.2	1.2	1.7^{5}	0.41^{8}
IXc	9.0	0.6	0.8^{6}	0.09^{6}

TABLE D.II (con't)

Animal & Brain Region	ΔF div. ±0.5	Dev. div.	Std .Dev. (s) div.	Rel. Std. Dev.
IXd	106.8	0.9	1.1^{5}	0.10^{1}
IXe	72.5	2.5	3.5^{3}	0.04^{8}
Xa	5.5	1.3	1.8^{0}	0.32^{7}
Xb	1.2	0.2	0.2^{9}	0.24^{2}
Xc	13.0	2.0	$2.^{5}$	0.1^{9}
Xd	26.3	2.5	3.5^{4}	0.13^{4}
Xe	1.0	0.0	0.0	0.0^{0}
XIa	2.8	0.7	1.0^{4}	0.37^{1}
XIb	2.0	0.5	0.7^{0}	0.35^{3}
XIc	9.0	0.3	0.5^{0}	0.05^{5}
XId	11.8	1.7	2.2^{5}	0.19^{1}
XIe	8.7	1.4	2.4^{6}	0.28^{3}
XIIa	4.5	0.6	0.8^{6}	0.19^{2}
XIIb	29.7	0.7	1.0^{4}	0.03^{5}
XIIc	29.3	1.7	2.3^{0}	0.07^{8}
XIId	54.3	1.4	1.8^{9}	0.03^{4}
XIIe	28.2	1.7	2.5^{1}	0.08^{9}
XIIIa	8.7	1.6	2.1^{7}	0.39^{6}
XIIIb	2.7	0.7	1.6^{0}	0.59^{5}
XIIIc	1.2	0.3	0.3^{6}	0.30^{0}
XIIId	1.7	0.2	0.2^{9}	0.17^{1}
XIIIe	8.2	0.8	1.0^{6}	0.12^{9}

TABLE D.II (con't)

Animal & Brain Region	ΔF div. ±0.5	Dev. div. ±	Std.Dev. (s) div.	Rel. Std. Dev.
XIVa	5.0	0.5	0.7^{0}	0.14^{1}
XIVb	21.7	1.2	1.6^{0}	0.07^{4}
XIVc	8.7	1.9	2.4^{6}	0.28^{3}
XIVd	51.5	0.7	1.0^{0}	0.01^{9}
XIVe	31.2	1.8	2.4^{8}	0.07^{9}

TABLE D.III

Effect of Ethanol on the Activity of L-Glutamic Acid Decarboxylase in Different Regions of Rat Brain

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles GABA/hr/ g wet brain $x10^{-5}$	Dev. ±	Std.Dev. (s)	Rel. Std.Dev.
Ia	34 ± 5	7.3	1.0	1.4_{1}	0.19_{8}
Ib	19 ± 4	5.2	0.4	0.5_{0}	0.09_{8}
Ic	29 ± 5	6.9	0.1	0.2_{4}	0.02_{0}
Id	38 ± 5	28.1	4.2	5.6_{6}	0.20_{1}
Ie	20 ± 4	18.0	0.9	1.6_{5}	0.09_{8}
IIa	48 ± 6	3.1	0.4	0.5_{2}	0.17_{6}
IIb	53 ± 7	2.9	0.1	0.1_{0}	0.04_{6}
IIc	43 ± 6	4.2	0.5	0.7_{0}	0.16_{6}
IId	29 ± 5	10.8	0.0	0.0_{4}	0.0_{5}
IIe	48 ± 6	7.5	0.5	0.6_{2}	0.08_{7}
IIIa	30 ± 5	11.5	1.9	2.6_{0}	0.22_{8}
IIIb	12 ± 4	3.9	0.3	0.5_{5}	0.12_{6}
IIIc	36 ± 5	12.3	1.2	1.5_{2}	0.12_{0}
IIId	55 ± 6	42.2	0.2	2.1	0.05_{0}
IIIe	56 ± 6	18.5	0.7	1.3	0.07_{0}
IVa	34 ± 5	5.5	0.9	1.2_{2}	0.22_{0}
IVb	30 ± 4	8.7	0.4	0.5_{2}	0.06_{0}
IVc	18 ± 4	5.4	0.2	0.2_{2}	0.04_{1}
IVd	30 ± 5	23.8	0.8	1.1_{3}	0.04_{7}
IVe	26 ± 4	20.1	1.3	1.7_{6}	0.08_{7}

