HUNTINGTON'S CHOREA AND SCHIZOPHRENIA: AMINO ACIDS IN THALAMUS

by

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ABSTRACT

Amino acids and other ninhydrin-positive compounds were measured in post-mortem thalamus from 25 Huntington's choreics, 10 schizophrenics, 5 schizophrenic-like psychotics, and 23 controls dying without neurological disease. Gamma-aminobutyric acid (GABA) was significantly reduced in choreic thalami, in accord with deficiencies found in other brain regions of choreics (Perry et al., 1973a,b). GABA was also significantly reduced in schizophrenic thalami, suggesting a biochemical link between these two diseases, and supporting the hypothesis of a defect in the GABA system in schizophrenia (Roberts, 1972). Homocarnosine, a GABA-containing dipeptide, was also low in choreic and 9 out of 10 schizophrenic thalami. One schizophrenic had extremely high homocarnosine. Glycerophosphoethanolamine was significantly elevated in Huntington's choreics, but not in schizophrenics.

A number of other variables were considered for their potential influence on amino acid concentrations in thalamus. The majority of amino acids were found to rise in a significantly linear fashion in the interval 3 to 49 hours post-mortem, although other models might have described the change better. GABA, ornithine, histidine and tyrosine were found to decrease significantly with increasing age between 21 and 80 years, in controls. The effects of pre-mortem hypoxia, regional variation within the thalamus, and neuroleptic drug treatment could not be rigorously tested with these data. Neuroleptics were unlikely to have been the cause of group differences in GABA concentration, since they failed to deplete GABA in brain of chronically treated rats. On the other hand, bronchopneumonia and other causes of pre-mortem hypoxia could not be ruled out as potential contributers to reduced GABA in thalamus.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACh..... acetyl choline

AChE.....acetylcholinesterase

ALA..... alanine

ANOVA..... Analysis of Variance

ARG..... arginine

ASN..... asparagine

ASP..... aspartic acid

BP.....bronchopneumonia

C.....controls (in tables)

CAT..... choline acetyl transferase

CPZ.....chlorpromazine

CSF.....cerebrospinal fluid

$(CYS)_2$.....cystine

CYSTA.....cystathionine

DA.....dopamine

DDC.....dopamine decarboxylase

df.....degrees of freedom

DOPAC.....dihydroxyphenylacetic acid

DZ.....dizygotic

EA.....ethanolamine

EPS.....extra-pyramidal system

ESR.....electron spin resonance

GABA.....gamma-amino butyric acid

GABA'.....expected value of GABA (from regression analysis)

GABA-LYS....gamma-amino butyryl lysine

GAD.....glutamic acid decarboxylase

GLU.....glutamic acid

GLYC-PEA....glycerophosphoethanolamine

GLY.....glycine

GP.....globus pallidus

GSH.....reduced glutathionine (in tables for this study, this abbreviation refers to total GSH (GSH + 2GS-SG))

GS-SG.....oxidized glutathione

HC.....Huntington's chorea

HCARN.....homocarnosine

HIS.....histidine

HP.....haloperidol

HVA.....homovanillic acid

ILE.... isoleucine

LEU.....leucine

ln.....natural logarithm

LYS.... lysine

MAO.... monoamine oxidase

MET.....methionine

MI.....myocardial infarction

MIF.... migration inhibition factor

MS.....multiple sclerosis

MZ.... monozygotic

NE.... norepinephrine

ORN... ornithine

PEA.... phosphoethanolamine

PHE.... phenylalanine

PMD... post-mortem delay

PRO.... proline

r^2..... coefficient of determination (= proportion of total variance accounted by regression with a given variable)

S..... schizophrenics (in tables)

SEM... standard error of the mean

SER.... serine

SL..... schizophrenic-like psychotics

SS..... sums of squares (in tables)

TAU... taurine

THR... threonine

TRP... tryptophan

TYR... tyrosine

VAL... valine

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CHAPTER 1

GENERAL INTRODUCTION

I HUNTINGTON'S CHOREA

A. Historical Background (Discussed by Myrianthopoulos, 1966; Heathfield, 1973; Vessie, 1939; Maltsgerger, 1961; Critchley, 1973; De Jong, 1973)

In 1872, George Huntington, a young New York physician, addressed the medical academy in Middleport, Ohio. Included in his talk was a description of hereditary chorea with dementia, thought at the time to be the first such report. He recognized 3 marked peculiarities of the disease: its hereditary nature, a tendency to insanity and suicide, and its manifestation as a grave disease only in adult life. Although it is now known that reports of hereditary chorea had appeared in the earlier literature (in particular, one by Waters in the 1840's), Huntington was the first to describe dementia as an essential feature (Maltsberger, 1961). His discussion was so succinct and lucid that the name association has been well justified.

Vessie (1939) traced most affected New England families to 3 British immigrants who arrived in America about 1632. Several of their descendants were notorious witches, burned for their curse. Nova Scotia families have been traced to the Huguenots who fled France after 1685, and Quebec families to a single ancestor who emigrated from France in 1645.

B. Prevalence and Social Impact

There is no evidence of racial, ethnic or geographic selectivity for Huntington's Chorea (HC). Most prevalence estimates range from 4 to 7 per 100,000 (Myrianthopoulos, 1973), although it is rare in Japan (0.4/100,000) and very prevalent in a few isolates such as the Moray Firth in Scotland (560/100,000) (Heathfield, 1973; Myrianthopoulos, 1966). In a Canadian study (Shokeir, 1975), prevalence in Saskatchewan and Manitoba was estimated at 8.4/100,000. At the end of 1977 there were 61 live cases of HC registered

with the British Columbia Health Surveillance Registry, however sources of ascertainment have been limited (Guy Renwick, personal communication) .

In one medical genetics clinic (Bird and Hall, 1978) HC has been the most frequent cause for referral, accounting for 11.4% of all initial visits. Clearly the impact weighing on families of afflicted individuals is tremendous, due to the chronic and insidious nature of the disease. Society too must share the financial burden of unemployment and the chronic hospitalization which is so frequently inevitable (Myrianthopoulos, 1966).

C. Pathology (Discussed by Heathfield, 1973; Myrianthopoulos, 1966; Klintworth, 1973)

The most conspicuous pathological feature is severe atrophy of the corpus striatum with (microscopically) loss of small neurons and accompanying astrocytic proliferation. The caudate is more severely affected than the putamen. Generalized shrinking of the brain is accompanied by dilatation of the lateral ventricles especially in the anterior horns, with flattening of the caudate. There is similar, but less marked involvement of cerebral cortex (especially frontal lobes), globus-pallidus (GP), substantia nigra (SN), thalamus, sub-thalamic nuclei, and dentate.

D. Clinical Features

There is considerable heterogeneity in the clinical expression of HC. Three recognizable forms are the classical, juvenile, and Westphal variants.

1) Classical HC (Discussed by Heathfield, 1973; Brackenridge, 1971; Maltsberger, 1961)

The classical form of the disease may be summarized as a disorder of movement, personality, and cognition. Movement is described as choreo-athetotic with hyperkinesia. Involuntary movements, initially difficult to distinguish from restlessness, become generalized so that they interfere with voluntary movement. Personality changes may begin with irritability

and irrational impulsive behavior which may progress to unexplained violent outbursts. Depression is common, and apathy and neglect characterize later stages. Psychotic episodes are not uncommon; frequently schizophrenia is the initial diagnosis. Cognitive impairment often presents as the first recognized symptom. The inability to remember, organize, and concentrate, and the loss of judgement may be the most debilitating symptoms. Generally there is no impairment of immediate recall; rather, difficulty with tasks requiring delayed recall and retrieval, similar to problems encountered in normal aging (Caine, 1978). Patients eventually become profoundly demented.

2) Juvenile Variant (Discussed by Byers, 1967; Barbeau, 1970; Myrianthopoulos, 1966, 1973; Heathfield, 1973)

About 8% of Huntington's choreics have onset before age 20, and 1-2% before age 10. This group has an almost distinct clinical syndrome, characterized by rigidity (rather than chorea), hypokinesia, parkinsonian tremor, convulsions, intellectual deterioration, cerebellar signs, and a rapidly progressive course. Pathology is typical of HC but there may be cerebellar disease also (Byers, 1967). Diagnosis of juvenile HC is generally based on a clear family history of classical HC, suggesting that this is not a genetically distinct variant. The specific modifying effect of an alternate allele seems unlikely, since Byers (1967) found a pair of half-sibs, both with juvenile HC. A repeated, but as yet unexplained finding has been the disproportionately high number of males among affected parents of juvenile cases.

3) Westphal Variant (Discussed by Myrianthopoulos, 1966; Heathfield, 1973)

The Westphal variant may represent a clinical intermediate between the classical and juvenile forms. It is characterized by prominent rigidity, possibly seizures, and adolescent or early adult onset. The pathology is again typical.

E. Age of Onset and Age at Death (Reviewed in Brackenridge, 1971a; Myrianthopoulos, 1966; Heathfield, 1973)

Estimates in the literature of the mean age of onset, or disease manifestation range from 33.8 years (Brackenridge, 1971a) to 44.0 years (Wendt, 1959). All such estimates must be interpreted in light of potential confounding variables. Onset in an individual is difficult to pin-point since early signs may not be distinguishable from a 'normal' behavior spectrum. Literature surveys (e.g. Brackenridge, 1971a) may yield an over-abundance of juvenile cases, due to their exceptional interest, which lower the calculated mean age of onset. Ascertainment of cases through affected offspring will bias the estimated mean toward later onset if earlier onset is associated with reduced genetic fitness. The artifact of 'anticipation' may arise from the failure to account for living individuals who could, at a later age, begin to manifest symptoms. This bias was avoided in the studies by Brackenridge (1971a) and Wendt (1959) by the exclusion of recent generations.

Age at death can be precisely determined, but calculated means are affected by similar factors of ascertainment as those just discussed. Duration of illness can be calculated by difference, and is usually claimed to be about 15 years.

F. Genetic Studies

HC is a classical example of a dominantly inherited disease with complete penetrance. There are, nonetheless, some genetic peculiarities. Most anomalous observations, such as 'anticipation' result from ascertainment bias. One that seems to be real, however, is patrilineal transmission, a phenomenon that could result from differential mortality and/or differential genetic fitness (Brackenridge, 1971a). Brackenridge (1971a) reported a significant correlation between age of onset and number of offspring, accounted for primarily by affected mothers; an observation that could lend support to this hypothesis. Also, a sex difference in age of onset could favour the male line of descent.

Could genetic mechanisms account for the observed phenotypic heterogeneity? Is variation the result of different alleles at the HC locus, the modifying effect of the alternate 'normal' allele, or the influence of genetic background?

The first approach to answering these questions is to determine whether there is familial clustering with respect to clinical subtypes, age of onset, and the pattern of neurological and psychiatric symptoms. Most studies have reported a significant sib-sib and parent-offspring correlation in quantitative traits such as age of onset and age at death (Brackenridge, 1072b; Myrianthopoulos, 1966). The concept of 'biotype', referring to the subjective impression of clinical consistency within families, has persisted in the literature (e.g. Wallace, 1972), and may apply to some families (Myrianthopoulos, 1966). The phenotypic pattern is related to age of onset (Brackenridge, 1971b; Myrianthopoulos, 1973).

The specific influence of the alternate allele in determining age of onset is unlikely, since correlation coefficients for this trait in sibs and half-sibs are both close to .5 (Brackenridge, 1972a). As previously discussed, the alternate allele is also unlikely to specifically cause the juvenile variant.

Wallace (1972) cited evidence to support the theory that genetic heterogeneity underlies phenotypic variability. Analysis of variance for age of onset and age at death showed significantly more variation between kindreds than within. Correlation coefficients for these traits in choreics did not decrease markedly from sib-sib through parent-offspring to cousin-cousin. On the other hand, Reed and Chandler (1958) found significantly more variation between sibships of a kindred than within sibships, supporting the theory that similarity in sibs is due to common background genes. Wallace proposed that there are at least 2 major (genetically distinct) groups of choreics, distinguished by relatively early or relatively late onset.

Brackenridge (1972b) and Myrianthopoulos (1966, 1973) suggested that the alternatives of a genetic continuum or genocopies, and a single main gene with modifiers cannot be distinguished with present data. The distinction might be made through biochemical studies.

II SCHIZOPHRENIA

A. Historical Background (Discussed by Stabenau, 1977; Ban and Lehmann, 1977; Baldessarini, 1977; Kety and Mathysse, 1972)

Kraepelin in 1896 lumped several previously discrete conditions under the name of 'dementia praecox' - impaired cognitive function with onset in early adulthood - and emphasized its inevitable malignant prognosis. In 1911, Bleuler reclassified 'schizophrenia' - fragmentation of mental function - as a group of disorders, enlarging the scope of the entity. His description of primary (core) and secondary (accessory) symptoms has retained its diagnostic value to the present day.

B. Prevalence and Social Impact (Discussed by Ban and Lehmann, 1977; Baldessarini, 1977)

Schizophrenia is probably the world's most important psychiatric disorder, and is one of the greatest public health problems in developed countries; The associated social stigma is of course profound. Prevalence is estimated at about 1% of the world population, with some variation associated primarily with criteria for diagnosis. As of 1977, approximately 20% of all schizophrenics in the United States were hospitalized (Baldessarini, 1977), accounting for the occupancy of up to half of all psychiatric beds (Ban and Lehmann, 1977) at tremendous cost. Those not hospitalized are also expensive to society, even considering unemployment alone.

