A Study on Morphology and Acetylcholinesterase Distribution in the Central Nervous System of Six Genera of Mice

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A STUDY ON MORPHOLOGY AND
ACETYLCOLINESTERASE DISTRIBUTION
IN THE CENTRAL NERVIOUS SYSTEM
OF SIX GENERA OF MICE

BY
ROY J. BETTI

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Faculty of the Graduate School of
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Master of Science

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To my parents for giving me this opportunity and to Mary Ann for her understanding and invaluable help without which this thesis could not have been written.
BIOGRAPHY

Roy J. Betti was born in Chicago, Illinois on October 14, 1943. He graduated from Fenwick High School (Oak Park, Illinois) in 1961 and, subsequently, from Loyola University (Chicago, Illinois) in 1965. During the last four years, he has been a student at Stritch School of Medicine (Chicago).

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Mr. Betti was married to the former Mary Ann Bandale in December of 1967.
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A. Introductory Remarks

"I have shown that the brains of domestic rabbits are considerably reduced in bulk, in comparison with those of the wild rabbit or hare; and this may be attributed to their having been closely confined during many generations, so that they have exerted their intellect, instincts, senses and voluntary movements but little."

Charles Darwin, 1874.

As shown by this quotation, it has long been speculated that the use of the brain might lead to changes in its size, in the interconnections of its cells, and in its chemical composition. In the last century such speculation led to research on brains of men attempting to correlate the anatomical with degree of intellectual attainment (Clark, 1895; Leeds, 1925). Gradually, however, it was realized that there were many exceptions to the rule that geniuses had large brains and idiots had small brains. The hypothesis of an intrinsic relation between brain size and cerebral exercise or ability was therefore generally abandoned. In its place the more modern theory that changes in neural interconnections or chemical mediators in the brain were the basis for behavioral differences. It is precisely with this hypothesis that this thesis is concerned. In part this is an attempt to clarify this
aspect of the cerebral mechanisms underlying psychological processes.

B. The Anatomical Considerations

1. Anatomy and Behavior

The study described here attempts to explore the association between brain anatomy and behavior. The literature on this subject is amazingly scanty. Studies on anatomical variations have been set aside in favor of those on brain chemistry. Nevertheless, it is the anatomical location which assumes prime importance in reference to the function and pathways of various brain enzymes. It is apparent that only anatomy and chemistry in combination will lead us to definitive answers.

Experiments in which there is controlled variation of the environment of animals (Rosenzweig, 1962) during the 30 days after weaning have shown that there is a significant concomitant alteration of the weight of the cerebral cortex. A number of investigators have speculated that repetitious sensory inputs may induce neurons to strengthen existing synaptic connections or to develop new ones (Hebb, 1957; Hebb and Krnjevic', 1962). A neuron with a larger number of functional connections would possibly require a more active perikaryon as the site for the production of more neural transmitters and breakdown
enzymes, and this might demand an increase in brain size. Any increase in the number of glia, size of the perikaryon, or perhaps also capillary diameter or number of neuronal branches could account for the increased bulk of the cortex seen in these experiments (Diamond, 1964).

The changes that take place in the organization of the cerebral cortex during development have been studied by many workers. For example, histological changes have been studied by Bolton (1903), Cajal (1911) and Lorente de Nó (1933), to name only a few classical cytologists. Haddara (1966) has shown that the increase in the volume of the cortex as age advances is accompanied by the spacing out of cortical neurons. He found that the packing density of the neurons of mice decreases rapidly between the third and seventh days after birth and henceforth more slowly. No change in packing density took place after the seventeenth day. These histological changes were rarely correlated with behavioral differences. It was not until the introduction of acetylcholine (Ach) and acetylcholinesterase (AchE) into brain research that behavior was talked about in terms of anatomy (Rosenzweig, 1960; Carlton, 1963).

2. **Anatomy and AchE**
The correlation between AchE activity and brain morphology has been previously reported upon by other investigators (Bennett et al., 1958; Diamond et al., 1963; Kling et al., 1965; Papp and Bozsik, 1966; and Pryor et al., 1966)

The enzyme AchE, which is known to catalyze the hydrolysis of Ach, is present in large quantities in the central nervous system. It is probably abundant in areas of the brain which normally have intense neural activity (Tower, 1958). It is concentrated at axonal endings (Geiger and Stone, 1962), probably along the synaptic membrane (de Roberts et al., 1963). Rosenzweig, Krech, and Bennett (1958) reported that in rats AchE activity is much the same in the two hemispheres, but varies significantly from area to area within the hemisphere. The motor cortex has higher AchE activity than the somatosensory cortex which in turn has higher activity than the visual area. These investigators also found significant individual differences in mean AchE activity. AchE activity increases with age, up to about 100 days in rats, and then declines, since brain weight increases progressively, there is no decline in total AchE activity (Bennett, Rosenzweig, Krech, Karlsson, Dye and Ohlander, 1958). The activity of AchE was reported per unit of tissue weight. Since the weight of the brain
continues to grow steadily, this does not necessarily imply that the total AchE activity of the cortex declines. If cortical AchE activity per unit weight is multiplied by cortical weight, these determinations indicate that the total cortical AchE activity remains essentially constant after 100 days. Since individual variation in brain chemistry is small (Jackson, 1913), these studies possibly might suffer from the fact that small differences may be attributed to errors of measuring brain weight and AchE activity. The change of Ach content with age in whole brain is asserted by Welsh and Hyde (1944) to be closely correlated with that of cholinesterase (ChE) activity. They as well as Crossland (1960) found that Ach doubles from 14 hours to 21 days and doubles again when the rat is "100+" days old. While the direction of change is the same for Ach and ChE, it appears that ChE activity increases more rapidly than Ach up to 21 days and then more slowly than Ach.

Bennett et al. (1958a) have shown that the rat brain AchE activity and brain weight differ according to genetic background, age and individual factors. Several workers (Elkers and Todrick, 1955; Cohn and Richter, 1956) have shown that subcortical centers reach their maximum AchE activity at an earlier age than does the cortex. These
investigators studied AchE activity in various areas of the developing rat brain at 8, 22, and 77 days. Of course, these investigators are not suggesting that AchE and "intelligence" are related genetically, but the possibility is open for further investigation.

Some of the areas studied by these authors were identical to those studied in this investigation, i.e., cerebral cortex, caudate nucleus, medulla, olfactory bulb, cerebellum, and thalamus. Within all these areas a rise in enzyme activity between the 8th and 22nd day occurs but between the 22nd and 77th day a different pattern emerges. The activity in caudate nucleus, olfactory bulb and cerebral cortex continues to rise. There is no change in the thalamus, and the medulla and cerebellum even show a fall in activity. The various layers of the cerebellar cortex for all species was shown to have a much lower activity than that of other structures of the nervous system (Goldberg and McCaman, 1967).

C. Brain Anatomy, AchE and Behavior

The study described here is an attempt to explore the association between brain anatomy, brain chemistry, and behavior. Perhaps differences in behavior between genera can to some extent be attributable to gross differences in brain structure, at least within the normal phenotypic variation
of behavior and brain structure. Since the behavior of an animal is in part controlled by the brain, it appears that the constituents of this structure should be investigated. However, it was probably the difficulty of clear results in this regard which brought the brain enzymes into serious consideration.

Previous studies have demonstrated the existence of reliably measureable individual, strain and age differences in AchE activity of the rat brain (Bennett, Rosenweig, Krech, Karlsson, Dye and Ohlander, 1958a). More importantly these individual differences in AchE activity have been shown to be related to the adaptive behavior of the rat (Rosenzweig, Krech and Bennett, 1958).

The role of genetic factors can only be speculative. For instance, the genetic predisposition which results in certain behavioral characteristics may be expressed by the neurochemistry of the brain (Stanburg et al., 1960; Harris, 1963; and Scudder et al., 1966a, 1966b).

In the earliest experiment involving the effects of cholinergic manipulations on behavior (Essig et al., 1950), found that intracarotid injections of DFP in cats, dogs, rabbits, and monkeys induce contraversive circling, i.e., turning away from the injected side. In all animals that
circle, ChE activity is found to be lower in the cortex and caudate nucleus on the injected side than on the other. Ach would thus be dominant in that hemisphere and could be expected to stimulate circling. Injections of atropine and scopolamine were found to eliminate the circling.

Several investigators have found that modifying the amount of experience in a single sense modality can effect rather specifically the brain regions serving that modality. Further results suggest that impairment of one sensory channel leads to greater use of other modalities and thus to greater cerebral development in the corresponding brain areas. For example, if blinded or light-deprived animals are raised in a complex environment, the somesthetic area of the cortex shows increase in weight and total acetylcholinesterase activity (Bennett et al., 1964). Liberman (1962) found that rats raised in darkness have significantly lower AchE activity in the retina than normal rats. Unilateral lesions in the visual and somesthetic cortex of rats are followed by a significant increase in the AchE activity in the contralateral hemisphere, perhaps due to a rerouting of brain activity (Krech, Rosenzweig, and Bennett, 1960). This then suggests the importance of sensory input with regard to the enzymatic development of the brain.
Chronically reduced ChE levels were maintained in rats (Russell, 1958, 1960) by placing "Systox", an organophosphorous anticholinesterase, in their food. He reported that behavior passed through several stages as ChE activity was progressively reduced: When activity was 60-100% of normal, no significant behavioral effects were observed. There was a suggestion of increased behavioral efficiency when ChE activity was 40-60% of normal, that is, the animals were more efficient in serial problem-solving, and more efficient in adjusting to stresses imposed by the environment. In other behavioral patterns, including locomotion, simple learning, instrumental conditioning, and visual discriminations, their performances did not differ significantly from those of control animals. Below 40%, there was a rapid loss of efficiency, followed by convulsions and death. Animals in the 20-40% range were reported to be slower in eliminating responses which had previously been learned and less efficient in adjusting to stresses imposed by the environment.