TABLE D.III (con't)

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles GABA/hr/ g wet brain x10^{-5}	Dev. ±	Std.Dev. (s)	Rel. Std .Dev.
Va	24 ± 4	2.2	0.3	0.3^{6}	0.16^{3}
Vb	21 ± 4	7.1	0.5	0.6^{4}	0.09^{0}
Vc	17 ± 3	4.7	0.8	1.0^{3}	0.22^{1}
Vd	23 ± 4	39.5	7.3	10.2^{5}	0.25^{9}
Ve	14 ± 3	27.5	1.7	2.3^{8}	0.08^{6}
VIa	31 ± 5	6.5	0.6	0.7^{7}	0.11^{9}
VIb	18 ± 4	6.7	0.4	0.5^{2}	0.07^{7}
VIc	19 ± 4	7.4	0.0	0.0	$0.^{0}$
VId	22 ± 4	41.4	3.3	4.4^{1}	0.10^{6}
VIe	27 ± 4	19.8	3.5	4.6^{7}	0.23^{5}
VIIa	27 ± 4	3.0	0.6	0.7_{1}	0.2^{6}
VIIb	23 ± 4	4.5	0.3	0.4^{0}	0.09^{0}
VIIc	19 ± 4	2.4	0.2	0.3^{0}	0.12^{5}
VIId	23 ± 4	32.4	0.2	0.2^{2}	0.06^{0}
VIIe	15 ± 4	26.7	4.9	6.9^{5}	0.26^{0}
VIIIa	18 ± 4	4.0	0.2	0.2^{2}	0.05^{5}
VIIIb	19 ± 4	3.2	0.8	1.0^{0}	0.31^{3}
VIIIc	18 ± 4	2.2	0.3	0.5^{0}	0.22^{7}
VIIId	21 ± 4	28.7	0.1	0.1^{2}	0.00^{4}
VIIIe	17 ± 4	26.5	5.1	6.8^{9}	0.26^{0}
IXa	17 ± 4	11.6	1.4	2.1^{1}	0.18^{1}
IXb	17 ± 4	4.6	0.9	1.2^{5}	0.27^{2}

TABLE D.III (con't)

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles GABA/hr/ g wet brain x10^{-5}	Dev. ±	Std.Dev. (s)	Rel. Std.Dev.
IXc	18 ± 4	7.5	0.4	0.5^{7}	0.07^{7}
IXd	27 ± 5	48.1	0.4	0.4^{6}	0.00^{9}
IXe	23 ± 4	38.8	1.3	1.8^{3}	0.04^{7}
Xa	28 ± 4	3.4	0.5	0.7^{7}	0.22^{8}
Xb	5 ± 7	8.5	0.5	0.6^{9}	0.08^{1}
Xc	28 ± 4	6.5	0.9	1.1^{0}	0.16^{9}
Xd	24 ± 4	14.2	1.3	1.7^{7}	0.12^{5}
Xe	24 ± 4	1.7	0.0	0.0	0.0^{0}
XIa	17 ± 4	3.6	0.5	0.7^{2}	0.20^{0}
XIb	25 ± 4	2.0	0.3	0.3^{6}	0.18^{5}
XIc	27 ± 4	4.9	0.1	0.2^{2}	0.04^{5}
XId	22 ± 4	7.6	0.8	1.2^{0}	0.15^{8}
XIe	21 ± 4	6.2	0.8	1.0^{7}	0.17^{4}
XIIa	22 ± 4	3.7	0.3	0.4^{6}	0.12^{6}
XIIb	30 ± 4	12.7	0.3	0.4^{8}	0.03^{7}
XIIc	29 ± 4	13.0	0.8	0.9^{8}	0.07^{5}
XIId	24 ± 4	28.1	0.7	0.9^{6}	0.03^{4}
XIIe	21 ± 4	17.3	1.1	1.4^{6}	0.08^{4}
XIIIa	26 ± 4	3.5	0.8	0.9^{8}	0.28^{2}
XIIIb	20 ± 4	3.0	0.5	0.6^{2}	0.20^{8}
XIIIc	26 ± 4	1.6	0.1	0.1^{4}	0.08^{8}
XIIId	23 ± 4	2.1	0.1	0.1^{2}	0.05^{7}
XIIIe	19 ± 4	6.6	0.5	0.7^{1}	0.10^{7}