C. Symptoms and Classification

Three approaches to disease classification have been: (1) phenomenology, (2) response to therapeutic intervention and (3) cause (Falek and Moser, 1975). These correspond to the level of understanding of the disease. For

schizophrenia, classification is still phenomenological, although drug response characteristics are beginning to be listed among criteria for diagnosis. 'Schizophrenia' is an operational definition, and it cannot be over-emphasized that biological homogeneity should not be assumed or even expected (Blass, 1977; Meltzer, 1976).

Bleuler's description of symptoms has been the basis for most diagnostic protocols. His core defining symptoms are: (1) a characteristic thought disorder or disturbance of associations, (2) inappropriate affect, (3) withdrawal from normal social interactions and (4) lack of contact with and interest in external reality. Accessory symptoms include (continuous or intermittent) persecutory, grandiose or somatic delusions, hallucinations, and catatonic symptoms (Kety and Matthysse, 1972). No one symptom is pathognomonic, and all are non-specific; thus it is not surprising that routine diagnostic methods, even those employing sophisticated computer analyses, are unacceptable (Meltzer, 1976; Falek and Moser, 1975). For research purposes, diagnostic uniformity is a minimum requirement that has not yet been achieved.

Two aspects of classification have been particularly controversial issues. One is the retention of Bleuler's sub-types (i.e. catatonic, paranoid, hebephrenic and simple) as discrete diagnostic entities (for discussion see Baldessarini, 1977; Falek and Moser, 1975). The other is Kety's 'spectrum concept' used to account for the various types of psychopathology (other than fully-developed schizophrenia) which may be present with greater frequency in first degree relatives of schizophrenics (Meltzer, 1976; Baldessarini, 1977).

D. Genetic Studies

Genetic studies of schizophrenia are confounded by diagnostic problems, ascertainment biases, and probably a complex and heterogeneous etiology. The most that can be said with certainty is that recent studies leave little doubt

as to the involvement of hereditary factors in the etiology of schizophrenia.

1) Evidence for Genetic Factors (Reviewed by Gottesman and Shields, 1973; DeFries and Plomin, 1978; Baldessarini, 1977; Kety, 1972; Kety and Matthysse, 1972; Tsuang, 1976)

Evidence for a genetic diathesis in schizophrenia runs along 3 major lines, which will simply be summarized. First, there is a higher prevalence of schizophrenia within families of schizophrenics than in the general population. There is also a correlation between the prevalence and degree of relation to the index case. Empiric risk to full sibs (including dizygotic (DZ) twins) and children is in the order of 10-15%. If two parents are schizophrenic the risk may be as high as 50%. Second, monozygotic (MZ) twins have a much higher concordance rate (more than 50%) than DZ twins, even if they are reared apart. Third, adoption studies (using various methodologies) indicate a higher prevalence of schizophrenia among biological relatives of index cases than among adoptive relatives. In fact, the environmental influence of being reared with a schizophrenic person is probably not relevant (with the corollary that common parenting practices may be irrelevant). No particular environmental factor has been demonstrated which will, even with moderate probability, induce schizophrenia. It is present in all countries that have been studied, covering a wide range of cultural influences. This does not imply, of course, that environmental factors are not relevant to the expression of schizophrenia. If so, the concordance rate for MZ twins would be 100%.

2) Possible Modes of Transmission (Reviewed by Kety and Matthysse, 1972; Gottesman and Shields, 1973; Tsuang, 1976; Baldessarini, 1977)

No simple Mendelian model can account for population data on schizophrenia. Any modification of a single recessive gene model can be ruled out since sibs are at no higher empiric risk than offspring. Genetic heterogeneity may be involved, helping to account for the high frequency of schizophrenia. There could be a single major dominant gene with reduced penetrance, but once the modifying effect of genes at the same or other loci

is allowed, such a model is difficult to distinguish from polygenic models. If several genes are involved, variation with respect to a schizophrenic diathesis could be either continuous or quasi-continuous. There seems to be general agreement among reviewers that either mono- or polygenic models will fit existing data. Cavalli-Sforza and Kidd have suggested that only with the aide of biochemistry is the genetic basis of schizophrenia likely to be elucidated (Kety and Matthysse, 1972).

CHAPTER 2

INTRODUCTION TO BIOCHEMICAL THEORIES AND FINDINGS

I SCHIZOPHRENIA

A. The Transmethylation Hypothesis (Reviewed by Nestoros et al., 1977; Smythies, 1976; Matthysse and Lipinski, 1975; Kety, 1972; Kety and Matthysse, 1972; Brodie, 1977; Ban and Lehmann, 1977)

Proposed by Osmond, Smythies and Harley-Mason over 25 years ago, the transmyethylation hypothesis still has heuristic value. Its basis was initially the structural similarity between the catecholamines and mescaline (a psychotomimetic drug). The suggestion was that a disturbance in methylation might lead to an excess of endogenous, hallucinogenic, methylated derivatives of the catecholamines (later extended to include indolamines). Attempts to find any potential 'schizotoxin' (methylated amines) present in higher amounts in schizophrenic tissues have failed. The most supportive finding has been that by Polin and co-workers in 1961 (and confirmed in a number of laboratories) that methionine[1] loading caused exacerbation of schizophrenic psychoses in some patients. It is not clear, however, whether the psychotogenic effect was an intensification of the schizophrenic process, or a superimposed toxic psychosis. Further, the effect could have resulted from other metabolic or pharmacological actions of methionine and/or its derivatives, not necessarily increased transmethylation. Attempts to ameliorate symptoms with a methyl acceptor (nicotinamide or nicotinic acid) have failed (e.g. Nestoros, 1977), however S-adenosyl methionine in brain is not effectively lowered by nicotinamide.

B. The Dopamine Hypothesis

Currently, the most popular hypothesis for a biochemical defect in schizophrenia is the 'dopamine hypothesis'. Very simply, it states that schizophrenia is related to a relative excess of dopaminergic activity in the brain's limbic system (particularly, the mesolimbic dopamine tract). Support for the theory is primarily pharmacological.

[1] Methionine is ultimately the source of methyl groups for transmethylation reactions, via S-adenosyl-methionine (SAM).

1) Evidence for Dopamine Involvement

a) Neuroleptic Drugs (Reviewed in Kety and Matthysse, 1972; Matthysse and Lipinski, 1975; Meltzer and Stahl, 1976; Baldessarini, 1977; Carlsson, 1978; Kety, 1972)

Anti-psychotic drugs which, unlike reserpine, do not deplete monoamine stores, belong to several chemically diverse classes, the major ones being the phenothiazines (e.g. chlorpromazine (CPZ) and fluphenazine), the thioxanthines, the butyrophenones (e.g. haloperidol (HP)) and clozapine. Not all members of these classes have anti-psychotic action, but those that do are functionally similar, possibly acting on a biological substrate common to many schizophrenics. Most, but not all of the anti-psychotic drugs cause extrapyramidal (parkinsonian) side effects (EPS effects); thus the term 'neuroleptic'. They are sometimes called major tranquilizers, however their action seems to be in relieving the fundamental manifestations of schizophrenia; they are truly anti-psychotic.

The neuroleptics inhibit dopamine (DA)-mediated synaptic transmission in a dose-dependent manner, probably through blackade of DA receptors. Evidence for this is indirect, but compelling in combination. First, DA stimulates a specific adenylate cyclase in the synaptic cell membrane, and the activation can be blocked by administration of CPZ or HP. Second, radio-ligand binding assays indicate that the ability of a drug to competitively inhibit binding of ^{3}H-DA and/or ^{3}H-HP correlates closely with its anti-psychotic potency.

The neuroleptic drugs cause an increase in DA turnover, with an increased rate of production of DA metabolites (dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)), an increased rate of conversion of tyrosine to DA, and an increased rate of firing of DA neurons. This is not true of the non-antipsychotic phenothiazines. The effect is thought to be a compensatory mechanism, secondary to the interruption of synaptic transmission and brought about by a decrease

in the 'long-loop' feedback inhibitory system.

Dopaminergic neurons have pre-synaptic autoreceptors that bind DA, resulting in feedback inhibition of firing ('short-loop'). This inhibition can be blocked by neuroleptics.

Motor side effects reminiscent of the symptoms of Parkinson's disease (for which a DA deficiency has been well established), are a major complication of antipsychotic drugs. Conversely, L-DOPA cannot be used to counteract the EPS effects because it causes psychotic exacerbation.

b) Alpha-methyl-tyrosine (Discussed by Carlsson, 1978; Meltzer and Stahl, 1976; Matthysse and Lipinski, 1975)

Alpha-methyl-tyrosine is a potent inhibitor of tyrosine hydroxylase (T-OH), the rate limiting enzyme in DA biosynthesis. Although nephrotoxicity precludes its use in very high doses, it can potentiate the antipsychotic effects of neuroleptics, lowering the dose requirement. Presumably it helps to avoid the almost self-defeating feedback effect of receptor blockade on DA turnover.

c) Amphetamine Psychosis (Discussed by Meltzer and Stahl 1976; Matthysse and Lipinski, 1975; Kety, 1972)

Amphetamine has long been known to possess psychotomimetic properties, capable of eliciting in non-schizophrenic individuals a clinical picture often indistinguishable from acute paranoid schizophrenia. The major pharmacological effect is believed to be induction of catecholamine release. It would seem, therefore, that schizophrenia might also involve an excess activity of catecholamines. Support for the involvement of DA, rather than norepinephrine (NE) comes from the observation that neuroleptics (which antagonize DA) ameliorate amphetamine psychosis. Further, amphetamine induces an increase in cerebro-spinal fluid (CSF) of HVA[1] without an increase in NE metabolites.

[1] measured following probenecid administration, to prevent loss of the metabolite to venous blood

No such increase, however, has been noted in schizophrenics.

2) The Limbic System

a) Anatomy (Discussed by Stevens, 1973; Meltzer and Stahl, 1976; Torrey and Peterson, 1974; Barr, 1974; Ingram, 1976)

The limbic system comprises frontal and medial temporal lobes lying above the brainstem, including the amygdala, hippocampus, septum, olfactory tubercle, and hippocampal gyri. It receives afferents from all parts of the brain, and is functionally associated with emotional aspects of behavior related to the survival of the individual and the species, and with memory. Efferents go to the hypothalamus, basal ganglia, mammillary bodies and thalamic nuclei[1]. Of particular interest is the 'limbic striatum' (Stevens, 1973), comprising the bed nucleus of the stria terminalis, the nucleus accumbens, and the olfactory tubercle. The latter structures contain the terminals of the major 'mesolimbic DA tract' which originates in the interpeduncular nucleus of the ventral tegmental area.

b) The Limbic System in Schizophrenia

Support for the involvement of the limbic system in schizophrenia comes from experiments both of nature and of man. Schizophrenic-like psychotic phenomena are frequently associated with psycho-motor (temporal lobe) epilepsy, encephalitis with predominance of temporal lobe involvement, brain tumors in limbic structures, and stimulation or ablation of the same (Torrey and Peterson, 1974; Stevens, 1973; Baldessarini, 1977). Attention is drawn to the 'limbic striatum' both because of its dopaminergic innervation and its "strategic interposition at the outflow of limbic structures subserving the primary adaptive processes disturbed in schizophrenia" (Stevens, 1974). There are some reports of EEG abnormalities in schizophrenics, particularly

[1] the hypothalamus, and anterior thalamic nuclei, being part of the 'limbic circuit' are considered by some to be parts of the limbic system

with electrodes implanted in limbic structures (Stevens, 1973; Meltzer and Stahl, 1976; Torrey and Peterson, 1974).

It is generally assumed that the antipsychotic activity of the neuroleptic drugs reflects interaction with DA synapses in the limbic striatum, while the EPS effects result from DA receptor blackade in the striatum[1] (Carlsson, 1978; Meltzer and Stahl, 1976; Snyder, 1972; Baldessarini, 1977).

3) Possible Causes of Dopaminergic Excess (Discussed by Meltzer, 1976; Meltzer and Stahl, 1976; Carlsson, 1978; Stevens, 1973; Matthysse and Lipinski, 1975; Kety, 1972)

The real cornerstone of the DA hypothesis is the evidence for DA blocking activity of the neuroleptics, but whether or not this is the action required for the antipsychotic effect remains to be demonstrated (Matthysse and Lipinski, 1975; Baldessarini, 1977). A relative increase in dopaminergic activity might come about by any of a number of means. Theoretically at least, there could be (1) an increase in absolute levels of DA due to increased amounts of precursor, increased activity of T-OH, decreased inactivation by degradative enzymes, or failure of control mechanisms for storage and release. Attempts to find alterations in any of these parameters have failed, although differences might be so narrowly localized as to be undetectable in large preparations. There could be (2) defective transport of DA from the synaptic cleft to pre- or post-synaptic cells, resulting in an impaired feedback system, (3) an increase in DA response, (4) an excess of excitatory stimulation on DA neurons, or (5) a deficiency of inhibitory input from an inhibitory transmitter such as gamma-aminobutyric acid (GABA).

a) GABA and the DA Hypothesis

Roberts (1972) proposed that the underlying defect in the etiology

[1] the nigrostriatal DA tract is the site of reduced dopaminergic activity in Parkinson's disease

of schizophrenia might be a lower-than-normal inhibitory effect of GABAergic neurons on other neurons. He suggested that neuronal systems are released, not driven; in other words, that excitatory neurons are usually held in check by the tonic action of inhibitory (such as GABAergic) neurons, and are released for discharge on demand, through disinhibition. The schizophrenic diathesis might involve a barely adequate inhibitory system which under 'stress' would be incapable of keeping excitatory neurons from excessive firing, and imbalances would arise. The problem could be reflected as a relative increase in, for example, dopaminergic activity, secondary to deficient inhibitory control (which in turn could be a primary or secondary phenomenon).

