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BRAIN SEROTONIN AND BEHAVIOR IN SELECTED STRAINS OF RATS

Berkeley, California

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Gordon T. Pryor

(Thesis)

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Department of Psychology and Lawrence Radiation Laboratory
University of California, Berkeley, California

January 1964

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CHAPTER 1

Brain Biochemistry and Behavior

> "Ever since physiology and psychology began to go on separate paths about ninety years ago, investigator after investigator has discovered with excitement what the other discipline had to offer. Such investigators have built a series of bridges from parts of the one discipline to parts of the other, without, however, uniting the two disciplines. Each bridge has permitted the exchange of methods and findings of great power and wealth, thus inevitably influencing the development of theory in both disciplines."
>
> (Rosenzweig, Krech, & Bennett, 1961)

A restatement of this quotation might be simply that problems suggested from the data and theory of one discipline require data and theory from other disciplines for their adequate solution; both disciplines being altered by the exchange. The reductionistic philosophy of the physiological psychologist requires that behavior, mental events, psychic phenomena, or what have you, be reduced to events of a physiological nature, which in turn require explanations in terms of anatomy, biochemistry, physics, molecular chemistry and perhaps even nuclear physics. The authors of the quotation cited above are apparently of this philosophy, being actively engaged in the delineation of anatomical and biochemical correlates of behavior. In this chapter the results of a decade of their research and theorizing will be reviewed.

Early statement of the research question and preliminary results

In 1953 the group composed of David Krech, Mark R. Rosenzweig and Edward L. Bennett with assistance from graduate students and technicians addressed themselves to the problem of establishing relations between measurable, overt behavior and brain biochemistry in the rat. They first selected as a behavioral measure the "hypotheses" formed by rats in a solutionless problem consisting of a four-choice light-dark, right-

left discrimination box (Krech Hypothesis Apparatus) in which both parameters were varied randomly, neither providing a reliable cue as to the "correct" choice. Krechevsky (1935) had shown that rats in this situation will consistently make either visual (light-dark) or spatial (left-right) "hypotheses" to guide their choices and that lesions in either the visual or somesthetic cortices would alter such behavior in a predictable fashion.

They chose as a suitable biochemical system in brain the choline acetylase (ChA)-acetylcholine (ACh)-acetylcholinesterase (AChE) system. This choice was based upon the current theory that ACh was released from neural terminals into the sub-synaptic space where it reacted with post-juctional receptors to change the permeability of the membrane. The change in membrane permeability led in turn to the establishment of an excitatory postsynaptic potential that contributed to the "spike" potential and its propagation down the next neuron. In other words ACh was considered a chemical transmitter at certain synapses. ChA is present as the last step in the synthesis of ACh, and AChE is present, presumably, to hydrolyse the liberated, "free" ACh thus terminating the transmission process and preserving some sort of discreteness (c.f. Crossland, 1960, for a review of ACh and other substances as transmitters in the central nervous system, and Koelle, 1962, for a revised theory of the role of ACh and AChE in this process).

Assuming such a role for ACh, these workers reasoned that due to its pivotal role in maintaining continuity throughout a neural network, its levels in brain might be reflected on a gross behavioral level, namely in adaptive behaviors or more specifically in learning and memory. Rather than measuring ACh directly, which is technically difficult due to its lability, they chose to measure AChE which is relatively stable and for which suitable instrumentation had recently been developed (Neilands & Cannon, 1955). They assumed, since there was a fairly high correlation

between the concentration of ACh and the activity of AChE throughout the brain, that the latter would be an adequate index of the former.

Using "hypothesis" formation as a behavioral measure and AChE as a biochemical measure, they proposed that rats with relatively higher AChE in the visual cortex would manifest visual "hypotheses" whereas rats with relatively higher AChE in the somesthetic cortex would show predominantly spatial "hypotheses." The results indicated that animals with spatial "hypotheses" had higher AChE activity throughout the dorsal cortex than animals with visual "hypotheses" (Krech, Rosenzweig, Bennett, and Krueckel, 1954). Although the specific hypothesis was shown experimentally to be incorrect, the results were extremely encouraging from the standpoint of establishing a relation between brain biochemistry and behavior.

Individual and genetic differences in behavior and brain biochemistry

Since most animals selected the lighted alley on their first trials in the Krech Hypothesis Apparatus, the assumption was made that visual cues were dominant and that in a solutionless problem, a shift to a spatial "hypothesis" represented a more adaptive response than maintaining the more obvious visual "hypothesis." Such an explanation was consistent with the biochemical results and allowed the hypothesis that animals with greater cortical AChE activity were more adaptive and hence better learners (Rosenzweig, Krech & Bennett, 1958). An empirical test of this hypothesis was available by measuring the cortical AChE in descendants of two strains of rats selectively bred for maze-behavior by Tryon (1940, 1942) and since maintained without further selection pressure in the Berkeley colony. During the seventh generation of selection, Krechevsky (1933) had found that the "maze-bright" strain tended to vary their hypotheses more and showed more spatial hypotheses

than the "maze-dull" rats who tended to stay with the more dominant visual cues. It was found that descendants of the "maze-bright" strain and descendants of the "maze-dull" strain, named respectively S_1 and S_3 to distinguish them from the original lines from which they may have changed considerably, did indeed differ in brain AChE. The S_1 strain had about 10 percent more cerebral AChE activity per unit weight than the S_3 strain (Rosenzweig, et al., 1958). In addition they found a statistically significant increase in AChE activity as one progresses from the posterior, visual cortex through the middle, somesthetic cortex to the anterior, motor cortex.

The differences between strains and cortical regions were immediately criticized by biochemists and physiologists as being biologically unimportant. Tower (1958) enumerated several criticisms that, in spite of numerous demonstrations to the contrary, have plagued this line of research until the present: (1) "the changes are close to, or within the probable limits of, error of sampling and analysis"; (2) "the method of measurement does not apparently take account of the type of cholinesterase"; (3) "cholinesterase in brain is apparently far in excess of requirements"; and (4) "the activity of the acetylcholine system is correlated with species brain size and probably with neuron density and/or size (p. 356)."

Answers to these criticisms have come partly from the Berkeley laboratories and partly from recourse to other experiments. First, it has been shown repeatedly that the techniques are reliable within $\pm$ 1-2 percent on duplicate runs of the same tissue sample and that the differences noted are consistent from experiment to experiment.

Second, cholinesterase (ChE, often called pseudo-cholinesterase[1])

---

[1]c.f. Standard nomenclature recommended by the Commission on Enzymes of the International Union of Biochemistry (1961).

accounts for less than 5 percent of the activity in rat brain measured by this technique (Bennett, Rosenzweig, Krech, Karlsson, Dye & Ohlander, 1958; Bennett, Krech & Rosenzweig, 1963).

Third, it is not at all apparent that AChE exists at functional sites in the central nervous system in excess. While it is true that at peripheral junctions a large reduction (up to 20 percent of normal) in AChE can be sustained without decrement in transmission (Bullock, Grundfest, Nachmansohn & Rothenberg, 1947), there is no evidence that the reduction occurred at functional sites (c.f. Koelle, 1962) or whether such a reduction would be as ineffective in the central nervous system. While Freedman, Willis, and Himwich (1949) reported no overt signs of toxicity after a 50 percent reduction in cerebral AChE, their behavioral measures were admittedly very crude. Sawyer (1955) found that almost any reduction in AChE was accompanied by some decrement in the swimming responsiveness of amblystoma.

Fourth, the S_3 strain has a larger brain than the S_1 strain (Bennett et al., 1958) suggesting that size of brain alone is not responsible for the observed difference in AChE activity per gram tissue between the strains. Further, preliminary data indicate that the S_3 strain has a denser network of neurons and glia in the cortex than the S_1 strain (Diamond, personal communication).

Returning to the question of age and strain differences, Bennett et al. (1958) published a report of extensive comparisons of the S_1 and S_3 strains on AChE activity and brain weight from 28 to 527 days of age. The cortical AChE values for both strains rise sharply until about 60 days, and then continue upward more gently, reaching a broad peak at about 100 days and declining slowly thereafter. In the subcortex AChE rises fairly rapidly to a sharp peak at about 80 days and declines thereafter. Total brain weight increases continuously in a negatively

accelerated fashion. The S_1 strain had higher AChE per unit weight than the S_3 strain at all points and for both cortical and subcortical values. On the other hand the S_3 strain had a heavier brain so that if total AChE activity is considered, the S_3 strain actually has more enzyme present due to its larger mass.

Two arguments might be raised against these results suggesting that higher AChE is associated with better learning. First, it may be that there is no generality in the behavioral measure (i.e., if the S_1 animals retained their superiority on the 17-unit T-maze for which their ancestors had been selectively bred, they may be inferior to the S_3 animals on other tasks). Second, the difference in AChE may be only one of a number of biochemical and physiological variables that could conceivably also account for the behavioral differences.

In order to test the generality of the behavioral superiority and to determine whether it still existed, animals from the two strains were compared on their performance in three mazes under food deprived conditions - the Lashley III maze, the Dashiell checkerboard maze, and the Hebb-Williams maze. The S_1 strain was superior in all respects to the S_3 strain, thus showing some generality in the behavioral differences (Rosenzweig et al., 1958). To be sure, the S_1 strain is not always superior to the S_3 strain on all tasks, as was shown by Searle (1949), but the exceptions are rare.

To determine whether the two strains differed generally in brain enzymes and/or metabolic rates, the glycolytic enzyme lactic dehydrogenase (LDH) was measured (Bennett, Krech, Rosenzweig, Karlsson, Dye & Ohlander, 1958). Although regional and age differences in LDH activity were noted, the pattern did not follow that of AChE, and there was no difference between the S_1 and S_3 strains. Later (Bennett, Rosenzweig, Krech, Ohlander and Morimoto, 1961) it was also shown that percent protein does not differentiate the two strains.

It now became important to find out whether the observed strain differences in brain AChE and maze performance were causally related or merely fortuitous. Two experiments were performed, both utilizing genetic principles. First, if the two variables were causally related, a negative correlation ought to be observed between error scores and brain AChE in crosses of the two strains. Instead, small positive correlations were found. Second, Roderick (1960) selectively bred two pairs of strains from different foundation stocks for cortical AChE. In both instances he was able to select a high (RDH and RCH) and a low (RDL and RCL) strain. Again, on the basis of the proposed behavior-AChE relation, animals of the high AChE lines should show superior maze performance. However, "in only one of the six comparisons was there any indication that this prediction held, and this difference was negligible and statistically insignificant. On the other hand, in four cases the low-ChE strain made fewer errors than the high-ChE strain" (Rosenzweig, Krech, & Bennett, 1960, p. 485).

To explain what appeared to be a paradox, the assumption that AChE is an index of ACh concentration was carefully reconsidered. If it were assumed instead that ACh is independent of AChE and under separate genetic control, the results would be consistent. The crossing of high and low strains would result in a random assortment of ACh and AChE so that in a group of offspring, animals having high AChE would tend to have a mean level of ACh and perhaps a lower synaptic transmission efficiency due to the too-rapid destruction of ACh. On the other hand, a group having low AChE would also tend to have a mean level of ACh and thus, perhaps, a higher synaptic transmission efficiency. Similar reasoning was suggested to explain the results of the selection experiment. To test this new hypothesis ACh was measured in whole brains of animals from the six strains; S_1-S_3, RDH-RDL, RCH-RCL. The prediction that the S_1

strain would have higher levels of ACh than the S_3 strain and that the other two pairs of strains would have almost equal levels of ACh was verified (Bennett, Crossland, Krech, & Rosenzweig, 1960).

Two further experiments to test this revised hypothesis have been undertaken. First, a selection program for animals having high and low ACh is underway. Due to induction effects these animals should also exhibit high and low AChE respectively and, if the hypothesis is correct, high and low maze performance respectively.

Second, a selection program similar to that of Tryon's selection for maze-bright and maze-dull rats was completed by Olson using errors on the Lashley III maze as the criterion for selecting high and low strains. In addition brain ACh and AChE were measured in each generation to see whether the biochemical variables segregated with the behavioral response. While a behavioral response was readily obtained, there was no separation of the lines on either ACh or AChE in the predicted direction.

It is premature to reject this hypothesis on the basis of the study by Olson alone. Clearly, his procedure has resulted in different strains biochemically than those of Tryon. But there are also quite evident behavioral differences apart from errors made on the Lashley III maze. The S_1 and S_3 strains are relatively tame whereas precautions are necessary in handling the Olson strains. Further, nothing is known about the generality of the superiority shown by the Olson bright line, and it may be quite specific. Just as likely, however, the biochemical events underlying learning are not unitary and involve a number of systems.

Effects of experience on AChE

Having demonstrated that there are individual and strain differences in brain AChE activity that can be related to certain measures of

adaptive behavior, the possibility of measurable biochemical changes as a result of learning was investigated. While the common belief was that changes in the central nervous system must occur during learning, demonstration of any such changes had not been reported. Several studies in the literature hinted at the possibility that changes in ACh and AChE might be found if the mechanisms underlying learning were involved with this system.

First, Boell, Greenfield and Shen (1955) reported that removal of one optic vesicle from frog larvae produced a decrease in AChE activity in the contralateral lobe. This deficiency was primarily restricted to regions with dense synaptic networks, and succinoxidase, an enzyme not specifically involved in neural transmission, was not affected. Second, Sperti & Sperti (1959) found that unilateral transection of the cerebellar peduncles in the rat led to a significant drop in AChE activity of the cerebellar cortex, the drop being greater on the side ipsilateral to the cut than on the contralateral side. Third, higher AChE activity was found in certain hypothalamic nuclei of lactating rats or rats on a high salt diet than control animals (Pepler & Pearse, 1957). Fourth, Burkhalter, Jones & Featherstone (1957) found that addition of ACh to a chick lung preparation increased the AChE activity , a phenomenon known as enzyme induction.

With these considerations in mind Rosenzweig, Krech & Bennett (1961) proposed that training involves increased neural transmission with an increased release of ACh which might be expected to induce a compensatory increase in AChE. An initial test of this hypothesis involved animals run in an umweg apparatus (Krechevsky, 1938). compared with untrained littermate controls. The trained animals had significantly higher cortical and subcortical AChE activity than their untrained littermates. A control experiment was conducted to test

whether the effects could be accounted for by differential handling, and it resulted in a negative answer. Since the animals trained on the umweg apparatus had been kept at 75 percent of their body weight, a second control experiment was conducted to test the effect of this variable on AChE activity. It was found that animals fed only once a day for 23 days and kept at 75 percent of their body weight showed a loss of about 4 percent of their brain weight and a small, insignificant rise in cortical AChE activity compared to normal controls. The subcortex showed no change in AChE activity.

To avoid the complicating effects of underfeeding a new experimental procedure was devised. One animal of a littermate pair was assigned at weaning (about 25 days) to an experimental group (Environmental Complexity and Training; ECT) and the other animal served as his control (Social Control; SC). The ECT condition consisted of housing 10 animals in a large, wire cage which had wooden "toys" that were changed daily, and allowing them to explore in small groups the Hebb-Williams apparatus (with a different pattern of barriers each day) for 30 minutes daily. In addition they were given training in the Lashley III maze, the Dashiell maze and the Krech Hypothesis Apparatus with a small glucose tablet as an incentive. Food and water were available ad lib. The SC condition consisted of housing three animals per cage under standard colony conditions. They received glucose tablets whenever their littermates did. After 80 days all animals were sacrificed for biochemical analysis under code numbers that did not reveal their group identity.

The results showed a significant increase in AChE activity per unit weight in the somesthetic cortex and the subcortex in the ECT group as compared to the SC group; the visual cortex also showed a rise in AChE per unit weight, but was not statistically significant (Rosenzweig et al., 1961).

Later in 1959 the results of four additional groups were reported using S_1 females, S_3 males, RDH males, and RDL males (Krech, Rosenzweig, Bennett & Longueill, 1959). In these experiments the control animals (Isolated Controls; IC) were kept one to a cage in 'solitary confinement' in an effort to maximize the effect on AChE activity. The IC animals were housed in special cages constructed of sheet metal. Only the front allowed visual access and it faced a blank wall. No visual contact between animals was permitted and handling was kept to a minimum. The results again showed a significant rise in subcortical AChE, but the ECT animals actually had lower cortical AChE activity per unit weight than their IC littermates! Percent protein was also measured in this experiment, but it did not change.

This rather puzzling result was replicated with a total of 177 animals from six strains comparing the ECT, SC and IC conditions (Krech, Rosenzweig & Bennett, 1960). In all strains the cortical AChE activity per unit weight was lower in the ECT animals than in the IC animals whereas the reverse was true for the subcortex. The SC condition resulted in AChE activity per unit weight intermediate between the ECT and IC conditions. When the ratio of cortical AChE activity to subcortical AChE activity was taken as an index, there was a negative monotonic relation between amount of stimulation and this index. To control for the possible effects of differential locomotor activity an experiment was conducted in which experimental S_1 animals had access to an activity wheel attached to their cages whereas control animals were prevented from entering the wheels. No significant differences in AChE activity were noted between animals in these two conditions.

Effects of environment on cortical weight

Clearly, there was a measurable effect of the ECT-SC-IC procedures

on AChE activity, but the cortical effect was not in the predicted direction, while the subcortical effect was. In a report published the following year (Rosenzweig, Krech, Bennett, & Diamond, 1960) these results were again replicated and a further analysis of the data was made that illuminated the earlier unexpected results. The expression of AChE activity as moles ACh x 10^{10} hydrolysed per minute per milligram of tissue (specific activity) did not reveal any changes that might also occur in tissue weight due to the procedures. When the separate components of this ratio, moles ACh x 10^{8} hydrolysed per minute (total activity) and wet weight of tissue, were scrutinized separately it became apparent that both were changing somewhat independently of each other. AChE total activity did indeed increase in both the cortex and the subcortex in ECT animals compared to SC or IC animals, just as had been predicted. Remarkably, however, the wet weight of the cortical sections also was higher in ECT than IC animals and by a greater percentage than the increase in activity. Accordingly AChE specific activity was lower in the cortex of ECT than IC littermates. The subcortex showed little if any weight change, and hence the increase in AChE total activity in ECT animals was also apparent when expressed as AChE specific activity for this section.

Previous experiments were reanalysed in terms of total activity and tissue weight and the results were quite similar in all respects. Subsequent experiments have also confirmed these findings (Krech, Rosenzweig, & Bennett, 1963; Zolman and Morimoto, 1962).

The unexpected finding of a morphological change as a consequence of environmental complexity and training was, perhaps, more intriguing than the predicted finding of a biochemical change. The current dogma would not lead to predictions of such anatomical changes whereas some sort of biochemical change could be assumed to occur, the problem being

to find and measure it.

Experiments utilizing anatomical techniques were conducted in an effort to elucidate these morphological changes. The depths of cortical sections in ECT and IC animals were measured, whereupon it was found that ECT animals had thicker cortices than IC animals, and that the percent difference corresponded quite closely to the weight difference (Diamond, Krech & Rosenzweig, in press). The increase in depth was greater in the visual cortex than in the somesthetic cortex as was true also of the weight difference. Further histological analysis revealed that there were less neurons, glia and capillaries per microscopic field in the ECT animals, indicating a greater amount of intercellular and intervascular substance, and the ECT animals had more large blood vessels and fewer small ones than the IC animals. The possibility that dendrite branching might account for the change was proposed and is in the process of being investigated.

Effects of experience on learning

Other investigators (e.g. Bingham & Griffiths, 1952; Forgays & Forgays, 1952; Hymovitch, 1952) had shown that training and opportunities for experience can produce changes in learning capacity. In the experiments comparing biochemical and morphological changes as a function of the amount of developmental stimulation from weaning to maturity, it was assumed that at least part of the observed change was due to an increased involvement of neural and biochemical elements involved in learning. However, it was necessary to demonstrate such superiority in performance of ECT animals over IC animals. To do this littermate S_1 male rats were placed in either an EC condition (same as ECT except without the formal training) or the IC condition for 30 days beginning at weaning. At the end of this period, the two groups were tested in a

series of reversal-discrimination problems in the Krech Hypothesis Apparatus. No difference was evident between the two groups on the initial light-correct problem, but on successive reversals the EC group showed a marked superiority over the IC group.

Additionally, it was found that the correlation between the cortical/subcortical weight ratio and the mean number of errors per reversal problem was -.77 for the EC group but only -.15 for the IC group; for specific AChE the respective correlations were .81 and .53. "It is clear from this correlational analysis that for animals raised in an enriched environment during infancy there is a substantial and stable relation between performance measures on the reversal discrimination problem and those morphological and biochemical measures of the rat's brain which are most affected by the enriched environment" (Krech, Rosenzweig & Bennett, 1962).

A 'critical period' for the effects of environmental stimulation?

Two questions arose once the effects of an 80 program of enriched environment *versus* an impoverished environment on brain biochemistry and morphology had been established. First, how much stimulation (or deprivation) was necessary to produce measurable effects? Second, did it matter when during the course of an animal's life the extra stimulation (or deprivation) was given?

Both questions were partially answered in an experiment investigating the effects of a 30 day training period. Zolman and Morimoto (1962) found that this shortened period of training was sufficient to produce significant increases in cortical weight of the ECT group compared to the IC group, but no significant differences were found in AChE total activity, although the means were in the expected direction in most comparisons. If the ECT animals were placed in isolation for 30 days after previously

having been exposed to the enriched environment for 30 days beginning at weaning, the differences tended to dissipate.

While animals exposed to the enriched environment from weaning showed the typical decrease in cortical specific AChE and increase in subcortical specific AChE, animals who began training at 55 days showed an increase in subcortical specific AChE only.

Reduction of the training period to 14, 7 and 3 days resulted in smaller increases in cortical weight of ECT animals compared to IC littermates, but even at the shorter period the increase was statistically significant if all three periods were combined. AChE total activity was not significantly affected by these shorter periods of exposure (Zolman, 1962).

To further test whether the effects of ECT versus IC depend critically on the age at which the animals are exposed, adult rats were tested (Rosenzweig, Krech & Bennett, 1963). Animals were assigned to the respective conditions at 105 days of age, the usual age of sacrifice in the earlier experiments. Rats of 105 days can be considered adult since they mature sexually at about 70 days and growth of the brain, which never stops completely in the rat, has fallen to a low rate before 100 days.

The differential experience afforded these adult rats by the 80 day ECT-SC-IC program resulted in biochemical changes as large or larger than seen in the younger animals. Thus, there did not appear to be a 'critical period' for altering either brain weight or AChE activity from the age of weaning on.

Investigation of the period prior to weaning has not been made with respect to effects of ECT or IC. However, the effects of 'handling' from daystwo through day ten, post partum, on animals of the S_3 strain indicated a moderate increase in cortical weight and a large

(10.6 percent) increase in subcortical weight in the experimental group (infants removed from the home cage, and placed for five minutes in a metal pan partly filled with shavings, and then returned to the home cage). Total AChE also increased in the sensory cortex and subcortex, but not in the remaining dorsal cortex (Tapp & Markowitz, 1963).