TABLE D.III (con't)

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles GABA/hr/ g wet brain $x10^{-5}$	Dev. ±	Std. Dev. (s)	Rel. Std. Dev.
XIVa	16 ± 4	5.4	0.4	0.5^{0}_{8}	0.09^{2}_{9}
XIVb	29 ± 4	9.8	0.5	0.6^{8}_{9}	0.06^{1}_{9}
XIVc	19 ± 4	6.9	1.2	1.5^{9}_{0}	0.23^{9}_{2}
XIVd	25 ± 4	25.6	0.3	0.5^{0}_{9}	0.01^{2}_{2}
XIVe	17 ± 4	23.5	1.2	1.6^{9}_{2}	0.07^{2}_{2}

XI. APPENDIX E

Experimental Data for

Acetylcholinesterase Assay

TABLE E.I

Spectrophotometric Measurement of Acetylcholinesterase Activity in Different Regions of Experimental and Control Rat Brain+

Date	Animal & Sample	Experimental Condition	ΔR %T ± 0.4%	Dev. %T
7-6-72	Ial	Ethanol induced one day off	8.0	0.2
	2		7.5	-0.3
	Av.		7.8	±0.3
	Ibl		3.5	0.0
	2		3.5	0.0
	Av.		3.5	±0.0
	Icl		3.0	-1.8
	2		6.5	1.7
	Av.		4.8	±1.8
	Idl		13.0	2.0
	2		9.0	-2.0
	Av.		11.0	±2.0
	Iel		2.5	1.0
	2		0.5	-1.0
	Av.		1.5	±1.0
7-6-72	IIal	Ethanol induced	46.5	0.7
	2		45.0	-0.8
	Av.		45.8	±0.8
	IIbl		44.0	0.0
	2		44.0	0.0
	Av.		44.0	±0.0
	IIcl		25.0	2.5
	2		20.0	-2.5
	Av.		22.5	±2.5
	IIdl		17.0	-26.5
	2		70.0	26.5
	Av.		43.5	±26.5

+ (I-XIV) denote animal no., (a-e) denote brain region no. and (1-2) denote enzyme assay no.

TABLE E.I (con't)

Date	Animal & Sample	Experimental Condition	ΔR %T ± 0.4%	Dev. %T
	IIe1		36.5	2.7
	2		31.0	-2.8
	Av.		33.8	±2.8
7-6-72	IIIa1	Control	8.5	0.0
	2		8.5	0.0
	Av.		8.5	±0.0
	IIIb1		13.5	6.2
	2		1.0	-6.3
	Av.		7.3	±6.3
	IIIc1		10.5	0.0
	2		10.5	0.0
	Av.		10.5	±0.0
	IIId1		19.0	2.0
	2		15.0	-2.0
	Av.		17.0	±2.0
	IIIe1		27.0	3.5
	2		20.0	-3.5
	Av.		23.5	±3.5
12-6-72	IVa1	Ethanol induced one day off	20.5	1.5
	2		17.5	-1.5
	Av.		19.0	±1.5
	IVb1		11.0	0.0
	2		11.0	0.0
	Av.		11.0	±0.0
	IVc1		3.5	-1.0
	2		3.5	1.0
	Av.		3.5	±1.0
	IVd1		16.0	4.2
	2		7.5	-4.3
	Av.		11.8	±4.3
	IVe1		6.5	0.0
	2		6.5	0.0
	Av.		6.5	±0.0

TABLE E.I (con't)

Date	Animal & Sample	Experimental Condition	ΔR %T ± 0.4%	Dev. %T
12-6-72	Val	Ethanol	21.0	7.2
	2	induced	6.5	-7.3
	Av.	one day off	13.8	±7.3
	Vbl		9.0	0.5
	2		8.0	-0.5
	Av.		8.5	±0.5
	Vcl		5.5	0.0
	2		5.5	0.0
	Av.		5.5	±0.0
	Vdl		9.5	0.0
	2		9.5	0.0
	Av.		9.5	±0.0
	Vel		4.5	1.0
	2		2.5	-1.0
	Av.		3.5	±1.0
12-6-72	VIal	Control	10.5	1.4
	2		7.8	-1.3
	Av.		9.1	±1.4
	VIbl		0.5	-0.3
	2		1.0	0.2
	Av.		0.8	±0.3
	VIcl		4.5	-0.3
	2		5.0	0.2
	Av.		4.8	±0.3
	VIdl		4.5	0.0
	2		4.5	0.0
	Av.		4.5	±0.0
	VIel		17.5	2.7
	2		12.0	-2.8
	Av.		14.8	±2.8
14-6-72	VIIal	Ethanol	12.0	2.0
	2	induced	8.0	-2.0
	Av.	one day off	10.0	±2.0