In light of the focus on limbic system involvement in schizophrenia, some pharmacological evidence for GABA influence on mesolimbic DA neurons supports the general hypothesis. Stevens et al. (1974) injected bicuculline (a putative GABA blocking agent) into the ventral tegmental area of cats. The resulting series of behaviors resembled the stereotypic activity of the animals following systemic DA potentiation. The findings supported the hypothesis that behavior characteristic of DA stereotypy resulted from blockade of GABA inhibition on DA neurons (although other explanations were possible).

C. Low Platelet MAO in Schizophrenia (Discussed by Wyatt and Murphy, 1976; Potkin et al., 1978; Berger et al., 1978; Baldessarini, 1978; Brodie, 1977; Meltzer, 1976)

A biochemical finding of current interest is that first reported by Murphy and Wyatt in 1972 of reduced monoamine oxidase (MAO)[1] in platelets of chronic schizophrenics. The finding has not always been confirmed, but the largest decrease may be in 'paraniod schizophrenics' (Potkin et al., 1978). The trait is not specific to these schizophrenics (patients with bipolar

[1] an important enzyme in the metabolic degradation of a variety of monoamines

depression also show reduced platelet MAO), and all attempts to reveal a deficiency in post-mortem brain have failed. Data from MZ twins discordant for schizophrenia suggest that the trait is genetically determined and thus a possible marker for susceptibility rather than a function of the disease state. Despite the complexities involved, studies of MAO may contribute to the understanding of the schizophrenic phenomenon.

D. Altered Transmitters and Related Enzymes

Recently, Bird et al. (1977) published a preliminary report on levels of DA, glutamic acid decarboxylase (GAD), and choline acetyl transferase (CAT), from patients dying with schizophrenia, schizophrenic-like psychoses, and controls[1]. They examined 3 parts of the limbic system (nucleus accumbens, amygdala, and hippocampus) as well as putamen. DA was found to be significantly increased in the combined psychotic group in nucleus accumbens but not putamen, and was not measurable in amygdala or hippocampus. GAD activity was significantly decreased in psychotics in all 4 regions. CAT was significantly lower in schizophrenics but not schizophrenic-like psychotics, only in nucleus accumbens.

About the same time, McGeer and McGeer (1977) published results of a similar study, in which they measured CAT, acetylcholinesterase (AChE), T-OH, dopamine decarboxylase (DDC), and GAD activities in numerous brain regions from 11 schizophrenics and 18 controls. All brains had been removed within 24 hours of death and cases involving pre-mortem coma had been excluded[2]. The main finding was increased CAT activity in caudate, putamen, nucleus accumbens and hippocampus of schizophrenic brains. GAD activity was not significantly different in any of these areas. The discrepancy in direction of change of CAT activity between this study and that of Bird et al, (1977) seems to have resulted from striking differences in the mean activities of the

[1] Synthesis of GABA from glutamic acid is catalysed by GAD. CAT is responsible for the synthesis of acetylcholine (ACh), another neurotransmitter

[2] See following sections, 'pre-mortem factors' and 'post mortem handling' for more detailed discussion of these points of concern

control groups, not the schizophrenics.

In light of considerable criticism in the Lancet[1] (Perry et al., 1978; Crow et al., 1978) following the report of Bird et al. (1977), the data were re-examined (Bird et al., 1978a,b). When only cases for which there was evidence of sudden death by natural causes were included, differences in GAD activity between controls and schizophrenics remained significant only in nucleus accumbens. A more extensive evaluation (Iversen et al., 1978), including only sudden death cases, demonstrated no significant difference between controls and schizophrenics for mean GAD activity in caudate, putamen or nucleus accumbens. (Significant reductions remained for Huntington's choreics).

Farley and co-workers (1978) have measured NE in 4 paranoid schizophrenics and 12 controls. Earlier studies indicated that NE levels did not vary with either age or post-mortem delay. Significant elevations of NE were found in 4 areas of limbic forebrain: nucleus accumbens, mammillary body, stria terminalis and ventral septum. Drug treatment with neuroleptics and/or cause of death were demonstrated to be unlikely factors to account for the differences. Several other limbic areas showed no significant difference between the 2 groups.

II HUNTINGTON'S CHOREA

A. Altered Transmitters and Related Enzymes

Results of studies on HC, in which measurements of GABA, GAD, and CAT were made are summarized in Table I. Following the initial reports of Perry et al. (1973a, 1973b) of a reduction of GABA in certain areas of the brain, studies were undertaken to measure GAD, because of the implication that a metabolic error in biosynthesis might be involved, and because the enzyme was thought to be more stable than GABA immediately after death. Most studies confirmed a decrease in GAD in HC brains, in areas which paralleled the GABA

[1] See following sections, 'pre-mortem factors' and 'post-mortem handling' for more detailed discussion of these points of concern

TABLE I: STUDIES OF NEUROTRANSMITTERS AND RELATED ENZYMES IN HC

AUTHOR	GABA	GAD	CAT	OTHER
Perry et al. (1973a)	-sig ↓ in SN -also ↓ in caudate, putamen-GP			- ↑ GLYC-PEA/PEA ratio
Perry et al. (1973b)	-striking ↓ in SN, caudate, putamen-GP -sig ↓ in OC, TC -no sig ↓ in FC, amygdala, thalamus, hypothalamus			- ↑ GLYC-PEA - ↓ HCARN
Bird et al. (1973)		- ↓ (85%) in caudate, putamen, GP -no ↓ in FC	- ↓ in caudate	
McGeer et al. (1973a)		- ↓ in caudate, putamen relative to that in thalamus, hippocampus, amygdala	-patchy ↓ in caudate, putamen	-T-OH normal -AChE normal
McGeer et al. (1973b)		-uniform ↓ (50-60%) in caudate, putamen, GP, SN	-patchy ↓	
Bird & Iversen (1974)	- ↓ in caudate, putamen	- ↓ in caudate, putamen -no ↓ in FC	-bimodality among pts: ½ had normal levels caudate, putamen; ½ had striking ↓	-DA normal in most ↓ in 6 rigid cases -T-OH normal
Stahl & Swanson (1974)		- ↓ (88-93%) in advanced HC, no in early HC (1 pt.)	- ↓ (73-99%) in striatum -no ↓ in CC -no ↓ in early HC (1 pt.)	-MAO ↓ (50%) in striatum of early HC
Urquhart et al. (1975)	-confirmed earlier studies (above)	-low in HC but also low in 2/5 control samples		- ↓ HCARN in GP, putamen, CC
McGeer et al. (1976a)		- ↓ in extrapyramidal structures	-patchy ↓ in neostriatum	

Abbreviations:

SN = substantia nigra
PEA = phosphoethanolamine
TC = temporal cortex
AChE = acetylcholinesterase
sig = significant
pt. = patient
GLYC-PEA = glycerophosphoethanolamine
OC = occipital cortex
T-OH = tyrosine hydroxylase
CC = cerebellar cortex
MAO = monoamine oxidase
HC = Huntington's chorea
GP = globus pallidus
HCARN = homocarnosine
FC = frontal cortex
DA = dopamine
↓ = decrease
↑ = increase

decrease. One study from Perry's laboratory, however, (Urquhart et al, 1975) demonstrated that GAD was also low in 2 of 5 control samples, a finding which could not be accounted for by pre-mortem factors[1]. Overall, the reduction in GABA has been most striking in the extrapyramidal system (caudate, putamen, GP and SN), a finding in keeping with the motor disturbance of HC.

Decreased CAT in HC has been described by McGeer as "patchy", since repeated sampling from caudate or putamen of a given individual may yield variable results.

GAD deficiency is not specific to HC. Bowen (1974, 1975) reported a fairly widespread but severe deficiency of GAD in brains of patients dying with senile dementia. Davies and Maloney (1976), on the other hand, found GAD levels within normal range in 3 Alzheimer's patients[2]. In Parkinson's disease, GAD deficiency may be secondary to treatment with anticholinergics (McGeer et al., 1973b). Clearly, carefully designed experiments need to be carried out in order to answer questions concerning the relevance of decreased GAD activity.

The finding of a parellel deficiency of the activity of an enzyme and its metabolic end product does not necessarily imply that a defective enzyme is primarily responsible for the loss of product. GAD is localized in inhibitory neurons that utilize GABA (Bird and Iversen, 1974) and destruction of such neurons would result in concomitant loss of enzyme and product. This is probably the case in HC (Perry et al., 1977). The observed neuronal degeneration, most marked in basal ganglia, may represent specific loss of GABAergic and possible small cholinergic neurons (Bird and Iversen, 1974). Clinical manifestations suggest DA hyperactivity, although no evidence of increased DA turnover has been demonstrated (except in 6 rigid cases as reported by Bird and Iversen (1974)). As discussed for schizophrenia, DA hyperactivity could result from a relative deficiency of GABA.

[1] See following section on 'pre-mortem factors'

[2] See also following section on 'age'

There are numerous possibilities for causes of selective cell death. McGeer and McGeer (1976c) have suggested, for example, that HC might be an excitotoxic phenomenon, resulting from chronic overstimulation of glutamate receptors, particularly in the caudate.

B. Immunological Findings

Barkely and co-workers (1977a,b, 1978) measured migration inhibition factor (MIF) activity (a correlate of delayed hypersensitivity reaction) in cultured lymphocyes confronted with brain tissue preparations from various individuals. Results are summarized in Table II:

TABLE II: Detectable MIF activity in cultured lymphocytes (Barkley et al., 1977a,b, 1978)

		SOURCE OF LYMPHOCYTES		
		Control	HC	MS
SOURCE OF BRAIN TISSUE (ANTIGEN)	Control	-	-	-
	HC	-	+	-
	MS	-	+	-
	AD	-	-	-
	Park.	-	-	-

HC = Huntington's Chorea
MS = Multiple Sclerosis
AD = Alzheimer's Disease
Park. = Parkinson's Disease

The authors hypothesized that the HC gene could be a partial viral genome, becoming active in middle life, and producing a gene product with some similarities to a virus that has been proposed for the etiology of multiple sclerosis (MS). Because of the late appearance of the gene product, it could elicit an immune response similar to that invoked by an infectious agent. MS lymphocytes might not respond similarly due to a different set of immune response genes. They did not comment on the possible relationship of these observations to pathogenesis in HC. They added that preliminary evidence demonstrated similar antigenicity of HC fibroblasts.

C. Evidence for Altered Membranes

Butterfield et al. (1977) studied electron spin resonance (ESR) characteristics of HC erythrocyte membranes. Differences from controls could be interpreted as due to (1) an altered membrane protein, (2) different amounts of a membrane component, (3) an altered consituent, organization or (4) a membrane-bound protein in HC, not found in controls. If a similar alteration were to exist in neuronal cells, it could be linked to the pathogenesis of HC.

Several studies have examined the behavior of HC fibroblasts in cell culture. Menkes (1973) found that HC fibroblasts performed relatively poorly in vitro. Gray and Dana (1977) found no uniform difference in growth characteristics between HC and non-HC cells. In contrast, Goetz et al. (1975), Kirk et al. (1977) and Leonardi et al. (1978) found an overall superiority of growth of HC fibroblasts. Methodology appears to have been an important variable; nonetheless, it seems that HC cells can reach higher saturation density, avoiding an initial lag phase and spending longer than normal in exponential growth. The abnormal behavior may be the result of an altered cell membrane.

D. GABA Receptors

^{3}H-GABA binding to synaptic membrane preparations can be studied in post-mortem brain. Findings with respect to altered density of GABA binding sites in HC are conflicting. Enna et al. (1976a,b) found no change in receptor density in basal ganglia of Huntington's choreics, whereas Lloyd et al. (1977) and Iversen et al. (1978) found a substantial decrease in binding site density in caudate and putamen. Iversen et al. (1978) reported, however, that results were quite variable, with some samples showing essentially normal binding and others very low values. If such heterogeneity can be confirmed, investigations should be undertaken to elucidate factors that might be associated with normal or deficient GABA receptors, because of the implications for therapeutic intervention.

CHAPTER 3

OTHER INFLUENTIAL VARIABLES

A. Post-mortem Handling

In order to interpret post-mortem measurements of amino acids and related enzymes, it is important to know how closely they represent levels that would have been present during life. The interval from death to freezing of brain tissue is a poorly controlled variable and may sometimes not be accurately estimated.

1) GAD

Iversen et al. (1978) recently carried out a carefully controlled study of GAD levels in mouse brain, with cooling conditions programmed to mimic those in human cadavers. With cooling to $4^{o}C$, they observed a rapid initial decline (during the first few hours) to 80% of initial values. Following this, a stable plateau was maintained up to 72 hours. This confirms results of earlier studies (McGeer et al., 1973a; Bird and Iversen, 1974: Urquhart et al., 1975; McGeer and McGeer, 1976a,b) which demonstrated relative stability of GAD during storage at $4^{o}C$. Decline was greater, however, at room temperature (Bird and Iversen, 1974; McGeer and McGeer, 1976) or if tissue had been frozen and then thawed (McGeer and McGeer, 1976a,b). The one discrepant finding has been that by Crow et al. (1978) of significantly reduced GAD in material obtained more than 48 hours post-mortem.