Effects of experience on blinded animals

Since one of the environmental variables that is clearly different in the ECT and IC conditions is the amount of visual stimulation provided, and since tissue samples from the visual cortex showed the greatest effects of these conditions in terms of both wet weight and AChE total activity, this modality was investigated further in an effort to delineate the relevant factors.

Experiments were performed with littermate triplets and quadruplets (Krech, Rosenzweig & Bennett, 1963). Animals were assigned to one of four conditions: Sighted-ECT; Blind-ECT; Sighted-IC; Blind-IC. Animals in the blind groups were enucleated under ether anaesthesia at weaning just prior to being placed in the experimental conditions.

The results showed the usual effects of the ECT-IC conditions on both the blinded and sighted animals. However, there were also effects produced by the blinding. First, the blinded animals had lower weights in all divisions of the brain except the somesthetic cortex, which increased. Second, AChE total activity was reduced in all sections of the brain except the somesthetic and visual cortices where it increased. The reduction in both total and specific AChE was especially marked in the superior colliculi.

It appeared as though the effects of blinding were similar in most respects to reducing stimulation as in the IC condition. The Blind-ECT animals had heavier brains and more AChE than the Sighted-IC

animals but less than the Sighted-ECT animals. Further, the Blind-IC animals were worse off in this respect than the Sighted-IC animals.

The striking exceptions in this pattern were tissue weight and AChE total activity of the somesthetic cortex which increased in the blinded animals, suggesting some sort of compensatory mechanism, and AChE total activity in the visual cortex which increased in the blinded group in spite of the fact that tissue weight actually decreased.

Two speculative hypotheses were presented to account for the increase in AChE total activity in the visual cortex of the rat resulting from blinding: (1) the visual cortex may actually be more active in blinded animals due to, perhaps, a loss of inhibitory signals; (2) the neurons affected by blinding are non-cholinergic and as a result of degeneration of their terminal endings neighboring cholinergic endings move in to make functional contact.

Effects of experience on brain ChE

As related earlier (pp. 4-5) a frequent criticism of the research reported in this chapter has been that the measure taken of AChE activity does not distinguish AChE from the non-specific ChE. Although it was shown that ChE accounts for less than 5 percent of the measure used (Bennett *et al.*, 1958) it was important to determine whether very large changes in ChE might account for the differences found as a result of the ECT-IC procedures.

Using a technique adapted from Ellman, Courtney, Androes, & Featherstone (1961) both AChE and ChE were determined using acetylthiocholine and butyrylthiocholine (in the presence of a specific inhibitor of AChE) as substrates respectively. The previous finding of less than 5 percent ChE in rat brain was confirmed (Bennett, Krech, & Rosenzweig, 1963). In an experiment comparing the effects of ECT and IC conditions,

a different pattern of change was found for ChE compared to that found for AChE. There was a general increase in ChE total activity in the cortex of ECT animals and little or no change in the subcortical section. The increase in the cortex was large enough so that specific ChE also showed an increase in the ECT group compared with a decrease in AChE specific activity. Thus, the changes in ChE cannot account for the observed changes in AChE.

The implications of these results are interesting. Morphologically, AChE is found in the brain primarily in neurons whereas ChE is also contained in glia. The recent interest in glia as perhaps being involved in learning may be of importance in this respect.(c.f. Hyden, 1961).

Summary of research on brain AChE and behavior

The results of the research attempting to relate brain AChE to behavior can be summarized as follows: (1) There are individual and strain differences in rat brain AChE that appear to be related to certain kinds of measurable behavior; (2) it is possible to select animals on the basis of cortical AChE but such selection is apparently independent of ACh; (3) varying amounts of environmental stimulation can produce measurable changes in rat brain AChE, ChE and wet weight of tissue in a predictable manner; (4) the visual system is only partly involved in the effects of an enriched versus an impoverished environment on AChE activity and tissue weight, other systems apparently compensating for loss of vision after blinding; (5) the biochemical and morphological changes brought about by varying the amount of environmental stimulation are not a function of the age at which animals are exposed to the stimulation, i.e, there is no "critical period".

Roles of other possible neurohumors

The preoccupation with AChE in this project is quite understandable,

at least during the early stages, for the following reasons: (1) ACh is the most respectable candidate for a transmitter role in the central nervous system; (2) AChE can be measured with a high degree of reproducibility which is extremely important in view of the small differences observed; (3) having been successful in initial attempts to relate this system to behavior, it was necessary to explore these leads further.

It is unlikely, though, that ACh is the only transmitter in the central nervous system or that it is the only such substance causally related to gross behavior. Control experiments have been reported establishing that observed differences in AChE are not merely a function of differences in general metabolic acitivity or protein content. However, this does not eliminate the involvement of other specific substrate-enzyme systems and especially other systems that may be involved in neural conduction and transmission.

The exploration of other substrate-enzyme systems in the brain seems justified now that the ACh-AChE system has gained a certain respectability in this respect. Clearly, AChE activity does not account for all of the variance observed in gross behavior of animals performing in mazes. Further, behavior is far from unitary and it is not unreasonable that different substrate-enzyme systems are involved with different kinds of behavior. In fact, it is clear that maze behavior is the resultant of a large number of factors including sensory discrimination, motivation, emotional components (i.e., autonomic responses), memory, etc. While these behavioral concepts fall short of precise definition, there seems to be enough intuitive meaning associated with them to distinguish one from another in most instances. The search for their physiological correlates provides the raison d'etre of research and theory in the field of physiological psychology.

CHAPTER II

The Role of Serotonin in Brain and its Possible Significance in Behavior

At the conclusion of Chapter I, it was suggested that non-cholinergic systems may also be related to measurable aspects of behavior. Even relatively simple behaviors are not unitary, and, consequently, several interrelated mechanisms probably provide their physiological basis. If this is translated into gross biochemical and behavioral measurements, the results should be a set of relations between levels or activities of certain substrates or enzymes and certain measurable sets of responses. Further, both the biochemical and behavioral variables will probably be found to be intercorrelated to a greater or lesser degree.

The purpose of this dissertation is to examine the role of brain serotonin in relation to measurable behaviors in several strains of rats. More specifically, three sets of experiments will be reported that attempt to discover such relations: (1) Chapter III will present the results of experiments in which several genetically distinct strains of rats have been measured with respect to brain serotonin and its enzymes of synthesis and degradation. Since these strains differ in maze performance, any differences in brain serotonin would provide a basis for further study of causal relations. (2) Chapter IV will give the results of experiments in which individual rats within two strains have been measured on a number of behavioral variables and their scores correlated with their individual levels of brain serotonin obtained after sacrifice. (3) Chapter V will present the results of experiments investigating the effects of an enriched <u>versus</u> an impoverished environment on brain serotonin in two strains of rats.

The selection of the serotonin system in brain for the study of

relations between a non-cholinergic system and behavior was based on a number of factors, including its current interest in the comparatively new field of psychopharmacology. It is a curious fact that a large number of agents that have a depressant or excitatory effect on animals and humans also alter the levels of peripheral and central serotonin. This suggests that serotonin may be involved in the regulation of normal patterns of neural activity.

A review of what is currently known about serotonin in the nervous system of mammals will be presented in this chapter. A short history of the discovery of serotonin in mammalian brain, and the theorizing that took place almost immediately thereafter will be followed by an examination of the techniques used to measure serotonin in tissue and the distribution of serotonin in nature. The biosynthesis and metabolism of serotonin will be examined, and it will be seen that this route is shared by other systems, such as noradrenaline, thus making unambiguous interpretations of the effects of agents that inhibit specific metabolic steps very difficult. Then, a survey will be made of a number of factors, both chemical and other, that alter the levels of serotonin in the brain. Finally, the effects of serotonin on neural activity and overt behavior will be followed by a discussion of the current conceptual status of serotonin and its possible role as a neural transmitter in the central nervous system.

Discovery of serotonin in mammalian brain

Interest in serotonin (5-hydroxytryptamine) in the mammalian nervous system can be traced to a series of independent events that took place in the early 1950s.

First, from the field of cardiovascular phammacology the search for an elusive substance of potent vasoconstrictor action led to the discovery

of serotonin by Rapoport, Green, & Page in 1947. Its synthesis in 1951 (Hamlin & Fischer) and commercial availability in 1952 allowed extensive investigation of physiological and pharmacological properties.

Second, Erspamer (1940) had discovered a substance that stimulated the smooth muscle of the intestine and uterus which he called enteramine. Enteramine was found to be identical with serotonin (Erspamer & Asero, 1952).

Third, serotonin was found in ganglia of certain invertebrates such as the clam, Venus mercenaria, where it behaved like a neurohumor (Welsh, 1954).

Fourth, Gaddum (1953) found that the action of serotonin on smooth muscle was antagonized by lysergic acid diethylamide (LSD), a substance having potent hallucinogenic properties.

Finally, Twarog & Page (1953), in the course of investigating the distribution of serotonin throughout the mammalian body, found, much to their surprise, relatively large concentrations of the amine in brain. This was confirmed by Amin, Crawford & Gaddum (1953, 1954) who also published a map of the regional distribution of serotonin in dog brain, which paralleled closely that found for sympathin (Vogt, 1954).

The discovery of so potent an agent as serotonin in brain, coupled with its uneven distribution and perhipheral antagonism by LSD could hardly fail to arouse the imagination of clinical investigator and theorist alike. The response was immediate and vigorous.

The rapid growth of research involving brain serotonin can be traced in terms of periodic reviews that have appeared during the last ten years. Page, in his 1954 review of serotonin listed 157 references, only two of which dealt with serotonin in brain. Four years later (1958), Page listed 529 references in his review of serotonin in the same journal. One-fourth of the text dealt with relations between serotonin and some

aspect of brain function or mental phenomena. In 1961, Erspamer presented a review "intended to give an account of the progress in the field of 5-hydroxytryptamine and related indolealkylamines in the last 6 to 7 years" (p. 144), that overlaps the first two reviews somewhat. It contains 1358 references, and is 187 pages in length. Erspamer further admits of having been selective in choosing from among over 2000 papers that had appeared during the interval. For a more recent, much less comprehensive discussion of the topic, the reader is directed to a review by Freedman & Giarman (1963) which includes a number of references from 1960 to 1963.

Speculation concerning the role of serotonin in brain

Woolley and Shaw (1954) boldly suggested that mental abnormalities (including schizophrenia) were related to faulty serotonin metabolism. The action of LSD was attributed to its displacement of serotonin molecules from functional receptor sites in the brain. These receptor sites were thought to be on neuroglia which were observed to exhibit a "pumping" motion *in vitro* in the presence of serotonin (Benitiz, Murray and Woolley, 1955).

Two groups suggested a transmitter role for serotonin. Marrazzi & Hart (1955) found that the transcallosally evoked potential was suppressed by serotonin injected into the carotid artery. They concluded that the activity of the brain is the resultant of an inhibitory system mediated by adrenergic-like compounds including serotonin and an excitatory system mediated by ACh.

Brodie & Shore (1957) proposed that serotonin was the chemical transmitter for the central representation of the parasympathetic nervous system. This view resulted from their discovery that reserpine depleted serotonin from brain and caused sedation. They attributed the effects of the drug to a constant release of serotonin from its storage

sites, thus making the active form of the amine available at its natural receptors.

More recently, Aprison (1962) has pointed out that serotonin may act as a neuromodulator of ACh. Noting that serotonin in high concentrations inhibits AChE, he suggests that serotonin may function in brain by competing with ACh at postjunctional receptor sites, thus modifying the action of ACh.

In view of the rather meager evidence on which these theories were based, it is not suprising that they have not been able to encompass the data that have accumulated since. After the first wave of enthusiasm for a quick solution to the role of serotonin in brain, research workers and theorists became engrossed in minor battles concerning how certain drugs that altered brain serotonin had their effects. Investigators relied on assumptions as to the actual role of serotonin in the brain in explaining the action of these drugs, rather than concentrating on validation of the assumptions. As a results, new theory has not kept pace with the gathering of data.

Procedures used to measure serotonin in brain

As is true in most fields, the methods used to measure serotonin largely determine the interpretations that can be made from the resulting data. Ideally, the procedure of choice should measure serotonin exclusively with high sensitivity and high reproducibility. Over the years a number of techniques have been developed for this purpose, but is is unfortunately the case that answers are not comparable from one technique to the other on similar material.

The measurement of serotonin in brain involves first extracting the amine and then either assaying its action on an appropriate biological preparation or measurement of the fluorescence emitted by the indole

nucleus of the molecule when excited by light of a specific wavelength (295 mμ).

Extraction of serotonin

Acetone or n-butanol can be used as an extracting medium. In the procedure of Amin et al. (1953)tissue is minced in several volumes of 80 to 100 percent acetone and extracted for 12 to 24 hours. The filtrate is removed and the residue is re-extracted. Both filtrates are combined, lyophilized, and the residue taken up in a small volume of acid for assay. Recoveries of added serotonin are partly a function of the concentration of acetone used. Towne, Ferster, and Schaefer (1961) describe a technique for preparing acetone that results in recoveries of 95 percent.

The use of butanol depends on the fact that the partition coefficient for serotonin between the aqueous and organic phases reaches 0.95 when the mixture is saturated with NaCl. In the procedure of Bogdanski, Pletscher, Brodie, & Udenfriend (1956), tissue is homogenized in 0.1 N HCl and extracted into ten volumes of n-butanol after saturation with NaCl and adjustment to pH 10 by addition of a borate buffer and solid sodium carbonate. After being washed twice with the borate buffer, the serotonin is returned to an aqueous phase by the addition of n-heptane. Chemical purity of the reagents is essential when estimation is by fluorometry. A modification of this technique is based on the observation that the method of Shore & Olin (1958) for catecholamines also extracts serotonin (Mead & Finger, 1961). This eliminates the buffer washes and makes it possible to simultaneously determine serotonin, adrenalin and noradrenaline in the same tissue sample. Recoveries of added serotonin are from 90 to 100 percent.

Both acetone and butanol extractions are inadequate to the extent

that unwanted compounds are also extracted. Quay (1963) described techniques whereby most substances that might contaminate the assay can be eliminated when butanol is used.

Assay of serotonin extracts

Bioassays were the earliest used and are still the most sensitive methods for measuring serotonin extracted from tissue. Preparations that have been used in this respect include beef and sheep carotid artery rings, the perfused vessels of the rabbit ear, the rat colon, the rat uterus, and the isolated heart of *Venus mercenaria* (Welsh, 1954; Page, 1954, 1958). In the hands of Freedman (1961) the clam heart was able to detect as little as one millimicrogram serotonin added to the bath. However, the preparations are hard to prepare and maintain, subject to tachyphylaxis and seasonal variations in sensitivity, have more variability than chemical methods, and are time consuming.

With the development of suitable fluorometric instruments, a fairly sensitive physico-chemical technique was developed for the determination of serotonin (Undenfriend, Weissbach & Clark, 1955; Udenfriend, Weissbach & Bogdanski, 1955). Serotonin and other 5-hydroxyindoles are maximally excited by ultraviolet light (295 mμ) and emit maximal fluorescence at 330 mμ when in 0.1 N HCl. The fluorescence maximum shifts to 540 mμ when the solution is made 3 N with respect to HCl. This shift provides for a relatively specific assay since substances that interfere at 330 mμ do not shift their peak in strong acid (e.g., catecholamines which are also extracted).

The bioassays do not give comparable results to the fluorometric technique in terms of absolute amounts of serotonin. In general it has been observed that considerably lower values are found using a bioassay. The presence of substances that have similar fluorescence characteristics to serotonin but are biologically inactive may account for the discrepancy.

The conclusion to be drawn from this survey of measurement techniques is that caution must be exercised when comparing results obtained by different techniques, used at different times, or by different investigators. As subtle a difference as the commercial source of reagents used can result in quantitatively different amounts of serotinin extracted. Or, the season of the year or source of biological assay material can produce quite different results in terms of absolute amounts of serotonin measured.

Distribution of serotonin in nature

In this section, the distribution of serotonin in nature will be reviewed, with the hope of obtaining some clues as to its function. First, phylogenetic comparisons will be made, followed by the regional distribution of serotonin in the brains of three mammalian species and its intracellular localization. Finally, the ontogenetic development of serotonin will be examined.

Serotonin is widely distributed in nature, being found in many plants as well as the animal kingdom. Also, neural tissue accounts for only a very small percentage of the serotonin found in vertebrates; gastric mucosa having up to 80 percent of the serotonin in most mammals. Non-neural serotonin will not be discussed further except to say that its function is largely a matter of speculation (c.f. Erspamer, 1961 for a review that includes serotonin in non-neural tissues).

Distribution of serotonin in vertebrates and invertebrates

Table 1 shows the concentration of serotonin in the brains and ganglia of a number of vertebrate and invertebrate species. Due to the different techniques used and the resulting overlap of values, it is not possible to draw many obvious conclusions regarding the phylogeny of serotonin. Vertebrates have from almost zero to 1.0 μg/gm serotonin

Table 1

Occurrence of Serotonin in Whole Brain or Ganglia

of a Number of Vertebrate and Invertebrate Species

	Serotonin (μg/g)			
	Single Species Reports	Studies Including Several Species		Range of Values Summarized by Erspamer's Review (1961)
		Karki, Kunztman & Brodie (1962)	Correale (1956)	
A. Vertebrates				
Dog			0.10	
Cat			0.03	
Rabbit	0.38[1] 0.54[2]	0.60	0.30	0.45-0.57
Guinea Pig	0.44[3]	0.33	0.30	0.58-0.68
Rat	0.54[4] 0.39[5] 0.63[6] 0.33[7] 0.59[8]	0.47	0.40	0.21-0.96
Mouse	0.66[9] 1.00[10]			0.66-1.00
Pigeon			0.15	
Hen			0.20	
Turkey			0.40	
Lizard			0.10	
Tortoise			0.10	
Water Snake			0.20	

1. Costa, Pscheidt, van Meter & Himwich, 1960
2. Pletscher, Shore & Brodie, 1956
3. Whittaker, 1959
4. Kato, 1960
5. Joyce, 1962
6. Skillen, Theines, & Cangelosi, 1961
7. Anderson & Bonnycastle, 1960
8. Heller, Harvey & Moore, 1962
9. Chessin, Dubnick, Leeson & Scott, 1959
10. Caspari, 1960

Table 1 (cont.)

	Serotonin (μg/g)
	Welsh & Morrehead (1962)
B. Invertebrates	
Molluscs	
Venus mercenaria	30–40
Loligigo peallii	0.7
Octopus vulgaris	0.8; 0.25
Crustaceans (several)	<0.10
Worms	
Lumbricus tessestris	10.4
Arenicola marina	3.1
Insects	
Blaberus gigantea	<0.02
Locusta migratoria	<0.02
Coelenterata	
Hydra (Whole body)	1.5
Flatworms	
Planaria	1.9

compared to the invertebrates where the range is from zero to as high as 30 to 40 μg/gm in ganglia of the clam.

Mollusks, worms and coelenterates have relatively large amounts of serotonin, whereas the crustaceans and insects are essentially without the amine. Hydra were the most primitive animals found to contain serotonin, and the value of 1.5 μg/gm is higher than almost any other whole animal studied. The head end of planaria appears to have more serotonin than the middle or tail end. A functional relation is suggested, since this is the most primitive animal that shows any marked cephalization of the nervous system.

The concentration of serotonin in the brains of a number of vertebrates analyzed by Correale (1956) suggests that there is an inverse relation in mammals between brain serotonin concentration and the cortical to subcortical weight ratio. This may be due in part to the lower values of serotonin generally found in cortical tissue.

Regional distribution of serotonin, 5-hydroxytryptophan decarboxylase and monoamine oxidase in dog, cat and human brain

Much of the interest in serotonin as a neurohumor arises from its uneven distribution in the mammalian brain. Table 2 shows the distribution of serotonin and its enzymes of synthesis and degradation, 5-hydroxytryptophan decarboxylase (5-HTPD) and monoamine oxidase (MAO), respectively, in a number of areas of the dog, cat and human nervous systems.

Several gross observations can be made. First, nuclei generally contain more serotonin than fiber tracts. Second, neocortical areas are generally lower in serotonin concentration than phylogenetically older cortical areas and subcortical nuclei. Third, there is a moderate correlation between serotonin concentration and the activity of its associated enzymes, especially 5-HTPD. Interspecies comparisons are

Table 2

Regional Distribution of Serotonin,

5-HTPD and MAO in Dog, Cat and Human Brain

Tissue	Serotonin (μg/g)			5-HTPD (μg serotonin formed/g/hr)		MAO (μg/hr/g)
	Dog*	Cat**	Human***	Dog*	Cat**	Dog*
Isocortex						
Grey and White	.17	.69		∿0×	∿0	∿0
Grey	.27	.68	.01-.08	7		819
Allocortex						
Cingulate g.		.41	.04-.10		∿0	
Hippocampal g.			.10-.30			
Uncus			.16-.30			
Subcallosal g.			.14-.60			1212
Ant. perforated substance	1.5	2.0	.41-1.51	109	264	926
Pyriform cortex	.94	1.4		16	20	
Hippocampus	.64		.06-.19	16		1176
Neostriatum						
Putamen			.23-.95			
Caudate n.	.72	1.6	.20-.70	306	300	935
Fornix	∿0		.22-.55	9		707
Corpus callosum	∿0		.20-.39	4		466
Amygdaloid n.	2.1	1.6	.24-.67	18	35	968
Thalamus	.5	.43	.23-2.70	38	50	940
Hypothalamus	1.7	2.45	.32-1.53	117	180	1624
Mammilary bodies			.27-.48			
Superior colliculi			.50-1.22			
Inferior colliculi		.76	.29-.81		40	
Midbrain	1.0			98		842
Substantia nigra			1.11-1.96			
Pons	.38	.33	.19-1.03	28	40	936
Pontine reticular f.		.89			85	
Medulla oblongata	.62		.20-.50	32		1117
Cerebellum	<.09		.01-.09	<9		930
Olfactory bulbs	.35			5		573
Spinal cord			.09-.20			

*Bogdanski et al., 1957
**Kuntzman et al., 1961
***Costa & Aprison, 1958
×Too low to measure

prohibited except on a relative basis since the results were obtained by different assay procedures. However, the pattern of distribution is similar in all three species. Those areas having the highest levels of serotonin from a group roughly composing the limbic system (Pribram & Kruger, 1954).

Intracellular distribution of serotonin

About 60 percent of the serotonin in ultracentrifuged homogenates of brain is in the mitochondrial fraction (Baker, 1958; Walaszek & Abood, 1958; Giarman & Schanberg, 1958). This has been equated with the bound form of the amine, whereas the remaining 40 percent is assumed to be in the free form.

Further separation of the mitochondrial fraction using a density gradient relative to sucrose has revealed a number of distinct subfractions (Hebb & Whittaker, 1958; de Robertis, 1962). Serotonin and ACh are found primarily in those subfractions rich in nerve endings, which have abundant synaptic vesicles (Whittaker, 1958, 1959; Michaelson & Whittaker, 1962, de Robertis, 1962). Further separation has not been accomplished, so that it is not known whether either substance is actually contained within the synaptic vesicles.

It can be tentatively concluded from this line of research that serotonin exists in at least two different cellular compartments. First, a cytoplasmic compartment that may correspond to the free form of the amine, and second, a particulate compartment that may represent the bound form of the amine. This latter compartment appears to be synaptic vesicles located mainly in nerve endings.