It has been postulated (Carlton, 1961, 1963) that a cholinergic system in the brain antagonizes a second system which activates behavior, and predicted therefore that increased activation and decreased cholinergic activity
should produce qualitatively similar effects. He found that amphetamine increased the response rate of rats in an operant shock-avoidance situation, and that atropine and scopolamine (anticholinergics) augmented the action of amphetamine.

D. Mouse Brain Atlases

A stereotaxic atlas of the mouse brain has been reported in abstract form only by Slotrick and Essman (1964) and Ehrlich (in preparation). The brain of the mouse is in many respects a smaller edition of the widely studied rat brain (Green, 1966). Guides to the rat brain (Zeman and Innes, 1963; Konig and Klippel, 1963) can be used for general orientation, but these are not accurate for stereotaxic placement of leisons or electrodes. The histological regions of the mouse cortex have been described (Rose, 1929) and detailed accounts have been given of the cellular arrangement in the entorhind cortex and the hippocampus (Lorente de No', 1933, 1934).
Materials and Methods

A. The Mice

1. Introductory Remarks and Ecological Description

The brains used in this study were from six different genera of mice: three strains of *Mus*, *Mus musculus* C57BL/6J, *Mus musculus* CF-1, and *Mus musculus* 'Mo.'; *Microtus ochrogaster*; *Onychomys leucogaster*; *Perognathus longimembris Bailli*; two strains of *Peromyscus*; *Peromyscus maniculatus Bairdii* and *Peromyscus maniculatus Colorado*; and *Reithrodontomys raviventris Raviventris*. *Microtus*, *Onychomys*, *Perognathus*, *Reithrodontomys*, and *Mus musculus 'Mo.'* mice were trapped by collectors, *Microtus* and *Mus* in a field in the vicinity of Columbia, Mo., *Onychomys* in the arid area near Tucson, Arizona, *Perognathus* from the prairies of Utah, and *Reithrodontomys* from the salt marshes of the San Francisco Bay area.

Phylogenetically, the genera *Reithrodontomys*, *Peromyscus*, and *Onychomys* appear most closely related to each other, belonging to the same Sub-family, Cricetinae (Hall, 1902). The genus, *Microtus*, a near relative, also belongs to the same Family as the three above genera, Cricetidae. However, *Perognathus* and *Mus* are more distantly related, each being
members of separate Families, the former to Heteromyidae and the latter to Muridae. All of the above genera are members of the Order, Rodentia, and the Sub-order, Sciuromorphae.*

The trapping period lasted about two weeks; as soon as they were trapped, the males were isolated from the females. Peromyscus and the inbred Mus strain C57BL/6J were obtained from Roscoe B. Jackson Memorial Laboratory. CF-1 mice were purchased from Carworth Farm.

All the mice were shipped to this laboratory by Air Express. Upon arrival, the mice were mated and treated routinely for ectoparasities. The mice were completely dipped into a solution of aramite (20 gms./1000 cc.) for several minutes. Following a two week post-reception period, they were placed, still paired, in an environmental chamber under controlled conditions of temperature (75°F ± 5°F) and humidity. They were housed, one pair, per cage, in transparent plastic 12 in. x 7 in. x 5 in. cages, with sawdust on the cage floor. Regular laboratory mouse pellets were provided in hampers, water was supplied ad libitum. The cages were cleaned weekly.

Both the domestic and feral strains of Mus are well

* We are indebted to Dr. D. M. Cameron, Jr., Dept. of Zoology, University of California, for taxonomic identification of these strains from the skulls and pelts which we provided.
known in the behavioral field. They are generally docile, easy to breed, and readily adaptable to laboratory studies. *Mus musculus C57BL/6J* is a shy and avoiding inbred strain, which is slightly more intelligent (Scudder et al., 1966b) than *Mus musculus C5-1*, an aggressive inbred strain. *Mus musculus "Mo."*, a feral strain, shows a high rate of avoidance conditioning and selective aggression.

*Microtus ochrogaster* (the meadow vole) is a large and heavy animal which was shown to be a poor learner in a previous paper from this laboratory (Scudder et al., 1968c). This genus usually prefers sparsely covered areas and is a hoarding communal species.

*Onychomys leucogaster* (the Northern Grasshopper mouse) was first described in detail by Bailey (1929). It is a carnivorous, insectivorous animal which is not colonial but is readily sociable. It will seldom fight members of the same species and paradoxically will either submit well to captivity or make violent attempts to escape. It does not breed readily in captivity.

*Perognathus longimembris* Bailli has received little behavioral study in the past. It is a jumpy, timid, solitary and feral mouse. This genus has been studied in most detail in reference to its high serotonin levels and radiation
resistance (See Scudder on amine levels).

*Peromyscus maniculatus Bairdii* and *P. m. Colorado* are both feral animals and ones that have been highly inbred in the laboratory. Both species show a large amount of variation in their behavior (Oligvic and Stenson, 1966). Emlen (1963) showed the importance of a circadian rhythm in these subspecies. Brant and Kavanau (1965) noted that when given no other alternative, *Peromyscus* will quickly explore a maze which they had previously avoided, and also that exploration, learning, and running are self-rewarding activities in this genus (King, 1958; King and Eleftheriou, 1959).

*Reithrodontomys raviventris Raviventris* also has received little behavioral study. It is a feral animal found around the salt water marshes along San Francisco Bay (Hooper, 1944). This species of all the animals investigated, had the highest total cholinesterase by automatic titration procedures (Scudder, unpublished data), and also the highest total biogenic amine level (Scudder et al., 1965a).

2. Behavioral Traits

a. General Approach

The behavioral traits of the mice have been compared to the behavior of an analogous machine (Scudder et al., 1965). Both can be considered mathematical abstractions; and if a
single quality such as speed is measured one machine being faster than another, and if speed is the goal to which a performance is directed, it follows that the fastest machine is best suited for the goal. Thus Microtus is a good avoidance machine while showing little learning. Otocolobus is a poor avoidance machine but learns well. All behavioral qualities will be discussed in this manner below.

b. Learning Patterns

In this reported study (Scudder, 1965, 1968c), an automated apparatus for measuring the rate of avoidance conditioning was used. The machine consisted of a connecting series of five inclined ramps and five chambers, arranged staircase-wise, and was electronically designed to present five identical environmental situations to an animal in temporal sequence without interference from the investigator. The animal's performance on this machine is interrupted as showing several characteristics of the learning or non-learning ability.

Learning patterns can be divided into concavity, convexity, erratic behavior, and straight-line behavior. Concavity represents early discovery of the correct solution and rapid attainment of the maximum in performance which is not further improved upon. Convexity refers to the initial
decrease in performance after which performance steadily increases. Erratic behavior interspaces gaps of improving performance with poor performance. Finally, straight-line behavior refers to the initial decrease in performance after which performance steadily improves.

If the assumption of homeostasis includes a goal directed behavior of avoiding shock and this behavior is arrived at by choice of possible paths in the nervous system, the animals with the more complex nervous system networks with many initially equal probable decisions will make more errors in trying out one major strategy after another than a comparatively simple system.

Concavity is the only pattern shared by mice of similar background since both *Onychomys* and *Mus musculus* 'Mo.' are feral, and straight-line behavior is characteristic of *Mus musculus* C57BL/6J, a highly inbred strain. *Peromyscus* appears to hit upon the solution to the climbing screen problem at once and steadily improves its performance. Its final behavior after 10 trials is not significantly different from most of the other genera. *Onychomys* and *Perognathus* also hit upon the correct solution but the performance of *Perognathus* did not improve as substantially as that of *Peromyscus* while that of *Onychomys* was quite erratic.
c. Escape

The order of the least to the greatest avoidance or escape tendency is as follows (Scudder et al., 1965): *Onychomys* < *Mus musculus* C57BL/6J < *Mus musculus* CF-1 < *Peromyscus maniculatus Bairdii* < *Perognathus* < *Microtus* < *Mus musculus* 'Mo.'. This evaluation gives no indication of the modulation of escape due to learning. Survival probably depends in a large measure on a good uniform escape or a well regulated processing of information leading to superior escape; and the wild forms, although they may not be highly inbred or homozygous for other traits show a strong tendency for uniform escape behavior. The exception to this generality is *Onychomys*, a carnivore. The ease of taming this mouse and its goal oriented aggression and fearlessness suggest (Bailey, 1929) that flight may not be a strong, readily learned characteristic of this genus. This in turn might explain their sparse population.

d. Forgetting

The order of increasing forgetting or variance is: *Mus musculus* 'Mo.' < *Microtus* < *Perognathus* < *Peromyscus* < *Mus musculus* C57BL/6J < *Mus musculus* CF-1 < *Onychomys*. The wild animals, the least inbred, generally show the most constant behavior (Scudder et al., 1965).
e. **Quality of Learning**

Although it is true that *Peromyscus* changed behavior more than any other genus, only *Mus musculus 'Mo.'* showed true avoidance of the conditioning stimulus suggesting that *Mus* individuals solved the problem in a qualitatively superior way. *Peromyscus* at the end of ten cycles through the machine had not learned to avoid any better than *Microtus* which showed no learning at all (Scudder et al., 1965).

f. **Quantity of Learning**

The amount of change in behavior during a trial period is expressed as quantity of learning. In amounts from the greatest to least the genera could be ordered: *Peromyscus* > *Mus musculus C57BL/6J* > *Mus musculus CF-1* > *Onychomys* > *Mus musculus 'Mo.'* > *Perognathus* > *Microtus* (Scudder et al., 1965). The following can be summarized in a form of a chart (See Table 1).

g. **Aggression**

From previous studies done in this laboratory (Scudder et al., 1968a), the animals rank in the following order with increasing aggression: *Peromyscus* < *Microtus* < *Onychomys* < *Mus musculus CF-1* < *Mus musculus C57BL/6J* < *Mus musculus 'Mo.'*.