2) Amino Acids

Perry and co-workers (1971 a,b) compared amino acid levels in biopsied human cerebral cortex (immediately frozen) with those in tissue obtained at autopsy and frozen 2.5-27 hours post-mortem. Most amino acids which were components of protein rose significantly after death, presumably due to proteolysis, and hydrolysis of N-acetylated amino acids. Ethanolamine (EA), GABA, and 2 GABA-containing dipeptides (homocarnosine (HCARN) and gamma-aminobutyryl lysine (GABA-LYS)) were also significantly elevated in the 5

post-mortem specimens. Concentrations of reduced and oxidized glutathione (GSH and GS-SG), on the other hand were significantly lower in autopsied Levels of a number of compounds[1] were not significantly different in biopsied and autopsied specimens, either because they were really not altered by post-mortem factors, or because the influence of this one variable was obscured by other large sources of variation.

Tews et al. (1963) demonstrated that GABA in dog cerebral cortex was elevated 51% by 20 to 23 minutes post-mortem. Minard and Mushahwar (1966) showed that GABA in rat brain rose to a plateau within 1-2 minutes post-mortem and then remained constant to the end of the test period (30 minutes). Glutamic acid (GLU) was significantly lower in brains not immediately frozen, but there were no comparable changes in the levels of aspartic acid (ASP) or the neutral amino acid fraction.

B. Pre-mortem Factors

There has recently been considerable discussion in the literature (e.g. Perry et al., 1978b; Crow et al., 1978) about the possible influence of cause of death on post-mortem GAD levels in the brain. In particular, there has been concern that conditions which lead to cerebral hypoxia cause a reduction in GAD activity. Since causes of death tend to be related to disease conditions, there is reason for legitimate concern when studying disease group differences.

One example may serve to illustrate the magnitude of this influence. McGeer et al. (1973a) measured GAD in several brain regions, from a few individuals. Results for the thalamus are summarized in Table III.

GAD activity (nmoles/gm/hour)		
	CONTROL	HC
no coma	7.30	2.90 4.43 4.01
coma	0.40	0.74

TABLE III: GAD Activity in Thalamus - Effect of Coma (McGeer et al., 1973a)

[1] enumerated in 'Discussion'

In larger studies (McGeer and McGeer, 1976a; Bowen et al., 1976) it was clearly demonstrated that GAD activity decreased following coma from either head injury or illness, but that there was some regional variation in the effect. The finding has been confirmed by Iversen et al. (1978) who compared GAD activity in 3 areas of brain from patients dying with bronchopneumonia to that in patients dying sudden deaths, and found lower mean GAD activity in the former group.

Little is known about the relevance of cause of death to brain amino acids. Tews et al. (1963) examined effects of pre-mortem anoxia in dog cerebral cortex. The increase of alanine was the most striking, but increases in GABA (28%) and several other amino acids were also significant.

C. Age

Numerous studies (McGeer et al., 1973; Bird and Iversen, 1974; Bowen, 1974, 1975; Bowen et al., 1976; McGeer and McGeer, 1976a,b; Perry et al., 1978a) have attempted to examine the effects of aging on neurotransmitter-related enzymes, particularly, GAD, CAT, T-OH, AChE, and DDC. Results are difficult to interpret since they are probably dependent on the following factors: (1) whether or not post-mortem changes are accounted for, (2) the age range examined, (3) whether or not terminally demented and non-demented patients are separated, (4) the region (s) of brain examined and (5) sample size.

Decreases have been reported, in various brain regions, for each of the enzymes listed above with increasing age. Senile dementia seems to correlate with more striking deficiencies of CAT (Perry et al., 1978). It is important to note that regional variation in these findings is considerable.

A report by McGeer and McGeer (1976b) is of particular interest for the present study. GAD activity was measured in 56 brain regions (where n is ≥ 5) from patients dying without pre-mortem coma or unconsciousness, and on whom autopsies were performed between 2 and 24 hours after death. In general,

the thalamic areas showed the greatest decline, in age ranges 5-20 years, and 20-50 years. Cortical and rhinencephalic areas followed, and basal ganglia showed relatively less decline with age.

The findings of decreased CAT with age in some regions, and more striking deficiencies with senile dementia support the pharmacological evidence (Drachman and Leavitt, 1974; Davis et al., 1978; Sitaram et al., 1978) for cholinergic involvement in memory storage. This function diminishes with aging (Drachman and Leavitt, 1974) and is deficient in HC (Caine, 1978).

D. Drugs

The vast majority of diagnosed schizophrenics and Huntington's choreics are treated with one or a combination of anti-psychotic drugs. Not only is this a variable that is almost inextricably linked to diagnosis, but the drugs act on the very neurochemical systems that are being investigated for potential differences.

One means of investigating drug response as a possible source of variation is to test the effect in a controlled animal experiment. Lloyd and Hornykiewicz (1977) treated rats both acutely and chronically with clozapine (chronic, 100 days) or HP (chronic, 167), and measured GABA and GAD in substantia nigra. Acute treatment with either drug caused a significant reduction in GABA (but not GAD) compared to saline-injected controls. Chronically treated rats, on the other hand, had GABA and GAD values not significantly different than controls.

In conjunction with the present investigation, a similar drug experiment was carried out[1]. Thirty rats were injected for 100 days; 10 with CPZ (20 mg/kg/day s.c.), 10 with HP (3 mg/kg/day s.c.) and 10 with 0.9% saline (equivalent volume). Following cervical dislocation brains were removed, grossly dissected and frozen in liquid nitrogen within 25-35 seconds. GABA concentrations were not significantly different in

[1] To be presented at NINCDS Huntington's Disease Symposium, San Diego, November 16-18, 1978

limbic forebrain among the three groups.

E. Regional Variation

There is considerable regional variation in the distribution of amino acids within the brain (Perry et al., 1971a). Similarly, there is regional variation in the distribution of enzymes such as GAD and CAT (McGeer and McGeer, 1976a). No breakdown has been done for amino acid distribution within the thalamus, however because of the presumed association between GAD and GABA, it is of interest to note the distribution of the enzyme within the thalamus. McGeer and McGeer (1976a) measured GAD activity in 7 thalamic areas, expressing results as a percentage of activity found in the caudate. Overall thalamus had 68% activity, with a range from 101% in anterior thalamus to 32% in ventral posterior thalamus.

CHAPTER 4

THE THALAMUS

The thalamus (Discussed by Barr, 1974; Ingram, 1976) is a large mass of grey matter making up most of the diencephalon. It may be subdivided into several nuclei on the basis of fibre connections and phylogeny. Some of these nuclei are 'specific', in that stimulation will evoke localized potentials in definite cortical areas. They receive specific sensory input and project to sensory areas of cerebral cortex. Stimulation of 'non-specific' nuclei, on the other hand, will evoke potentials over wide neocortical areas of both hemispheres. These are functionally related to association areas of cortex, and participate in emotional response to sensory stimuli. In general, the thalamus is a relay station, modulating and controlling contacts between cerebral cortex and the outside world.

The anterior nucleus receives afferents via the mammillothalamic tract and projects to the cingulate gyri. It is therefore included in the limbic system. The ventral lateral nucleus is important for distribution of impulses from basal ganglia to motor areas of frontal lobe, thereby controlling voluntary movement. The dorso-medial nucleus contributes to mood and related motor responses, and appears to play a role in memory.

Thalamic lesions may yield elevated sensory thresholds, with abnormal responses beyond the threshold. There may be spontaneous pain as well as emotional instability.

CHAPTER 5

PURPOSE AND RATIONALE OF THE PRESENT INVESTIGATION

In this study, amino acids and other ninhydrin-positive compounds were measured in autopsied brain from patients dying with Huntington's Chorea, with schizophrenia or schizophrenic-like psychoses, and from controls dying without evidence of neurological illness.

A deficiency of GABA had been noted in some (but not all) regions of brain in patients dying with Huntington's Chorea (Perry et al., 1973a,b). The thalamus had not been thoroughly examined. Knowledge of the distribution of the biochemical alteration could contribute to an understanding of the pathogenesis underlying this disease.

Schizophrenic brain was examined as well for two reasons. First, Huntington's chorea and schizophrenia share certain clinical features. It seemed possible, therefore, that they might share a biochemical alteration. Second, it had been proposed (Roberts, 1972) that a deficiency of GABAergic activity might underly the elevated dopaminergic activity that is thought by many to be a key feature of schizophrenia. It seemed appropriate, therefore, to look for differences in GABA levels amongh these groups. Striking differences in any of the other amino acids being measured concommitantly would of course be of interest as well.

A number of other independent variables, besides disease status, are likely to influence amino acid levels in the brain. These have been examined and accounted for, as much as possible within the confines of the available material.

CHAPTER 6

MATERIALS AND METHODS

A. Sources of Brain Tissue

The majority of material used was obtained from Dr. E.D. Bird, MRC Neurochemical Pharmacology Unit, University of Cambridge, England. Data for thalamus of 5 controls and 6 Huntington's choreics had been obtained in Dr. Perry's laboratory, during a period of time when thalamus was routinely analysed. These data were included in the study. Thalamus from a further 4 choreics had been in storage here and available for analysis, and that from 2 more HC patients and 5 controls was made available during the course of the study. Most of the Vancouver material was from patients who had died at Riverview Hospital or the Vancouver General Hospital, but some had been provided through donations from other North American centres. Brain dissections were performed in England (for Dr. Bird's material) or in Vancouver. Data from a total of 70 samples were used for the study.

B. Handling (Bird and Iversen, 1974; Bird et al., 1977)

Material from England was handled in the following manner: Whole brains obtained at necropsy were frozen at -20°C. Frozen brains were transported on dry ice, stored at -20°C and then at -10°C for 12 hours before dissection. Dissections were carried out at -5°C. Dissected material was then chopped and mixed, refrozen for storage, then transported to Vancouver on dry ice where it was stored at -80°C.

In all cases but one, Vancouver material was handled in the following manner: Whole or half brains were obtained at necropsy, immediately dissected and parts frozen on dry ice. They were transported frozen to storage at 80°C. In one case, the whole brain was stored at -80°C until dissection could be performed, and then parts were re-frozen and stored.

C. Preparation of Tissue for Amino Acid Analysis

A portion of frozen brain, usually approximating 200 mg was weighed, suspended in cold 0.4 M perchloric acid (0.5 ml per 100 mg), then homogenized in a tissue grinder with 100 strokes of a motor-driven Teflon pestle. The homogenate was centrifuged at 21,000 x g for 10 minutes, the supernatant removed and retained. The pellet was resuspended in 0.4 M perchloric acid (0.3 ml per 100 mg of original tissue), and rehomogenized (40 strokes). This was recentrifuged for 10 minutes at 21,000 x g, the supernatant removed and combined with the first supernatant. The pooled supernatant was adjusted to pH 2.5-3.0 with KOH and centrifuged for 10 minutes at 21,000 x g to precipitate the potassium perchlorate. The supernatant was removed, volume measured, and concentration calculated using initial wet weight. Samples were stored frozen at -80^{o}C until amino acid analysis could be carried out.

D. Amino Acid Analysis

Amino acids, small peptides and other ninhydrin-positive compounds were separated on a Technicon automatic amino acid analyser (Perry et al., 1968), adjusted for simultaneous analysis of 2 samples. Molar concentrations of the amino acids were calculated from the chromatograms using a Technicon integrator-calculator.

E. Statistical Methods (Sokal and Rholf, 1969)

Standard methods were used for the following statistical tests: Paired t Test, One-way Analysis of Variance (ANOVA), Linear Regression Analysis, Multiple Linear Regression Analysis, Regression on ln of one variable, and Multiple Regression using ln of one variable. If ANOVA yielded significant results, Scheffe's Test for aposteriori comparisons was used to localize differences.

F. Data Used in Statistical Analyses

The majority of analyses were carried out using 63 of the original 70 samples. Exclusions were made for the following reasons:

1) Three samples from one individual and two from another were analysed to see whether there were striking regional differences within the thalamus for any amino acids. (No marked differences were noted.) Only one sample (anterior) for each individual was included in calculations.
2) Two 'controls' had been treated with neuroleptics. Their control status was therefore questionable.
3) The diagnosis for one choreic was in question.
4) One Huntington's choreic was an extreme outlyer on a computer analysis that involved grouping individuals on the basis of all amino acids. Examination of notes revealed that this brain had been lost in transit and arrived in Vancouver completely thawed. Its exclusion therefore seemed justified.

In all, there were maxima of 23 controls (C), 25 Huntington's choreics (HC), 10 schizophrenics (S), and 5 patients with schizophrenic-like psychosis (SL) for any given analysis. The latter two groups were not pooled since "(those) who were placed in the schizophrenia-like group ranged from those who narrowly failed to meet the criteria for schizophrenia, to those in whom the diagnosis of schizophrenia seemed inappropriate" (Bird et al., 1977). The SL group was a poor one to deal with because it was small, and there were several unknowns among the independent variables. Although it was included in some analyses, no conclusions about it should be drawn from this sample.

G. The Independent Variables

Information concerning the following independent variables was available, for most individuals included in the study: age, interval between death and freezing of brain tissue (post-mortem delay (PMD)), region of the thalamus

sampled, immediate cause of death, diagnostic category, and some drug history.