Ontogenetic development of serotonin

Serotonin concentration increases in the brains of rats from early foetal life to adulthood in a somewhat irregular manner (Kato, 1959;

Karki, Kuntzman & Brodie, 1962; Nachmias, 1960). At birth, levels of serotonin are about one-third adult levels and increase only slightly for the first ten days of post-natal life. Then, corresponding to a rapid behavioral, physiological and morphological transition, adult levels are rapidly approached during the next five to ten days. A similar pattern of development occurs in the rabbit, but the new born guinea pig which is well developed at birth has adult levels of serotonin. This correspondence of serotonin levels and behavioral development led Karki et al. (1962) to suggest that serotonin is involved in primitive physiological processes.

However, goat fetuses taken 15 to 25 days before birth had higher brain levels of serotonin than their mothers (Pepeu & Giarman, 1962). In addition neocortical areas were richer in serotonin than certain subcortical nuclei, the reverse of the adult pattern. This finding, if substantiated, is puzzling indeed.

MAO parallels quite closely the development of serotonin (Nachmias, 1960; Karki et al., 1962). No evidence of MAO activity was found in 15-day old rat fetuses using a histochemical technique (Shimizu & Morikawa, 1959). Guinea pigs were found to have adult activities at birth. On the other hand, the capacity to synthesize serotonin is well developed at birth in both rats and guinea pigs (Karki et al., 1962), 5-HTPD activity increasing only slightly post-natally.

The ontogenetic development of the serotonin system in the species studied lacks the consistencies necessary to deduce its physiological role. Perhaps most important in this respect is the lack of correspondence between serotonin present and the capacity to synthesize serotonin. This suggests that mechanisms involved in transport and energy utilization in general are responsible for the delayed appearance of serotonin in neonatal rats. The parallel development of serotonin

with primitive behavioral patterns may be secondary rather than causal.

Biochemistry of serotonin

In this section the main route of synthesis and metabolism of serotonin will be discussed. The importance of this discussion lies in the fact that endogenous serotonin can be altered by interfering with any of the steps in the metabolic pathway of serotonin. A large amount of research has been directed towards the discovery of inhibitors of critical enzymes in the synthesis and metabolism of serotonin and the subsequent observation of changes in behavior. The efforts of these studies have been thwarted to a large extent by the discovery that the enzymes involved are not exclusively utilized by the serotonin system. Therefore, interference with serotonin levels also results in alterations of other naturally occuring substances, and it has not been possible to completely separate the effects of one from the other.

The major pathway in the synthesis and metabolism of serotonin is shown in Figure 1. Brain tissue *in vitro* has been observed to catalyze all of the reactions except the conversion of dietary tryptophan to 5-HTP which may occur in the liver or gut.

The relatively small amount of serotonin in brain is offset to some extent by its extremely rapid turnover in this organ. The estimated half-life of serotonin in brain is 10 to 30 minutes compared to 33 to 48 hours in blood platelets and spleen, 17 hours in stomach mucosa, and 11 hours in intestinal mucosa (Udenfriend & Weissbach, 1958). Such a rapid synthesis and metabolism suggests an important function.

Biosynthesis of serotonin

An enzyme system capable of converting dietary tryptophan to 5-HTP has been isolated from the intestinal mucosa of rats and guinea pigs (Cooper & Melcer, 1961). Freedland, Wadzinski, & Waisman (1961) also found

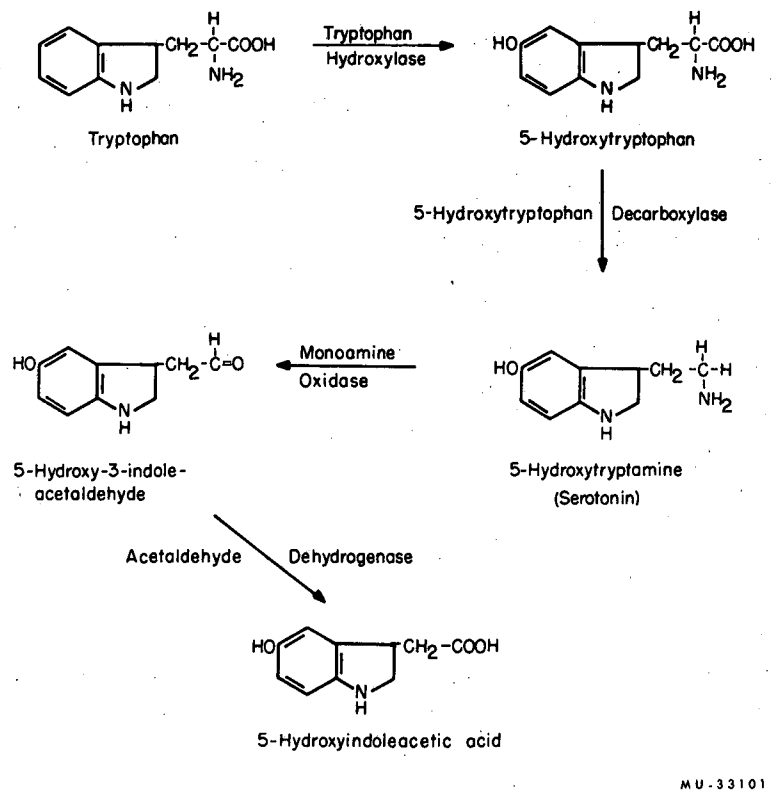

Fig. 1. Major pathway in the metabolism of serotonin.

such an enzyme in liver, but the affinity for serotonin is very low. This liver enzyme has tentatively been identified as phenylalanine hydroxylase (Renson, Goodwin, Weissbach, & Udenfriend, 1961).

Uptake of 5-HTP by brain

The product of hydroxylation of tryptophan, 5-HTP, which is not normally detectable in the circulation, is apparently transported into brain tissue by an active mechanism (Schanberg & Giarman, 1960; Schanberg, McIlroy, & Giarman, 1961; von Wartburg, 1962). This is indicated by: (1) an enhancement of uptake of 5-HTP by brain slices in the presence of oxygen and glucose, (2) the interference of this uptake by dinitrophenol, and (3) a substantially diminished incorporation of 5-HTP by brain slices at 0°C. Areas of rat brain known to be rich in serotonin and 5-HTP are most active in taking up 5-HTP-^{14}C. A number of agents that alter serotonin metabolism or brain metabolism in general do not affect this transport, whereas L-tryptophan, L-tyrosine and DL-3,4-dihydroxyphenylalanine (DOPA) caused a marked inhibition. Phenylalanine, α-methyl DOPA, glutamic acid and γ-aminobutyric acid were also ineffective.

Decarboxylation of 5-HTP to serotonin

Once in the brain 5-HTP is decarboxylated to serotonin by 5-HTPD (Clark, Weissbach & Udenfriend, 1954). This enzyme was originally thought to be quite specific, but recent evidence suggests otherwise (Yuiller, Geller & Eidusen, 1959, 1960; Werle & Aures, 1959; Rosengren, 1960; Lovenberg, Weissbach & Udenfriend, 1962). In fact, it is now agreed that the metabolism of 5-HTP and DOPA make use of the same enzyme. This discovery has resulted in the solution of several problems, but it has created several more. Identity of 5-HTPD and DOPAD means that the levels of products formed by this enzyme are a

function of other variables which must be determined. Second, the results of administering precursors are made uninterpretable since tissue devoid of the product normally may synthesize it under these conditions. Third, the results of inhibiting the enzyme are ambiguous since more than one product is affected.

Distribution of 5-HTPD

5-HTPD is found in most parenchymatous tissues. Its regional distribution is shown for cat and dog brain in Table 2. The enzyme has been found exclusively in the non-particulate fraction of ultracentrifuged homogenates (Hagen, Weiner, Ono, & Lee, 1960; Giarman, 1956) and requires pyridoxal-5-phosphate as a coenzyme (Buxton & Sinclair, 1956; Buzard & Nytch, 1957).

Agents that inhibit 5-HTPD

A large number of agents inhibit 5-HTPD *in vitro* and *in vivo* (Clark, 1959). There is a reciprocal competition for the enzyme by 5-HTP and DOPA (Bertler & Rosengren, 1959; Rosengren, 1960; Yuwiller *et al.*, 1959) as well as by other catecholamines (Smith, 1960). Phenylalanine and its metabolites inhibit the enzyme, and the resulting alteration in serotonin levels may be important in the behavioral symptoms of the disorder known as phenylketonuria (Fellman, 1956; Davison &Sandler, 1958). Various degrees of inhibition are noted with α-methylamines. They apparently act by inactivation of the coenzyme (Sourkes, 1954). Unfortunately, the α-methylamines also produce other effects such as releasing noradrenaline which limits their usefulness in the study of the functional role of serotonin considerably.

Storage and release of serotonin

The final step in the synthesis of serotonin could be thought of

as its storage or binding. Theoretically, the bound amine is physiologically inert and protected from enzymatic degradation. Then, after an adequate stimulus, it is thought to be released into the free form in which state it is physiologically active and may combine at some receptor site or be oxidized by MAO. Operationally, two notions have been prevalent. To the pharmacologist binding means that the substance is not available at an active site as reflected by the absence of an observable pharmacological response. To the biochemist who is intent on measuring the amine in the two states, binding means the combination of the substance with cell particles as reflected by the amount of serotonin found in the particulate fractions of ultracentrifuged homogenates.

A combination of results from both fields suggests that there are actually two bound forms: one, a reserve pool in which the amine is complexed with intracellular components, and a second, mobile pool in equilibrium with the first from which the amine is released according to physiological need. The mobile pool is assumed to be maintained by a specialized transport system whereas the slower mixing between mobile and reserve pools probably takes place by simple diffusion (Brodie, 1962; Shore, 1962; Green, 1961). For example, mild disruption of the particulate fraction containing ACh and serotonin releases about 50 percent of both amines whereas more drastic measures are needed to remove the remaining 50 percent (Whittaker, 1959).

Release of serotonin by reserpine

The discovery that serotonin is depleted from all body depots by reserpine (Brodie, Pletscher, & Shore, 1955) led to the proposal that this was a major action of this potent tranquilizer. The course of sedation caused by reserpine paralleled quite closely the depletion of serotonin. Brodie & Shore (1957) assumed that reserpine released serotonin from the bound

form and that the free amine then acted on neural receptors of the parasympathetic nervous system.

This hypothesis has been widely criticized. Most damaging is the fact that reserpine alters the levels of a number of naturally occurring substances in brain. Adrenaline, noradrenaline, dopamine, γ-aminobutyric acid, adenosine triphosphate and adrenocorticotrophin are all reduced by reserpine, whereas ACh is increased (Erspamer, 1961).

The more favorably met alternative theory that the sedation accompanying reserpine was due to depletion of noradrenaline (Carlsson & Hillarp, 1956; Holzbauer & Vogt, 1956) has not been verified. Indeed, the reduction of noradrenaline per se does not cause sedation. Adminstration of α-methylmetatyrosine produces a transient fall in serotonin, correspoinding to its capacity to inhibit 5-HTPD and a long-lasting, complete depletion of noradrenaline resulting from the blockade of this amine's storage sites, but no sedation results. In such a noradrenaline-depleted animal, subsequent administration of reserpine immediately produces sedation with a corresponding loss of serotonin (Gessa, Costa, Kuntzman, & Brodie, 1962).

Further objection to the Brodie & Shore hypothesis that reserpine causes sedation by depleting serotonin arose from the observation that 5-HTP is a poor antagonist to reserpine whereas DOPA has strong awakening effects on reserpine-treated mice (Blascko & Chrusciel 1960; Carlsson, Lindquist & Magnusson, 1957). However, it would seem that just such a prediction would be made by Brodie & Shore, since they postulate that free serotonin stimulates parasympathetic certers whereas noradrenaline stimulates sympathetic centers, the function of which would include arousal.

Metabolism of serotonin

Serotonin is converted to 5-hydroxyindoleacetaldehyde (5-HIAAl) by

MAO and is then further degraded to 5-hydroxyindoleacetic acid (5-HIAA) by aldehyde dehydrogenase or oxidized to pigments. Other routes of metabolism exist, but their participation in mammalian brain has not been demonstrated and in all probability is quite small or non-existent (Erspamer, 1961).

The conversion of the acetaldehyde to the acid is rate limiting. When purified aldehyde dehydrogenase is added to a mitochondrial MAO system there is increased 5-HIAA formation with a decrease in pigment formation. MAO is a promptly acting enzyme with no known coenzyme requirements.

Although the investigation of MAO has been primarily *in vitro* using the liver enzyme, this route of metabolism occurs in brain *in vivo*. Rabbit brain stem contains 0.5 to 0.94 μg 5-HIAA per gram which increases considerably after administration of 5-HTP or reserpine, both of which result in more free serotonin being available for attack by MAO (Roos, 1962; Roos and Werdinius, 1962).

Relative specificity of MAO

MAO is not a very specific enzyme, being capable of oxidizing tyramine, catecholamines and aliphatic amines. Therefore, it cannot be simply concluded that MAO serves a unitary function in any given tissue where more than one of these substrates are present. However, it appears that the relative specificity of the enzyme may not be unique in this respect. The relative specificity of MAO for a number of substrates is quite different depending on the source of the enzyme (Weiner, 1960 a,b; Blaschko, Richter & Schlossman, 1937). This may be true because the enzymes are not chemically identical, as Weiner (1960b) has suggested, or the tissue composition may be different allowing more or less access of a given substrate to the enzyme.

Distribution of MAO

MAO is widely distributed in nature, being found in nearly all

parenchymatous tissues of vertebrates and a number of tissues of invertebrates (Blaschko, 1957). Its regional distribution in dog brain (Table 2) is much more ubiquitous than that of serotonin or 5-HTPD.

The question of intracellular localization of MAO is important in view of the suggestion that MAO has a function in neural transmission analogous to AChE (Udenfriend et al., 1957). All evidence to date points to a predominantly mitochondrial locus for this enzyme (Crotzias & Dole, 1951; Hawkins, 1952; de Lorez Araiz & de Robertis, 1962). Thus it does not appear to meet the anatomic requirements for terminating synaptic transmission. AChE is definitely not mitochondrial (de Robertis, 1962), thus distinguishing these two systems at least as to possible sites of action. A revised theory of synaptic transmission such as proposed by Koelle (1961) and Burn & Rand (1962) where two stages are assumed might accomodate the non-membranous MAO system.

Drugs that inhibit MAO

Drugs that inhibit MAO can be classified as to their relative potency, whether they are fast or slow acting and whether they are reversible or nonreversible. Iproniazid (Marsilid) is the prototype of the slow-acting, nonreversible inhibitors. It produces a relatively slow rise of cerebral serotonin which is hardly detectable after two hours (Shore & Brodie, 1957), reaches it maximum after 6 to 8 hours, and lasts several days. (Pletscher, 1956; Paasonen & Giarman, 1958; Brodie, Spector, & Shore, 1957).

Alpha-methylphenethylhydrazine (Catron; JB516) is a non-reversible, fast-acting inhibitor which is equipotent to iproniazid on liver MAO but is 50 times more active on brain MAO. After only 15 minutes there is a 65 percent inhibition of brain MAO; the inhibition reaches a maximum of almost 100 percent after one hour and lasts 12 to 15 days (Chessin,

Dubnick, Kramer, & Scott, 1959; Brodie, Spector, Kuntzman, & Shore, 1958).

Finally, the harmala alkaloids are very potent, very fast, reversible inhibitors of MAO. Harmine and harmaline are 100 times as effective as iproniazid. A conspicuous rise of serotonin in rat brain occurs within ten minutes, and the effects are gone after only six hours (Udenfriend, Witkop, Redfield, & Weissbach, 1958).

The early enthusiasm for MAO inhibitors in studying the functional role of serotonin in brain has had to be tempered with extreme caution. As was pointed out, MAO is very non-specific so that its inhibition affects a number of systems. In addition, the inhibitors themselves are not specific, so that unambiguous interpretations cannot be made. These facts have for the most part been ignored with the consequence that undue speculation as to the mode by which these drugs produce their behavioral effects has resulted.

It is clear from this discussion of the pathways involved in serotonin synthesis and metabolism that what appears in Figure 1 to be a relatively simple, straightforward system is actually part of a much more complex set of systems. For this reason, the investigation of enzymes in this system for the purpose of discovering the functional role of serotonin in neural tissue has not been successful.

Factors affecting endogenous brain serotonin: chemical

Drugs that interfere with the synthesis, storage or metabolism of serotonin were discussed in connection with the enzymes or mechanisms involved. In addition, a number of other substances alter brain levels of serotonin, but the mechanisms by which they act are largely unknown.

Profound alterations in consciousness accompany microgram quantities of LSD. The proposal that LSD acts by displacing serotonin molecules in brain (Woolley & Shaw, 1954) was discredited with the discovery that the

bromine derivative of LSD was without psychic effects in spite of its antagonism to serotonin on smooth muscle (Gaddum & Hameed, 1954).

No changes in rat brain serotonin were observed after LSD by two groups of investigators (Brodie, Shore, & Pletscher, 1956; Paasonen & Giarman, 1958). However, Freedman (1961) using more animals and a more refined analytical technique has reported that as little as 130 μg/kg LSD produces a small but significant rise in serotonin within 10 to 120 minutes following injection. The effect was more pronounced in animals pretreated 22 hours earlier with reserpine and depended on the doses of both drugs. Since there were no measurable effects on 5-HTPD or MAO, and since the increase was restricted to the particulate fraction, it was concluded that LSD induced binding of serotonin.

Chlorpromazine does not alter normal levels of brain serotonin but antagonizes the rise of serotonin and noradrenaline after treatment with iproniazid (Ehringer, Hornikiewiez, & Lichner, 1960; Pletscher & Gey, 1960; Morpurgo, 1962) and the depletion after reserpine (Costa, Garattini, & Valzelli, 1960). The suggestion that chlorpromazine affects the permeability of the monoamine storage granules was questioned by Bartlett (1962) who found a lowered excretion of 5-HIAA which suggested a reduced turnover. Further, the reduced accumulation of serotonin was paralleled by hypothermia (Morpurgo, 1962). When hypothermia was prevented the effect did not occur.

A large number of chemically unrelated central nervous system depressants cause a significant rise in brain serotonin. These depressants include chloral hydrate, ethyl alcohol, diethyl ether, cloralose and also the anticonvulsants diphenylhydantoin, pentobarbitol, phenobarbitol, and sodium bromide (Bonnycastle, Giarman, & Paasonen, 1957; Bonnycastle, Anderson, & Bonnycastle, 1959).

Pentobarbital produced a 100 per cent increase in brain serotonin after only five minutes, followed by an exponential return to normal. Serotonin rose more slowly after phenobarbital, reaching a peak after two hours and subsiding slowly thereafter. A comparable increase required 20 minutes with ether anesthesia. To determine whether the respiratory depressant properties of these drugs might account for the results, rats were exposed for 20 minutes to 6.75 per cent CO_2, 8 to 10 per cent O_2 or a mixture of the two with negative results. Rats sacrificed immediately after loss of the righting reflex from pentobarbital had normal levels of serotonin, suggesting that the change in serotonin was secondary rather than causal.

Anderson & Bonnycastle (1960) suggest that "the decreased central activity caused by these depressant compounds. . .simply decreases the amount of serotonin being released" and thus allows it to accumulate. Support for this view is given by Türker and Akcasu (1962) who found a depletion of serotonin in cat brain after morphine. In the cat morphine causes excitation. The stimulant properties of amphetamine are well-known and this drug in high doses also causes a decrease in serotonin (Paasonen & Vogt, 1956).

Ethanol injection causes a small increase in brain serotonin in the rat (Paasonen & Giarman, 1958) but a similar treatment results in a marked decrease in brain serotonin in the rabbit (Gursey, Vester,& Olson, 1959). Lower doses of ethanol given to rabbits for seven days have the same depleting effect on brain serotonin (Gursey & Olson, 1960). No changes in brain serotonin of C57/BLCrgl mice were observed after three months' forced drinking of a 10 per cent ethanol solution,although a marked increase in liver alcohol dehydrogenase occurred (Schlesinger & Pryor, un-published observations).

Just as was the case in the last section, definitive answers to the question of the role of serotonin in brain are not available from studies using agents that alter brain serotonin. What is becoming increasingly more evident, however, is that any agent that results in an alteration of normal levels of serotonin, also has a profound behavioral consequence, although the reverse may not always be true. The question then becomes one of discovering whether the changes in serotonin are secondary or causal. Evidence of this nature has been lacking for the most part.

Factors affecting endogenous serotonin: physiological-environmental

In this section, physiological and environmental factors that alter brain serotonin will be discussed. These range from such drastic measures as electro-shock and adrenalectomy to such subtle events as the time of day.

It has been reported that adrenalectomy increases (Sofer & Gubler, 1962), leaves unaltered (Towne & Sherman, 1960) or decreases cerebral serotonin (Put & Meduski, 1962). The discrepancy in these results may lie in the time course of the changes and, consequently, when after adrenalectomy serotonin was measured since Put & Meduski (1962) found the response to be dual in nature. First, they found an increased turnover of serotonin, detected as an increased urinary excretion of 5-HIAA, and perhaps reflecting a response to stress in general. No change in brain serotonin need occur during this phase which might account for the negative results of Towne & Sherman (1960). Second, they found a decrease in serotonin which they attributed to a loss in the binding capacity of brain tissue which could be prevented by corticosteroid therapy.

Thyroidectomy is without measurable effects on brain serotonin, 5-HTPD activity or MAO activity in rats. Nor does feeding animals a diet containing 0.15 per cent desiccated thyroid or 0.15 per cent propylthiouracil lead to changes in brain levels of the substrate or its enzymes (Skillen, Thienes, & Strain, 1961).

A small, though significant, decrease (12 to 15 per cent) in rat brain serotonin was detected 35 days following electrolytic lesions that interfered with fibers of the medial forebrain bundle (Heller, Harvey, & Moore, 1962). The decrease in serotonin was not correlated with postoperative irritibility and could not be accounted for by the volume of tissue destroyed. The authors suggest the existence of central 'serotonergic' fibers whose sectioning results in 'central denervation' analogous to that seen in peripheral adrenergic nerves (Cannon & Lissak, 1939; Goodall, 1951; von Euler & Purkhold, 1951). Toh (1960) found no effect on brain serotonin in rats sacrificed three days after having "bilateral electrolytic lesions placed in the hypothalamus." This suggests that the time course of the effects is critical, just as was the case with adrenalectomy.

The effects of convulsions on brain serotonin are uncertain. A conspicuous increase (200 to 300 per cent) in rat brain serotonin has been reported following electroshock or leptazol convulsions (Garattini & Valzalli, 1956, 1957; Fresia, Genovese, Valsecchi, & Valzelli, 1957; Jori, Velsecchi, & Volzelli, 1957; Garattini, 1958). Much less striking affects were reported by Bertaccini (1959) after similar treatments (20-30 per cent increase), whereas Bonnycastle *et al.* (1957) were unable to detect any change. Convulsions in rabbits produced by insulin hypoglycemia led to an increase of serotonin in the hippocampus and telencephalon (Costa & Himwich, 1959).