TABLE E.I (con't)

Date	Animal & Sample	Experimental Condition	ΔR %T ± 0.4%	Dev. %T
	VIIb1		8.5	0.0
	2		8.5	0.0
	Av.		8.5	±0.0
	VIIc1		7.0	0.5
	2		6.0	-0.5
	Av.		6.5	±0.5
	VIId1		7.0	0.0
	2		7.0	0.0
	Av.		7.0	±0.0
	VIIe1		5.0	0.0
	2		5.0	0.0
	Av.		5.0	±0.0
14-6-72	VIIIa1	Ethanol induced one day off	6.0	-1.3
	2		8.5	1.2
	Av.		7.3	±1.3
	VIIIb1		8.0	-1.3
	2		10.5	1.2
	Av.		9.3	±1.3
	VIIIc1		10.0	0.2
	2		9.5	-0.3
	Av.		9.8	±0.3
	VIIId1		10.5	0.5
	2		9.5	-0.5
	Av.		10.0	±0.5
	VIIIe1		3.5	0.0
	2		3.5	0.0
	Av.		3.5	±0.0
14-6-72	IXa1	Control	3.5	0.0
	2		3.5	0.0
	Av.		3.5	±0.0
	IXb1		4.5	-0.8
	2		6.0	0.7
	Av.		5.3	±0.8

TABLE E.I (con't)

Date	Animal & Sample	Experimental Condition	ΔR %T ± 0.4%	Dev. %T
	IXc1		7.0	0.2
	2		6.5	-0.3
	Av.		6.8	±0.3
	IXd1		7.0	0.0
	2		7.0	0.0
	Av.		7.0	±0.0
	IXe1		10.0	1.2
	2		7.5	-1.3
	Av.		8.8	±1.3
20-6-72	Xa1	Ethanol	13.5	-0.3
	2	induced	14.0	0.2
	Av.		13.8	±0.3
	Xb1		6.5	2.7
	2		1.0	-2.8
	Av.		3.8	±2.8
	Xc1		7.5	-0.5
	2		8.5	0.5
	Av.		8.0	±0.5
	Xd1		7.0	0.0
	2		7.0	0.0
	Av.		7.0	±0.0
	Xe1		7.0	-0.5
	2		8.0	0.5
	Av.		7.5	±0.5
20-6-72	XIa1	Ethanol	2.5	0.7
	2	induced	1.0	-0.8
	Av.		1.8	±0.8
	XIb1		7.0	0.5
	2		6.0	-0.5
	Av.		6.5	±0.5
	XIc1		9.0	0.0
	2		9.0	0.0
	Av.		9.0	±0.0

TABLE E.I (con't)

Date	Animal & Sample	Experimental Condition	ΔR %T ± 0.4%	Dev. %T
	XIdl		11.0	1.2
	2		8.5	-1.3
	Av.		9.8	±1.3
	XIel		8.0	0.0
	2		8.0	0.0
	Av.		8.0	±0.0
20-6-72	XIIal	Control	6.0	-1.3
	2		8.5	1.2
	Av.		7.3	±1.3
	XIIbl		14.0	0.7
	2		12.5	-0.8
	Av.		13.3	±0.8
	XIIcl		11.0	-0.5
	2		12.0	0.5
	Av.		11.5	±0.5
	XIIdl		10.0	1.5
	2		7.0	-1.5
	Av.		8.5	±1.5
	XIIel		2.0	-1.5
	2		5.0	1.5
	Av.		3.5	±1.5
22-6-72	XIIIal	Ethanol induced	8.5	0.0
	2		8.5	0.0
	Av.		8.5	±0.0
	XIIIbl		7.5	0.2
	2		7.0	-0.3
	Av.		7.3	±0.3
	XIIIcl		6.5	-0.5
	2		7.5	0.5
	Av.		7.0	±0.5
	XIIIdl		6.0	0.2
	2		5.5	-0.3
	Av.		5.8	±0.3