The males of the three strains of *Mus* showed the highest incidence of aggression. The males of *Mus musculus 'Mo.'*
### Table 1
Comparison of Learning Patterns Among the Mice Studied

The above numbers rank the genera from greater to lesser: e.g., the most stable animal showing the greatest quantity of learning, greatest quality of learning, and most escaping animal (average base time) is listed as one, the second best, etc.

<table>
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<tr>
<th></th>
<th>'No.'</th>
<th>C57BL/6J</th>
<th>Onychomys</th>
<th>Microtus</th>
<th>Perognathus</th>
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<td><strong>Stability</strong></td>
<td>1 6 5 7 2 3 4</td>
<td></td>
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<tr>
<td><strong>Quantity</strong></td>
<td>4 3 2 5 7 6 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Quality</strong></td>
<td>1 2 5 7 4 7 3</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Escape</strong></td>
<td>1 3 2 7 6 5 4</td>
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were particularly aggressive, they averaged two or three times as many attacks as the males of *Mus musculus* C57BL/6J and *Mus musculus* OF-1, respectively. Another indication of the highly aggressive character of the strains of *Mus* is that these mice did not settle down with time. The aggressive activity continues without change in frequency for the duration of the test even when run for several days. The highly aggressive *Mus* strains were noncompetitive as they do not relate to any goal. It is interesting that Banks (1959, 1962) has shown that noncompetitive fighting is directly related to the amount of exploratory and investigative activity of the mice.

As with aggression, the incidence of exploratory behavior was far greater for the three strains of *Mus* than for the other genera, these differences being statistically significant. The exploring behavior of *Mus* may be related in part to the level of illumination (Wilmer, 1964; Ross, 1966). The strains of *Mus* which have pigmented eyes are generally more active under white light than are the albino animals such as *Mus musculus* OF-1 (McLearn, 1960).

It is important to note that aggression may not be a unitary phenomenon but that possibly there are several kinds of aggression each of which has a particular neuroendocrine
basis (Karczmar and Scudder, 1968). Several other authors (Scott, 1958; Bevan et al., 1960) have made the obvious point that aggression has many causes and that this term is applied to a wide variety of behaviors.

h. Activity

In previous studies (Scudder et al., 1966c) activity was recorded in a constant environment chamber by means of either Woodard Transistorized Photoactometers or mouse wheel cages (Acme Metal Products). The latter was provided with the standard side-well from which the mice could enter or leave the wheel ad lib. In this study it was noted that solitary animal activity may be different from group activity.

Photoactometer studies were particularly useful in the determination of circadian rhythms in the various mouse strains. At least two strains, Microtus and Mus musculus C57Bl/6J, were markedly nocturnal.

Obviously, the two tests differ in their behavioral meaning. The photoactometers record spontaneous activity which is not influenced, after the initial exploratory period, by the setup; running in the wheel involves a feedback from the revolving system, and it may be learned, self-perpetuating, and stereotypic.
It is of interest that *Peromyscus* strains that exhibited high degrees of wheel running were the only ones that also exhibited a high degree of stereotypic activity in a test previously conducted in this laboratory, which involved social interaction between strains.

Finally it was suggested that in a social environment high exploratory activity increases the chance of encounters and thus leads to aggression, of course provided that the strain in question is at all aggressive. It is not surprising, therefore, that not only the sequence of exploration as measured in a social milieu but also exploration and curiosity parallel the sequence of aggressive activity as previously observed.

*Mus musculus C57BL/6J* exhibits the highest activity with regard to these four parameters; it is followed by *Mus musculus CF-1* and *Mus musculus 'No.'* in this sequence. The following chart (See Figure 1) ranks the individual genera and strains with regard to photoactivity.

1. Curiosity

The method employed in the studies of curiosity was essentially that of Glickman and Sroges (1956). Through a perforated metal roof of the cage four objects were inserted: a string, a drinking straw, a brown metal-tipped shoelace,
Figure 1  Photoactivity

Relationship of activity existing among the mice studied.
and a q-tip. These items were replaced for each test. The cage was washed after each test. Each test was run for five minutes in the morning. At five second intervals it was noted whether the mouse either touched or looked at any of the four objects. Only deliberate investigation of some kind, and not accidental touchings, were recorded (Scudder et al., 1968b). The following graph (See Figure 2) ranks the mice according to visual and tactile curiosity.

J. Stereotypy

From past studies conducted in this laboratory (Scudder et al., 1968a and b) curiosity, exploration and aggression emerge as traits opposite to those of stereotypy and wheel activity. Moreover, the genus Mus emerges from these studies as curious, exploratory, aggressive and relatively free of stereotypic or self-rewarding activities. This may fit well with the concept of Mus as being from the point of view of evolution particularly successful as a genus and capable of adapting itself to diverse environmental and social situations (Scudder et al., 1966b).

3. Neuromuscular Activity

The climbing times of these animals reflect neuromuscular coordination, innate geotropism, escape tendencies and learning. From the best to the worst climber there exists the
Figure 2  Curiosity

Relationship present among the various mice studied to the behavioral characteristics of visual and tactile curiosity.
following rank (Scudder et al., 1965): Mus musculus 'Mo.' > Onychomys > Mus musculus CF-1 > Mus musculus 057BL/6J > Peromyscus > Microtus > Perognathus. Figure 3 represents average time spent on a balancing wheel by several of the genera and strains under investigation.

Data obtained during the first few days of life measured the ability of the very young pups to cling to the screen; once this ability was present, the mice could exhibit the preference for either climbing up or jumping off the wheel. The wheel data are concerned, therefore, with the physical and behavioral development of the mice.

4. Amine and AChE Levels
   a. Biogenic Amines

   The levels of norepinephrine, epinephrine, dopamine, and serotonin and acetylcholine run parallel in the various strains, a strain with a high level of one amine exhibited a high level of the other amines (Richardson et al., 1968). When values for catecholamines and for serotonin were combined, the highest level exhibited by Reithrodontomys was almost twice that found in Mus musculus 'Mo.'. The following graph (See Figure 4) relates in progressive order the combined biogenic amine concentration of the various genera
Figure 3  Balancing Wheel

Average time spent on the balancing wheel representing the physical and behavioral development of the mice studied.
Figure 4  Amine Concentration

The relationship of combined biogenic amine concentrations present among the mice studied. Each barograph represents, in micrograms per gram, wet brain weight, combined values of serotonin, dopa, dopamine, norepinephrine, and epinephrine.
b. Acetylcholine and Cholinesterase

The following graphs (See Figures 5 & 6) represent the amount of acetylcholine and cholinesterase in the various genera and strains studied (Sobotka et al., 1968). Relative cholinesterase levels of whole mice brains were determined in an acetylcholine substrate, which determines both the acetylcholinesterase and pseudocholinesterase present. In this method, sodium hydroxide is titrated to the amount of acetic acid released by the enzymatic breakdown of acetylcholine. In the same study, it was also shown that the relative distribution among the genera and strains of mice is identical when methylcholine is used, which is a specific substrate for acetylcholinesterase.