A number of treatments that might be regarded generally as stressful have been investigated. No consistent effect on brain serotonin resulted from anoxia, sleep, food or water deprivation, conditioned avoidance training or aversive doses of electroshock to the feet (Freedman, Barchas, & Schoenbrun, 1962). Opposed to this is the report that rats given "light electrical shocks...by means of an inductorium" for 15 minutes show a slight increase in brain serotonin"(Toh, 1960). Also, rats given randomly spaced shocks to the feet in conjunction with a buzzer and light for 30 minutes show a significant rise in brain serotonin that reaches a peak 30 to 45 minutes following termination of the treatment (Pryor, un-published observation).

Exhaustion induced in rats by 4 to 6 hours swim at 23° C or 30 to 40 minutes at 15° C resulted in a 15 to 20 per cent increase in brain serotonin and a 20 per cent decrease in noradrenaline (Freedman et al., 1962). Exposure to extreme heat (40° to 50° C) or extreme cold (1° C) for 30 minutes reduces brain serotonin in rats (Toh, 1960).

In addition to the changes in brain serotonin seen after the rather extreme physiological and environmental conditions reported above, it is apparently true that there is a cylic rise and fall in brain levels of the amine corresponding to some aspects of the normal 24-hour period (Albrecht, Visscher, Bittner, & Halberg, 1956). A small but significant decrease was noted in mice just prior to the usual period of onset of increased activity (beginning of the dark period). Highest levels of brain serotonin occurred during the middle of the light cycle. The authors suggest a relation between brain levels of serotonin and the behavioral periodicity via the adrenal-pituitary axis; the adrenal glands show an increased activation at the same time that levels of brain serotonin decrease.

There is some support for this hypothesis. Reserpine causes a 75 per cent loss of ACTH from the pituitary within 20 hours resulting in "a biological picture almost indistinguishable from the classical stress response evoked by cold" (Westerman, Maickel, & Brodie, 1962). This response was shown not to be a result of the accompanying depletion of noradrenaline.

Three conclusions are tentatively warranted from the results of the studies presented in this section. First, there appears to be a short term response of brain serotonin to conditions that are characterized by increased neural activity. Second, there appears to be a long term response of brain serotonin to conditions that chronically reduce specific patterns of neural or hormonal activity. Third, serotonin may be implicated in the regulation of certain cyclic phenomena, either as a secondary factor or in a causal capacity.

Effects of serotonin on neural activity

In previous sections alterations in brain serotonin were reported to accompany increased or decreased neural activity. The implication was that these changes are related in some way to neural function. In this section the reverse approach will be taken, and studies investigating the effects of serotonin on neural activity will be reviewed. Starting with the peripheral nervous system and working into the spinal cord and brain, it will be shown that serotonin can affect neural activity at all levels of the nervous system. However, it will also be pointed out and emphasized that the effects of serotonin on neural activity have not been studied in such a way as to distinguish unambiguously direct from indirect actions of the amine. Why this is so can be summarized briefly.

Serotonin is able to elicit physiological responses from a large number of non-neural tissues primarily through its action on smooth muscle. This makes the study of the effects of serotonin on neural tissue extremely difficult since the amine is usually administered parenterally. The difficulty is compounded by that fact that serotonin enters the brain only very slowly due to the blood-brain barrier, necessitating large doses that flood the periphery causing an unphysiologic environment (c.f. Erspamer, 1961).

Effects of serotonin on peripheral and spinal nerves

The most common resonse of peripheral nerves and ganglia to serotonin is excitation or potentiation. Large bursts of impulses are elicited from vagal afferent fibers of the cat lung (Paintal, 1955) and the gastro-intestinal tract (Douglas & Ritchie, 1957), but this may reflect non-neural receptors. A potentiation of the response of the superior cervical ganglion of the cat to the sub-maximum pre-ganglionic stimulation and to injections of ACh is seen after intra-arterial injection of serotonin (Trendelenburg, 1957). This and the increased spontaneous electrical activity of post-synaptic fibers of the inferior mesenteric ganglia (Gyermek, 1961; Gyermek & Bindler, 1962) is due to non-cholinergic receptors, but does not reflect normal activity since serotonin has not been detected in peripheral ganglia.

Several studies indicate that myelinated afferent somatic nerves involved with pain and mechanoreceptors are sensitive to serotonin but non un-myelinated fibers from mechanoreceptors, heat or cold receptors (Armstrong, 1957; Fiallbrandt & Iggo, 1959). No effect is seen on the excitability or conduction of the frog and rat sciatic nerves (Casella & Rapuzzi, 1957 a, b, c).

Serotonin produces a marked transient inhibition of the patellar reflex in unanesthetized, vagotomized high spinal cats followed by facilitation, but the effect is more irregular in intact animals (Kissel & Domino, 1957). An initial increase in amplitude and tone of the flexion reflex is followed by a gradual, marked depression in lightly anesthetized low spinal cats (Little, de Stefano, & Leary, 1957) or cats with the cord divided at both C-2 and T-1 (Slater, Davis, Leary, & Boyd, 1955).

Close intra-arterial injections of serotonin have a predominantly depressant effect on reflexes from dorsal roots to corresponding ventral roots (Curtis, Eccles, & Eccles, 1955; Carels, 1962). The amplitude of the strech reflex from the hind legs of cats is affected by serotonin given into the carotid artery which Koella & Czicman (1961) interpret as evidence that serotonin modulates the downward discharge from the reticular formation.

Effects of serotonin on evoked potentials of the brain

As little as 1.0 mg/kg serotonin injected into the common carotid artery of the lightly anesthetized cat was found to inhibit the transcallosally evoked potential recorded on the side of injection (Marrazzi & Hart, 1955). The same picture was seen after endogenous serotonin was increased by inhibition of MAO with iproniazid (Gluckman, Ross, Hart, & Marrazzi, 1957). Serotonin was found to be much more effective than adrenaline or noradrenaline in producing the effect. Neither carotid body nor carotid sinus interfered in the production of the depression (Rodriguez, Hart, & Marrazzi, 1961). As a result of these experiments Marrazzi and co-workers have argued that serotonin is an inhibitory transmitter in brain.

The results of Marrazzi & Hart (1955) have been confirmed by Koella, Smythies, & Bull (1959, 1960) with optically evoked potentials recorded from both visual cortices. They concluded that serotonin injected into the carotid artery had three sites of action; the carotid sinus receptors, centers in the brain stem reticular formation and the cortical synapses themselves. The depression seen by Marrazzi & Hart is thought to be the resultant of these three separate effects.

Intraventricular injection of 50 μg serotonin decreased the amplitude of cortical surface negative waves from stimulation of cutaneous nerves in lightly anesthetized cats. A biphasis response--depression followed by enhancement--resulted from a much larger dose (300 μg). Since there was also marked dilation of the vessels of the exposed cortex and edema, the direct effects of serotonin cannot be assessed (Malcolm, 1958).

Several groups of investigators have reported a decrease in evoked potentials from isolated cortex of cats, rabbits and dogs as a result of intravenous, intracarotid or topically applied serotonin (de Stefano, Leary, & Feldman, 1956; Ochs, Booker, & Aprison, 1960; Rech & Domino, 1960). The possible indirect effects on oxygen tension and systemic blood pressure were also investigated by de Stefano et al. While serotonin inevitably caused a fall in both depression of the evoked potential did not occur until after they had returned to normal.

Effects of serotonin on the electroencephalogram

Effects of serotonin on the electroencephalogram (EEG) range from enhancement of slow waves to arousal to no effect, depending on a number of parameters.. Usually the response is complex-sometimes desynchronization followed by synchronization, or sometimes just the opposite.

Rothballer (1957) found first a brief activation of the cat EEG, then a longer, predominating period of deactivation, sometimes with abnormal slow waves. This was followed by a second brief period of activation when higher doses of serotonin were used. A biphasic response lasting for a longer period of time was also described by Monnie & Tissot (1958) for the rabbit. This response is accompanied by an increase in excitability of the intralaminary thalamic system and a decrease in excitability of the reticular ascending system.

No effect of serotonin was seen on the desynchronized activity of the 'encephale isole' or the 'cerveau isole'. However, after synchronization following bilateral section of the trigemini, doses of serotonin as low as 0.25 to 1.0 μg produce an evident arousal reaction that persists after denervation of the carotid sinus or section of the brain stem at the distal end of the pons. Section of the brain stem at the rostral end of the pons abolishes the effect, suggesting the involvement of nuclei in the pontine or bulbar section of the reticular formation (Mantegazzini, 1957).

No changes in the EEG of conscious cats were seen after 75 to 100 μg serotonin injected into the lateral ventricles (Schwarz, Wakim, & Bickford, 1956), but larger doses (200 to 250 μg) cause an increase in slow rhythms of the cortex (Bradley & Hance, 1956; Vogt, 1957). No changes were apparent in the 'encephale isole' at any doses used (Bradley, 1957).

The response to 5-HTP, which readily enters brain tissue to be decarboxylated to serotonin, is similar in one respect to that seen after serotonin; namely it is usually complex. Costa and Rinaldi (1958) observed that 5-HTP injected intravenously produces a pattern characterized by monorhythmic, diffuse, high voltage activity with the disappearance of cortical fast rhythms and hippocampal theta waves.

Even more complex responses were obtained depending on the dose of 5-HTP used (Monnier & Tissot, 1958; Monnier & Graber, 1960). A progressive disruption of the normal cortical activity leading to a practically isoelectric tracing was seen after high doses of 5-HTP given repeatedly to unanesthetized rabbits (Domer & Longo, 1962).

The intravenous injection of potent MAO inhibitors (JB516 or SKF385) to rabbits was observed to induce a persistent desynchronization of the EEG (Costa, Pscheidt, van Meter, & Himwich, 1960). No effect was seen after iproniazid in rabbits or normal monkeys (Costa et al., 1960; Wada, 1961), but a marked activation of spontaneously occurring epileptic abnormalities was seen in monkeys given focal lesions with aluminum cream (Wada, 1961).

Discussion of the effects of serotonin on neural activity

If the goal of the studies reviewed thus far on the effects of serotonin on neural activity has been to delineate the functional role of serotonin in the mammalian nervous system, it must be concluded that all, taken singly or together, have failed to a greater or lesser degree. Some of the reasons for this failure are apparent.

First, the site or sites of action of serotonin injected into the general circulation cannot be controlled or predicted regardless of the route of administration. Since the amine has an effect on so many tissues and especially on the smooth muscle lining blood vessels, it is virtually impossible to separate indirect vascular actions from direct action on the neural tissue itself. In addition, many of the non-neural tissues affected by serotonin contribute afferent inflow to the central nervous system which may interfere with the response being measured. Denervation is useful to some extent in this respect but does not completely solve the problem.

Close intra-arterial injection or injection into the cerebrospinal fluid is also inadequate since local blood supply and permeability changes prohibit unambiguous interpretation.

Second, serotonin enters the brain only very slowly, and in addition, there are regional differences in this respect. To overcome this difficulty experimenters have employed extremely high doses of serotonin, thus flooding the periphery with unphysiological levels of the amine. Others have used 5-HTP, which passes readily into brain. The general assumption is that 5-HTP has its effects only after being converted to serotonin, but this assumption is unproven. More serious limitations in the use of 5-HTP are that it may be decarboxylated to serotonin in regions naturally devoid of the amine, or that it may compete with other substances for receptor sites, thus changing the concentration of these substances. The net result in either case is the disruption of a complex environment rather than the intended result.

Third, inhibitors of 5-HTP or MAO have been used to alter endogenous levels of serotonin. As was pointed out earlier, neither the inhibitors nor the enzymes are very specific so that very little unambiguous information is gained by their use. Indeed, results may be quite misleading unless all of the factors involved are taken into account which they can't be at present because they are largely unknown.

The net result of these limitations is that there has been no adequate way of getting physiological amounts of serotonin to possible natural receptors in the brain. The alternatives used have been indirect and as it turns out inherently incapable of answering the questions asked!

Effects of serotonin on single neurons in the central nervous system

The problems encountered in trying to discover the functional role of serotonin in the central nervous system are by no means unique to this substance. They are due to the complex structure of the nervous system itself. A technique that shows promise of circumventing many of these problems has been used recently in the search for central nervous system transmitters. Multiple glass microelectrodes can inject substances ionophoretically onto the surface of, or within, single neurons while simultaneously recording the electrical behavior of the same neuron through another barrel (Curtis, 1961, 1962).

Using this technique Curtis (1961, 1962) and Krnjevic & Phillis (1961) found that: (1) ACh excites some neurons in the spinal cord and cortex in a manner similar to a peripheral transmitter substance; (2) a whole series of naturally occurring amino acids either depolarize or hyperpolarize central neurons depending on whether the molecule is structurally related to glutamic acid or GABA, respectively; however, other considerations argue against any of these substances being actual transmitters; (3) no neurons explored thus far in the spinal cord or brain stem respond in any way to serotonin, adrenaline or noradrenaline; however, (4) neurons in the lateral geniculate are responsive to all three, but not in a transmitter-like manner.

Neurons in the lateral geniculate offer the only positive evidence concerning serotonin, and even here its role must be inferred. In this experiment Curtis & Davis (1961) passed serotonin as cations from a saturated aqueous solution (pH 3 to 4) into the intra-cellular fluid surrounding neurons in this nucleus while recording from an impaled neuron with one of the other barrels.

They found suppression of the orthodromic response to stimulation of the optic nerve. The latency of the suppression was inversely related to the magnitude of the electrophoretic current. Antidromic spikes from stimulation of the optic radiations or the excitation elicited by glutamic acid applied from another barrel, however, were unaffected by serotonin. Other compounds including adrenaline and noradrenaline acted like serotonin but were less effective. The conclusion reached was that since serotonin does not modify membrane conductance of these neurons, it is unlikely that the amine was combining with sub-synaptic receptors of inhibitory neurons or that it was depressing the electrically excitable membrane of the neurons in some other fashion. It is probable that the suppression was due to competitive interaction of serotonin with the natural transmitter at excitatory synapses, or that the release of the transmitter was inhibited.

Effects of serotonin on brain enzymes

No action is seen on rat brain alkaline phosphatase or lactic or malic dehydrogenase *in vitro* (Clark, Fox, Marin & Benington, 1956) nor on oxidative phosphorylation *in vivo* (Lingjaerde & Skaug, 1958).

The hydrolysis of ACh, butyrylcholine, procaine and murexine by plasma cholinesterase is inhibited by high concentrations of serotonin (10^{-3} M) *in vitro* (Erdös, Baart, Foldes, & Zsigmond, 1957) but markedly potentiated by lower concentrations (5×10^{-6} M) (Fried & Antopol, 1957).

Serotonin inhibits AChE from rabbit caudate nuclei *in vitro* from 29 per cent to 100 per cent at final concentrations of 10^{-3} M to 10^{-2} M (Aprison, 1960).

Effects of serotonin on gross behavior

Much of the work studying the effects of serotonin on behavior has been done by people unsophisticated in the analysis of behavior. In spite of the fact that casual observation has been the rule, a number of gross effects have been confirmed.

In general it has been found that serotonin or its precursor administered in relatively low doses results in a state resembling sedation and depression, whereas higher doses lead to excitation and restlessness. For example, slight drowsiness, hypokinesis, less prompt reaction to stimuli and decreased attention reaction is seen in rabbits given 0.1 to 1.0 μg/kg serotonin, but an opposite picture occurs with higher doses (Monnier & Tissot, 1958). Low doses cause mild sedation and a reduction of motor activity in rats (Shore, Silver, & Brodie, 1955; Shore, Pletscher, Tomich, Carlsson, Kuntzman, & Brodie, 1957).

A similar picture of depression results in dogs given low doses of 5-HTP. However, 30 to 100 mg/kg 5-HTP causes excitement, disorientation, loss of light reflex and contact plantar reflex, tremors, ataxia, apparent blindness, and unresponsiveness to alarming stimuli. In addition, there is mydrosis, piloerection, sexual excitation, tachycardia, salivation, lacrimation, tachypnoea, vomiting and increased intestinal motility (Bogdanski, Weissbach, Uderfriend, 1958; Uderfriend, Weissbach, & Bogdanski, 1957; Monnier & Tissot, 1958; Costa, Himwich, Goldstein, Canham, & Himwich, 1959). A picture resembling catalepsy is seen in dogs given 40 to 900 μg/kg serotonin into the cisterna magna after a latency of 10 to 60 minutes and lasting up to six hours (Sacchi, Garello, Dolce, & Bonamini, 1955).

These extreme states are not very conducive to delimiting the role of serotonin!

Cats respond to either intravenous (Bradley, 1957) or intraventricular (Feldberg & Sherwood, 1954) serotonin with a tendency to sit or lie down, but do not sleep or appear drowsy. The eyelids remain wide open and bursts of profuse salivation are observed.

Serotonin injected directly into cerebral tissue of mice produces tachypnea micturation, defecation, piloerection, central depression and scratching (Haley, 1957 a, b). Rats respond to injection or self-injection of serotonin into the hypothalamus by loss of muscular tone and by apparently going to sleep (Olds & Olds, 1958).

Effects of serotonin on conditioning and maze performance

Cook & Weidley (1957) have studied the effects of a number of drugs, including serotonin, on a conditioned avoidance resonse. Using an apparatus consisting of a grid floor and a pole they trained rats to respond to a buzzer and shock through the grid by climbing the pole. After reaching a satisfactory criterion of escaping the shock, the animals were tested with just the buzzer for 30 seconds, followed by shock if the avoidance response had not been made during this interval. While some drugs such as barbital and methylparafynol blocked both the avoidance and the escape responses, serotonin (10 mg/kg, s.c.) selectively blocked only the avoidance response as measured by the proportion of animals not responding during the 30-second interval of buzzer alone.

Aprison and co-workers (Aprison & Ferster, 1961 a, b, c; Aprison, Wolf, Poulos, & Folkerth, 1962; Hingtgen & Aprison, 1963) have begun an investigation of neurochemical correlates of behavior, and they have chosen the serotonin system as a starting point.

Using pigeons trained to peck at a disk on a multiple fixed-interval, fixed-ratio schedule (Fernster & Skinner, 1957), they have evaluated the effect of artificially raising or lowering endogenous brain levels of serotonin on this behavior.

Increasing brain levels of serotonin by injecting 5-HTP into the breast muscle was found to depress responding as measured by the time taken to emit one-half the number of responses emitted during control sessions. Furthermore, there was a linear dose-response curve from 25 to 75 μg/kg (Aprison & Ferster, 1961 a). The same effect was found using serotonin but with much smaller doses, which the authors attribute to the effects of serotonin on peripheral circulation and intestinal motility (Aprison & Ferster, 1961 c).

Injections of saline or iproniazid phosphate were without effect on the pecking response. However, pretreatment with three 50 mg/kg doses of iproniazid spaced over a period of 40 hours markedly enhanced the behavioral effect of 5-HTP. This enhancement decreased over a period of 47 days and was inversely correlated with the recovery of brain MAO activity. The authors concluded that the behavioral effects can be explained in terms of free serotonin present in the brain after 5-HTP injections (Aprison & Ferster, 1961 b).

In an attempt to correlate endogenous levels of serotonin with the observed behavior, the amine was measured in four brain sections of trained pigeons given 50 mg/kg 5-HTP and sacrificed at various intervals following the injection. A close relation was found between the time course of serotonin changes in the telencephalon and diencephalon following 5-HTP and the time course of the behavioral response to 5-HTP noted in the same birds during testing prior to sacrifice (Aprison *et al.*, 1962).

Reduction of endogenous levels of serotonin was also found to depress the pecking response in pigeons (Hingtgen & Aprison, 1963). This was accomplished by administering α-methylmetatyrosine which reduces serotonin levels for about nine hours and in addition depletes noradrenaline for at least 4 to 7 days in the pigeon (Aprison & Hingtgen, 1963). The depression of the behavioral response coincided with reduction of serotonin and not with the reduction of noradrenaline. Although the time course of the behavioral effects due to 5-HTP and α-methymetatyrosine are distinctly different, it appears that with both compounds more free serotonin is made available than in the normal state.

Different results have been obtained using mice and rats, other kinds of performance measures and different methods of altering brain serotonin. Woolley & van der Hoeven (1963) reported a decrement in performance by mice in a simple T-maze after serotonin was increased using 5-HTP plus an MAO inhibitor. They found a decrease in performance in mice raised on a phenylalanine plus tyrosine diet from weaning to adulthood which was attributed to decreased brain serotonin. On the other hand, the same authors reported that when serotonin was reduced by administration of reserpine performance was enhanced.

Louttit (1962) found that rats made phenylketonuric (and who consequently had reduced levels of brain serotonin) by a diet of phenylalanine, or whose brain serotonin was raised by isocarboxazid scored more errors on the Hebb-Williams tests than control groups but were superior on a successive discrimination problem.

The conclusions that can be drawn from studies investigating the effects of altered brain serotonin on behavior are severely limited by the lack of specific knowledge as to the actual sites of action of serotonin.

It is clear that gross alterations in brain serotonin produce observable changes in behavior of a rather severe nature. However, the methods used to alter the amine do not allow a clear separation of central from peripheral effects. Further, the changes in behavior cannot be correlated with specific neural mechanisms within the central nervous system. Aprison and co-workers(pp.58-60) have found that the changes in the pecking response are correlated with changes in serotonin in the telencephalon and diencephalon, but further delimitation of the effects has not been made.

Serotonin as a neurotransmitter

Shortly after the discovery of serotonin in mammalian brain, its role as a neurotransmitter in the central nervous system was proposed by a number of workers. Marrazzi & Hart (1955) nominated the amine as an inhibitory transmitter, whereas Brodie & Shore (1957) proposed that it functioned as an excitatory substance at synapses mediating the central representation of the parasympathetic nervous system. The conclusions of both gorups were based on rather weak, indirect evidence and assumptions; consequently few have been convinced by their respective arguments (c.f. Erspamer, 1961).

Rather than evaluating the merits of each separate hypothesis made by these and other investigators, an examination of the criteria for accepting a substance as being a neurotransmitter in the central nervous system will be made, and the evidence that serotonin meets these criteria will be examined.

Criteria for assessing a substance as being a neurotransmitter: evidence regarding serotonin

The criteria that have been suggested for assessing a substance

as being a neurotransmitter are based primarily upon the facts that are known about the mode of release, action and inactivation of Ach at peripheral junction. While some investigators have relaxed these criteria somewhat in studying possible central transmitters, the final identification of a substance as a transmitter requires that all criteria be satisfied. A distillation of the criteria suggested by Crossland (1960) and Patton (1958) is presented below along with the evidence showing the extent to which serotonin meets these criteria in the central nervous system of mammals.

1. It must be shown that there are enzymes within the presynaptic neuron capable of synthesizing the substance.

The existence of such an enzyme (5-HTPD) has been shown in brain for serotonin although it is not very specific. Ultracentrifugation of tissue homogenates suggests a cytoplasmic origin for 5-HTPD although definitive histological evidence is lacking.

2. It must be shown that the substance, after being formed, is stored or 'bound' in some appropriate cellular compartment.