TABLE E.I (con't)

Date	Animal & Sample	Experimental Condition	ΔR %T ± 0.4%	Dev. %T
	XIIIe1		7.5	-0.5
	2		8.5	0.5
	Av.		7.0	±0.5
22-6-72	XIVa1	Control	0.5	0.0
	2		0.5	0.0
	Av.		0.5	±0.0
	XIVb1		29.6	7.3
	2		15.0	-7.3
	Av.		22.3	±7.3
	XIVc1		4.5	0.1
	2		4.3	-0.1
	Av.		4.4	±0.1
	XIVd1		8.0	-6.0
	2		20.0	6.0
	Av.		14.0	±6.0
	XIVe1		5.0	0.7
	2		3.5	-0.8
	Av.		4.3	±0.8

TABLE E.II

Average Fluorimetric Measurements of Acetylcholinesterase Activity in Different Regions of Experimental and Control Rat Brain

Animal & Brain Region	t min	ΔR %T ±0.4%	Dev. %T ±	Std.Dev. (s) %T	Rel. Std. Dev.
Ia	3.2	7.8	0.3	0.3^{6}	0.04^{6}
Ib	7.2	3.5	0.0	0.0	0.0^{0}
Ic	4.7	4.8	1.8	2.4^{8}	0.51^{5}
Id	2.4	11.0	2.0	$2.^{8}$	0.2^{5}
Ie	13.5	1.5	1.0	$1.^{4}$	0.9^{4}
IIa	0.7	45.8	0.8	1.0^{6}	0.02^{3}
IIb	0.8	44.0	0.0	0.0	0.0^{0}
IIc	1.5	22.5	2.5	3.5^{4}	0.15^{7}
IId	1.2	43.5	26.5	$37.^{4}$	0.8^{6}
IIe	1.0	33.8	2.8	3.8^{9}	0.11^{5}
IIIa	3.0	8.5	0.0	0.0	0.0^{0}
IIIb	1.8	7.3	6.3	$8.^{8}$	1.2^{1}
IIIc	2.4	10.5	0.0	0.0	0.0^{0}
IIId	1.5	17.0	2.0	$2.^{8}$	0.1^{6}
IIIe	1.1	23.5	3.5	4.9^{5}	0.21^{0}
VIa	1.3	19.0	1.5	$2.^{1}$	0.1^{1}
VIb	2.3	11.0	0.0	0.0	0.0^{0}
VIc	5.9	4.5	1.0	$1.^{4}$	0.3^{1}
VId	2.5	11.8	4.3	6.0^{1}	0.50^{9}
VIe	3.9	6.5	0.0	0.0	0.0^{0}

TABLE E.II (con't)

Animal & Brain Region	t min	ΔR %T ± 0.4%	Dev. %T ±	Std. Dev. (s) %T	Rel. Std. Dev.
Va	2.6	13.8	7.3	10.2^{5}	0.74^{2}
Vb	3.0	8.5	0.5	0.7^{1}	0.08^{3}
Vc	4.6	5.5	0.0	0.0	0.0^{0}
Vd	2.6	9.5	0.0	0.0	0.0^{0}
Ve	7.8	3.5	1.0	1.4^{1}	0.40^{4}
VIa	2.8	9.1	1.4	1.9^{1}	0.20^{9}
VIb	37.5	0.8	0.3	$0.^{3}$	0.4^{5}
VIc	5.3	4.8	0.3	0.3^{6}	0.07^{5}
VId	5.6	4.5	0.0	0.0	0.0^{0}
VIe	1.8	14.8	2.8	3.8^{9}	0.26^{2}
VIIa	2.6	10.0	2.0	$2.^{8}$	0.2^{8}
VIIb	3.0	8.5	0.0	0.0	0.0^{0}
VIIc	3.9	6.5	0.5	0.7^{1}	0.10^{8}
VIId	3.6	7.0	0.0	0.0	0.0^{0}
VIIe	5.0	5.0	0.0	0.0	0.0^{0}
VIIIa	3.6	7.3	1.3	1.7^{7}	0.24^{2}
VIIIb	1.9	9.3	1.3	1.3^{9}	0.14^{9}
VIIIc	2.2	9.8	0.3	0.3^{6}	0.03^{6}
VIIId	2.5	10.0	0.5	0.7^{6}	0.07^{7}
VIIIe	4.8	3.5	0.0	0.0	0.0^{0}
IXa	7.2	3.5	0.0	0.0	0.0^{0}
IXb	4.9	5.3	0.8	1.0^{6}	0.20^{0}
IXc	3.8	6.8	0.3	0.3^{6}	0.05^{2}