B. Histochemistry

1. Koelle Technique

The histochemical technique used for the localization of AchE in this study was that of Koelle (1951), modified (Koelle, 1955, 1963), to separate butyrocholinesterase (BuChE) from AchE. All solutions were prepared according to Koelle (1951), and contained maleate buffer, copper glycinate, Na₂SO₄ (24-28%), MgCl₂, and traces of copper thiocholine at
Figure 5  Acetylcholine

A comparison of acetylcholine levels made per gram of whole mouse brain in the mice studied.
Figure 6 Cholinesterase

A comparison of cholinesterase made in whole mouse brains among the mice studied using an acetylcholine substrate.
at pH 6. The brains to be studied were frozen immediately on the microtome block, and sagittal sections were cut at 16 micra and placed on slides. As soon as the sections had thawed and the excess moisture had evaporated, the slides were placed in the appropriate storage solution, Koelle (1951) also indicated that diffusion of both specific and non-specific rat ChE was minimized by the incorporation of 24% Na$_2$SO$_4$ in the incubation media, and that non-specific rat ChE was inactivated relatively selectively by 30 minutes incubation with 2.5 x 10$^{-8}$ M di-isopropyl fluoroophosphate (DFP) in 24% Na$_2$SO$_4$. Since it has been previously shown by Shute and Lewis (1963), Koelle (1954), Gerebtzoff (1959), and in this laboratory (Scudder, unpublished data) that BuChE is relatively an insignificant enzyme in the central nervous system, both specific and non-specific cholinesterases were localized in this procedure. Therefore, an inhibitor was not used. The storage solution consisted of H$_2$O and 40% Na$_2$SO$_4$ adjusted to pH 6. Storage solutions were prepared in Coplin jars and kept in a dry oven at 37°C. In order to minimize spontaneous hydrolysis, acetylcholine was added immediately before the solutions were used. The solutions were stirred, filtered through Whatman No. 2 filter paper
and placed in Coplin jars previously warmed in the incubator.
Following the precipitation of copper thiocholine at sites of enzymatic activity, the mercaptide was converted to CuS by immersion in (NH₄)₂S solution (Malmgren and Sylven, 1955). They were then transferred to rinse solution 1 (20% Na₂SO₄, saturated with CuThCh) for five minutes, and immersed for approximately two minutes in rinse solution 2 (10% Na₂SO₄, saturated with CuThCh). Preincubation for 30 minutes appeared to be sufficient, and incubation periods of 30 minutes all gave good results. The sites of enzymatic activity were stained brown to brownish black. Following the developer solution (developing time of 30 seconds proved satisfactory), the slides were rinsed rapidly in water, fixed in CuS-saturated 10% formalin, dehydrated through alcohol and xylol, and mounted in Permount without counterstaining.

The same concentration of acetylthiocholine was used for all genera and strains studied, and no attempt was made to find the optimal concentration of this substrate for a particular genus. In carrying out this procedure, therefore, it is possible that not all of the brain AchE of a certain genus is stained.

2. Kluver and Barrera Technique

It is well known that the various components of the
neuron are chemically different and therefore expected to be more or less selectively stained by appropriate histological techniques. The perikaryon (cell body) contains large quantities of ribonucleic acids (RNA) and therefore can be easily stained with basic aniline dyes. The myelin sheath of the myelinated nerve fibers requires a different staining technique which is based on the formation of a metal-dye linkage with the lipoprotein component of the myelin sheath. Kluver and Barrera (1953) succeeded in developing a staining technique (using copper phthalocyanines), which demonstrates both cell bodies and myelin sheaths, the so-called luxol fast blue-cresyl-violet stain.

With this technique, myelin is stained greenish-blue. Neurons and glial nuclei are stained pink to violet and Nissal substance appears a faint pink to clear within the neuronal cytoplasm. Although not mentioned in the original article of Kluver and Barrera, it is possible that only a small proportion of neurons and glial cells are stained by this method.

The brains were fixed in 10% formalin for 48 hours, dehydrated through alcohols, and embedded in paraffin. Care was taken to keep the long axis of the brain parallel to the side of the block and the bottom of both cerebral hemispheres
touching the bottom of the embedding box, to avoid tilting of the brain so as to obtain true sagittal sections. The sections were cut at 16 micra and mounted on albuminized slides, which were left at room temperature over night. The paraffin wax was removed with xylol and the slides were then passed through absolute and 95% alcohols. They were then incubated in a 0.1% Luxol Fast Blue MBS (Dupont) solution for 16 hours, at 57°C in an oven, taking precautions to prevent the loss of alcohol through evaporation from the staining solution. Luxol Fast Blue is an alcohol soluble amine salt of sulfonated copper tetrabenzotetrazoporphrin. At this point, differentiation was begun by a quick immersion in 0.05% Li₂CO₃ solution. The differentiation was continued in 70% alcohol until the gray and white matter could be distinguished, care was taken not to over differentiate. When differentiation was completed, the white matter was stained greenish-blue in sharp contrast with the colorless gray matter. Immersion in cresyl-fast-violet solution at 57°C (previously warmed and filtered) for six minutes served as a counterstain. The slides were cleared in xylol and mounted in Permount.

Although porphyrin is probably present in other body
tissues, since it is a precursor of hemoglobin, by spectroscopic examination it was found to be present only in the central nervous system (Kluver and Barrera, 1953). On the basis of Kluver's work, it became apparent that porphyrins have a selective affinity for myelin.

C. Comparative Study Methods of AchE Location

The histological structures investigated were grouped into the following regions: telencephalon, diencephalon, mesencephalon, metencephalon, myelencephalon, and cerebellum. Similar structures were examined in all the animals and evaluated for AchE. These anatomical areas were investigated with regards to the intensity of AchE stain. A subjective evaluation was made by frequent referrals to a standard slide which was previously rated on a 0 to 4+ scale. Any peculiarities in structure or staining were also noticed at this time. For precise localization of these areas it was necessary to make an atlas for each of the animals under investigation. These atlases were prepared from the Kluver-Barrera stained slides. For each phase of the study, mice of similar sex, age and approximate weight were sacrificed. All animals were killed by decapitation. For the AchE study, the brains were immediately removed and quick-frozen in an International-
Harris cryostat at -25°C. Cross-sections, 16 micra in thickness, were taken from alternate slices, air-dried slightly, and placed on slides. For the histological study, the brains were similarly removed, suitably fixed, and embedded in paraffin. An International Rotary Microtome was used and serial sections, 16 micra in thickness, were made. In all cases gross observations of the brain were made immediately upon removal.

Serial sections of brain made of each mouse by the histological technique of Kluver and Barrera were photographed. The photomicrographs were enlarged 100X with a Solar Enlarger, and the brain section itself served as the photographic negative. Kodak Kolabromide Photographic Paper was used as the positive plate. Therefore, with this procedure, dark staining areas such as nuclei and fiber tracts appeared light on the photomicrographs (See Figure 7). The photomicrographs were then arranged sequentially and labelled. They were traced on onion skin paper and the tracings were labelled. In the atlas this labelled sheet was pasted opposite the photomicrograph. Several existing rat atlases (Konig, 1963; Zeman, 1963), and a discussion of the finer anatomy of the central nervous system in the rat (Cragie, 1925), were of considerable help. Double photographs of each section were taken. One was pasted on plain white paper, the other kept loose. Since the
Figure 7  Mouse Brain Cross-Section

A typical photomicrograph of mouse brain used in the construction of the atlases.
magnification was known and the thickness of the sections was constant, areas and volumes of certain anatomical structures could then be determined.

D. Experimental Design

The mice were similarly sacrificed and their brains were histochemically fixed. The brains of the various genera and strains were compared anatomically, both grossly and histologically. Grossly, differences were noted in brain weights, sizes of brain parts were compared, and any unusual findings were itemized. Histologically, lengths of fiber tracts were compared, differences in volume of important nuclei were noted, and again differences were itemized.

The histological distribution of AchE was qualitatively compared among the individual genera and strains. The anatomical structures were located and the amount of AchE was graded by referring to the Reference Slide which was evaluated from 0 to ++++, e.g., from virtually no AchE to a maximum concentration of ++++.

The behavioral aspects of these animals were then correlated with the amount and distribution of AchE found. It was the basic assumption of this work that neurochemical mediators are one means of determining behavioral patterns of animals.
Results

A. Anatomical Differences and Similarities among the Different Genera of Mice

1. Gross Observations

The following data was obtained from the freshly dissected brains of all the mice: body wt./brain wt., length of cerebrum, length of cerebellum, length of olfactory bulbs, and length of hypothalamus. In computing these figures, the mathematical average of ten mice was used for each genus and strain investigated. These results can be found in Table 2.

According to the body wt./brain wt. ratio the mice distribute themselves into three distinct categories. The animals with the greatest brain weight compared to body weight and classified in the first category are Peromyscus maniculatus Bairdii, Peromyscus maniculatus Colorado, and Reithrodontomys. The middle group includes: Mus musculus 'Mo.', Microtus ochrogaster, Perognathus longimembris Bailli and Onychomys leucogaster. The third group, those mice which have the lowest brain weight comparable to body weight, are Mus musculus CF-1 and Mus musculus C57BL/6J.

Throughout this discussion, when referring to the fact that one mouse has a larger structure than another, it is
### Mouse Investigated

<table>
<thead>
<tr>
<th>Mouse Investigated</th>
<th>% brain wt.</th>
<th>length of cerebrum</th>
<th>length of cerebellum</th>
<th>length of olfactory bulbs</th>
<th>length of hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mus m. C57BL/6J</em></td>
<td>1.4</td>
<td>8.0</td>
<td>5.0</td>
<td>1.8</td>
<td>3.0</td>
</tr>
<tr>
<td><em>Mus m. CF-1</em></td>
<td>1.4</td>
<td>11.0</td>
<td>4.5</td>
<td>4.2</td>
<td>3.0</td>
</tr>
<tr>
<td>*Mus m. <em>Mo.</em></td>
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<td>8.0</td>
<td>4.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
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<td><em>Microtus ochrogaster</em></td>
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<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td><em>Onychomyus leucogaster</em></td>
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<td>3.0</td>
<td>3.0</td>
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<td>4.3</td>
<td>2.8</td>
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<td><em>Peromyscus maniculatus Colorado</em></td>
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<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td><em>Reithrodontomyx</em></td>
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<td>3.0</td>
<td>1.5</td>
<td>2.0</td>
</tr>
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**Table 2 Gross Brain Measurements**

Average values for gross anatomical measurements in mm. based on ten mice for each genus and strain studied.
meant that according to the sections either the absolute length of this structure is longer or its approximated volume is greater. These figures then are approximations of absolute anatomical dimensions and provide a general indication of the size of various structures.