The location of serotonin in the particulate fraction of ultracentrifuged brain homogenates and, further, in the subfraction containing synaptic vesicles comes close to satisfying this criterion in brain. It is still necessary, however, to show that the presence of serotonin and synaptic visicles in the same subfraction is not fortuitous.

3. It must be shown that the substance is released into the synaptic space upon stimulation of the presynaptic fibers.

Two reports bear on this criterion. Angelucci (1956) found a substance in the perfusate of the frog spinal cord after stimulation of the skin that had actions on the clam heart resembling serotonin,

but positive identification was not made. Later, Benetato, Oprisiu, Tomas, Bubuianu, & Iluiter (1959) reported that serotonin, ACh and adrenaline were found in the perfused head of a dog after stimulation of the central ends of the cut vagus and Hering nerves. Even assuming in both cases that the substance was serotonin, there is no way of determining its origin.

4. It must be shown that the substance mimics the action of the transmitter on postsynaptic receptors when introduced in physiological amounts at these sites.

The only direct evidence for serotonin regarding this criterion in the central nervous system is mostly negative. It will be recalled that serotonin injected ionophoretically in the near proximity of single neurons in the spinal cord and brain stem was without effect. In the lateral geniculate nucleus synapses were found that were depressed by serotonin. However, the conclusion was that serotonin had this effect by competing with the natural transmitter for post-synaptic receptor sites, or by depressing release of the natural transmitter from pre-synaptic terminals. In either case, there was no evidence for a direct transmitter role for serotonin.

5. It must be shown that enzymes capable of rapidly destroying the substance are present at or near the postmembrane junction to insure discrete transmission.

There is an enzyme present in brain capable of degrading serotonin, namely MAO, but its location on or within mitochondria is not compatible with this criterion. This means that serotonin would somehow have to get back into the cell after reacting with the postsynaptic site before it was destroyed enzmatically, or rely on diffusion processes to terminate the effects of serotonin. In either case

discrete transmission would most likely be lost.

6. Drugs that block the action of the naturally occurring transmitter should block the action of the candidate substance when applied locally; drugs that interfere with the synthesis of the substance should lead to failure of the neuron to act on the postsynaptic cell; drugs that interfere with the inactivation of the substance should block transmission to the postsynaptic cell.

None of these criteria can be evaluated at present for serotonin in the central nervous system, since they require considerably more intimacy with single synaptic events than has heretofore been possible.

Of the six criteria serotonin only reasonably meets 1 and 2. Little or no satisfactory evidence is available for 3, 4 or 6 and slightly damaging evidence exists for 5. Therefore, using these criteria, serotonin cannot be considered a transmitter substance at present. Other authors have used less stringent criteria often requiring only indirect or circumstantial evidence, but eventually any nominee must satisfy all six criteria listed above. As yet, no substance completely qualifies in this respect.

Present conceptual status of serotonin in the central nervous system

It seems unlikely that so potent a substance as serotonin has only a minor role in brain function. The actions of the so-called psychotomimetic, tranquilizers and psychic energizers all affect serotonin and the results are striking, to say the least. It remains to be seen, however, whether these agents produce their effects through their action on serotonin, or whether the changes in serotonin are merely fortuitous or a small part of a much larger, more complex picture. If it is assumed for the moment that this relation is indeed causal, the task of determining the exact mechanisms remains.

As was just seen, it is not possible at present to ascribe a neurotransmitter role to serotonin in the central nervous system of mammals. On the other hand, this possibility cannot be abandoned. The evidence for such a role seems much more convincing in some invertebrates (Welsh, 1957).

A second possibility is that serotonin functions as a modulator of synaptic activity either: (1) by competing with the true transmitter for postsynaptic receptor sites but not exerting an action itself, or (2) by somehow regulating the amount of transmitter released, or (3) by interfering with the synthesis of destruction of the transmitter. The evidence for this possibility is slightly stronger than that given for the role of serotonin as a neurotransmitter. The experiment by Curtis & Davis (1961) showing just such a modulatory effect on sites in the lateral geniculate by serotonin add support to this hypothesis. Further, the fact that MAO is mitochondrial is not incompatible with this interpretation.

CHAPTER III

Strain Differences in Non-Cholinergic Systems

In Chapter I evidence was presented suggesting that the ACh-AChE system is related to measurable aspects of behavior in the rat. In this chapter certain non-cholinergic systems will be investigated in this respect. This will be done by comparing different strains of rats, known to differ in maze performance, as to levels of serotonin and activities of the enzymes DOPA decarboxylase (DOPAD), monoamine oxidase (MAO), and glutamic acid decarboxylase (GAD) in brain tissue.

A difference in brain biochemistry between strains of animals that also exhibit a measurable behavioral difference is suggestive evidence that the two variables are causally related. For this reason, strain differences can be used as a starting point in such an investigation. The strain comparison approach was heavily relied upon in establishing even a nominal relation between AChE and learning as discussed in Chapter I.

Evidence for considering serotonin to be importantly involved in neural function was presented in Chapter II. While the exact role of serotonin in the central nervous system is uncertain at present, the evidence suggests an involvement of the serotonin system in behavior characterized by a large autonomic component. Brodie & Shore's (1957) hypothesis that serotonin is the chemical transmitter for the central representation of the parasympathetic nervous system has considerable indirect support. Even if a transmitter role for serotonin is not assumed, the striking changes seen in the behavior of animals after injection of drugs that raise or lower brain serotonin lead one to suspect that this amine is involved in some way with the regulation of what may loosely be termed "emotional behavior".

Three reports of strain differences in brain serotonin have been made. (1) Caspari (1960) reported that C57/BLIOJ mice had a higher mean concentration of serotonin in whole brains than BALB/cJ mice. (2) This could not be confirmed by Maas (1963) using whole brains, but (3) when a section including diencephalon, mesencephalon, and pons was analyzed separately, the BALB/cj mice were found to have higher levels of serotonin than the C57/BLIOJ mice (Maas, 1962). The BALB/c strain has been reported by several investigators to be more emotional (as measured by defecation scores in an open field) than the C57/BL strain (Maas, 1963; McClearn, 1959; Thompson, 1953; Thiessen, 1961).

The discrepancy between Caspari's results and those of Maas prohibit the interpretation of a relation between brain serotonin and emotional behavior. To further investigate such a possible relation Pryor, Schlesinger, & Calhoun (unpublished observations) measured the levels of brain serotonin in Berkeley lines of these two strains along with four other inbred strains of mice that were available: A/Crgl, C3H/2Crgl, DBA/2Crgl, and RIII/Crgl. Animals from the six strains had previously been rated on an "emotionality scale." The rater was instructed to catch and handle the animal and return it to its home cage, then he was asked to judge the animal on a 0 to 5 scale as to: (1) difficulty in catching the animal, (2) difficulty in handling, (3) muscular tension, (4) amount of defecation and urination while being handled, and (5) reaction to probing with a pencil. This admittedly naive measure of "emotionality" was quite reliable between raters (coefficients of concordance in three replications were 0.60, 0.72, 0.72; $p \leq .01$) and between groups of animals (coefficient of concordance between the mean ranks of the six strains for three replications was 0.77; $p \leq .01$).

Reliable differences between the six strains with respect to total serotonin, wet weight of tissue and serotonin concentration were found, although the four middle strains did not differ among themselves. There were no differences between the C57/BLCrgl mice and the BALB/cCrgl mice in whole brain serotonin thus supporting Maas (1963). When the ranks of the means of brain serotonin were compared with the mean ranks obtained on the emotionality scale, a high negative correlation was found (rho= 0.94) suggesting an inverse relation between brain levels of serotonin and this measure of emotionality.

Three experiments investigating strain differences in rats will be reported in this chapter. First, a comparison will be made between the S_1 and S_3 strains at two different ages with respect to brain serotonin. Second, other groups of S_1 and S_3 rats will again be compared with respect to brain serotonin, and in addition, they will be compared with respect to the brain enzymes ChE, AChE, dihydroxphenylalanine decarboxylase (DOPAD; the enzyme responsible for the synthesis of serotonin), monoamine oxidase (MAO; the enzyme responsible for the metabolism of serotonin) and L-glutamic acid decarboxylase (GAD; the enzyme responsible for the synthesis of γ-aminobutyric acid). Third, the S_1 and S_3 strains, the RDH and RDL strains, and the OMB and OMD strains will be compared with respect to brain serotonin and the five enzymes just mentioned.

Experiment I

Comparison of S_1 and S_3 strains of rats with respect to brain serotonin

As a beginning in the investigation of possible non-cholinergic systems and behavior in rats, brain serotonin concentration was compared in the S_1 and S_3 strains of rats. There were several reasons for

selecting these two strains. First, it was known that they differed with respect to brain ACh and AChE (c.f. Chapter I) and ChE (Bennett, Krech, & Rosenzweig, 1963). Second, it was known that they differed in brain weight ($S_3 > S_1$). Third, it was known that they differed on a variety of behavioral measures , including emotionality (S arle, 1949; Rosenzweig, 1963). Fourth, it was known that the strains did not differ in the glycolytic enzyme LDH (Bennett et al., 1958) or per cent protein in the brain (Bennett, et al., 1961).

Thus, with these two strains about which so much was already known, both behaviorally and biochemically, the serotonin assay would provide a partial test of whether non-cholinergic systems were involved in behavior. If the two strains did not differ in brain serotonin, then this system could be considered doubtful as a biochemical mediator of normal behavior. If, on the other hand, the two strains did differ in brain serotonin, then a further investigation of this system would be justified.

In this first experiment brain serotonin was measured in animals from the S_1 and S_3 strains at two different ages. The animals were being used in other experiments in progress at the time and the chemical analyses were performed at widely separated times. Since results from experiments performed at different times did not appear to give comparable absolute values, only the strain comparisons will be considered and not the effects of age. Animals from both strains within a given group were comparable in age.

METHOD

Subjects

The subjects were male rats of the S_1 and S_3 strains, descendents of Tryon's (1940, 1942) maze-bright (S_1) and maze-dull (S_3) strains.

Animals in Group I were approximately 40 days old when sacrificed and were part of an experiment in which the effects of cross-fostering on AChE activity and serotonin was being conducted. At birth some of the animals had been placed with mothers of the other strain from which part or all of the pups had been removed. Other animals from each strain were raised by their natural mothers as controls. Due to differential survival of the pups under the various conditions, the effects of cross-fostering could not be reliably assessed and, therefore, only results dealing with strain comparisons will be reported here or elsewhere. Animals in Group II were approximately 155 days old when sacrificed and had spent 20 days in activity wheels (from 95 to 115 days) in an experiment comparing the two strains on this variable.

Sacrifice and dissection

All animals were sacrificed by decapitation. Their brains were rapidly removed, weighed to the nearest 0.1 mg using a "semi-micro", direct reading, analytical balance (Sartorius Selecta), frozen on dry ice and stored at -22°C until they were analysed for serotonin.

Gross dissection of the brains of animals in Group I was as follows: The dorsal cortex was peeled off and constituted the section labeled Dorsal Cortex (DC); the ventral cortex, including amygdala, hippocampus and corpus callosum was dissected free and constituted the section labeled Ventral Cortex (VC); the cerebellum was removed, weighed and used to carry tissue standards; the remaining tissue including olfactory bulbs, caudate nuclei, thalamus, hypothalamus, other basal ganglia and brain stem constituted the section labeled Subcortex I (SC I).

Gross dissection of the brains of animals in group II was as follows: The cerebellum was removed, weighed and used to carry tissue standards; the rest of the brain constituted the section labeled Total

Brain I (TB I).

Due to different coat colors of the S_1 and S_3 strains, sacrifice could not be done blind. All chemical determinations were carried out using code numbers that did not reveal the strain of the animal.

Chemical analysis

Serotonin was extracted using a modification of the procedure described by Shore and Olin (1957) for catecholamines which also extracts serotonin (Mead & Finger, 1961). Analysis of the extract was done according to the method of Bogdanski *et al.* (1957).

Tissues were homogenized in 1 to 2 ml 0.1 N HCL that had been saturated with NaCl. Homogenization was performed in 50 ml lucite or glass centrifuge tubes using a motor driven teflon tissue homogenizer fitted to the tubes. Fifteen ml n-butanol (washed with 1.0 N HCL, 1.0 N NaOH and distilled H_2O, and saturated with NaCl) were added to the mixture and homogenization was continued. One gram of solid NaCl was added to insure saturation. The tube was capped and shaken vigorously by hand for 1 to 2 minutes after which the aqueous and organic phases were separated by centrifugation at 2500 rpm for ten minutes.

A 15 ml aliquot of the organic phase was transferred to a glass-stoppered, 50 ml conical, glass, centrifuge tube using a glass syringe and number 19 stainless steel needle. The centrifuge tube contained 1.0 ml 0.1 N HCL and 30 ml n-heptane. After vigorous shaking by hand for 1 to 2 minutes, the phases were separated by centrifugation at 1200 rpm for 10 minutes. The organic phase was aspirated off and discarded.

A 600 μl aliquot of the aqueous phase was transferred to a one-half dram vial and 200 μl concentrated HCL were added just prior to analysis.

For analysis an aliquot (0.3 to 0.5 ml) of the acidified mixture (now 3N with respect to HCL) was transferred by a disposable glass pipette to a round quartz cuvette and placed in the cell compartment of an Aminco-Bowman spectrophotofluorometer. The same cuvette was used for all analyses (being washed thoroughly between samples with distilled H_2O and acetone) and was carefully positioned using a plastic set-screw.

The mixture was excited by 305 mμ light from a xenon source. The resulting fluorescence was read in relative fluorescence units at 550 mμ. The cuvette compartment was maintained at room temperature(±0.1°C) by a circulating water bath. The light path was directed in and out of the cell compartment by slit arrangement number 3 (American Instrument Co., Instruction and Service Manual No. 768). A yellow filter was inserted in the shutter in front of the photomultiplier tube to cut out scattered light that overlapped the serotonin fluorescence. The filter did not interfere with the fluorescence of the sample. After being positioned in the cell compartment, the shutter was opened and the resulting fluorescence read immediately to avoid loss from photo-decomposition.

Cerebelli were pooled and used as tissue blanks and to carry known amounts of serotonin through the entire procedure.

RESULTS

When serotonin is measured in 0.1 N HCL the peak fluorescence is at 350 mμ. A number of other substances found in brain and extracted by this method also fluoresce at this wavelength. Reduction of pH causes a shift in the fluorescence of 5-hydroxyindoles but does not affect the fluorescence characteristics of the other compounds (Bogdanski et al., 1957). This shift in the fluorescence of serotonin as a function of pH is shown in Figure 2.

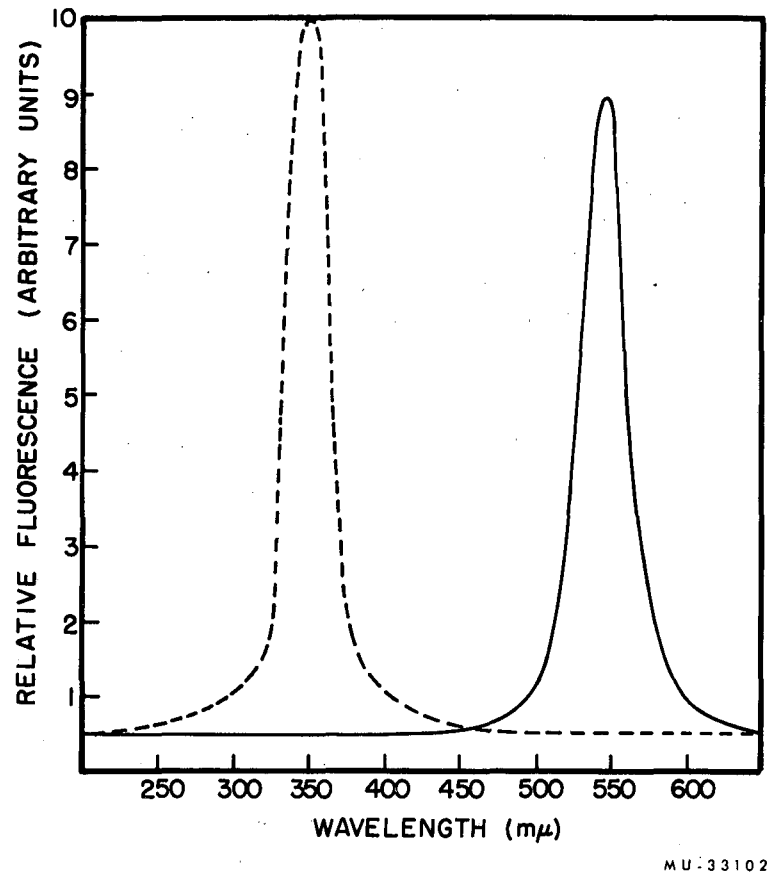

Fig. 2. Shift in the fluorescence of serotonin as a function of pH (exciting wavelength, 295 mμ).

———————— serotonin measured in 4 N HCl

---------- serotonin measured in 0.1 N HCl.

The procedure used here also extracts 5-HTP which has very similar fluorescence properties to serotonin. Bogdanski et al., (1957) removed 5-HTP by washing the acid-butanol mixture with a borate buffer adjusted to pH 10. The quantities of 5-HTP in brain are extremely small. Preliminary experiments showed no detectable differences in measured serotonin whether the borate wash was used or not, and therefore, the borate buffer wash was eliminated.

Fluorescence spectra of authentic serotonin are shown in Figure 3 for several concentrations together with the spectrum of a brain sample extracted by the method used here. Fluorescence of authentic serotonin was linear over a considerable range of concentrations. Figure 4 shows a standard curve obtained by carrying known amounts of serotonin through the extraction procedure in tissue. The linearity seen in this standard curve was not always evident, especially at the low concentrations. The reason for the frequent departure from linearity is believed to be poor extraction due to insufficient shaking during the extraction. In later experiments, such as the one from which the curve in Figure 4 was obtained, this difficulty was overcome. The problem did not arise when actual samples were extracted since part of the homogenization was performed in the presence of butanol. For this reason values obtained from some experiments are probably too high thus prohibiting the comparison of separate experiments on an absolute basis. Nevertheless, within-experiment comparisons remain fully justified.

These procedures permit recoveries of added serotonin ranging from 90 to 100 per cent. Table 3 gives the results of an experiment that shows that the methods of extraction and analysis permit a fairly high degree of reproducibility even at relatively low concentrations.

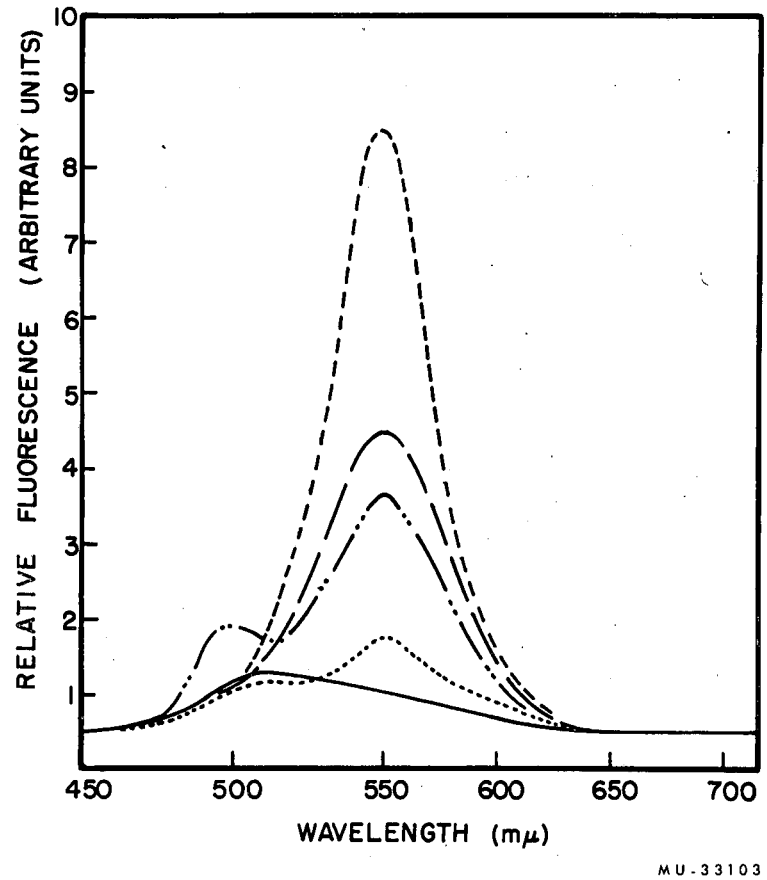

Fig. 3. Fluorescence spectra of several concentrations of authentic serotonin and extract of rat brain (exciting wavelength, 295 mμ).

——— H_2O blank — — 500 ng serotonin/ml

------ 100 ng serotonin/ml — — — 1000 ng serotonin/ml

—·· — rat brain extract (1.5g)

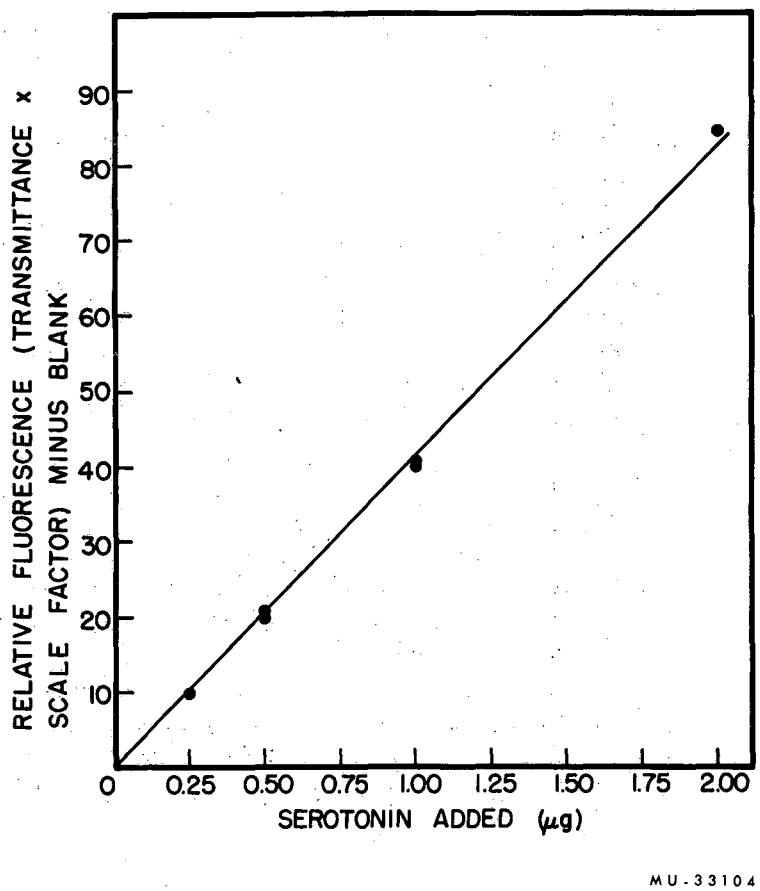

Fig. 4. Standard curve obtained by carrying known amounts of authentic serotonin through the extraction in cerebelli homogenates.