TABLE E.II (con't)

Animal & Brain Region	t min	ΔR %T ± 0.4%	Dev. %T ±	Std. Dev. (s) %T	Rel. Std. Dev.
IXd	2.4	7.0	0.0	0.0	0.0^{0}
IXe	2.9	8.8	1.3	1.7^{7}	0.20^{1}
Xa	1.5	13.8	0.3	0.3^{6}	0.02^{6}
Xb	14.5	3.8	2.8	$3.^{8}$	1.0^{2}
Xc	2.1	8.0	0.5	0.7^{1}	0.08^{8}
Xd	3.6	7.0	0.0	0.0	0.0^{0}
Xe	3.4	7.5	0.5	0.7^{1}	0.09^{4}
XIa	17.5	1.8	0.8	$1.^{0}$	0.5^{9}
XIb	3.9	6.5	0.5	0.7^{1}	0.10^{8}
XIc	2.3	9.0	0.0	0.0	0.0^{0}
XId	2.7	9.8	1.3	1.7^{7}	0.18^{0}
XIe	3.1	8.0	0.0	0.0	0.0^{0}
XIIa	3.6	7.3	1.3	1.7^{7}	0.24^{2}
XIIb	1.9	13.3	0.8	1.0^{6}	0.07^{9}
XIIc	2.2	11.5	0.5	0.7^{1}	0.06^{1}
XIId	2.5	8.5	1.5	2.1^{2}	0.24^{9}
XIIe	10.9	3.5	1.5	2.1^{2}	0.60^{6}
XIIIa	3.0	8.5	0.0	0.0	0.0^{0}
XIIIb	3.5	7.3	0.3	0.3^{6}	0.04^{9}
XIIIc	3.6	7.0	0.5	0.7^{1}	0.10^{1}
XIIId	4.4	5.8	0.3	0.3^{6}	0.06^{2}
XIIIe	3.2	8.0	0.5	0.7^{1}	0.08^{8}

TABLE E.II (con't)

Animal & Brain Region	t min	ΔR %T ± 0.4%	Dev. %T ±	Std .Dev. (s) %T	Rel. Std. Dev.
XIVa	4.9	0.5	0.0	0.0	0.0^{0}
XIVb	1.2	22.3	7.3	10.3^{2}	0.46^{2}
XIVc	5.7	4.4	0.1	0.1^{4}	0.03^{2}
XIVd	1.7	14.0	6.0	$8.^{4}$	0.60^{6}
XIVe	5.3	4.3	0.8	1.0^{6}	0.24^{7}

TABLE E.III

Effect of Ethanol on the Activity of Acetylcholinesterase in Different Regions of Rat Brain

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles Acetic Acid/hr/g wet brain x 10^{-5}	Dev. ±	Std. Dev. (s)	Rel. Std. Dev.
Ia	3.4 ± 0.5	6.1	0.2	0.2^{8}	0.04^{6}
Ib	1.9 ± 0.4	4.9	0.0	0.0_{7}	0.0^{0}
Ic	2.9 ± 0.5	5.1	1.0	1.2^{5}	0.24^{9}
Id	3.8 ± 0.5	7.8	1.5	2.0^{1}	0.26^{3}
Ie	2.0 ± 0.4	1.9	1.5	$2.^{1}$	1.1^{1}
IIa	4.8 ± 0.6	19.2	0.3	0.4^{2}	0.02^{2}
IIb	5.3 ± 0.7	16.7	0.0	0.0_{3}	0.0^{0}
IIc	4.3 ± 0.6	10.6	1.2	1.6^{3}	0.15^{3}
IId	2.9 ± 0.5	18.6	7.0	$9.^{5}$	0.5^{1}
IIe	4.8 ± 0.6	14.2	1.2	1.6^{3}	0.11^{4}
IIIa	3.0 ± 0.5	7.6	0.0	0.0	0.0^{0}
IIIb	1.2 ± 0.4	16.8*			
IIIc	3.6 ± 0.5	7.8	0.0	0.0_{1}	0.0^{0}
IIId	5.5 ± 0.6	8.3	1.0	1.4^{1}	0.17^{0}
IIIe	5.6 ± 0.6	11.3	1.7	2.3^{4}	0.20^{6}
IVa	3.4 ± 0.5	15.0	1.2	1.6^{9}	0.11^{3}
IVb	3.0 ± 0.4	9.8	0.0	0.0_{2}	0.0^{0}
IVc	1.8 ± 0.4	6.7	1.5	2.1^{2}	0.31^{6}
IVd	3.0 ± 0.5	12.5	5.8	$8.^{2}$	0.6^{5}
IVe	2.6 ± 0.4	6.7	0.0	0.0	0.0^{0}