Age and PMD are continuously distributed variables, and therefore readily examinable for differences among groups, and for their potential influence on the dependent variables (i.e. amino acids).

Diagnostic categories were, of course, part of the experimental design.

Drug history was a difficult variable to deal with. First, it was highly confounded with diagnosis, since most schizophrenics and choreics had been treated with neuroleptics, and controls had not. Second, each individual's treatment pattern was likely to have been different, and the reliability of recorded information was questionable. A division was made somewhat arbitrarily, between those who had been treated with neuroleptics and those who had not. No rigorous analysis of potential drug effect could be carried out on this sample.

Similarly, no rigorous analysis of the other discrete variables, region of thalamus and cause of death, could be carried out. Details were simply tabulated.

H. The Dependent Variables

Thirty amino acids and other ninhydrin-positive compounds were examined statistically. Others had been measured but were present in amounts too small to be quantitated accurately.

I. Statistical Protocol

For each amino acid, the following protocol was carried out: Control data were first submitted to a Multiple Linear Regression Analysis of amino acid on age and PMD. If this regression was not significant, it was assumed that these 2 independent variables did not contribute markedly to the variation, and a straight ANOVA was performed for 4 groups. If the ANOVA was significant, group differences were localized using Scheffe's Test

(a highly conservative aposteriori test for differences between means taken from a larger group of means). If the ANOVA was not significant, data for all 4 groups were pooled and submitted to a Multiple Linear and Quadratic Regression Analysis (by computer) as a more powerful test of the effects of age and PMD.

If the initial Multiple Linear Regression was significant, the relative contributions of age and PMD were quantitated, and significance determined. If group differences for these amino acids were to be analysed, correction would have to be made for the influence of the (significant) independent variable(s). Such analyses were not carried out, except for GABA. The rationale for the further analysis of GABA is described under 'Results'.

CHAPTER 7

RESULTS

A. The Independent Variables

1) Age (See Figure 1)

There was no significant difference in mean age among controls, Huntington's choreics, and schizophrenics (see Table IV). Schizophrenics, however, tended to be distributed towards the older end of the age spectrum.

TABLE IV: Mean Age (years) of Controls, Huntington's Choreics and Schizophrenics

	C	HC	S
n	23	25	10
mean	56.2	54.6	64.9
SEM	±3.5	±2.3	±3.7

ANOVA on 3 means: $F_{2,55} = 1.95$ (NS)

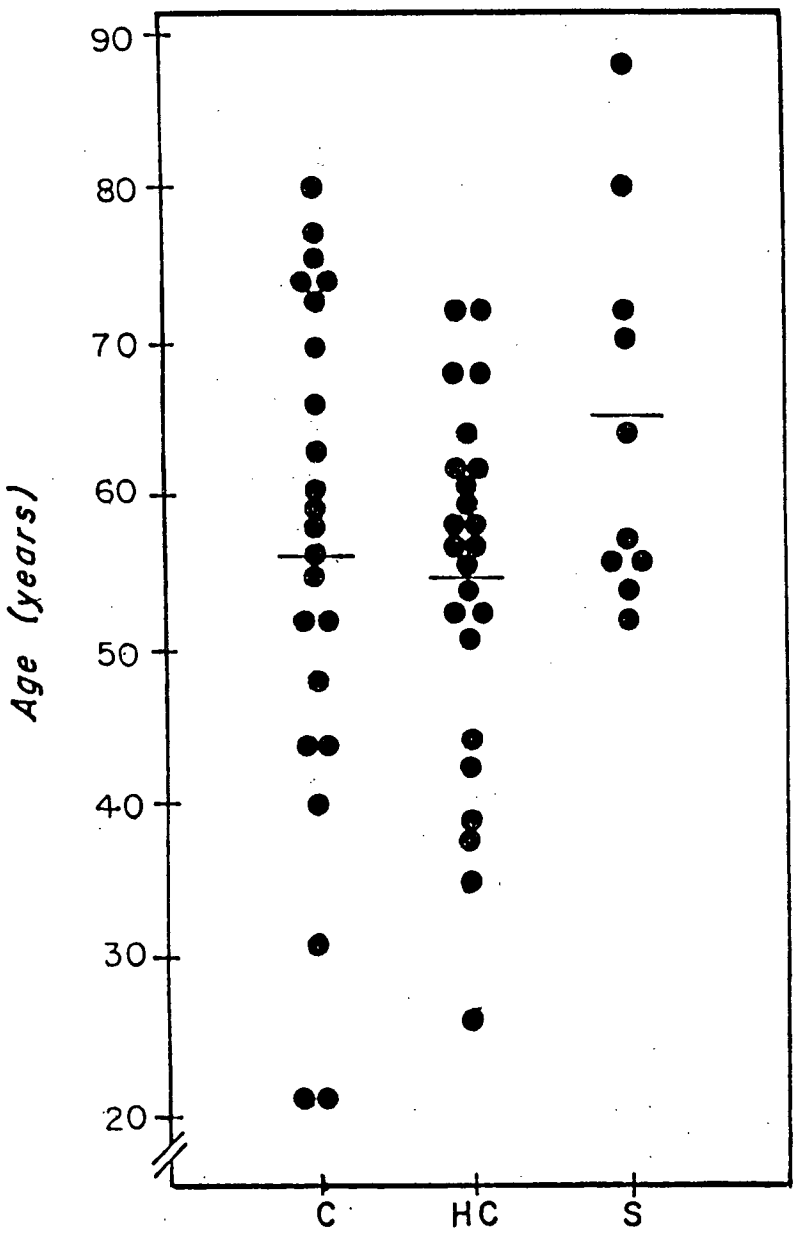
2) Post-mortem Delay (PMD)

a) Estimates of PMD

PMD was unknown for a number of samples, (2 controls, 7 choreics and 2 schizophrenic-like psychotics). Since this variable influences the levels of most amino acids, it was useful to have an estimate of PMD for those unknowns. Total GSH and ILE were chosen to base predictions on, since a preliminary analysis indicated that they were the compounds most highly correlated with PMD, without being confounded by other variables. A natural log transformation of PMD allowed the best fit of data to a multiple regression ($n = 49$[1], $r^2 = .661$, $F_{2,46} = 44.95$, $p < .001$). From the multiple regression, the equation for prediction of PMD was: $\ln(PMD)' = 2.4668 - 1.0234(GSH) + 2.0301(ILE)$. See Table V for the estimates of PMD.

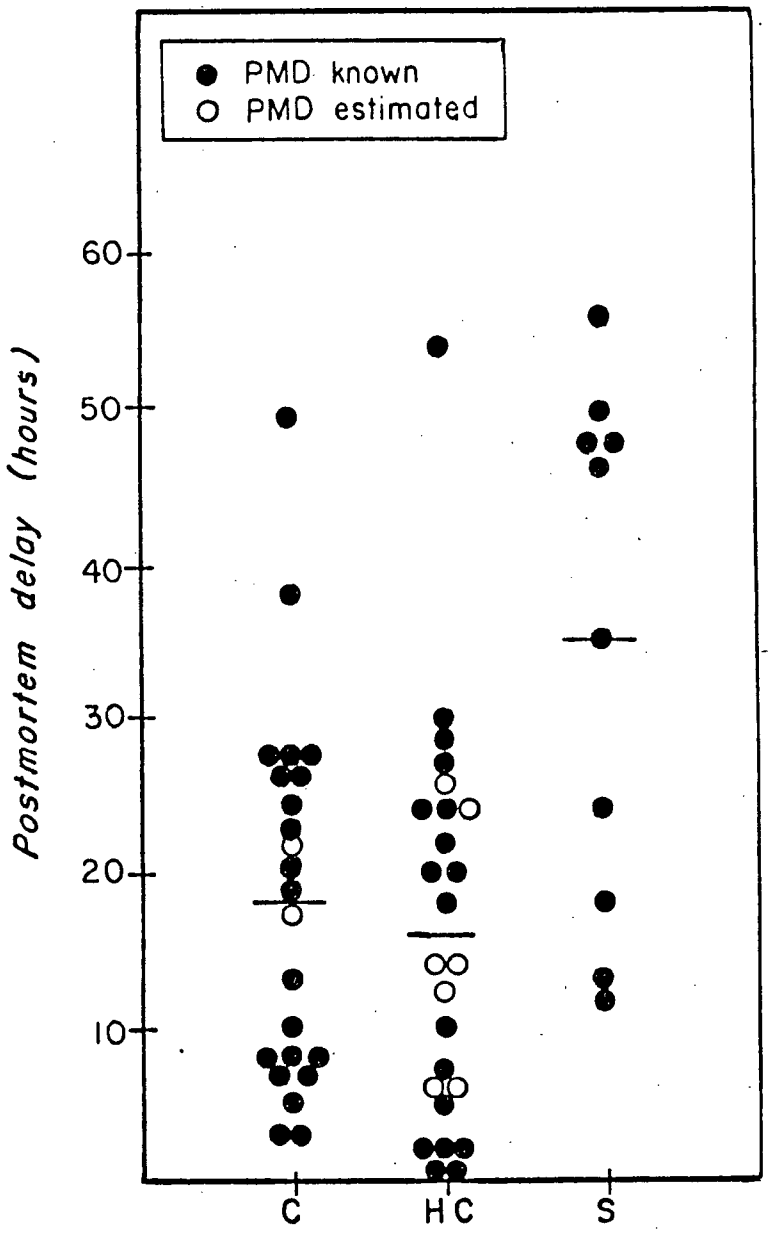
[1] including all individuals for whom both ILE and total GSH were known, except #39 - an extreme outlyer for ILE

FIGURE I.



Age distribution in controls, Huntington's choreics and schizophrenics

FIGURE II.



Postmortem delay distribution in controls, Huntington's choreics and schizophrenics

TABLE V: Estimates of PMD for 11 Individuals			
ID#	GSH*	ILE*	PMD' (hrs)
(C) 11	0.31	0.33	17
(C) 67	0.19	0.41	22
(HC) 21	0.97	0.19	6
(HC) 22	1.09	0.22	6
(HC) 31	0.55	0.29	12
(HC) 32	0.59	0.37	14
(HC) 44	0.25	0.22	14
(HC) 51	0.18	0.45	24
(HC) 53	0.65	0.51	26
(SL) 13	?	0.44	35**
(SL) 37	0	0.67	46

* μmoles/gm wet weight
** PMD estimated from regression of ILE on ln(PMD)

b) PMD - Group Differences

Calculations of mean PMD and comparisons among groups were made both with and without individuals for whom PMD had been estimated. Either way, schizophrenics had a significantly longer mean PMD than controls or Huntington's choreics. The latter 2 groups were not significantly different with respect to this variable. (See figure II and Tables VI and VII.)

TABLE VI: Mean PMD (hours) for Controls, Huntington's Choreics and Schizophrenics. (Without estimates of PMD)			
	C	HC	S
n	21	18	10
mean	18.0	16.4	35.0
SEM	±2.6	±3.2	±5.0
ANOVA on 3 means: $F_{2,46} = 6.48$ ($p < .005$)			
S vs. C ($P < .025$); S vs. HC ($p < .01$)			

TABLE VII: Mean PMD (hours) for Controls, Huntington's Choreics and Schizophrenics (Including estimates PMD)			
	C	HC	S
n	23	25	10
mean	18.1	15.9	35.0
SEM	±2.4	±2.4	±5.0
ANOVA on 3 means: $F_{2,55} = 8.07$ ($p < .001$)			
S vs. C ($p < .025$); S vs. HC ($p < .01$)			

3) Drug Histories (Summarized in Table VIII)

a) Controls

All 23 controls used for statistical analyses had been treated without drugs, or with diuretics or 'others'. All are placed under '-' in Table VIII.

b) Huntington's choreics

Four patients had been treated with tetrabenezines (similar to reserpine), lithium, valium, or miscellaneous drugs but not neuroleptics. These are placed under '-' in Table VIII. Nineteen patients had been treated with phenothiazines and/or HP, and are placed under '+' in Table VIII. One patient had been treated with phenothiazines for 2 years, but these had been discontinued for the last 6 years of life. This patient is listed as '+/-' in Table VIII. For one patient, nothing was known about the last 2 years of life, but at the time of death drugs were listed as 'none'. This patient is listed as '?' in Table VIII.

c) Schizophrenics

Nine patients had been treated with phenothiazines and/or HP for at least 1 year, and are placed under '+' in Table VIII. One patient was treated without neuroleptics and is listed as '-'.

d) Schizophrenic-like Psychotics

Three patients had been treated with phenothiazines and/or HP, and are listed under '+' in Table VIII. One patient had been treated with sedatives only, and is listed as '-'. For one patient, drug history was unknown.

4) Causes of Death

Causes of death for patients in each diagnostic group are tabulated in Table IX. Those listed toward the top are more likely to have involved a rapid death without prolonged hypoxia. Bronchopneumonia and hepatic coma would certainly have involved prolonged hypoxia.

5) Regions of Thalamus

Most samples had been taken from anterior medial thalamus, but some were from other regions. For several individuals, the region of thalamus sampled was not specified. The regions sampled, for individuals in each diagnostic group, are tabulated in Table X.