Table 3

Reproducibility of Methods Used for Extraction and Analysis of Serotonin in Rat Brain

	γg Serotonin Added to 1.0 ml Aliquots of Rat Brain (356 mg/ml)		
	0.0	0.065	0.13
Relative Fluorescence (arbitrary units)	38.0	53.0	65.0
	42.0	50.5	61.0
	41.0	55.0	60.0
	43.0		
	41.0		
	40.5		
Mean	40.9	52.8	62.0
Mean Minus Tissue Blank	——	11.9	21.1

Comparison of S_1 and S_3 rats

Table 4 shows the means and standard deviations with respect to wet weight of tissue and serotonin concentration for both groups of S_1 and S_3 rats.

The adult S_1 rats had a significantly higher concentration of brain serotonin than the adult S_3 rats in TB I. None of the differences in brain serotonin between the 40 day old rats were statistically significant, although the means of the S_1s were higher in all three brain sections than the means of the corresponding sections of the S_3s.

In both the young and adult groups, the S_3 animals had significantly heavier brain weights than the S_1 animals. In spite of the significantly lighter brain weights of the adult S_1s, they had over ten per cent more total serotonin than the S_3s.

DISCUSSION

The results of this experiment indicate that at least by the time S_1 and S_3 rats reach adulthood their brains differ in serotonin concentration with the S_1 strain having significantly more serotonin per gram tissue than the S_3 strain. It may be that this difference appears earlier, but due to the much greater variability present in the younger animals, such a conclusion was prohibited at this time.

Comparisons between younger and older animals were not made in this experiment since age was completely confounded with time of sacrifice and time of analysis. Other experiments have indicated that absolute values from analyses performed at widely separated times cannot be compared.

Table 4

Means and Standard Deviations of S_1 and S_3 Strains with Respect to Wet Weight of Brain Tissue and Serotonin Concentration

Age	Section	S_1 Strain						S_3 Strain					
		Wet Weight of Tissue (mg)			Serotonin (ng/g)			Wet Weight of Tissue (mg)			Serotonin (ng/g)		
		$\bar{x}$	(n)	SD	$\bar{x}$	(n)	SD	$\bar{x}$	(n)	SD	$\bar{x}$	(n)	SD
40 days	DC	374**	(18)	17.8	329	(17)	131.0	402	(11)	9.7	306	(9)	131.0
	VC	273*	(19)	14.6	321	(17)	74.0	291	(11)	19.4	310	(11)	91.6
	SC I	500**	(18)	20.3	704	(18)	91.8	538	(11)	20.4	700	(11)	79.7
110 days	TB I	1380**	(9)	33.3	594**	(9)	55.6	1538	(8)	39.0	478	(8)	47.0

* $p \leq .05$

** $p \leq .01$

The difference in brain weight between the S_1 and S_3 animals was as expected and conformed to that found by Bennett *et al.* (1958).

Interpretation of the differences in brain serotonin between the two strains cannot be made without recourse to experiments to be reported later. It appears, however, that biochemical differences between the S_1 and S_3 strains are not restricted to the ACh-AChE system.

Experiment II

Comparison of S_1 and S_3 rats with respect to brain serotonin, AChE, ChE, DOPAD, GAD and MAO

The results of Experiment I showed that by adulthood the S_1 strain of rats differs from the S strains in levels of brain serotonin. Two experiments were performed to confirm and extend this finding. First, serotonin was again measured in the S_1 and S_3 strains, and in addition, the enzymes of synthesis (DOPAD) and metabolism (MAO) of serotonin were measured together with GAD, AChE and ChE. Second, having found a difference in brain serotonin between the S_1 and S_3 strains, two other pairs of strains were investigated with respect to brain serotonin and these five brain enzymes. Comparisons between the S_1 and S_3 strains only will be reported here; comparisons involving other strains will be reported in Experiment III.

Analysis of serotonin prohibits the simultaneous analysis of most enzymes, since the amine is extracted in 0.1 N HCL in order to prevent its metabolism by MAO. For this reason littermates were used in this experiment; one animal was analysed for AChE, ChE, DOPAD, GAD and MAO. It was assumed that levels of serotonin and enzyme activities were very similar between littermates.

GAD has not been discussed heretofore except in passing. This enzyme metabolizes L-glutamic acid to γ-aminobutyric acid (GABA). GABA has a unique occurence in the central nervous systems of vertebrates and may play a direct or indirect role in the regulation of neural activity. For this reason it, as well as serotonin, is a candidate for a non-cholinergic neurotransmitter role in the central nervous system. It has been suggested that GABA causes an increase in the membrane conductance of neurons related to an increase in permeability to chloride and/or potassium ions. Low concentrations of GABA mimic the effects of stimulation of the inhibitory nerve on the crayfish stretch receptor, (Roberts, & Eidelberg, 1960).

METHOD

Subjects

The subjects used in this experiment were 10 littermate pairs of male S_1 rats and 13 littermate pairs of male S_3 rats. One animal of each pair was assigned at weaning to either a Social Control (SC) condition or a Social Testing (ST) condition. Littermates were housed together, two per cage with food and water available *ad lib*.

ST animals were tested in a variety of situations beginning at 95 days of age. The testing program included measures of activity, emotionality and discrimination-reversal learning; details of this testing program will be given in Chapter IV. SC animals were not handled except when being weighed or during cage cleaning.

All animals were 114 days of age when sacrificed for biochemical analysis. Brains of the ST animals were analysed for serotonin, and brains of the SC animals were analysed for DOPAD, GAD, MAO, AChE, and ChE.

Sacrifice and Dissection

Animals were sacrificed by decapitation after which their brains were rapidly removed, dissected and weighed to the nearest 0.1 mg. The dissections were performed as follows: After removal of the calvarium, a specially designed miniature plastic T-square was placed on the brain to delimit the samples to be removed from the visual and somesthetic areas of the cortex (Figure 5). The boundaries of each cortical section were first circumscribed with a scalpel, and then the cortical tissue was carefully peeled free of the white matter. Samples were taken from both hemispheres and constituted the sections labeled Visual Cortex (V) and Somesthetic Cortex (S). These sections were placed on dry ice and stored in a deep freeze at -22°C until analysis for AChE and ChE. The cerebelli were then removed, weighed and used to carry tissue standards through the serotonin procedure. The remaining brain including olfactory bulbs, medulla and pons constituted the section labeled Total Brain II (TB II). For animals in the ST group the TB II section was immediately extracted and analyzed for serotonin. For animals in the SC group the TB II section was frozen on dry ice and stored between two blocks of dry ice until the following day when they were analyzed for DOPAD, GAD, MAO, AChE and ChE.

Chemical analyses

Serotonin

Serotonin was determined in TB II sections from animals in the ST group by the procedures described in Experiment I.

Enzyme essays

TB II sections from animals in the SC group were homogenized in 0.03 M potassium phosphate buffer (pH 7.0) to a final concentration

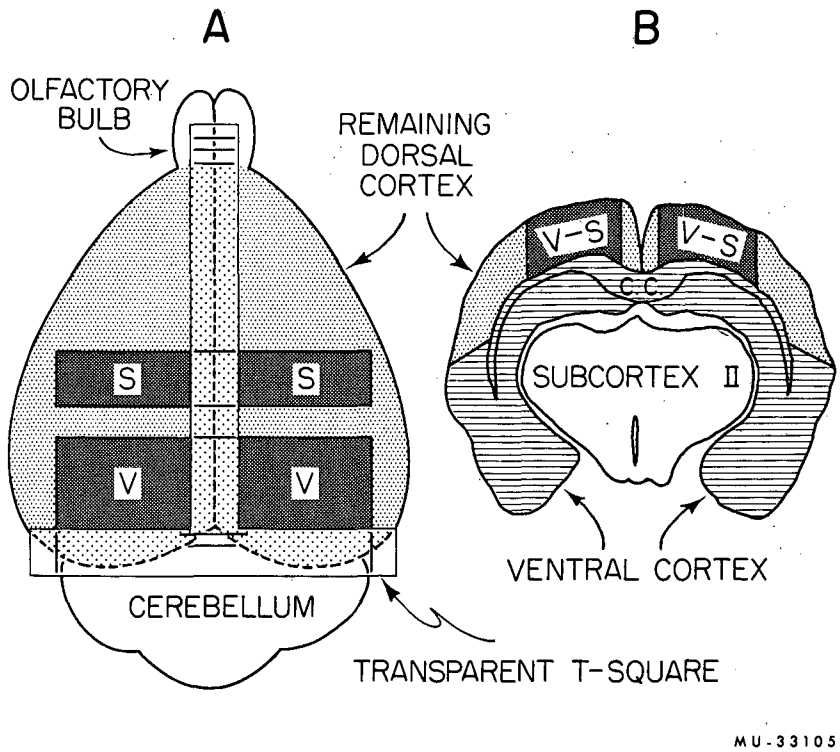

Fig. 5. Left, a diagram of the dorsal aspect of the rat brain, showing how the samples of the visual area (V) and of the somesthetic area (S) are dissected, guided by a small plastic T-square. Right, a diagramatic representation of a sagittal section of the rat brain (from Rosenzweig et al., 1962). Note — Subcortex II in this figure has been defined as Subcortex I throughout this dissertation.

of 20 mg/ml. Appropriate aliquots were taken for simultaneous determinations of DOPAD, GAD, MAO, AChE and ChE activities. V and S sections from animals in both groups were homogenized in 0.1 M potassium phosphate buffer (pH 7.9) to a final concentration of 3 mg/ml. Only AChE and ChE activities were determined on these sections. Duplicate determinations were made for all assays wherever possible.

AChE and ChE

AChE and ChE activities were determined by the spectrophotometric method described by Ellman, Courtney, Androes & Featherstone (1961) as modified by Bennett *et al.*, (1963).

For AChE, acetylthiocholine iodide (AcSCh iodide) was used as substrate in the presence of 5,5' dithio-bis-(2-nitrobenzoic acid) or DTNB. Thiocholine, the enzymatic product, reacts with DTNB forming the yellow anion, 2-nitrothiobenzoate, which absorbs maximally at 412 mμ.

For ChE, butyrylthiocholine iodide (BuSch iodide) was used as substrate in the presence of DTNB and a selective inhibitor of AChE, 1:5-bis-(4-trimethylammonium-phenyl) pentane-3-one diiodide (Burroughs Wellcome Code number 62C47).

All assays were done using a Beckman DU spectrophotometer equipped with a Gilford Automatic Cuvette positioner. The samples were preincubated for 10 minutes at 37°C, the substrate added and the reaction rates recorded for 10 minutes. Four samples were analyzed simultaneously and their absorbances, as a function of time, were recorded on a strip chart. Blanks were obtained by substituting buffer in place of the samples and the non-enzymatic rates of hydrolysis recorded.

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Specific amounts of reagents and tissue used in this experiment were as follows:

AChE - V and S sections

Tissues were homogenized in 0.1 M potassium phosphate buffer (pH 7.9) to a final concentration of 3 mg/ml. An aliquot containing 1.8 mg tissue was transferred to 1 cm light-path quartz cuvette containing 2.57 ml 0.1 M potassium phosphate buffer. Fifty µl of a 4 mg/ml solution of DTNB (40 mg DTNB plus 18 mg $NaHCO_3$ dissolved in 10 ml 0.1 M sodium phosphate buffer, pH 7.0) were added and the mixture preincubated for ten minutes at 37°C. Fifty µl of a 10.4 mg/ml solution of AcSCh iodide were added and the reaction rate recorded for ten minutes.

ChE-V and S sections

Three ml of the homogenate were transferred to a 1 cm light-path quartz cuvette. Fifty µl of a 2 mg/ml solution of DTNB containing 7.1 mg/ml 62C47 (specific inhibitor ofAChE) were added and the mixture preincubated for 10 minutes at 37°C. Fifty µl of a 200 mg/ml solution of BuSCh iodide were added and the reaction rate recorded for ten minutes.

AChE - TB II sections

Tissues were homogenized in 0.03 M potassium phosphate buffer (pH 7.0) to a final concentration of 20 mg/ml. An aliquot containing 2 mg tissue was transferred to a 1 cm light-path cuvette containing 2.57 ml 0.45 M potassium phosphate buffer (pH 7.53) making the final pH of the mixture 7.5. Fifty µl of a 4 mg/ml solution of DTNB were added and the mixture preincubated for ten minutes at 37°C. Fifty µl of a 10.4 mg/ml solution of AcSCh iodide were added and the reaction rate recorded for ten minutes.

ChE - TB II sections

One ml of the homogenate containing 20 mg tissue was transferred to a 1 cm light-path cuvette containing 2 ml 0.03 M potassium phosphate buffer (pH 7.0). Fifty µl of a 2 mg/ml solution of DTNB with inhibitor were added and the mixture preincubated for ten minutes at 37°C. Fifty µl of a 200 mg/ml solution of BuSCh iodide were added and the reaction rate recorded for ten minutes.

Calculation of AChE and ChE activities

Reaction rates for AChE and ChE were calculated using the following formula:

$$\text{Moles substrate hydrolysed/min/mg} = \frac{\Delta OD\text{-Blank}}{t} \times \frac{1}{E} \times \frac{TV}{W}$$

Where,

ΔOD = Change in optical density of the solution
t = Time (normally 10 minutes)
E = Extinction coefficient of reaction product = 13,600
TV = Total volume of the incubation mixture (l)
W = mg sample used

Decarboxylase activities

Decarboxylations were run in special one-arm flasks having a total volume of approximately 6 ml (Figure 6). For assays, 0.1 ml of stock substrate solution* was added to the side arm of the cell and 0.1 ml pyridoxal phosphate to the main compartment. Homogenate plus buffer were added to the main compartment to a total volume of 1.2 ml. The side arm was capped with a rubber serum cap (#F-1) and the cell attached to an evacuating apparatus (Figure 7) by a 2 inch length of rubber tubing. Cells were evacuated to 2 to 3 cm Hg pressure and the gaseous phase replaced by N_2. The evacuation was repeated twice.

*The stock substrate solution contained equal amounts of ^{14}C labeled D and L isomers, but only the L isomer was a substrate for these enzymes.

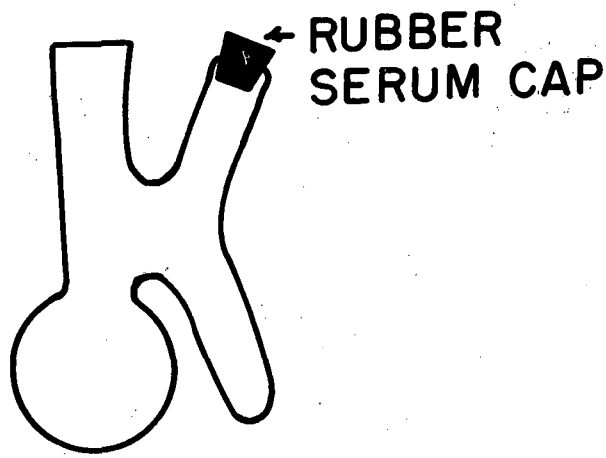

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Fig. 6. Diagram of special one-arm flasks used to run carboxylations.

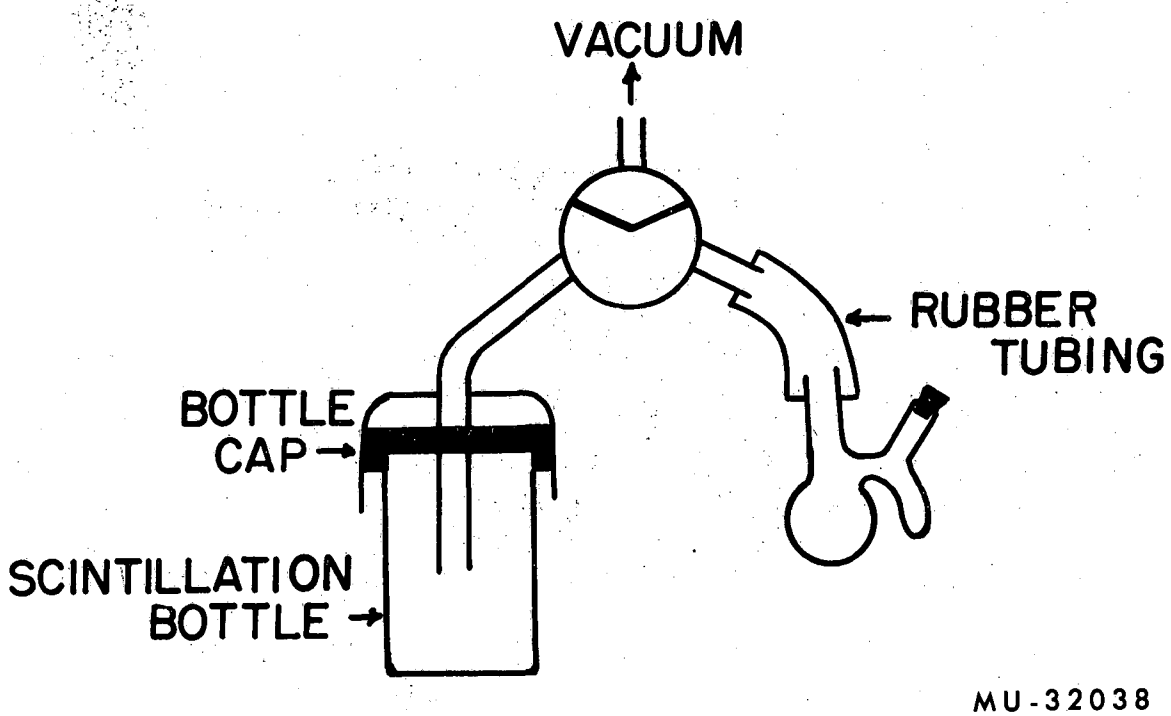

Fig. 7. Diagram of special evacuating apparatus used in decarboxylase assays.

Each cell was then placed under a slight vacuum and sealed by a screw clamp on the rubber tubing. After incubation at 37°C, the enzymatic reaction was terminated by addition of 0.3 ml 5N H_2SO_4 using a syringe and number 23 stainless steel needle.

To collect evolved CO_2, 2 ml of phenethylamine reagent (Woeller, 1961) were added to scintillation bottles and the bottles evacuated with the evacuating apparatus. The incubation cells and scintillator bottles were then interconnected by means of a three-way stopcock. After 5 minutes, 0.5 mM of $NaHCO_3$ were added to the acid-killed homogenates with a syringe and number 23 stainless steel needle through the serum cap. Nitrogen gas was then passed through the cell to insure complete recovery of CO_2. The entire collection period for ten cells required 25 minutes. Scintillation fluid number 2 (toluene, dioxane, ethanol, napthalene, PPO and POPOP prepared as described by Woeller, (1961) was added and the samples counted in a Packard liquid scintillation counter.

DOPAD

The fact that DOPAD and 5-HTPD are identical was discussed in Chapter II. Since the rate of decarboxylation of DOPA by this enzyme is 6.4 times that of 5-HTP, DOPA was used as substrate. Optimal conditions for this enzyme were pH 7.0 and a final concentration of DL- DOPA of 5×10^{-4} M. For assay 0.2 μc of DL-DOPA-1-^{14}C was used per cell. Pyridoxal phosphate was required as a cofactor with 0.1 mg giving maximal activity. Under these conditions, activity decreased after two hours to approximately 75 per cent of the initial rate. No reduction in decarboxylase activity was observed under an atmosphere of air, but reactions were routinely run in a nitrogen atmosphere at

37°C in a metabolic shaker (117 strokes per minute). All cells were given a two minute preincubation and the reaction initiated by mixing the DOPA with the homogenates. The reaction was stopped after two hours by addition of 0.3 ml 5 N H_2SO_4.

GAD

Optimal conditions for this enzyme were pH 8.0 and a final concentration of DL-glutamic acid of 1×10^{-3}M. For assay 0.2 µc of DL-glutamic acid -1-^{14}C was used per cell. Pyridoxal phosphate was required as a cofactor with 15 µg giving maximal activity. In an atmosphere of air initial activity was 40 per cent of the activity in a nitrogen atmosphere. Activity of the enzyme was not stable when stored for one week at -22°C. No loss in activity was apparent if tissues were kept on dry ice overnight. Assays were routinely run at 37°C and a pH of 7.0 in an atmosphere of N_2. All cells were given a two minute preincubation and the reaction initiated by mixing the glutamic acid with the homogenate. The reaction was stopped after one hour by addition of 0.3 ml 5 N H_2SO_4.

Calculation of decarboxylase activities

Reaction rates for DOPAD and GAD were calculated using the following formula:

$$\mu g \text{ substrate decarboxylated/hr/g} = \frac{\text{net dpm of evolved } CO_2}{\text{net dpm of substrate in incubation mixture}} \times \frac{\mu g \text{ substrate in incubation mixture}}{\text{tissue used (g)}}$$

Where,

net dpm of evolved CO_2 = cpm of evolved CO_2 - blank

$$\text{net dpm of substrate in incubation mixture} = \frac{\text{cpm of 100 } \mu\text{l stock substrate solution} - \text{background}^{*}}{2}$$

blank = cpm of non-enzymatic decarboxylation

background = cpm of scintillation fluid plus trapping agent only

MAO

An aliquot of homogenate containing 5 to 10 mg tissue was transferred to a 25 ml glass-stoppered Erlenmeyer flask and made up to a total volume of 1.0 ml with 0.03 M potassium phosphate buffer (pH 7). After a two minute pre-incubation period in a metabolic shaker, 20 μg 5-hydroxytryptamine-2-^{14}C-oxalate (specific activity of 0.94 mc/mg; New England Nuclear Corp.) were added. After a one hour incubation period at 37°C (35 strokes/minute), the reaction was terminated by transferring the mixture to a preheated 12 ml centrifuge tube in a boiling water bath. The reaction mixture was held at 100°C for five minutes after which the tube was centrifuged for 20 minutes at 3,000 rpm. An aliquot (0.1 ml) of the supernatant was spotted along the long axis of an 18-1/4 x 22-1/2 inch sheet of Whatman Number 1 paper. Spots were placed two inches apart (eight spots per sheet). It was found in preliminary experiments that much of the radioactivity remained at the origin on subsequent chromatography if the spots were dried by the conventional warm air method. Therefore, the spots were dried by drawing room temperature air through the paper, which resulted in a greater amount of the radioactivity leaving the origins. Sheets were developed in butanol:acetic acid:water (5:1:4) and autoradiographs prepared by exposure of the paper to x-ray film for one week to ten days. In this solvent system serotonin has a Rf value of 0.51 and its oxidative, deaminated

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*The stock substrate solution contained equal amounts of both the D and L isomers, but only the L isomer was a substrate for these enzymes, necessitating the division by 2.

product, 5-HIAA, has a Rf of 0.82. Internal standards were used with each experiment and were carried in boiled tissue. Radioactivity was counted using an automatic spot counter (Moses, Lonberg-Holm, 1963).