* These values were rejected by 4S method.

TABLE E.III (con't)

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles Acetic Acid/hr/g wet brain x 10^{-5}	Dev. ±	Std.Dev. (s)	Rel. Std.Dev.
Va	2.4 ± 0.4	15.4	8.1	$1.^{1}_{2}$	0.7^{4}
Vb	2.1 ± 0.4	10.9	0.7	0.9^{2}	0.08^{4}
Vc	1.7 ± 0.3	8.7	0.0	0.0	0.0^{0}
Vd	2.3 ± 0.4	11.1	0.0	0.0_{4}	0.0^{0}
Ve	1.4 ± 0.3	5.8	1.0	1.3^{4}	0.23^{1}
VIa	3.1 ± 0.5	7.9	1.2	1.6^{9}	0.21^{4}
VIb	1.8 ± 0.4	1.1	0.4	$0.^{4}$	0.3^{6}
VIc	1.9 ± 0.4	6.7	0.4	0.5^{0}	0.07^{4}
VId	2.2 ± 0.4	5.5	0.0	$0.0_{}$	0.0^{0}
VIe	2.7 ± 0.4	14.7	2.8	3.8^{9}	0.26^{4}
VIIa	2.7 ± 0.4	9.9	2.0	2.8^{2}	0.28^{5}
VIIb	2.3 ± 0.4	9.9	0.0	0.0_{9}	0.0^{0}
VIIc	1.9 ± 0.4	9.2	0.7	$0.9^{}$	0.10^{7}
VIId	2.3 ± 0.4	8.2	0.0	0.0	0.0^{0}
VIIe	1.5 ± 0.4	8.9	0.0	0.0	0.0^{0}
VIIIa	1.8 ± 0.4	10.8	1.9	2.6^{8}	0.24^{8}
VIIIb	1.9 ± 0.4	15.9	1.1	1.5^{5}	0.09^{7}
VIIIc	1.8 ± 0.4	14.6	0.4	0.5^{0}	0.03^{4}
VIIId	2.1 ± 0.4	19.2	1.0	1.3^{4}	0.07^{0}
VIIIe	1.7 ± 0.4	8.3	0.0	0.0	0.0^{0}
IXa	1.7 ± 0.4	5.5	0.0	0.0_{9}	0.0^{0}
IXb	1.7 ± 0.4	8.3	1.2	$1.6^{}$	0.20^{4}
IXc	1.8 ± 0.4	10.1	0.4	0.5^{0}	0.04^{9}
IXd	2.7 ± 0.5	10.4	0.0	0.0_{2}	0.0^{0}
IXe	2.3 ± 0.4	10.2	1.5	$2.1^{}$	0.20^{7}

TABLE E.III (con't)