Perognathus and Microtus have the longest cerebellums, the cerebellum of Perognathus being slightly larger. These genera also have relatively long olfactory bulbs, the olfactory bulbs of Perognathus being slightly longer. They also present with the greatest cerebellar lobulation of any of the mice studied. The vermis of the cerebellum was only distinctly demarcated in Perognathus and Microtus. In proportion to the size of the olfactory bulbs, the size of the cerebral hemispheres of Microtus is much smaller than those of the other genera. The longest over all length of brain (from olfactory bulbs to medulla oblongata) is seen in Perognathus, followed by Microtus. These two genera also have the most prominent optic chiasma. Figure 8 demonstrates the cerebrum and cerebellum of both of these genera.

Onychomys, the two subspecies of Peromyscus and Reithrodonontomys have both their inferior and superior colliculi covered, so these structures are not visible from a dorsal view unless the cerebrum and cerebellum are pushed aside.
Figure 8  Cerebrum and Cerebellum

Comparison of the relative similarity in size of the cerebrum and cerebellum existing between *Perognathus* and *Microtus*. 25X
This is in distinction to the strains of Mus in which both colliculi are clearly visible (See Figure 9).

Surprisingly the two strains of Peromyscus differ widely in the dimensions of their olfactory bulbs. Peromyscus maniculatus Bairdii has an average length of 4.3 mm. compared to 3.0 mm. for Peromyscus maniculatus Colorado. These figures were also derived by taking the average of ten mice for each strain (See Figure 10).

Comparing the three strains of Mus, Mus musculus CF-1 has the longest olfactory bulbs, and it also has the longest cerebrum and cerebellum. In this group, Mus musculus C57BL/6J has the least prominent pons. Since both of these strains belong to the group with the smallest body/brain weight ratio, it is surprising that the longest cerebrum and cerebellum were present in Mus musculus CF-1 rather than Mus musculus 'Mo.' which has a relatively greater brain weight.

Microtus has the largest and most clearly defined olfactory tracts. In Figure 11, it is compared with Reithrodontomyys where the olfactory tract is barely visible. (These brains were from comparably similar age mice, approximately 70 days old.)

2. Histological Observations

The quantitative histological measurements of the
Mus

Peromyscus

Figure 9 Colliculi

Difference in anatomical exposure of the colliculi demonstrated between the three subspecies of Mus and the two subspecies of Peromyscus. 25X
Comparison of olfactory bulbs in the two subspecies of *Peromyscus* illustrating the difference in length. 25X
Comparison in the prominence of the olfactory tracts existing between *Microtus* and *Reithrodontomys*. 25X
various genera and strains studied are listed in Table 3. The following points were found to be markedly significant:

a. The greatest depth of the neocortex at the diencephalic-telencephalic junction at the level of the commissure fornix was present in Mus musculus 'Mo.' It measured 1.45 mm. (See Figure 12).

b. The longest extension of the hippocampus was present in Mus musculus 'Mo.' The anterior-posterior extension of this structure measured 3.82 mm. (See Figure 13).

c. The approximate volume occupied by the reticular formation was greatest in Mus musculus 'Mo.' followed by Peromyscus maniculatus Colorado.

d. The nuclei of the thalamus have the greatest extension in Perognathus followed by Microtus ochrogaster.

e. The olfactory bulbs of Peromyscus maniculatus Bairdii were found to be the largest. A typical cross-section is shown in the following figure (See Figure 14).

f. Perognathus on gross observation was found to have the longest cerebellum, extending 6 mm. A cross-section from this structure is demonstrated in Figure 15.

g. The anterior-posterior extension of the lamina glomerulosa bubli olfactorii is the longest in Perognathus. A
<table>
<thead>
<tr>
<th>Mouse Investigated</th>
<th>length of hippocampus</th>
<th>length of globus pallidus</th>
<th>neocortex (max. thickness)</th>
<th>thalamus</th>
<th>reticular form* (approx. volume)</th>
<th>length of lamina gielgouloua bulbi</th>
<th>length of nuc.olfactorius</th>
<th>length of nuc. olfactorius volume (approx.)</th>
<th>length of genu corporis callosi</th>
<th>length of genu amygdaloides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mus m. C57BL/6J</td>
<td>2.50</td>
<td>0.82</td>
<td>0.90</td>
<td>1.44</td>
<td>1.45</td>
<td>1.92</td>
<td>0.96</td>
<td>2.08</td>
<td>3.76</td>
<td>2.94</td>
</tr>
<tr>
<td>Mus m. CF-1</td>
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<td>0.98</td>
<td>1.05</td>
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<td>1.38</td>
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<td>0.89</td>
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<td>2.96</td>
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<td>1.92</td>
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<td>Peromyscus maniculatus Bairdii</td>
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<td>0.94</td>
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<tr>
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<td>1.92</td>
<td>0.90</td>
<td>2.24</td>
<td>4.90</td>
<td>2.60</td>
</tr>
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</table>

Table 3 Histological Brain Observations

Values given either in mm. or mm.³ representing histological differences derived from the mouse atlases based on one mouse for each genus and strain studied.
Figure 12  Neocortex

Cross-section of the neocortex of Mus musculus 'Mo.' demonstrating its maximum depth. 100X
Figure 13 Hippocampus

Cross-section showing the hippocampus of *Mus musculus* 'Mo.'. 100X
Figure 14  Olfactory Bulbs

Cross-section of olfactory bulbs taken from *Peromyscus maniculatus Bairdii*. 100X
Figure 15  Cerebellum

Cross-section of the cerebellum of *Perognathus* sp. demonstrating its relatively large size. 100X
cross-section from this fiber tract is shown (See Figure 16).

B. Location, Description, and Relative Intensity of AchE

1. General Remarks

For anatomical reference the results of brain AchE distribution have been divided into telencephalon, diencephalon, mesencephalon, myelencephalon and cerebellum. The structures most significantly stained for AchE were noted in each region and compared among the various mice. Important differences and similarities were noted in each section.

2. Telencephalon

The important structures in this region and their relative AchE concentrations were compared among the various genera and strains (See Table 4).

The lamina glomerulosa bubli olfactorii of Mus musculus CF-1 and Mus musculus 'Mo.' had the greatest amount of AchE (See Figure 17). The genera showing the least were Microtus, Onychomys and Perognathus, while Mus musculus 057BL/6J and Reithrodonotomys were intermediate.

The nucleus amygdaloideus of Microtus contained the most AchE (See Figure 18), while none was present in Mus musculus 057BL/6J. All the remaining genera had an identical minimal amount of AchE in the amygdaloid.
Figure 16  Olfactory Tracts

Cross-section showing the lamina glomerulosa bulbi olfactorii in *Perognathus* sp. 100X
Table 4 AchE in Telencephalon

Table of important structures in the telencephalon stained for AchE in the genera and strains studied.
Figure 17  Olfactory Tracts

Maximal amount of AchE shown in the lamina glomerulosa bulb olfactorii of *Mus musculus* CF-1. 250X
Figure 18  Nucleus Amygdaloideus

Difference in intensity of AchE in the nucleus amygdaloideus present between Microtus and Perognathus. 250X
The caudate nucleus as a general rule was abundant in AchE in all genera. Nevertheless, a striking difference was present among *Microtus*, *Mus musculus* CF-1 and *Perognathus* (See Figure 19). *Microtus* contained an extremely dense amount of AchE while it was minimal in *Mus musculus* CF-1 and intermediate in *Perognathus*.

The greatest amount of AchE in the globus pallidus was seen in both subspecies of *Peromyscus*. The least was present in *Mus musculus* C57BL/6J, *Onychomys* and *Perognathus*.

The AchE distribution of the tractus olfactorius lateralis showed a unique phenomenon. The two subspecies of *Peromyscus* and *Reithrodonotomys* had the greatest and equal amount followed by the three subspecies of *Mus*; followed by *Microtus*, *Onychomys* and *Perognathus* which had the least and equal amounts.

The distribution of AchE in the nucleus septi medialis showed a similar phenomenon. The greatest amount of AchE was present in the three subspecies of *Mus*, followed by the two subspecies of *Peromyscus* and *Reithrodonotomys*, and *Microtus*, *Onychomys* and *Perognathus* had the least and equal amounts (See Figure 20).

The telencephalic distribution of AchE was identical in *Peromyscus maniculatus Bairdii* and *Peromyscus maniculatus*
Figure 19  Nucleus Caudate

Comparison of intensity of AchE in the nucleus caudate of three genera of mice studied.  250X
Comparison in intensity of AchE in the nucleus septi medialis demonstrated between Mus and Microtus. 250X

* Identical pattern for all three subspecies of Mus.
Colorado. The three subspecies of Mus varied slightly from each other and for the most part were quite identical in their AchE content. Microtus, Onychomys and Perognathus formed another group which for the most part were identical in their AchE content. Reithrodontomys was more closely related to Peromyscus than to the others.