Calculation of MAO activity

Four sources of radioactivity were counted for each sample (Figure 8): A - the origin which accounted for about 10 to 20 per cent of the total radioactivity; B - a minor spot (less than 5 per cent of the total radioactivity) assumed to be a reaction product, possible corresponding to 5-hydroxyindoleacetaldehyde; C - the spot associated with unmetabolized serotonin which usually accounted for over 50 per cent of the total radioactivity; and D - the spot associated with the major enzymatic product of metabolism, 5-HIAA, which accounted for 20 to 40 per cent of the total radioactivity. A fifth spot, accounting for less than 5 per cent of the total activity, can be seen above the D-spot but was not counted since its identity was unknown. The reaction rate of MAO was calculated as follows:

$$\text{per cent serotonin/hr/gm metabolized} = \frac{\text{cpm } B' + \text{cpm } D'}{\text{cpm } A' + \text{cpm } B' + \text{cpm } C' + \text{cpm } D'} \times \frac{1}{\text{tissue used (g)}}$$

Where,

cpm X' = cpm X - background

background = cpm of mylar film with no spot.

Results

Chemical

The methods used for the determination of ChE and AChE were modified slightly from those of Ellman *et al.* (1961) and Bennett *et al.* (1963) in order to be compatible with the DOPAD, GAD and MAO assays. For this reason the results of some preliminary

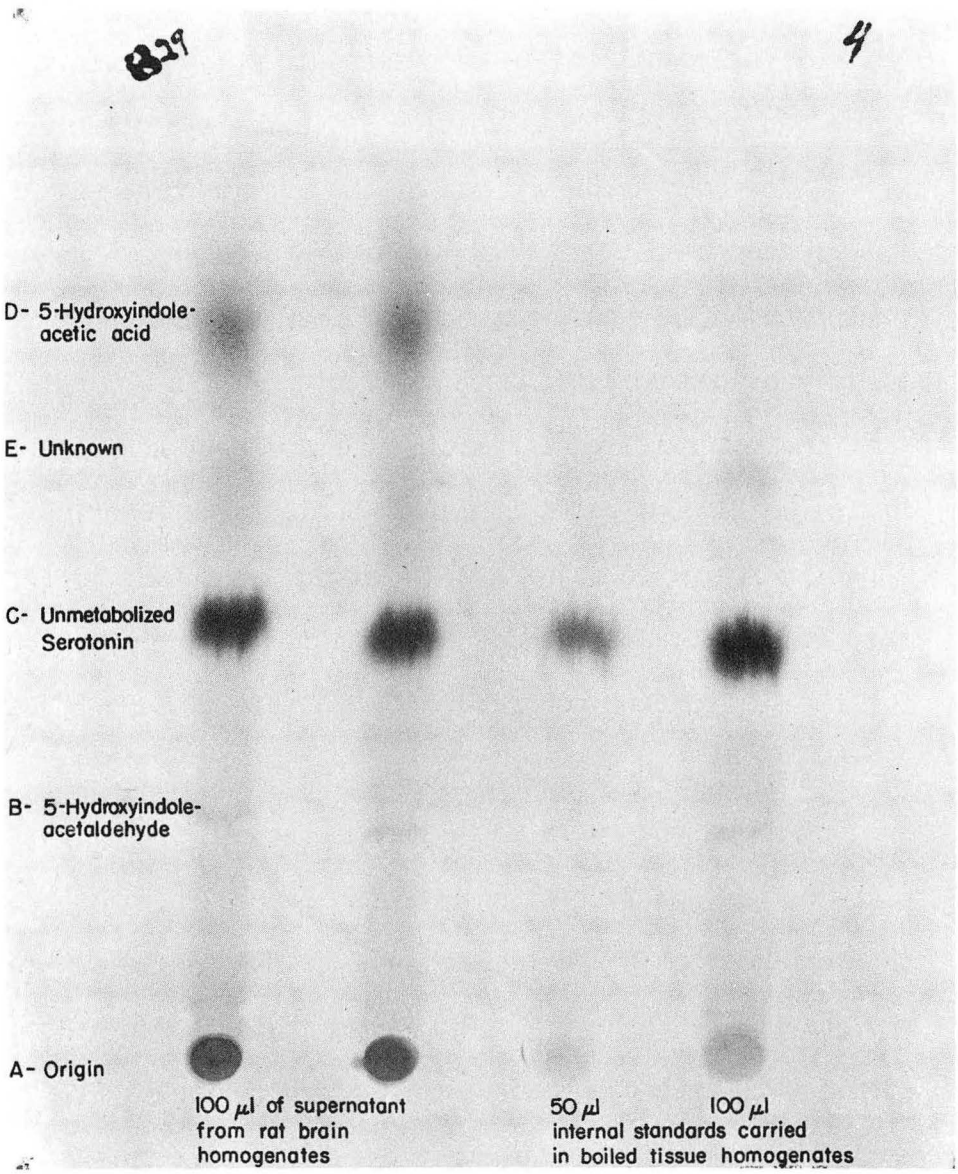

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Fig. 8. Autoradiogram obtained from chromatogram of two samples of rat brain homogenates, and two internal standards carried in boiled rat brain homogenates showing metabolism of serotonin by MAO.

biochemical experiments will be reported in which buffer and pH conditions were varied. The results of these experiments determined the final choice of conditions used during routine assays. Figure 9 shows the results of an experiment in which non-enzymatic hydrolysis was compared as a function of pH for two different buffers at 37°C. For both AcSCh and BuSCh the rate of non-enzymatic hydrolysis was greater when 0.1 M Tris buffer was used than when 0.2 M sodium phosphate buffer was used, and for both buffers the rates increased rapidly beyond pH 7.4.

The net rates of enzymatic hydrolysis of AcSCh (using 2 mg rat brain) and BuSCh (using 20 mg rat brain) as a function of pH are shown in Figure 10 using a 0.2 M sodium phosphate buffer. The net rate of hydrolysis of AcSCh increased in a liner fashion from pH 6.5 to 8.2, but the net rate of hydrolysis of BuSCh dropped off quite rapidly beyond pH 8.0.

Since tissues were to be homogenized in 0.03 M potassium phosphate buffer (pH 7.0) to accomodate the decarboxylase assays, a less than optimal* pH was selected for the AChE analysis. Table 5 shows the results of comparing a 0.15 M potassium phosphate buffer (pH 7.4) or a 0.45 M potassium phosphate buffer (pH 7.538) with a 0.2 M sodium phosphate buffer (pH 7.3 or 7.1) on the rate of AcSCh hydrolysis. Also included is a comparison of the rates of hydrolysis of BuSCh in several strengths of buffer.

There was no appreciable difference in the rate of hydrolysis of BuSCh over these ranges so that the ChE assay was run in the same buffer in which tissues were homogenized.

* In the sense that a higher net rate of hydrolysis could be obtained at a different pH.

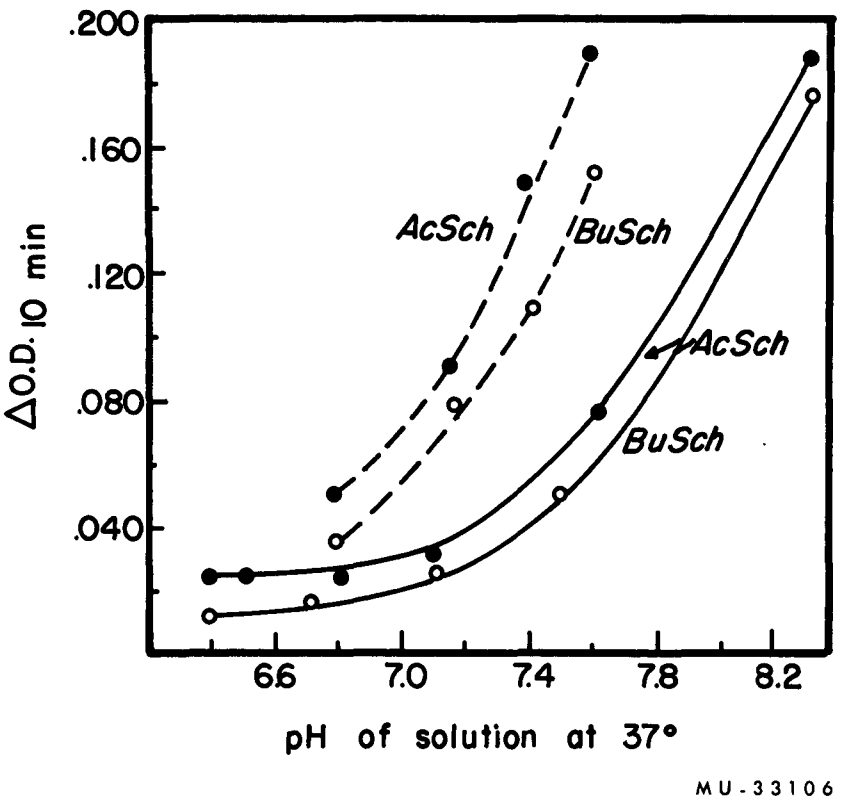

Fig. 9. Rate of non-enzymic hydrolysis of AcSCh and BuSCh as a function of pH in two buffers. • AcSCh; o BuSCh; —— 0.2 M sodium phosphate buffer; - - - 0.1 M tris buffer.

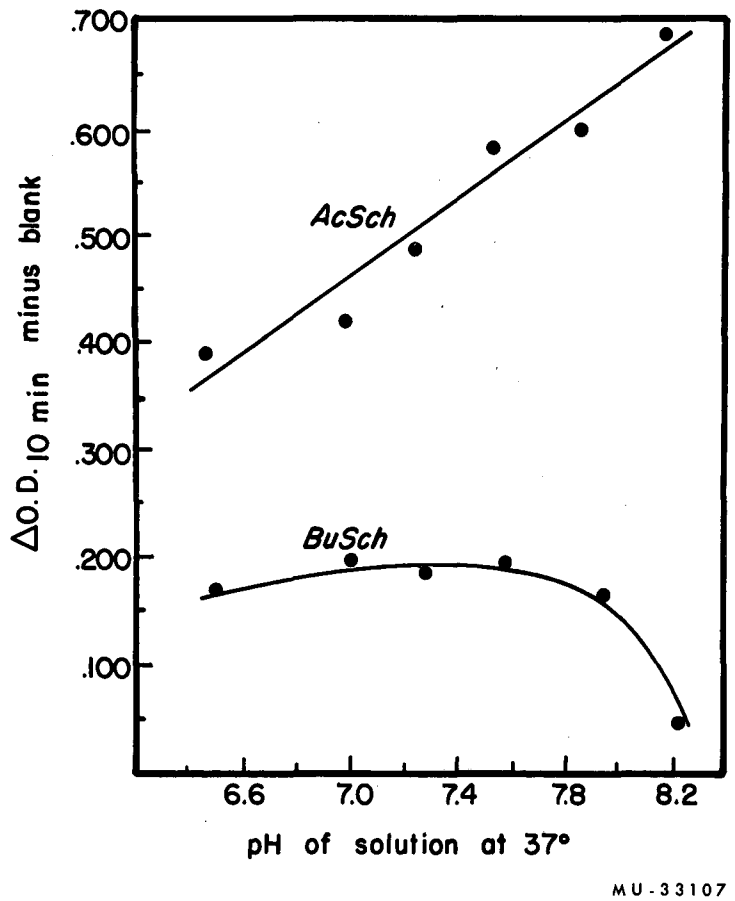

Fig. 10. Net rate of hydrolysis of AcSCh and BuSCh by 2 mg rat brain in 0.2 M sodium phosphate buffer as a function of pH.

Table 5

Rates of Hydrolysis of AcSCh and BuSCh as a Function of Buffer, Molarity and pH

Buffer		Sodium Phosphate		Potassium Phosphate		
Molarity		0.2	0.2	0.03	0.15	0.45
pH		7.3	7.6	7.0	7.4	7.53
Net Rate of Hydrolysis ($\Delta O.D._{10\ min}$)	AcSCh	483	500	-	499	534
	BuSCh	201	-	198	-	195

The 0.45 M potassium phosphate buffer (pH 7.53) allowed the highest net rate of hydrolysis of AcSCh while maintaining a constant pH against addition of at least 250 µl 0.03 M potassium phosphate buffer per 2.7 ml, including 2 to 6 mg tissue.

Strain comparisons - total serotonin and enzyme total activities

The animals used in this experiment were divided into two groups, SC and ST. Comparisons between strains, however, will be made only within a given group, since possible differences between conditions would increase the variance within strains and tend to mask between strain differences.

The means and standard deviations for the S_1 and S_3 strains with respect to body weight, brain weights, total serotonin content and enzyme total activities are shown in Table 6. The S_1 animals were heavier in body weight than the S_3 animals but had lighter brains. In the sensory cortex (V and S) the S_1s had higher AChE and ChE total activities than the S_3s in spite of the fact that the S_3s had heavier tissues, although not significantly so ($p > .10$). There were no significant differences between the two strains in the total activities of ChE and AChE in TB II.

Total activities of DOPAD and GAD did not significantly differentiate the two strains, but that of MAO did with the S_3s having significantly higher total activities than the S_1s ($p \leq .05$). The S_1s had 7.6 per cent more total serotonin in the TB II section than the S_3s but this difference was not statistically significant.

Two attempts were made to compare the strains on a purely biochemical basis, uncontaminated (in some sense) by tissue weight. First, the traditional ratio of serotonin or total enzymatic activity

per unit weight was used. Second, serotonin and enzyme total activities were compared between strains after the covariance associated with tissue weights were partialled out.

Strain comparisons - serotonin concentration and enzyme specific activities

A comparison of the two strains on serotonin concentration and enzyme specific activities (i.e. activity per unit weight) is shown in Table 7. In all comparisons, the S_1s had higher means than the S_3s. Individual tests of significance (Mann-Whitney U statistic) showed these differences to be statistically significant for all variables except DOPAD, GAD and MAO specific activities. Serotonin was higher in the S_1 strain than the S_3 strain by 18.2 per cent ($p \leq .002$). The usual differences between the two strains in AChE specific activities were apparent in all three brain sections. Similar differences were found for ChE specific activity.

Strain comparisons - discriminant function analysis of total serotonin and enzyme total activities.

The previous analysis, using the ratio of serotonin or enzyme total activity to tissue weight, was resorted to because the weights of tissue sections are usually not equal from one section to another, or from one animal to another. It was assumed that if one section has more tissue, where "more" is defined in terms of wet weight, then it ought to have more serotonin or enzyme solely by virtue of this fact. To compensate for the differences in weight, (due to minor inaccuracies during dissection and to morphological differences between section and animals), the measured amounts of serotonin or enzyme activities were scaled to presumably comparable units by using wet weight of the tissue as the divisor.

Table 6

Comparison of S_1 and S_3 Strains with Respect to Body Weight, Brain Weights, Total Serotonin and AChE, ChE, DOPAD, GAD and MAO Total Activities

Variable	Section	S_1		S_3	
		$\bar{x}$	S.D.	$\bar{x}$	S.D.
Body Weight (g)		335***	31.1	298	30.3
Brain Weights (mg)	V	76.7	4.48	79.2	4.84
	S	57.9	3.55	59.4	3.30
	TB II	1,308***	77.7	11,435	67.0
Serotonin (ng)	TB II	851	72.7	789	137.1
AChE (M x 10^8/min)	V	4,898***	326	3,918	417
	S	3,962*	292	3,433	301
	TB II	183,350	8,570	187,760	8,060
ChE (M x 10^8/min)	V	256	18.7	242	17.7
	S	201**	18.7	188	10.1
	TB II	4,771	273	4,840	325
DOPAD (μg/2 hr)	TB II	685	74.0	735	71.8
GAD (μg/hr)	TB II	973	209	928	227
MAO (percent metabolized/hr)	TB II	5,714**	655	6,169	318

* $p \leq .10$; two tailed test using the Mann-Whitney U statistic
** $p \leq .05$; " "
*** $p \leq .02$; " "

Table 7

Comparison of S_1 and S_3 Strains with Respect to Serotonin Concentration and AChE, ChE, DOPAD, GAD and MAO Specific Activities

Variable	Section	S_1		S_3	
		$\bar{x}$	S.D.	$\bar{x}$	S.D.
Serotonin (ng/g)	TB II	651***	43.5	551	98.3
AChE (M x 10^{10}/min/mg)	V	61.3***	3.05	52.9	4.91
	S	68.5***	3.43	62.5	4.59
	TB II	141.3***	4.96	132.4	3.22
ChE (M x 10^{10}/min/mg)	V	3.34*	0.17	3.06	0.13
	S	3.49***	0.22	3.17	0.16
	TB II	3.71***	0.12	3.46	0.16
DOPAD (μg/2 hr/g)	TB II	534	57.0	527	49.5
GAD (μg/hr/g)	TB II	756	157	666	166
MAO (percent/hr/mg)	TB II	4.45	0.41	4.42	0.23

* $p \leq .10$; two tailed test using the Mann-Whitney U statistic
** $p \leq .05$; " "
*** $p \leq .02$; " "

The result was a 'concentration' or 'specific activity' for that section which could be compared directly to the concentration or specific activity of another section. The following two assumptions are usually not considered in this common way of expressing substrate levels or enzyme activities: (1) that the tissue is homogeneous with respect to the substrate or enzyme activity being measured and (2) that a linear relation exists between the substrate or enzyme activity and the weight of the tissue. Thus, two samples would differ in concentration only if the slopes of the regression between substrate or enzyme activity and tissue weight differed.

If the first assumption of homogeneity is correct for a given tissue section, then it should be possible to check the second assumption by examining the correlation between a substrate or enzyme measured in that section and the wet weight of the section in animals having the same regression slope. Due to sampling errors and individual differences this correlation would not be expected to be perfect but it should be quite high. A low, zero or negative correlation, however, would suggest that the ratio be used only cautiously, if at all.

Table 8 shows the correlations between tissue weights and total serotonin or enzyme activities for the S_1 and S_3 strains in this experiment. Only AChE and ChE had correlations with tissue weight that were significant beyond the .05 level of confidence, and these correlations only accounted for between 36 and 70 per cent of the variance. Many of the other correlations were very low, including those for AChE and ChE in the S section. Assuming the tissue weights to be accurate, either the methods of measurement were inadequate, or the assumptions discussed above were not realized.

Table 8

Correlations Between Total Serotonin, Enzyme Total Activities and Wet Weight of Tissues for S_1 and S_3 Strains

		V		S		TB II_a		TB II_b	
		S_1+	S_3++	S_1	S_3	S_1	S_3	S_1	S_3
Serotonin -	TB II_a					.59	.06		
AChE	V	.71**	.39						
	S			.07	.38				
	TB II_b							.69**	.66**
ChE	V	.72**	.83**						
	S			.57	-.02				
	TB II_b							.84**	.74**
DOPAD	TB II_b							.28	.26
GAD	TB II_b							.31	-.12
MAO	TB II_b							.60*	.36

a and b for littermates, ie., a littermate analyzed for serotonin and b littermate analyzed for enzymes.

* $p \leq .05$

** $p \leq .01$

+ n = 10 for S_1s throughout

++ n = 13 for S_3s throughout

In any case, a technique that does not require these assumptions or is based upon the observed correlations may be preferred. The multivariate technique known as discriminant function analysis (c.f. Anderson, 1960) meets these requirements. Clearly, however, no statistical technique can overcome inadequate measurement, and to the extent that inadequate measurement was responsible for the observed low correlations, the results and conclusions must suffer accordingly.

Another, perhaps more important, reason for resorting to multivariate analysis should be mentioned. This concerns tests of significance made on a number of variables that are not statistically independent. Unlike the situations where multiple comparisons are made on a single dependent variable among several groups, or where a single variable is measured a number of times on the same subject (i.e., repeated measures designs), this problem occurs when several dependent variables have been measured on the same experimental material and these variables are interrelated to some degree.

Clearly, if two highly correlated variables are measured in the same material and the means compared between two groups, both will differ in the same way. Interpretation of the differences, however, may not be apparent when separate univariate tests have been made. Techniques are needed that will take into account the intercorrelations between variables. Multivariate techniques do just this and therefore will be relied upon heavily here.

Appendix A shows the computational steps for computing the discriminant function and Wilks' (1932) Lamda test of the significance of the discriminant. Such a discriminant function was computed between the S_1s and S_3s for weights of TB II<u>a</u>, TB II<u>b</u> (<u>a</u> and <u>b</u> for littermates i.e., littermate <u>a</u> was analyzed for serotonin and littermate <u>b</u> was analyzed for the five enzymes), total serotonin and total

activities of AChE, ChE, DOPAD, GAD and MAO. The Lamda test resulted in an $F_{8, 14} = 4.36$ ($p \leq .01$). Thus, these variables taken together were able to discriminate the two strains when the difference between mean vectors was maximized by an appropriate weighting of the variables dependent upon the respective intercorrelations, even though only MAO total activity, by itself, was significantly different between strains.

The question was then asked whether the biochemical variables taken alone could discriminate the two strains after the covariance associated with tissue weight was partialled out (computational procedures are in Appendix B). The Lambda test of the resulting discriminant was associated with an $F_{6, 16} = 2.07$ ($.10 \leq p \leq .20$). Table 9 shows the original mean vectors and the adjusted mean vectors after tissue weights had been partialled out. The S_1s have higher adjusted mean AChE, ChE and DOPAD activities and total serotonin than the S_3s, as was found when specific activities and concentration were used.

Univariate comparisons on the adjusted means showed ChE to be significantly different ($p \leq .01$) between strains after weight was partialled out even though the six variables taken together were not.

Correlations between weight, substrate and enzymes

Table 10 shows the correlations between tissue weights and the seven biochemical variables (total activities) for the S_1 and S_3 strains in this experiment. Above the diagonal are the full set of correlations and below the diagonal are the partial correlations among the biochemical variables with the tissue weights held constant (see Appendix C for computational procedures).

Table 9

Means and Adjusted Means for S_1 and S_3 Strains with Respect to Tissue Weight, Total Serotonin and Total Activities of AChE, ChE, DOPAD, GAD and Mao

Variable	Original Means Strain		Adjusted Means Strain	
	S_1	S_3	S_1	S_3
TB II_a (mg)	1,307**	1,434	-	-
TB II_b (mg)	1,285**	1,397	-	-
Serotonin (ng)	851	789	873	767
AChE (M x 10^6/ min)	1,833	1,877	1,879	1,832
ChE (M x 10^8/ min)	4,771	4,840	4,991**	4,617
DOPAD (µg/2 hr)	685	735	706	713
GAD (µg/hr)	973	928	989	910
MAO (per cent/ hr)	5,714*	6,169	5,963	6,009

* $p \leq .05$

** $p \leq .01$

Table 10

Correlations[1] and Partial Correlations[2] Between Tissue Weights and Biochemical Variables for the S_1 and S_3 Strains

		TB II$_a$	TB II$_b$	AChE	ChE	Serotonin	DOPAD	GAD	MAO
TB II$_a$	S_1[3]		.85**	.52	.77**	.59	.35	.15	.46
	S_3[4]		.69**	.28	.60*	.06	.31	.06	.05
TB II$_b$	S_1			.69*	.84**	.58	.28	.31	.60
	S_3			.66*	.73**	-.01	.26	-.12	.36
AChE	S_1				.57	.62	.12	-.09	.31
	S_3				.39	.27	-.10	-.20	.38
ChE	S_1			.01		.33	.59	.55	.31
	S_3			-.13		.33	.43	.20	.43
Serotonin	S_1			.42	-.43		.14	-.02	.58
	S_3			.42	.49		.14	.06	.58*
DOPAD	S_1			-.07	.68*	.08		.77**	-.13
	S_3			-.34	.34	.13		.66**	.60*
GAD	S_1			-.50	.65*	-.22	.84**		.07
	S_3			-.11	.40	.04	.71**		.09
MAO	S_1			-.19	-.44	.39	-.37	-.18	
	S_3			.11	.34	.68**	.65*	.22	

1 Above the diagonal

2 Below the diagonal

3 n = 10

4 n = 13

* $p \leq .05$
** $p \leq .01$

The correlations between tissue weight and enzyme total activities have already been discussed (p. 102). The partial correlations among the biochemical variables indicate somewhat different patterns for the two strains. The essentially zero correlations between AChE and ChE for both strains ($r = .01$ for the S_1s and $r = -.13$ for the S_3s) suggest that these two enzymes operate independently of each other.