Animal & Brain Region	mg wet brain per sample ± m.p.e.	Moles Acetic Acid/hr/g wet brain x 10^{-5}	Dev. ±	Std.Dev. (s)	Rel. Std.Dev.
Xa	2.8 ± 0.4	16.4	3.0	4.2^{4}	0.25^{8}
Xb	0.5 ± 0.7	20.1*			
Xc	2.8 ± 0.4	11.5	0.7	0.7^{0}	0.06^{0}
Xd	2.4 ± 0.4	7.8	0.0	0.0^{0}	0.0^{0}
Xe	2.4 ± 0.4	8.4	0.5	0.7^{8}	0.11^{1}
XIa	1.7 ± 0.4	2.8	1.2	1.6^{8}	0.5^{1}
XIb	2.5 ± 0.4	7.0	0.6	0.7^{8}	0.11^{4}
XIc	2.7 ± 0.4	11.2	2.3	3.1^{2}	0.28^{8}
XId	2.2 ± 0.4	11.9	1.5	2.1^{2}	0.17^{0}
XIe	2.1 ± 0.4	10.2	0.0	0.0	0.0
XIIa	2.2 ± 0.4	10.1	0.4	0.5^{0}	0.04^{9}
XIIb	3.0 ± 0.4	11.9	0.7	0.9^{2}	0.07^{7}
XIIc	2.9 ± 0.4	10.7	0.5	0.6^{4}	0.05^{9}
XIId	2.4 ± 0.4	11.5	0.3	0.3^{6}	0.03^{1}
XIIe	2.1 ± 0.4	4.2	2.3	$3.^{1}$	0.7^{5}
XIIIa	2.6 ± 0.4	8.8	0.0	0.0	0.0^{0}
XIIIb	2.0 ± 0.4	7.8	0.4	0.5^{0}	0.06^{4}
XIIIc	2.6 ± 0.4	8.3	0.6	0.7^{8}	0.09^{4}
XIIId	2.3 ± 0.4	8.2	0.6	0.7^{8}	0.09^{5}
XIIIe	1.9 ± 0.4	10.3	0.4	0.5^{0}	0.04^{8}
XIVa	1.6 ± 0.4	0.8	0.0	0.0	0.0^{0}
XIVb	2.9 ± 0.4	24.1	3.3	4.5^{9}	0.19^{0}
XIVc	1.9 ± 0.4	6.3	0.2	0.2^{2}	0.03^{5}
XIVd	2.5 ± 0.4	17.1	4.3	6.0^{1}	0.35^{1}
XIVe	1.7 ± 0.4	8.7	3.2	4.4^{5}	0.51^{2}

TABLE E.IV

Spectrophotometric Measurement of Acetylcholinesterase from Electric Eel

AChE Volume µl	Run No.	ΔR %T ±0.4%	Dev. %T	Std. Dev. (s) %T	Rel. Std. Dev.
0.50 ± 0.02	1	1.5	0.0		
	2	1.5	0.0		
	Av.	1.5	±0.0	0.0	0.0^0
1.00 ± 0.02	1	2.5	-0.7		
	2	4.0	0.8		
	Av.	3.2	±0.8	1.0^6	0.33^2
2.00 ± 0.02	1	7.0	0.5		
	2	6.0	-0.5		
	Av.	6.5	±0.5	0.7^1	0.10^8
3.00 ± 0.02	1	9.0	0.0		
	2	9.0	0.0		
	Av.	9.0	±0.0	0.0	0.0^0
4.00 ± 0.02	1	11.5	-0.3		
	2	12.0	0.2		
	Av.	11.8	±0.3	0.3^6	0.03^0
5.00 ± 0.02	1	14.0	-0.3		
	2	14.5	0.2		
	Av.	14.3	±0.3	0.3^6	0.02^5
10.00 ± 0.02	1	28.5	-2.3		
	2	33.0	2.2		
	Av.	30.8	±2.8	3.1^8	0.10^3

TABLE E.V

Production of Acetic Acid from Acetylcholine Bromide by the Action of Acetylcholinesterase Extracted from Electric Eel

AChE Volume µl	Nanomoles Enzyme Units ± m.p.e.	Moles Acetic Acid/hr x10^{9}	Dev. ±	Std.Dev. (s)	Rel. Std. Dev.	% Error
0.50 ± 0.02	0.8 ± 0.2	0.8	0.0	0.0	0.0^{0}	0
1.00 ± 0.02	1.6 ± 0.3	1.8	0.5	0.6^{4}	0.35^{0}	12
2.00 ± 0.02	3.2 ± 0.6	3.5	0.3	0.4^{2}	0.12^{0}	9
3.00 ± 0.02	4.8 ± 0.9	4.9	0.0	0.0	0.0^{0}	2
4.00 ± 0.02	6.4 ± 1.2	6.2	0.0	0.0	0.0^{0}	3
5.00 ± 0.02	8.0 ± 1.6	7.3	0.0	0.0	0.0^{0}	8
10.00 ± 0.02	16.0 ± 3.1	17.4	1.3	1.7^{6}	0.10^{0}	9

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