3. Diencephalon

The important structures in this region and their relative AchE concentrations were compared among the various genera and strains (See Table 5).

AchE located in the hypothalamus was most prominent in Mus musculus 'Mo.', Mus musculus CF-1, Microtus and Reithrodontomys. None was found in Mus musculus C57BL/6J and Onychomys, and both subspecies of Peromyscus had a relatively low content.

The columna forniciis was most prominent in Reithrodontomys, all other genera having a relatively low content.

The stria terminalis of Mus musculus CF-1 and Microtus had the highest AchE content. All other genera had a moderate amount except Mus musculus C57BL/6J and Reithrodontomys which were low.

The nucleus ventralis thalami of Reithrodontomys had the
### Table 5 AchE in Diencephalon

Table of important structures in the diencephalon stained for AchE in the genera and strains studied.

<table>
<thead>
<tr>
<th>Anatomical Structure</th>
<th>Mus mus. C57BL/6J</th>
<th>Mus mus. CF-1</th>
<th>Mus mus. 'No. 1'</th>
<th>Microtus</th>
<th>Orychomys leucogaster</th>
<th>Peromyscus maniculatus Bairdii</th>
<th>Peromyscus maniculatus Colorado</th>
<th>Reithrodontomys</th>
</tr>
</thead>
<tbody>
<tr>
<td>columna fornicis</td>
<td>1 1 2</td>
<td>1 1 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
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<td>3 2 2</td>
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<td>stria terminalis</td>
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</tbody>
</table>

4= intense AchE staining  
3= moderate AchE staining  
2= light AchE staining   
1= slight AchE staining   
0= no AchE staining
highest AchE content, while Mus musculus C57BL/6J had none. Mus musculus CF-1, Mus musculus 'Mo.', Onychomys and Perognathus had a moderate amount, whereas Microtus and the two subspecies of Peromyscus had a low amount.

The nucleus habenulae lateralis was most prominent in AchE in Perognathus. This was followed by Mus musculus C57BL/6J and Mus musculus 'Mo.'. It was interesting to note that Microtus had a very low content (See Figure 21).

The stria medullaris thalami was well represented with AchE in all genera, being especially prominent in the two subspecies of Peromyscus.

The width of the neocortex was taken at the diencephalic-telencephalic junction at the level of the commissure fornici. A representative section from this area is shown in Figure 22. All genera showed approximately the same distribution, i.e., a poor concentration of AchE.

It is also interesting to note that the hippocampus of all genera had a relatively low AchE content (See Figure 23).

It was more difficult grouping the genera into general categories in the diencephalic region; nevertheless, both subspecies of Peromyscus had identical AchE content. The subspecies of Mus showed some variations from one another and no pattern could be established for the remaining genera.
Microtus  Mus musculus 'Mo.'  Perognathus

Figure 21  Nucleus Habenulæ

Comparison in intensity of AchE in the nucleus habenulæ lateralis of three genera of mice studied. 250X
Figure 22  Neocortex

AchE content and distribution in the neocortex demonstrating a low concentration for all genera and strains. 250X
Figure 23  Hippocampus

AchE shown in the hippocampus demonstrating a low concentration for all genera and strains. 250X
4. Mesencephalon

The important structures in this region and their relative AchE concentrations were compared among the various genera and strains (See Table 6).

In the fasciculus longitudinalis medialis Onychomys and Perognathus had the highest AchE content followed by the two subspecies of Peromyscus and Reithrodontomys. Mus musculus CF-1 and Mus musculus 'Mo.' had no detectable AchE in this region.

In the fasciculus retroflexus Mus musculus C57BL/6J and Mus musculus 'Mo.' had the highest content followed by Mus musculus CF-1 and the two subspecies of Peromyscus. The remaining genera had a scanty amount of AchE in this region.

In the nucleus Edinger-Westphalia the two subspecies of Peromyscus had a moderate amount of AchE, and all the other genera were shown to have minimal amounts.

In the nucleus interpenduncularis Mus musculus CF-1 had the highest level followed by Onychomys, Perognathus and Reithrodontomys, which were moderate.

In the nucleus posterior thalami all three subspecies of Mus, the two subspecies of Peromyscus and Reithrodontomys had a high content. These were followed by Microtus, Onychomys and Perognathus which all had moderate amounts.
<table>
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<tr>
<th>Anatomical Structure</th>
<th>Mus m. C57BL/6J</th>
<th>Mus m. CP-1</th>
<th>Mus m. 'Mo.'</th>
<th>Microtus ochrofaster</th>
<th>Onychonyx leucofaster</th>
<th>Perognathus</th>
<th>Peromyscus maniculatus Bairdii</th>
<th>Peromyscus maniculatus Colorado</th>
<th>Peromyscus reticulata</th>
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4 = intense AchE staining
3 = moderate AchE staining
2 = light AchE staining
1 = slight AchE staining
0 = no AchE staining

Table 6 AchE in Mesencephalon

Table of important structures in the mesencephalon stained for AchE in the genera and strains studied.
Again in this region of the brain the two subspecies of *Peromyscus* had identical distributions of AchE which was closely paralleled by *Reithrodontomys*. No other patterns of similarity were noted in the remaining genera, e.g., the three subspecies of *Mus* varied quite markedly from each other in this region.

5. **Myelencephalon**

The important structures in this region and their relative AchE concentrations were compared among the various genera and strains (See Table 7).

The corpus trapezoideum contained a marked degree of AchE in all genera except *Microtus* and *Onychomys* which showed little stain.

In the formatio reticularis *Perognathus* had the highest level followed by *Mus musculus* CF-1. The remaining genera and strains either had a low level or were completely devoid of AchE.

The tractus solitarius had the highest AchE content in *Onychomys* and *Perognathus*, all other genera and strains having low amounts.

The only similarities present among the genera and strains were: 1) the two subspecies of *Peromyscus* again had an identical distribution of AchE and 2) all three subspecies of *Mus*
### Anatomical Structure

<table>
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<tr>
<th></th>
<th>Mus mus. C57Bl/6J</th>
<th>Mus mus. C3H</th>
<th>Mus mus. 'No.1'</th>
<th>Microtus</th>
<th>Onychomys longicaudatus</th>
<th>Peromyscus maniculatus Bairdii</th>
<th>Peromyscus maniculatus Colorado</th>
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<td>tractus solitarius</td>
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</tr>
</tbody>
</table>

4 = intense AchE staining  
3 = moderate AchE staining  
2 = light AchE staining  
1 = slight AchE staining  
0 = no AchE staining

#### Table 7 AchE in Myelencephalon

Table of important structures in the myelencephalon stained for AchE in the genera and strains studied.
had an identical distribution in the corpus trapezoideum and tractus solitarius.

6. **Cerebellum**

The cerebellum is of interest by the fact that all genera and strains had an identical distribution of AchE. The cerebellar nuclei showed a scanty amount of AchE and the Purkinje cells were found devoid of AchE.
Discussion and Speculations

A. Introductory Remarks

The following discussion is intended in part by the design of the problem to be speculative in nature. At times, correlations are made not with the absolute certitude of cause and effect relationships, but with the idea of fostering further possibilities in pharmacological brain research. In several instances the correlations among behaviors, AchE concentration, and brain anatomy are highly suggestive of definite interaction but are not intended to be taken as conclusively proven facts. As noted before, in the study of animal behavior the variables become so numerous as to make the experimental design difficult.

The results of the present study indicate that there exist differences in the amount and distribution of AchE in the brains of various genera and strains studied. The results also indicate gross anatomical and histological differences which together with the AchE distribution correlated with certain behavioral characteristics of the mice.

One of the major and as yet unsolved problems in this study is to determine the neural basis of memory and intelligence. It would appear that some structural change must
occur, but thus far their value have not been ascertained. Since synaptic transmission is now widely accepted as being chemically mediated, much work has been done in an attempt to find what the transmitter(s) in the central nervous system may be. It appears that evidence for Ach has been accumulating in the neurochemical literature (Armett and Ritchie, 1961; Mitchell, 1963). As it has been referred to previously, AchE is also present in large quantities in the central nervous system, and it is particularly abundant in the areas of the brain which normally have intense neural activity (Tower, 1958). The levels of AchE do not necessarily correlate with the levels of Ach, each being genetically independent (Rosenzweig, 1960). Nevertheless, Reithrodontomys and Mus musculus 'Mo.' have been shown in this laboratory (Scudder, unpublished data) to be high in both Ach and AchE levels.

B. Anatomy and Behavior
1. Gross Anatomy

It was previously noted that the mice studied in this investigation arranged themselves into three distinct groups, according to their total body weight/total brain weight ratio. Nevertheless, the behavior of these mice, even within a particular group was varied (Scudder et al., 1965, 1966a,
that correlations between brain weight and behavior could not be made for all facets of the animal's behavior. Nonetheless, it will later be speculated that the superior performance of Reithrodontomys and Peromyscus, both of which have the relatively largest brain weights, may be accounted by this fact. Perhaps the idea offered by Darwin that the brains of domestic rabbits are reduced in bulk might also hold true for mice, e.g., the inbred strains of Mus, Mus musculus C57BL/6J and Mus musculus CF-1, were noted to have relatively lower brain weights.