TABLE VIII: SUMMARY OF NEUROLEPTIC DRUG HISTORIES

	C	HC	S	SL
No neuroleptics (-).....	23	4	1	1
Treated with neuroleptics (+).....		19	9	3
(+/-) (see text).....		1		
History unknown (?).....		1		1
	23	25	10	5

TABLE IX: CAUSES OF DEATH

	C	HC	S	SL
Myocardial Infarction (MI)	6	2	1	1
Heart Attack	5			1
Aortic Aneurism	1			
Coronary Thrombosis	1			
Suicide (hanging)	1			1
Pulmonary Embolism	1		1	
Asphyxia		1	1	
Accident	1			
Peritonitis		1		
Asthma	1			
Congestive Heart Failure	1			
Cancer (caecum)	1			
Bronchopneumonia (BP)		17	3	1
Hepatic Coma	3			
"Others"			3	1
Unknown	1	4	1	
	23	25	10	5

TABLE X: REGIONS OF THALAMUS SAMPLED

	C	HC	S	SL
Anterior Medial (AM)	10	12	9	4
Anterior (A)		2		
Medial (M)	5		1	1
Lateral (Lat)	2			
Posterior Lateral (PL)		1		
Posterior (Post)		1		
Unknown (?)	6	9		
	23	25	10	5

B. The Amino Acids

1) GABA

a) Data Uncorrected for Age and PMD

The mean GABA concentration in the thalamus of schizophrenics and of Huntington's choreics was significantly lower than that of controls, when no correction was made for the effects of age and PMD. Schizophrenics and choreics did not differ significantly from each other. (See Table XI and Figure III.)

TABLE XI: Mean GABA Concentration (μmoles/gm wet weight) of Controls, Huntington's Choreics and Schizophrenics

	C	HC	S
n	23	25	10
mean	2.16	1.64	1.59
SEM	±.13	±.10	±.11

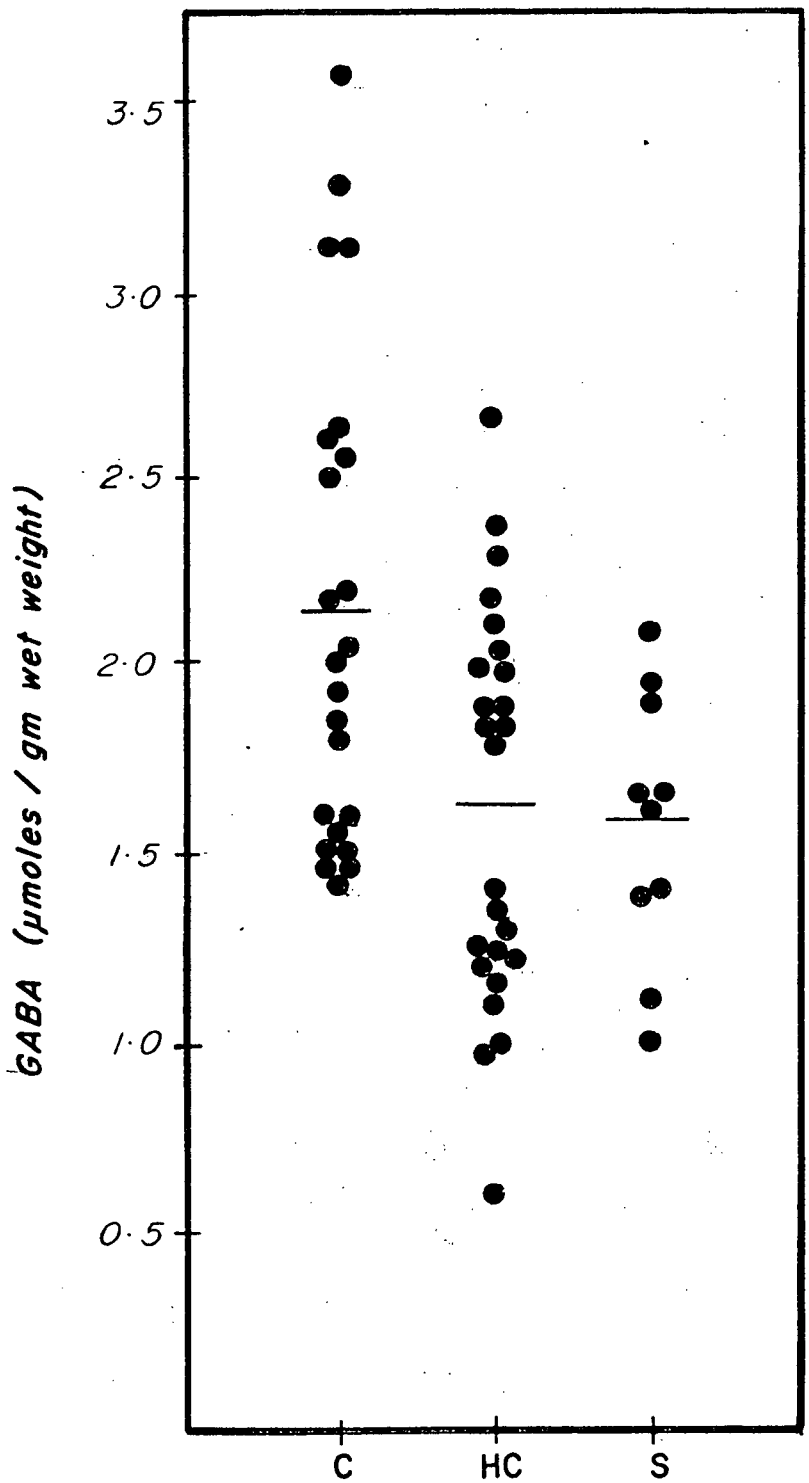
ANOVA on 3 means: $F_{2,55} = 6.41$ ($p < .005$)

C vs. HC ($p < .01$); C vs. S ($p < .001$); HC vs. S (NS)

b) Effects of Age and PMD on GABA

Initially, a multiple linear regression of GABA on age and PMD was carried out, and fit to this model was significant. It was decided, however, to do a log transformation of PMD, even though fit of control data to this model was not quite as good. The reasons were the following: (1) Analyses of pooled data for ILE and GSH had indicated that fit of data for these compounds to a natural log curve was better than to a linear curve. (2) Observations have suggested that GABA increases very rapidly in the first hour post-mortem, and very little after that (T.L. Perry, personal communication). It would make little biological sense for GABA to continue increasing at a constant rate post-mortem. (3) Linear and natural log curves are not very different

FIGURE III.



GABA concentration (uncorrected data) in controls, Huntington's choreics and schizophrenics

in the middle range of PMD values. See, for example Figure VI, a plot of THR vs. PMD with the best fitting linear and natural log curves through control data. The biggest differences between the two plots are in the extremes of the PMD distribution, and the linear curve is higher in both regions. Since a number of choreics had a very short PMD, and a number of schizophrenics had a very long PMD, their amino acid values might seem excessively low in relation to the linear plot through controls. The natural log transformation was seen as a more conservative estimate of the control mean. The regression analysis is shown in Table XII.

TABLE XII: Multiple Regression Analysis of GABA vs. Age and ln(PMD) in Controls (n = 21) for whom Age and PMD were Known

Source of Variation	df	SS	MS	F	r^2
Total	20	8.476			
Multiple Regression	2	3.221	1.610	5.516 ($p < .025$)	.380
Regression with Age	1	1.860	1.860	6.370 ($p < .025$)	.219
Regression with ln(PMD)	1	1.361	1.361	4.661 ($p < .05$)	.161
Residual	18	5.255	0.292		

The best fitting plane through GABA values, plotted against age and ln(PMD) was defined by the equation:

$$GABA' = 2.582 - .023(age) + .330(\ln(PMD))$$

The fit of this plane to observed GABA values was significant at the 2.5% level. Both independant variables (age and ln(PMD)) contributed significantly to the variation (linear decrease with age, logarithmic increase with PMD). The variables together accounted for 38% of the variation in GABA values in controls.

c) GABA - Differences Among Groups, Accounting for Age and PMD

Using the equation generated from the multiple regression analysis

for controls, an expected (control) value for GABA (GABA') was calculated for each individual. This value would lie on the plane, and would be the best estimate of GABA for an age- and PMD-matched control. Deviations of observed GABA values from GABA' were calculated. A negative deviation means that the observed value is less than the mean for an age- and PMD-matched control; that the observed value lies below the plane. The converse is true for a positive deviation. Table XIII has tabulated individuals for each diagnostic group, in order of magnitude of deviations from the plane. Details of all the independent variables are tabulated as well, for a visual inspection of potential relationships with high or low GABA.

Two types of analyses were carried out, each with and without individuals for whom PMD had been estimated. The first was an analysis of variance on deviations from the control mean (GABA-GABA') among 3 groups (C, HC, S). The second was a paired t test, between GABA and GABA', as if GABA' represented a matched control for each individual. Results are summarized in Tables XIV and XV. With or without individuals for whom PMD was estimated, there were significant negative deviations of HC and schizophrenic GABA values from values expected of controls. From the ANOVA, there were significant differences in mean deviation from expected, among the 3 groups. The differences were localized to between controls and choreics, and between controls and schizophrenics. Accounting for age and PMD, Huntington's choreics and schizophrenics have significantly lower mean GABA than controls.

TABLE XIII: Tabulated Variables for Each Individual, and Deviations of GABA Values from Expected GABA Values

	#	AGE (yrs)	PMD (hrs)	GABA μmoles gm w.w.	GABA' μmoles gm w.w.	GABA-GABA' μmoles gm w.w.	CAUSE OF DEATH	PART OF THAL	Rx
CONTROLS	8	75	26	3.12	1.96	1.16	MI	AM	-
	60	48	3	2.63	1.86	0.77	?	?	-
	63	40	27	3.29	2.77	0.52	MI	?	-
	61	21	5	3.12	2.64	0.48	suicide(hanging)	?	-
	3	56	10	2.51	2.08	0.43	MI	AM	-
	2	21	38	3.59	3.31	0.28	accident	AM	-
	65	63	7	2.05	1.80	0.25	aortic aneurism	MED	-
	7	70	26	2.21	2.08	0.13	heart attack	AM	-
	5	53	24	2.56	2.46	0.10	MI	AM	-
	10	55	49	2.62	2.63	-0.01	heart attack	AM	-
	4	74	20	1.87	1.90	-0.03	asthma	AM	-
	69	44	3	1.81	1.95	-0.14	hepatic coma	?	-
	59	59	13	1.93	2.10	-0.17	cong. heart	?	-
	62	66	8	1.61	1.78	-0.17	heart attack	?	-
	1	60	19	2.03	2.20	-0.17	coronary thromb.	MED	-
	67*	80	22*	1.49	1.80*	-0.31*	MI	AM	-
	11*	77	17*	1.47	1.78*	-0.31*	MI	AM	-
	70	58	8	1.61	1.96	-0.35	hepatic coma	MED	-
	68	74	23	1.49	1.95	-0.46	heart attack	LAT	-
	6	44	27	2.19	2.68	-0.49	heart attack	LAT	-
	66	52	7	1.55	2.05	-0.50	Ca (caecum)	MED	-
	9	73	27	1.48	2.01	-0.54	pulmonary embol.	AM	-
	64	31	8	1.45	2.57	-1.12	hepatic coma	MED	-

* PMD estimated

Abbreviations:

w.w. = wet weight

GABA' = expected GABA concentration for control with given age and PMD
= 2.582 - .023(age) + .330 (ln(PMD))

THAL = Thalamus

AM = anterior medial, P = posterior,

MED = medial, A = anterior, PL = posterior lateral

Rx = treatment with neuroleptics

cong. heart = congestive heart failure

Ca = cancer

pulmonary embol. = pulmonary embolism

coronary thromb. = coronary thrombosis

TABLE XIII: continued

	#	AGE (yrs)	PMD (hrs)	GABA µmoles gm w.w.	GABA' µmoles gm w.w.	GABA-GABA' µmoles gm w.w.	CAUSE OF DEATH	PART OF THAL	Rx
HUNTINGTON'S CHOREICS	33	64	1	2.30	1.14	1.16	BP	?	+
	45	57	1	2.19	1.29	0.90	BP	?	+
	21*	72	6*	2.39	1.55*	0.84*	?	AM	-
	54	68	5	2.13	1.58	0.55	BP	?	?
	31*	60	12*	2.05	2.05*	0.00*	BP	AM	-
	25	68	20	2.00	2.04	-0.04	peritonitis	AM	-
	22*	55	6*	1.89	1.93*	-0.04*	BP	AM	+
	27	72	28	1.85	2.06	-0.21	MI	AM	+
	52	26	24	2.67	3.04	-0.37	?	P	+
	53*	55	26*	2.01	2.42*	-0.41*	asphyxia	?	+
	28	57	22	1.81	2.32	-0.51	BP	AM	+
	57	44	2	1.24	1.82	-0.58	BP	?	+
	49	35	7	1.85	2.43	-0.58	BP	A	+
	46	39	2	1.24	1.93	-0.69	BP	?	+
	44*	62	14*	1.25	2.05*	-0.80*	MI	AM	-
	58	61	2	0.63	1.43	-0.80	?	?	+
	55	51	54	1.89	2.75	-0.86	BP	?	+
	24	62	18	1.26	2.14	-0.88	?	AM	+
	32*	54	14*	1.34	2.23*	-0.89*	BP	AM	+
	42	58	10	1.11	2.03	-0.92	BP	AM	+
	51*	53	24*	1.40	2.43*	-1.03*	BP	AM	+
	29	58	27	1.30	2.36	-1.06	BP	A	+
	30	53	29	1.18	2.50	-1.32	BP	AM	+
	26	43	24	0.99	2.66	-1.67	BP	PL	+
	47	38	20	1.01	2.71	-1.70	BP	?	+/-

* PMD estimated

TABLE XIII: continued

	#	AGE (yrs)	PMD (hrs)	GABA µmoles gm w.w.	GABA' µmoles gm w.w.	GABA-GABA' µmoles gm w.w.	CAUSE OF DEATH	PART OF THAL	Rx
SCHIZOPHRENICS	16	70	12	2.11	1.82	0.29	others	AM	+
	40	88	18	1.39	1.55	-0.16	others	AM	-
	18	80	35	1.67	1.95	-0.28	BP	AM	+
	15	64	13	1.62	1.98	-0.36	pulmonary embol.	AM	+
	19	57	50	1.96	2.57	-0.63	asphyxia	AM	+
	50	52	24	1.67	2.46	-0.79	MI	M	+
	17	54	48	1.91	2.64	-0.73	others	AM	+
	20	72	56	1.42	2.29	-0.87	BP	AM	+
	12	56	48	1.13	2.60	-1.47	BP	AM	+
	14	56	46	1.02	2.58	-1.56	?	AM	+
PSYCHOTICS	41	92	8	2.04	1.19	0.85	MI	AM	-
	36	64	10	1.98	1.90	-0.16	heart attack	AM	+
	35	44	17	2.36	2.52	-0.16	others	AM	+
	37*	47	45*	1.74	2.78*	-1.04*	suicide	AM	+
	13*	52	35*	1.03	2.58*	-1.55*	BP	M ED	?