The (length of cerebellum)/(length of total brain) ratio of Perognathus and Microtus is the highest of all the animals studied. Since Perognathus is bipedal, a large majority of time, this perhaps would correlate with its relatively large cerebellum. The well known and classical function of the cerebellum is coordination of the action of muscle groups, and at times their contractions, so that movements are performed smoothly and accurately. There is no doubt that the cerebellum is more developed in bipedal animals who require more muscular coordination (Fadiga and Pupilli, 1964; Partridge and Glaser, 1960). It might be surmised that perhaps Microtus has a larger cerebellum to compensate for its smaller cerebrum. In other words, it is possible that Microtus
might be an immature genera intellectually and have poor cerebral development, but to survive in its environment, it has developed the necessary cerebellar functions. *Microtus* is a poor learner but has the greatest total activity and a rather high tactile curiosity (Scudder et al., 1968b). Although a cause and effect relationship cannot be proven, it is not unreasonable to suggest that *Microtus* is a poor learner because it has a smaller cerebrum in comparison to the other mice, and its total activity may be due to its larger cerebellum which would endow it with motor skill and is responsible for muscle synergy throughout the body.

The length of the olfactory bulbs was longer in *Mus musculus CF-1* than in the other mice studied, and this subspecies also had the greatest amount of AchE concentrated in the lamina glomerulosa bulbi olfactorii. Perhaps the high degree of curiosity and exploratory behavior of this mouse (Scudder et al., 1968b) could be correlated with its larger olfactory fiber tract. *Reithrodontomys* had the smallest olfactory bulb length with only an intermediate amount of AchE in its lamina glomerulosa bulbi olfactorii and by the same studies as mentioned above had poor exploratory behavior and minimal curiosity. Again it can be postulated that smaller olfactory bulbs might put a rodent at a disadvantage in certain
behavioral characteristics such as curiosity and exploratory behavior. The possibility also exists that in its natural environment, salt marshes, *Reithrodontomys* is exposed to a minimal number of natural enemies and lacks proper stimulation to develop large olfactory bulbs.

As previously noted, the different genera and strains of mice studied arranged themselves into three distinct groups when the ratio of body weight/brain weight was considered. This classification correlates very closely to the phylogenetic origin of these genera, the only exception being *Onychomys* which belongs to the same sub-family as *Reithrodontomys* and *Peromyscus* but in comparison has less of a brain weight. It is interesting to note that it is *Onychomys* which shows erratic behavior on the climbing screen and is far less superior in "learning" to *Reithrodontomys* and *Peromyscus* (Scudder et al., 1965).

It appears conceivable at this point to speculate that the larger "relative" brain weight of *Reithrodontomys* and *Peromyscus* may account for its superior performance. Several investigators (Diamond et al., 1965, 1966) were able to show anatomical changes in the brains of rats which were "more intelligent". Such changes were an actual increase in the cortical depth and glial cells in the brain. The size of
gross anatomical structures of the three subspecies of Mus are approximately identical, as they are for the two subspecies of Peromyscus. The only exception is the relative brain weight of Mus musculus 'Mo.' as compared to Mus musculus C57BL/6J and Mus musculus CF-1. This correlates with the behavioral qualities of the two subspecies of Peromyscus which are practically identical as are those of Mus musculus C57BL/6J and Mus musculus CF-1. However, Mus musculus 'Mo.' has been shown to be superior in some ways, e.g., greatest avoidance or escape tendency and the least forgetting (Scudder et al., 1965). Although the correlation is not absolute, this could possibly be an example of different brain anatomy representing different behavior.

2. Histological Anatomy

Various environmental situations and their correlations with the histological anatomy of the rat brain has been investigated by several authors (Krech et al., 1960; Diamond et al., 1964). Throughout this particular study several histological structures were noted to be significantly enlarged or reduced from the other genera and strains and possible behavioral correlations are postulated below.

Both Microtus and the subspecies of Peromyscus have been
found to have the largest volume of caudate nucleus. It is true that *Peromyscus* belongs to the group of mice with the greatest brain weight/body weight, but *Microtus* does not. Therefore, it is reasonable to assume that there might be more of a correlation in the large size of the caudate nucleus than to merely large brain size. Both of these genera demonstrate high degrees of total activity and wheel cage activity (Scudder, unpublished data). One theory states that wheel cage activity perhaps reflects the immaturity of the animal. Repetitive acts perhaps can be speculated to be a form of immaturity. They possibly involve the same neural circuits without deviating to other varieties of behavior which, of course, would mean more complicated neural interrelationships. Animals which are prone to this behavior might derive self-satisfaction from it and seek to repeat it. If one considers a form of maturity to be more of an interaction with the environment, these animals have not yet attained this state. Those animals which perform this activity with repetition are possibly "playing". Nevertheless, it can also be argued that perhaps "play" represents a discrete act and as such is a sign of maturity as opposed to chaotic motion which is infantile. It can be speculated that such activity may involve or necessitate a significant component of the extrapyramidal
tract, such as the caudate nucleus. It is interesting to note that *Peromyscus* and *Microtus* had approximately the same levels of biogenic amines (Scudder et al., 1966a) which possibly could explain their high degree of total activity.

Within the genera of *Mus* there was a marked difference between *Mus musculus* 'No.' and *Mus musculus* CF-1 as to the approximated volume of the reticular formation. The brain space occupied by this structure was far greater in *Mus musculus* 'No.'. Although the function of the reticular formation is indeed complex and largely unknown, here it will be assumed to be a structure which affects consciousness, attention, and learning by alerting the cerebral cortex (Ushiyama et al., 1960). Considered in this light, the more prominent reticular formation of *Mus musculus* 'No.' might in part explain its greater capacity to learn. It is interesting to note that *Mus musculus* 'No.' in comparison to the other genera and strains exhibits minimal nocturnal and diurnal sleep patterns. The question could be asked, is this due to a hyperactive reticular formation? However, to carry these correlations further, from previous studies (Scudder et al., 1965), *Mus musculus* CF-1 was shown to be a poor learner but its sleeping time was even less than *Mus musculus* 'No.' Nevertheless, *Mus musculus* CF-1, contrary to what might be expected from the hypothesis above,
has a relatively small volume of reticular formation. Therefore a correlation at this level is inconsistent because of the ubiquitous variations in anatomy and behavior.

To give us some insight into neural circuits and the possible correlation between one brain part and another, it is interesting to postulate a functional relationship between two histological areas which both appear to be excessively large in comparison to the other mice studied. According to D. O. Hebb's (1949) theory of neural circuits, if inputs activate loops of neurons often enough, some sort of structural change will occur. The globus pallidus of Microtus is much longer than any mouse in this study, and it was also shown to have a large caudate nucleus. It would appear that the occurrence of both of these structures being large is more than chance alone. The globus pallidus is one of the nuclei of the extrapyramidal system, and the caudate nucleus and putamen are presumed to "tone down" or inhibit its activity (Denny-Brown, 1962). It would be conceivable that the overactivity of one structure would necessitate the hypertrophy of the other to keep it in check.

Two other structures which show somewhat of a similar correlation are the hippocampus and hypothalamus. One of the major sources of input signals to the hypothalamus and its
related structures is the hippocampus (Pampiglione and Falconer, 1960). On this basis, it is often stated that the principle role of the hippocampus is to associate the different incoming sensory signals in a manner to excite appropriate hypothalamic reactions (Olds, 1958; Green, 1964). Also it has been suggested that the hippocampus plays the role of associating the affective characteristics of different sensory signals, and then in turn transmitting the correlated information into reward or punishment areas of the hypothalamus and other centers to help control the information that a person will or will not learn. In other words, if a stimulus strikes a reward area it will be learned or reinforced, but if a stimulus strikes a punishment area it will be forgotten or repressed. The correlation can be made between the hippocampus of Mus musculus 'Mo.', which was the largest, and the fact that this mouse had the best avoidance conditioning response (Scudder et al., 1965). All of the genera and strains, including Mus musculus 'Mo.', had a relatively low concentration of AchE in the hippocampus, but several of the mice including Mus musculus 'Mo.' had an abundant concentration of AchE in the hypothalamus. Perhaps a reciprocal Ach-AchE relationship can be postulated in the neural circuits of these two structures.

It has been reported (Diamond et al., 1964) that an
enriched environment produces an increased depth of the rat cerebral cortex. In the genera and strains studied in this investigation, it was surprising to discover a wide range of cortical depths.

The greatest depth of the neocortex at the diencephalic-telencephalic junction of the genera and strains studied was present in *Mus musculus* 'Mo.' and *Peromyscus maniculatus Bairdii*. On the other extreme the least neocortical depth was found in *Mus musculus* C57BL/6J and *Microtus*. The three subspecies of *Mus* and the two subspecies of *Peromyscus*, therefore, were found not to be identical as might be expected, but significantly different in this regard. If we assume that the cerebral cortex is the center of learning and "intellection", it is not surprising that *Peromyscus maniculatus Bairdii* and *Mus musculus 'Mo.'* display the greatest quality and quantity of learning (Scudder et al., 1965). On the other hand, *Microtus* and *Mus musculus* C57BL/6J were far inferior in the learning situation and this is possibly reflected in their cortical depths. It might be speculated that the highly inbred strains perhaps receive less challenging stimuli from their environment which results in the disuse and atrophy of their cortical structures. In *Mus musculus 'Mo.'* this cortical depth correlates very nicely with its high levels of AchE per gram of brain weight.
Although it would be foolish at this point to state that AchE activity is responsible for superior learning, the possibility of such a relationship is suggested.