*PMD estimated

TABLE XIV: Mean GABA Deviations (umoles/gm wet weight) of Controls, Huntington's Choreics and Schizophrenics (Without estimates of PMD)

	C	HC	S
n	21	18	10
mean	0.0	-0.53	-0.66
SEM	$\pm$.11	$\pm$.18	$\pm$.17

ANOVA on 3 means: $F_{2,46} = 5.16$ ($p < .01$)

C vs. HC ($p < .05$); C vs. S ($p < .025$); HC vs. S (NS)

Paired t Test (GABA vs. GABA') C: $t_{20} = -0.01$ (NS)
HC: $t_{17} = -2.87$ ($p < .01$)
S: $t_{9} = -3.63$ ($p < .01$)

TABLE XV: Mean GABA Deviations (μmoles/gm wet weight) of Controls, Huntington's Choreics and Schizophrenics (Including Estimates of PMD)

	C	HC	S
n	23	25	10
mean	-0.03	-0.48	-0.66
SEM	$\pm$.10	$\pm$.15	$\pm$.17

ANOVA on 3 means: $F_{2,55} = 4.88$ ($p < .025$)

C vs. HC ($p < .05$); C vs. S ($p < .05$); HC vs. S (NS)

Paired t Test (GABA vs. GABA') C: $t_{22} = -.269$ (NS)
HC: $t_{24} = -3.20$ ($p < .01$)
S: $t_{10} = -3.63$ ($p < .01$)

2) Glycerophosphoethanolamine (GLYC-PEA)

A multiple linear regression of GLYC-PEA on age and PMD was non-significant for controls, therefore comparisons were made among groups with uncorrected data. Huntington's choreics had a mean GLYC-PEA concentration significantly higher than that of controls. The mean for schizophrenics was intermediate between controls and choreics, not

significantly different from either. (See Table XVI and Figure IV)

TABLE XVI: Mean GLYC-PEA Concentration (μmoles/gm wet weight) in Controls, Huntington's Choreics, Schizophrenics and Schizophrenic-Like Psychotics

	C	HC	S	SL
n	23	25	10	5
mean	0.79	1.25	0.92	0.70
SEM	±.12	±.08	±.07	±.03

ANOVA on 4 means: $F_{3,59} = 7.51$ ($p < .001$)

C vs. HC ($p < .001$); all other pairwise comparisons NS

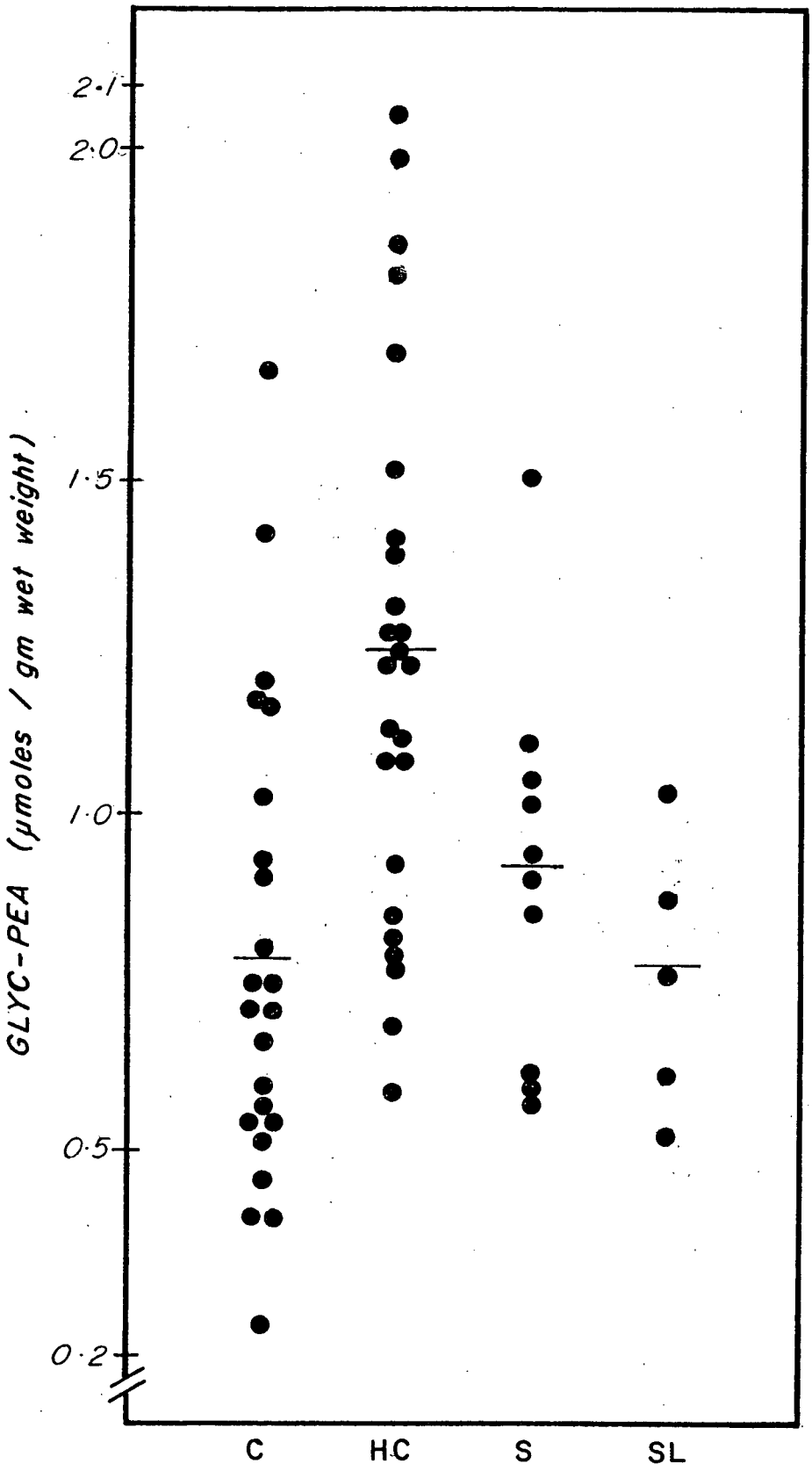
3) Homocarnosine (HCARN)

A multiple linear regression of HCARN on age and PMD for controls was non-significant, therefore comparisons were made among groups with uncorrected data. One schizophrenic patient had a value for HCARN more than 3 standard deviations beyond the mean for controls (1.58 umoles/gm wet weight; see Figure V). Analyses were carried out with and without this value. The latter is summarized in Table XVII. With the outlyer, the schizophrenic mean was elevated to 0.49 (± .12) which was not significantly different from controls. The overall ANOVA on 4 means was correspondingly less significant ($F_{3,58} = 3.68$ ($p < .01$)). The mean HCARN concentration for Huntington's choreics and for 9 out of 10 schizophrenics was significantly lower than that for controls.

4) Amino Acids Showing no Significant Linear Change in Controls (n = 21) with Age (range, 21-80 years) or PMD (range, 3-49 hours) and no Significant Differences Among Diagnostic Groups

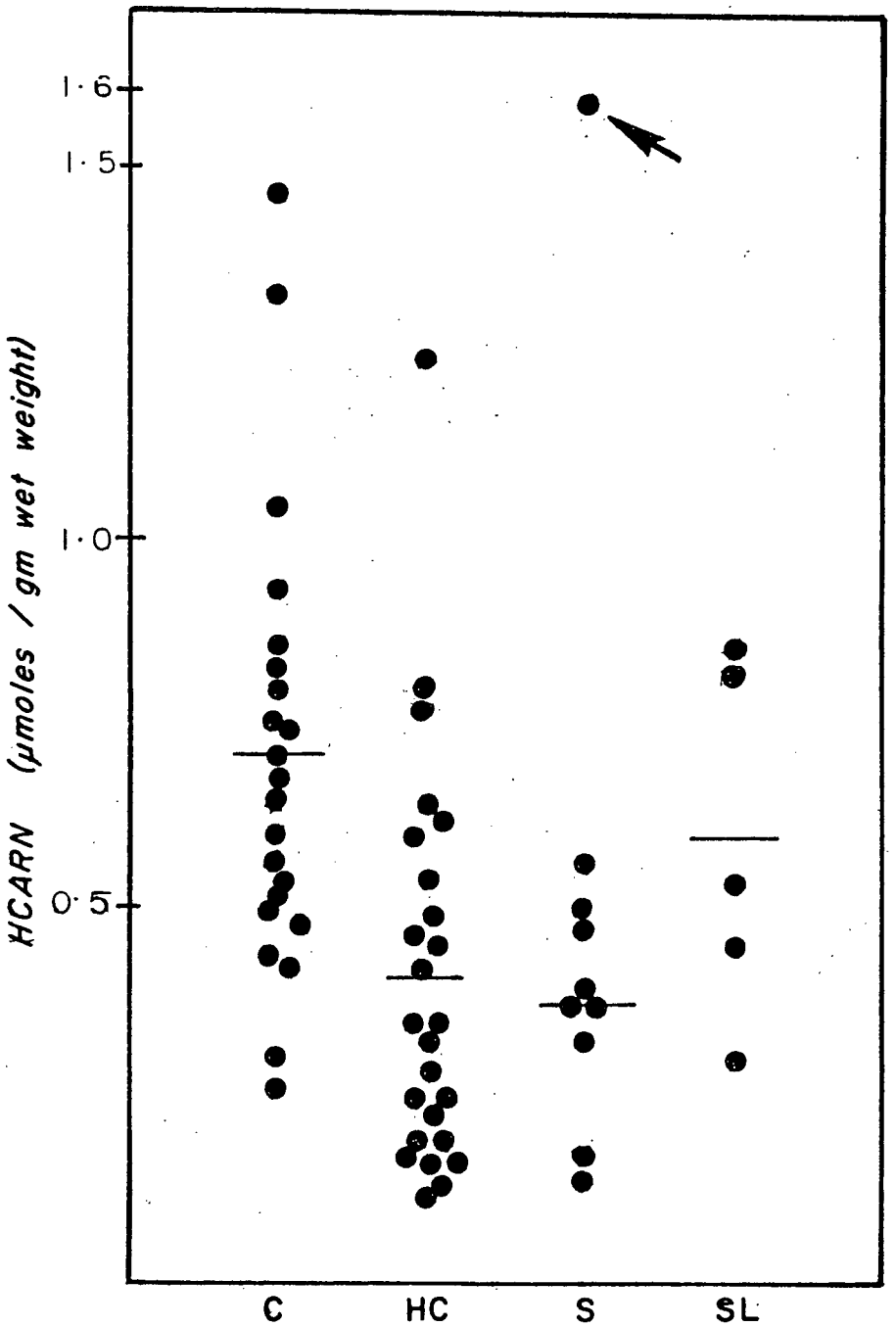
The multiple linear regression in controls was non-significant for several other amino acids, therefore comparisons among groups were made with uncorrected data. There were no significant differences among the 4 diagnostic groups for TAU, GLU, CYSTA, $(CYS)_2$, PHE, TRP, or

FIGURE IV.



GLYP-PEA concentration in controls, Huntington's choreics, schizophrenics and psychotics.

FIGURE V.



Homocarnosine (HCARN) concentration in controls, Huntington choreics, schizophrenics and psychotics.

TABLE XVII: Mean HCARN Concentration (μmoles/gm wet weight) in Controls, Huntington's Choreics, Schizophrenics and Schizophrenic-like Psychotics

	C	HC	S	SL
n	22	25	9	5
mean	0.70	0.41	0.37	0.59
SEM	±.06	±.05	±.04	±.09

ANOVA on 4 means: $F_{3,57} = 5.939$ ($p < .005$)

C vs. HC ($p < .01$); C vs. S ($p < .025$); all other pairwise comparisons NS

GABA-LYS. Means are summarized in Table XVIII. Since there were no no significant differences among groups, data for these amino acids were pooled and submitted to a linear/quadratic regression analysis. With this, $(CYS)_2$ showed a combined linear increase and quadratic decrease with PMD ($r^2 = .26$), suggesting that it increased initially and then plateaued. PHE showed a straight linear increase with PMD ($r^2 = .29$). Other amino acids of this group still showed no significant change with age (range, 21-92 years) or PMD (range, 1-56 hours).