C. Behavior and AchE Distribution

From what has previously been stated about neurotransmitters and the fact that different anatomy can be correlated with different behavior, it was not too surprising to show that different areas in the mouse brain have different concentrations of AchE. Going one step beyond, it was also reasonable to find different concentrations of AchE in identical areas of different genera of mice, since these mice run a wide gamut of behavior patterns.

Several investigators (Aprison et al., 1956; Krech et al., 1960; Pierce, 1959; Rosenzweig et al., 1962) have previously related different behavioral patterns with AchE activity. Many of the investigations indicate that environmental stimuli may produce selective regional changes in the enzymatic (AchE) development of the brain, the direction of the response being affected by the age of the animal and other concomitant stimuli. In general, the effect of environmental stimuli in the pre-weaning period has been a suppression of the enzymatic development (AchE) of the cortical structures and an acceleration of
that of the subcortical structures (Kling et al., 1964). It has been shown earlier (Rosenzweig et al., 1962) that in the post-weaning rat, an enriched environment increases the AchE activity of both the cortical and subcortical structures. Another investigation (Liberman, 1962) found that rats raised in darkness have significantly lower ChE activity in the retina than normal rats.

Before discussing several of the more significant correlations between AchE location and behavior in these mice, I would briefly like to raise the possibility of genetic predisposition and AchE. The two subspecies of Peromyscus had identical amounts and distribution of AchE, and for the most part, the three subspecies of Mus, except for minor variations, also had identical amounts and distribution of AchE. The exceptions, when they did occur, were present in Mus musculus 'Mq.' which can be explained by the fact that the other two subspecies of Mus are highly inbred and perhaps more genetically predispositioned to be alike. Nevertheless, the fact that variability can also be genetically endowed cannot be disregarded nor can the concept that inbreeding may produce a tendency for variance. The one factor which still remains a mystery in these animals is their various behavior in the light
of identical AchE distributions. Therefore, the concept that
the level and distribution of AchE could be genetically pre-
determined in any given genus is brought forth.

The possibility exists that areas in the mouse brain
which appear to have high concentrations of AchE are not spec-
ific for this enzyme, but rather are endowed with a high neuro-
humeral content and activity indicating a variety of trans-
mitters. Perhaps these areas are high in general metabolic
activity which might suggest increased levels of ATPase. Some
support for this statement comes from several investigators.
In an autoradiographic study of the distribution of $^{14}\text{C}$-nor-
epinephrine in the brain of the rat (Reivich and Glowinski,
1967), the most striking finding was that almost all of the
components of the limbic system were markedly labelled. Most
of the hypothalamic nuclei were intensely labelled as was the
caudate nucleus. This correlates with the general findings
of this study that the majority of mice had high levels of
AchE confined to these areas. Other areas of close correlation
were the marked labelling of $^{14}\text{C}$-norepinephrine in the medial
longitudinal fasciculus, medial septal nucleus and fasciculus
retroflexus. These areas were found to have moderate AchE
concentrations. Very little or no radioactivity was present
in the cerebral cortex or subcortical white matter, and these
areas were also shown to have a low concentration of AchE. To further substantiate the possibility of general activity at these sites, a few correlations can be made with the distribution and intensity of autofluorescence in the mouse brain (Sobotka, unpublished data). In Peromyscus maniculatus Bairdii the globus pallidus was found to have a high intensity of autofluorescence, it also contains a high level of AchE. The lamina glomerulosa bubli olfactorii in Mus musculus SC-1 was also shown to have high intensity autofluorescence, and in this study, Mus musculus CF-1 and Mus musculus 'Mo.' were found to have high AchE concentration in this structure. Therefore, although the design of this study was to demonstrate AchE concentration in various brain parts, other enzymes or transmitters are present at the same locations, and naturally any correlations related to one enzyme relate equally to another.

In several instances the amount and distribution of AchE in certain anatomical regions was far greater in one or several genera in comparison to the others.

The greatest amount of AchE concentrated in the hypothalamus was present in Mus musculus 'Mo.' and Mus musculus CF-1, both of which we have previously stated to demonstrate aggressive behavior. The least aggressive mice, the two subspecies
of Peromyscus, had a relatively low AchE content in their hypothalami. It is a well established physiological phenomena (Bard, 1928; Ranson, 1937) that stimulation of certain areas of the hypothalamus causes higher animals to become extremely excited, plus development of elevated blood pressure, dilated pupils, and symptoms of rage, such as hissing, arching back and assuming a stance ready to attack. Perhaps the higher levels of hypothalamic AchE in these mice is a necessary component for increased input and output from this center.

The hypothalamus, being the center of the limbic system, is primarily concerned with the affective nature of sensory sensation, i.e., with whether the sensations are pleasant or painful. From well documented experiments (Bard and Mountcastle, 1947; Brady, 1960), it appears that major reward centers are in the hypothalamus. Everything learned depends on reward and punishment. If a behavior is rewarding, it persists; if it is punishing, it ceases. Therefore, the reward and punishment centers, undoubtedly, constitute one of the most important of all the controllers of integrated activities. It would appear that increased activity in hypothalamic centers can be a valuable tool in learning and reinforcement. This, perhaps, would correlate with the fact
that was previously mentioned, *Mus musculus 'Mo.'* is a good "learning" animal.

It was previously stated that *Microtus* had the largest volume of caudate nucleus, and this is also correlated with the fact that *Microtus* had the highest concentration of AchE in its caudate nucleus. Again this may be another example where the use or overuse of a particular center may necessitate higher levels of AchE.

*Perognathus* and *Mus musculus CF-1* have the longest length of olfactory bulbs and also have a high concentration of AchE located in their nuclei habenulae lateralis. The nuclei habenulae are olfactosomatic reflex centers receiving impulses by way of the striae and discharging them to the interpeduncular ganglion (Peele, 1961). It would be interesting to speculate that the greater need to use the sense of olfaction in these mice has led to an increase in the size of the bulbs and in greater activity of the nuclei directing impulses from this site. Perhaps one could argue that these animals are more immature from the standpoint of being poorer learners and consequently their sense of olfaction is more developed. It is interesting to note that *Mus musculus CF-1* is almost blind, *Perognathus* may follow desert trails long distances (Scudder, personal communication). There is no doubt that a more
perceptive sense of olfaction would be useful to both of these animals.
Summary and Conclusions

This study has been an attempt to establish correlations in various genera and strains of mice between 1) brain functional anatomy, 2) concentration and localization of AchE, and 3) behavior. Both differences and similarities between strains and genera, based on data obtained by other investigators at the Institute for the Study of Mind, Drugs, and Behavior, were noted in these parameters. AchE was localized qualitatively by the author utilizing the histochemical method of Koelle, and the anatomy of the various types of brains was studied from microphotographs of serial sections throughout the entire brain for each genus and strain studied.

Both grossly and histologically, many of the genera were found to vary between one another considerably. Furthermore, AchE varied in amount and distribution and was found to be localized in different regions in different genera.

In several instances, subspecies were found to have approximately identical distributions of AchE while exemplifying varied behavioral characteristics. The three subspecies of Mus and the two subspecies of Peromyscus are
examples. However, even though the distribution and concentration of AchE in the three subspecies was identical, their microanatomy was found to vary. For example, it was found that the approximated volume of the reticular formation was far greater in Mus musculus 'Mo.' as compared to Mus musculus C57BL/6J and Mus musculus CF-1.

Some genera of mice with relatively smaller brain weights as compared to their body weights were also found to have certain histological structures larger than the mice with larger brains. For example, both Microtus and the subspecies of Peromyscus have the largest volume of caudate nucleus. Peromyscus belongs to the group of mice which in this study were found to have the greatest brain/body weight, but Microtus does not.

An interesting observation was made throughout this study that two histological areas or nuclei that possibly are related functionally by common neural circuits were often found to be both enlarged. For example, the globus pallidus and caudate nucleus of Microtus were both relatively larger than those of the other mice. This was also true of the hippocampus and hypothalamus of Mus musculus 'Mo.'

Although the design of this study concerns primarily the importance of AchE in behavior, nevertheless, the possibility
exists, based on these studies, that areas in the mouse brain which appeared to have high levels of AchE activity were not necessarily specific for this enzyme, but rather may be endowed with a high neurohumeral content and general metabolic activity indicating a variety of transmitters. Thus in several instances AchE concentrations in certain areas correlated very closely with the norepinephrine distribution in these similar areas found by others, and this fact alone underscores the danger in accepting the correlations and hypotheses of this thesis as more than speculations of heuristic value for further research.


Approval Sheet

The thesis submitted by Roy J. Betti has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is, therefore, accepted in partial fulfillment of the requirements for the Degree of Masters of Science.

Date: 9/1968
Signature of Advisor: [Signature]