TABLE XVIII: Amino Acids Showing no Significant Linear Change in Controls (n = 21) with Age (range, 21-80 years) or PMD (range 3-49 hours) and no Significant Differences Among Diagnostic Groups

	Mean Concentration (± SEM) (μmoles/gm wet weight)			
Amino Acid	C	HC	S	SL
TAU	0.83 (±.06)	1.03 (±.09)	0.88 (±.07)	0.93 (±.09)
GLU	7.94 (±.21)	8.38 (±.53)	8.10 (±.29)	8.80 (±.19)
CYSTA	0.89*(±.07)	1.01 (±.12)	1.09 (±.14)	1.35 (±.19)
$(CYS)_2$	0.23 (±.02)	0.19 (±.02)	0.25 (±.02)	0.32 (±.01)
PHE	0.34 (±.02)	0.28 (±.02)	0.34 (±.02)	0.38 (±.03)
TRP	0.07 (±.01)	0.07 (±.01)	0.09 (±.01)	0.10 (±.01)
GABA-LYS	0.07 (±.01)	0.05 (±.02)	0.02 (±.01)	0.03 (±.01)

* excluding 3 individuals who died in hepatic coma

5) Amino Acids Showing a Significant Linear Change in Controls (n = 21) with PMD (range, 3-49 hours) but not with Age (range, 21-80 years)

The majority of amino acids showed a significant linear increase in controls between 3 and 49 hours post-mortem. These are listed in Table XIX, in decreaseing order of r^2 (the coefficient of determination, which indicates the proportion of the total variance accounted for by a linear regression with PMD). Total GSH and PEA showed significant linear decreases in this range. If group differences were to be examined for these amino acids, correction would have to be made for the influence of PMD on amino acid concentrations. Figure VI illustrates the change of THR with PMD.

6) Amino Acids Showing Significant Linear Changes in Controls (n =21) With Both PMD (range, 3-49 hours) and Age (range, 21-80 years)

Both age and PMD contributed significnatly to the control variation in ORN, HIS, and TYR concentrations (as well as GABA). Coefficients of determination are listed for each variable, for each amino acid, in Table XX.

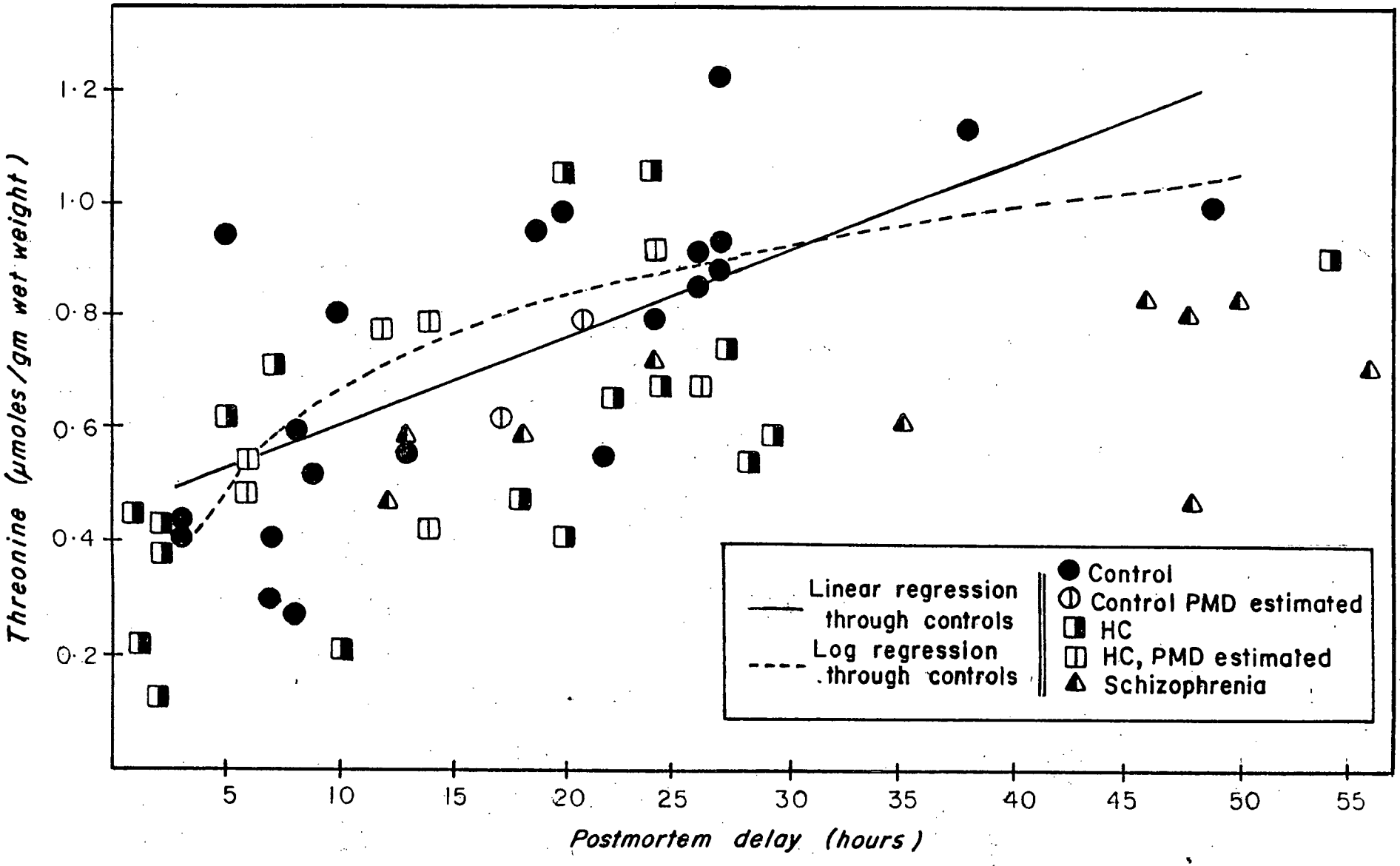
TABLE XIX: Amino Acids Showing a Significant Linear Change in Controls (n = 21) with PMD (range, 3-49 hours) but not with Age (range, 21-80 years)

	Amino Acid	Coefficient of Determination (r^2)	Null Probability (p)
Linear Increase	EA	.737	< .001
	VAL	.571	< .001
	PRO	.550	< .001
	SER	.506	< .001
	THR	.505	< .001
	LEU	.502	< .001
	ILE	.486	< .001
	MET	.461	< .001
	LYS	.449	< .001
	GLY	.398	< .005
	ASP	.375	< .005
	ALA	.268	< .025
	ASN*	.192	> .05
	GLN*	.190	> .05
	ARG*	.166	> .05
Linear Decrease	GSH (TOTAL)	.432	< .005
	PEA	.254	< .025

TABLE XX Amino Acids Showing Significant Linear Changes in Controls (n = 21) with Both PMD (range, 3-49 hours) and Age (range, 21-80 years)

Amino Acid	Linear Decrease With Age		Linear Increase With PMD		Multiple Linear Regression	
	r^2	p <	r^2	p <	r^2	p <
ORN	.229	.005	.416	.001	.645	.001
HIS	.222	.005	.222	.005	.445	.005
TYR	.153	.05	.235	.025	.388	.025

FIGURE VI. Threonine vs. postmortem delay



CHAPTER 8

DISCUSSION

The key findings of this study were (1) a deficiency of GABA in the thalamus of Huntington's choreics and schizophrenics, (2) a deficiency of the GABA-containing dipeptide, homocarnosine, in choreics and 9 out of 10 schizophrenics (1 schizophrenic had excessively high HCARN) and (3) an elevated concentration of GLYC-PEA in Huntington's choreics. Compounds which were examined, but showed no differences among groups were: TAU, GLU, CYSTA, $(CYS)_2$, PHE, TRP, and GABA-LYS. There were indications that GABA-LYS might correlate with GABA and HCARN, and thus be reduced in choreics and schizophrenics as well, but its mean concentration was too low for group differences to be extracted with these techniques.

All of the findings with respect to HC are in accord with studies of amino acids in other parts of HC brain (Perry et al., 1973a,b; Urquhart et al., 1975). No other studies of amino acids in schizophrenic brain have been published to date. A concurrent study of nucleus accumbens demonstrated a similar, but more striking deficiency of GABA in schizophrenics and choreics[1].

Unfortunately, human material in general, and autopsied brain in particular comprises a poor system for experimental design. It is extremely difficult to control for a number of variables, besides diseases being studied, that may influence the biochemical parameters under investigation. Statistics can, in some cases, mimic the controls of experimental design, but problems are encountered when independent variables are not distributed homogeneously among groups.

The first independent variable considered was age. Significant linear decreases with age, in control thalami, were observed for GABA, ORN, HIS, and TYR. Similar decreases of other amino acids may have been masked by the

[1] to be presented at NINCDS Huntington's Disease Symposium, San Diego, November 16 - 18, 1978

over-riding influence of PMD. In light of the finding of decreased GABA with age, data for a number of other brain regions were examined. Linear regression of GABA with age was significant in frontal cortex, and not in occipital cortex, caudate, putamen-GP, or SN. It is interesting to compare these findings to those of McGeer and McGeer (1976b) who found the most striking decrease of GAD with age to be in the thalamus.

The second variable was post-mortem delay. Significant linear increases with PMD in controls were observed for the majority of amino acids: EA, VAL, PRO, SER, THR, LEU, ILE, MET, LYS, GLY, ASP, ALA, ORN. HIS, TYR, and GABA. In a pooled sample, $(CYS)_2$ and PHE also increased significantly. Significant decreases with PMD were observed for total GSH and PEA. There were no significant linear changes in TAU, GLU, CYSTA, TRP, GABA-LYS, GLYC-PEA or HCARN between 1 and 56 hours post-mortem. ASN, GLU, and ARG showed nearly significant linear decreases with PMD in controls (range, 3-49 hours).

These findings with respect to post-mortem changes do not correspond exactly with findings of Perry et al. (1971b), who compared biopsied and autopsied cortex specimens. They noted no significant differences for GLYC-PEA, TAU, PEA, TRP, ORN, and CYSTA, between 8 biopsied and 5 autopsied specimens. GSH and GLN were lower in autopsied cortex, while other amino acids were strikingly higher. The differences between these studies may stem from from the different delay periods being examined, and the nature of the change in any given amino acid. The delay in the present study (for controls) ranged from 3 to 49 hours. A rapid and marked change during the first 3 hours, followed by a relative plateau, would appear as no significant change with PMD in this analysis. Further, other models, such as logarithmic transformations or power curve fits were not tested for most amino acids. Such other models would likely be more appropriate, but a linear model is probably not a bad approximation in most cases. It is a matter of concern that the distribution of PMD was not the same in the 3 diagnostic groups.

Extrapolation of the control curve to account for choreics and schizophrenics in the extremes of the distribution was not entirely appropriate. On the other hand, a log transformation was seen as a conservative means of coping with the variable, for reasons previously discussed, and also because a large change in PMD at the higher end of the distribution would make little difference in the correction factor for GABA. Clearly an experiment designed to test the change in amino acid concentrations with PMD, without other confounding variables, needs to be carried out.

Differences in cause of death among the 3 groups are also a matter of concern, particularly following the results of Bird et al. (1977) and Iversen et al. (1978) which suggested that decreased GAD in various parts of schizophrenic brain was no longer significant when cases involving pre-mortem hypoxia were excluded. No conclusions can be drawn from the present study about the possible effect of pre-mortem hypoxia on amino acids, but it is quite possible that this variable is influential, and could account for part of the observed group differences.

The effect of neuroleptic drugs was seen as a potential candidate for causing differences in GABA concentrations among groups. As discussed previously, this variable was highly confounded with diagnosis, and could not be separated adequately. The chronic drug experiments on rats (Lloyd and Hornykiewicz, 1977; Perry et al. [1]) suggested, however, that CPZ and HP are not the cause of decreased GABA in choreics and schizophrenics.

Were this experiment to be done again, it would be better to restrict samples to one part of the thalamus, or to use a homogenate of whole thalamus.

[1] to be presented at NINCDS Huntington's Disease Symposium, San Diego, November 16-18, 1978.

Most samples were from anterior medial thalamus. Those that were not may have contributed to the variation in amino acids, but were sufficiently few that they would not likely have been the cause of group differences.

In conclusion, GABA concentration was significantly decreased in the thalamus of schizophrenics and Huntington's choreics, whether or not variation with age and post-mortem delay were accounted for. This decrease could be a result of the disease process, but could also appear because of the high frequency of bronchopneumonia (and therefore pre-mortem hypoxia) as cause of death in these groups. The possible effect of neuroleptic drugs can also not be entirely ruled out. Homocarnosine was also lower in these 2 groups, although one schizophrenic had an extremely elevated concentration. Glycerophosphoethanolamine was elevated in choreics.

It is hoped that these findings will contribute to an understanding of the biochemical nature of these two horrendous diseases, and of the possible relationship between them. That may in turn contribute to an understanding of etiological and pathogenetic factors, and suggest appropriate intervention.

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