Serotonin was positively correlated with AChE and MAO for both strains, but little relation was apparent between serotonin and DOPAD or GAD.

Serotonin was positively correlated with ChE in the S_3 strain ($r = .49$) but negatively correlated in the S_1 strain ($r = -.43$). Neither of these correlations were, however, statistically significant. ChE was moderately correlated with DOPAD ($r = .68$ for the S_1s and $r = .34$ for the S_3s) and GAD ($r = .65$ for the S_1s and $r = .40$ for the S_3s). AChE was slightly negatively correlated with these enzymes. The highest correlations were between DOPAD and GAD ($r = .84$ for the S_1s and $r = .71$ for the S_3s).

DISCUSSION

The difference between brain serotonin concentration in the S_1s and S_3s found in Experiment I was successfully replicated in this experiment. The S_1s had about 18 per cent more serotonin per gram tissue than the S_3s. When total serotonin was considered, the S_1s still had more serotonin than the S_3s but not significantly so. The same result was seen when tissue weight was partialled out statistically, but again the difference was not significant.

Somewhat similar results were found for the enzymes AChE, ChE, DOPAD, GAD and MAO. In all comparisons the S_1s had higher enzyme

specific activities than the S_3s, but when total activity was considered the differences decreased or even reversed depending on the particular correlations between enzyme total activities and tissue weight. When tissue weight was partialled out statistically, the S_1s tended to have higher AChE, ChE and GAD activities but lower MAO activity than the S_3s.

Considering the serotonin system alone, it appeared that the capacity for synthesizing the amine is about equal for both strains as reflected by the adjusted mean DOPAD activities (Table 9: S_1, 989; S_3, 910). The S_3s, however, had a greater capacity for metabolizing serotonin as reflected by the higher MAO activity (S_1, 5,963; S_3, 6,009), and the lesser amount of unmetabolized serotonin (S_1, 873; S_3, 767). Nothing can be inferred as to the relative amounts of bound and free serotonin in the two strains, but it appears that the S_1 strain may have more functional serotonin available than the S_3 strain.

The correlations between serotonin and AChE are interesting in view of the suggestion by Aprison (1961) that serotonin may compete with ACh for neural receptor sites. If his hypothesis were correct, the fact that the S_1 strain has higher values than the S_3 strain in both the ACh-AChE system and the serotonin system might reflect a causal relation between the two neurohumoral systems.

Experiment III

Comparison of Six Strains of Rats with Respect to Brain Serotonin, AChE, ChE, DOPAD, GAD and MAO

In Experiments I and II a difference in brain serotonin was found between the S_1 and S_3 strains. In Experiment II the cholinergic

enzymes, AChE and ChE, and the non-cholinergic enzymes DOPAD, GAD and MAO were investigated in these strains. This experiment extends this investigation to two other pairs of strains of rats. In addition, the regional distributions of these enzymes will be investigated.

The three pairs of strains chosen for this experiment were the S_1 -S_3, RDH-RDL and OMB-OMD strains.* Reasons for these choices were as follows: First, the S_1 -S_3 strains were known to differ in AChE, ACh and maze-performance. The S_1 strain has higher brain ACh levels, higher AChE specific activity and is superior on most learning tasks to the S_3 strain. These facts were mainly responsible for the hypothesis that the ACh-AChE system was positively related to learning. Second, the RDH strain (Roderick Dempter High AChE) was selectively bred for high cortical AChE compared to the RDL strain (Roderick Dempter Low AChE; Roderick, 1960). When tested for maze performance the RDL strain was found to be slightly superior to the RDH strain (Rosenzweig et al., 1960). These facts were mainly responsible for the hypothesis that the ratio of ACh to AChE was positively related to learning (c.f. Chapter I). Third, the OMB strains (Olson Maze-Bright) were selected for performance on the Lashley III maze and thus are superior to the OMD strains (Olson Maze-Dull) in this respect, but in one of the lines (line V), the two strains did not differ with respect to either ACh or AChE and in the other line (line II), the OMD strain was actually higher in brain ACh

* OMB and OMD strains were selected by Richard Olson in a joint project with the Department of Psychology and Genetics, University of California, Berkeley. Results of this project have not been published as yet.

concentration and AChE specific activity than the OMB strain (R. Olson, personal communication). These facts prohibited a simple, general hypothesis that the ACh-AChE system was positively related to all aspects of learning.

Failure of the OMB and OMD strains to differ as expected in ACh or AChE does not automatically invalidate the proposed ACh-AChE ratio hypothesis. It may be that the Lashley III maze was not demanding enough to require changes in the ratio of ACh to AChE that would result in high and low strains on these biochemical variables, or other factors related to learning may have been inadvertently selected for, e.g., differential response to deprivation, exploratory tendencies, reaction to novelty, emotional responses to the apparatus, etc. Investigation of these variables has not been made with animals from these two strains.

On the other hand, it is doubtful that the neural and biochemical events underlying an animal's performance in a maze can ever be completely separated into independent components corresponding to these rather artificial categories. Many neural and biochemical events undoubtedly occur that are closely interrelated and are grossly reflected in the errors an animal makes and how fast he performs.

The purpose of this experiment was to see whether serotonin and the enzymes DOPAD, GAD and MAO showed the same differences among these three pairs strains as does AChE. Correlations of these enzymes with AChE found in Experiment II suggest that different patterns will be found. If such is the case, then the question will be asked whether the three strains that are superior in maze performance have anything in common biochemically that

includes these non-cholinergic systems.

METHOD

Subjects

The subjects in this experiment were ten littermate pairs of male rats from each of the following six strains: S_1, S_3, RDH, RDL, OMB and OMD. A special breeding schedule was arranged so that animals from all six strains were born at approximately the same time. This resulted in animals between strains being matched for age within 1 to 3 days. All animals were weaned at about 25 days of age, ear-punched for identification, and placed 3 to 4 per cage under colony conditions, including food and water *ad lib*. Males were housed separately and strains were kept segregated. All animals were moved from Life Sciences Building where they were born to Tolman Hall at about 60 days of age.

Littermate pairs were selected prior to sacrifice on the basis of matching weights and general health. Ten litters were sampled from each of the S_1, S_3, RDH and RDL strains. Breeding stock from both the II and V lines of the OMB and OMD strains were obtained with the intention of selecting ten littermate pairs from each of the four strains. Reproduction was so poor, however, that this plan had to be abandoned. None of the OMB pups from the II line survived, so that only OMB animals from the V line were available. Ten pairs of males were selected from the five OMB litters of the V line that survived. Five pairs of males were selected from the three OMD litters of the V line that survived and five pairs of males were selected from the four OMD litters of the II line that survived. This resulted in the pairing of five sets of OMB and OMD animals of the V line and five

sets of OMB animals of the V line with five sets of OMD animals of the II line, a procedure that is methodologically inadequate since the two lines were selected from different parental populations. One littermate was assayed for serotonin and the other littermate was assayed for enzymes.

Order of sacrifice

Group I - Four littermate pairs of animals from each of the six strains were brought from the colony to a room near the biochemistry laboratory two days prior to sacrifice. This was done to avoid any alteration in brain serotonin that might occur due to an abrupt change in environment. Animals were sacrificed for serotonin analysis in the morning in blocks of six, including one animal from each strain. Cerebelli were removed, weighed and used to carry tissue standards through the serotonin procedure. The whole brain, less cerebellum (TB I), was removed, weighed to the nearest 0.1 mg and immediately extracted for serotonin. Littermates of these animals were sacrificed the same afternoon in blocks of six, including one animal from each strain. After decapitation the brains were rapidly dissected, weighed to the nearest 0.1 mg and frozen on dry ice. The sections were stored at -22°C until they were analyzed for the five enzymes. Animals in Group I were approximately 87 days old.

Group II - After Group I had been sacrificed, it was discovered that one of the enzymes, GAD, was not stable when stored frozen for periods over several days. Consequently, it was decided to sacrifice animals for enzyme analyses not more than 24 hours prior to analysis. The remaining 36 animals for serotonin analysis were sacrificed as in Group I and the extraction was

performed immediately. They were approximately 90 days old and constituted Group II.

Group III - Littermates of animals in group II were sacrificed as follows. Six animals, one from each strain were sacrificed per day. Their brains were rapidly removed, dissected, weighed and frozen on dry ice. Half of the sections were analyzed the same day and the other half were stored between two blocks of dry ice until the following day when they were analyzed. This schedule made it impossible to analyze more than two blocks of animals per week, and consequently these animals ranged in age from 95 to 123 days, with a mean of 108 days. Animals within a block were approximately the same age.

Dissection of brains for enzyme analyses

Brains were dissected for enzyme analyses as follows. Using a miniature, plastic T-square a sample of tissue from both hemispheres was removed from the visual and somesthetic areas of the cortex as described in Experiment II and constituted the sections labeled Visual Cortex (V) and Somesthetic Cortex (S). Remaining dorsal cortex was peeled off down to the temporal ridge and constituted the section labeled Remaining Dorsal Cortex (RDC). Ventral cortex and surrounding structures, including hippocampus, amygdala and corpus callosum were separated and constituted the section labeled Ventral Cortex (VC). The cerebellum was removed by severing its peduncles and constituted the section labeled Cerebellum (Ce). A section labeled Hypothalamus (H), was taken by circumscribing the area bounded by the optic chiasm anteriorly the optic tracts laterally and the

posterior aspect of the mamillary body posteriorly with a scalpel and removing the tissue down to a depth of the anterior commissure anteriorly and a parallel depth posteriorly. Finally, a section consisting of Medulla and Pons (M+P) was separated by sectioning the brain stem just posterior to the inferior colliculi. The remaining tissue, including thalamus, caudate nuclei, other basal ganglia, olfactory tubercles, and olfactory bulbs constituted the section labeled Remaining Subcortical Brain (RSB).

Chemical analyses

All chemical analyses were performed according to the techniques described in Experiment II. The concentrations of homogenates and amounts of tissue used in this experiment are shown in Table 11.

RESULTS

Animals in this experiment varied in age, time of biochemical analysis and duration of tissue storage. Therefore, these sources of variance were removed prior to statistical analysis. Blocks of animals, one animal from each of the six strains, were processed together so that an adjustment of the data was possible. The mean for each block of six animals was computed and subtracted from the grand mean computed over all ten blocks. These deviations from the grand mean were then added to the values of each animal within that block. This adjustment procedure equalized the means for blocks while leavning the means for strains unaffected. Variances within strains was, of course, reduced accordingly.

Table 11

Concentration of Homogenates and Amounts of Tissue Used in Experiment III

Section	Concentration of Homogenates*	Amount of Tissue Used per Assay (mg)				
		AChE**	ChE***	DOPAD	GAD	Mao
V	10	2	6	-	10	10
S	10	2	6	-	10	10
RDC	20	2	20	20	10	10
VC	20	2	20	20	10	10
H	5	1	10	5	5	5
M + P	20	2	20	20	10	10
RSB	10	1	20	10	10	5
Ce	20	3	20	-	10	10

* All tissues were homogenized in 0.03 M potassium phosphate buffer, pH 7.0.

** 0.45 M potassium phosphate buffer, pH 7.53.

*** 0.03 M potassium phosphate buffer, pH 7.0.

Some data were lost during the experiment due to errors in biochemical procedures. Within strain means were substituted for these missing data in the statistical analyses. Four blocks of tissue were unanalyzeable for GAD due to too lengthy storage, but in this case no substitution for missing data was made, separate statistical analyses being computed with only six subjects per strain.

Body weights and brain tissue weights

Mean body weights and brain tissue weights are shown for the six strains in Table 12. The means are based on ten cases in each strain except for the M+P and RSB sections where only six animals were available. In the other four animals M+P and RSB sections were not divided, constituting a single section.

The S_1 and S_3 strains showed the usual differences in brain weight found earlier (Experiments I and II, Bennett *et al.*, 1958), even though they did not differ in body weight. For all eight sections the S_3s had heavier brain tissue weights than the S_1s. The RDLs were heavier in body weight and brain weights than the RDHs, and the OMBs were heavier in body weight and brain weights than the OMDs.

Serotonin

Table 13 shows the means and standard deviations for the six strains with respect to TB I tissue weight, total serotonin and serotonin concentration in TB I. The S_1s had significantly more serotonin per gram tissue than the S_3s (10 per cent). The RDHs had a higher mean concentration of serotonin (815 ng/g) than the RDLs (795 ng/g), and the OMDs had a higher mean concentration of serotonin (740 ng/g) than the OMBs (719 ng/g). Neither of these latter comparisons reached an acceptable level of statistical significance ($p > .05$).

Table 12

Means and Standard Deviations of S_1, S_3, RDH, RDL, OMB and OMD Strains with Respect to Body Weight and Brain Tissue Weights

Variable	Strains											
	S_1		S_3		RDH		RDL		OMB		OMD	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Body Weight* (g)	302	26.6	298	30.5	234	44.4	324	25.3	372	42.2	312	18.7
V* (mg)	66.2	5.1	72.7	5.4	66.2	6.1	73.2	3.3	75.5	4.4	72.6	4.7
S* (mg)	52.9	2.9	55.6	3.0	50.5	3.8	55.1	2.2	56.2	5.0	56.1	3.3
RDC* (mg)	264.2	16.6	295.5	12.3	257.8	16.8	297.8	11.6	313.5	16.6	300.6	20.0
VC* (mg)	318.8	26.5	339.8	32.6	304.8	38.2	346.2	20.8	368.5	17.7	348.2	19.1
Total Cortex (mg)	702	37.1	763	34.7	679	41.8	772	25.4	814	28.7	778	39.4
H* (mg)	60.0	5.2	61.1	6.5	53.9	4.8	62.5	7.1	63.3	6.1	59.4	6.3
M + P** (mg)	168.0	9.1	197.6	9.2	158.9	10.9	194.0	9.7	192.4	11.1	188.4	19.1
RSB** (mg)	433.5	34.6	482.0	41.1	417.7	19.5	461.0	15.3	534.9	20.5	488.1	24.3
Total Sub-Cortex (mg)	661	47.6	719	43.7	625	56.2	707	25.1	760	27.3	718	43.9
Ce* (mg)	237.6	20.4	269.9	20.0	235.3	18.9	269.7	7.0	276.5	18.5	256.3	20.6
Total Brain (mg)	1611	99.0	1752	93.1	1538	90.2	1749	41.5	1850	64.1	1754	99.7
Cortex/Sub-Cortex	1.06	0.06	1.06	0.03	1.09	0.10	1.09	0.05	1.07	0.04	1.08	0.04

* $n_i = 10$

** $n_i = 6$

Table 13

Means and Standard Deviations of S_1, S_3, RDH, RDL, OMB AND OMD Strains with Respect to Total Serotonin and Serotonin Concentration in TB I

Variable	Strain											
	S_1		S_3		RDH		RDL		OMB		OMD	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Wet Weight of TB I (mg)	1399*	70.4	1570	81.7	1366*	55.2	1480	87.3	1597	55.8	1526	61.5
Total Serotonin in TB I (ng)	1147	49.6	1157	32.1	1100*	41.1	1160	35.3	1133	55.2	1114	43.4
Serotonin conc. in TB I (ng/g)	829*	31.7	752	45.6	815	22.7	795	64.3	719	50.2	740	43.5

* $p \leq .02$, two-tailed test using Wilcoxon matched-pairs test of difference between pairs of means

Enzyme total and specific activities

Appendices D_1 to D_{13} show the means and standard deviations for the six strains with respect to enzyme total and specific activities in the eight brain sections. Because of the large number of variables and the high intercorrelations of many of the variables, some means for reducing this number was sought. A principal axis factor analysis was obtained from the correlation matrix of tissue weights and enzyme total activities for all six strains combined. GAD total activities were not included in this analysis since four of the ten samples for each section were lost. M+P and RSB sections were combined into one section for purposes of this analysis.

The factor analysis resulted in six factors or dimensions that accounted for over 95 per cent of the covariance between variables. Table 14 shows the factor loadings of body weight, brain tissue weights and enzyme total activities on these six dimensions. The first factor is apparently a weight dimension that includes both body weight and brain tissue weights. Many of the enzyme total activities were also highly correlated with this factor. The second factor is common to the VC and H sections. Both tissue weights and enzyme total activities for the VC and H sections were highly correlated with it. The remaining four factors appear to be specific to total enzyme activities with relatively little relation to tissue weights.

On the basis of this factor analysis the following variables and linear composites of variables were retained as accounting for nearly all the information contained in the original 54 variables: Body weight; tissue weights - VC, H, V + S+ DC (Dorsal Cortex;DC), M + P + RSB (Subcortex II SC;II); and the corresponding enzyme total activities.

Table 14

Factor Loadings of Body Weight, Brain Tissue Weights and Enzyme Total Activities on First Six Dimensions Obtained from Principal Axis Factor Analysis

Variable		Dimension					
		1	2	3	4	5	6
Body Weight		-.78	.11	.06	.30	.03	-.24
Weight of	V	-.83	-.10	.10	-.20	.01	-.25
	S	-.73	-.14	-.02	-.44	-.09	-.14
	RDC	-.85	-.05	-.09	.27	.04	.13
	VC	-.62	.22	.18	.29	-.41	-.14
	H	-.45	.76	-.13	-.24	.20	-.04
	Ce	-.89	-.02	.27	.08	.06	-.08
	M+P+RSB	-.93	-.15	.01	.09	.17	-.08
ChE	V	-.27	-.18	.14	-.71	-.12	.15
	S	.07	-.10	-.17	-.65	-.23	.27
	RDC	-.40	-.11	-.26	.26	.15	.46
	VC	.10	-.33	-.02	-.10	.14	-.37
	H	-.17	.65	-.03	-.16	.17	.47
	Ce	-.38	-.26	.48	-.26	-.23	.45
	M+P+RSB	-.60	-.38	.13	-.04	-.14	.34
AChE	V	-.31	-.06	-.62	-.29	-.27	-.13
	S	-.23	-.11	-.80	-.21	-.25	-.16
	RDC	-.50	-.62	-.72	.28	-.16	.14
	VC	.07	.63	-.10	.17	-.54	-.11
	H	-.16	.83	-.19	-.27	.20	-.07
	Ce	-.75	-.06	-.06	.02	-.37	.24
	M+P+RSB	-.71	-.16	-.29	.01	.28	-.00
DOPAD	DC	-.38	.09	.45	.04	.15	.14
	VC	.04	.57	.28	.10	-.01	-.01
	H	-.49	.59	.10	-.05	.18	.18
	M+P+RSB	-.63	.15	.17	.05	-.16	-.16
MAO	V	-.63	-.26	.14	-.21	-.13	-.13
	S	-.74	-.13	.01	-.30	-.07	-.07
	RDC	-.77	-.00	-.19	.26	.16	.16
	VC	-.33	-.06	.21	.22	-.41	-.41
	H	-.33	.76	-.06	-.24	-.06	-.06
	Ce	-.75	.04	.38	-.06	-.07	-.07
	M+P+RSB	-.82	-.23	-.07	.06	-.07	-.07
Serotonin		-.23	-.02	.07	-.45	-.29	-.29

V and S sections were not successfully analyzed for DOPAD, so that only RDC values were available for this enzyme. Composite sections were obtained by adding the component weights or enzyme total activities assuming, in the latter case, a linear relation between enzyme total activities and tissue weights. Specific activities for these composites were computed by dividing the sum of the total activities by the sum of the component weights.

Comparison of S_1 and S_3 strains - enzyme total activities

Table 15 shows the means and standard deviations of the S_1 and S_3 strains with respect to VC, H, DC and SC II brain tissue weights and the corresponding enzyme total activities. The two strains did not differ significantly in VC or H tissue weights. The S_3s, however, were ten per cent heavier than the S_1s in DC and SC II sections ($p \leq 0.05$).

Enzyme total activities did not differ significantly between the S_1s and S_3s in any of the brain sections. The means for all five enzyme total activities in the DC and SC II sections were, however, slightly higher in the S_3s than in the S_1s. These differences probably reflect the significantly greater tissue weights of these sections in the S_3s compared to the S_1s.

A discriminant function was computed for the two strains composed of body weight, DC and SC II tissue weights and the corresponding enzyme total activities of ChE, AChE, DOPAD, and MAO. The test of significance of the discriminant resulted in an $F_{11,8} = 3.33$ ($p \leq .05$). To determine whether the significant discrimination reflected only tissue weight differences, a second discriminant function was computed with body weight and tissue weights partialled out. The $F_{8,11} = 2.00$ was not significant ($p > .05$), but the adjusted means were higher for the

Table 15

Means and Standard Deviations of S_1 and S_3 Strains with Respect to VC, H, DC and SC II Tissue Weights and Corresponding Enzyme Total Activities

Variable	Section	S_1		S_3		$F_{1,N-2}$ #
		$\bar{x}$	SD	$\bar{x}$	SD	
Brain Weights	VC	319	27.9	340	34.4	2.22
(mg) n_i = 10	H	60	5.4	61	6.9	.13
	DC	383	22.5	423	17.2	20.01***
	SC II	601	50.8	658	45.7	6.76**
ChE Total	VC	910	180	926	190	.04
Activity	H	365	53	350	45	.50
(M x 10^8/min)	DC	1138	69.8	1194	76.6	2.83
n_i = 10	SC II	3192	436	3352	281	.95
AChE Total	VC	(x10^{-1})3774	892	3445	830	.75
Activity	H	5613	606	5366	8836	.60
(M x 10^8/min	DC	(x10^{-1})2139	104	2179	145	.43
n_i = 10	SC II	(x10^{-2})1177	119	1246	100	1.96
DOPAD Total	VC	122	43.3	111	43.4	.33
Activity	H	54.8	2.8	577	789	.09
(µg/2 hr)	RDC	69	9.2	71	18.0	1.21
n_i = 10	SC II	600	62.5	634	78.5	1.15
MAO Total	VC	1658	159	1739	181	1.09
Activity	H	430	29	468	55	3.72*
(per cent/hr)	DC	1757	117	18.78	156	3.85*
n_i = 10	SC II	3658	349	3880	309	2.52
GAD Total	VC	239	29.4	242	19.1	.00
Activity	H	58.0	7.5	58.7	7.0	.00
(µg/hr)	DC	418	17.6	427	38.8	.27
n_i = 6	SC II	749	124.9	758	141.1	.01

$F_{1,N-2} = \frac{(N-2)\ (1-\Lambda)}{\Lambda}$, where $\Lambda = \frac{Wii}{Tii}$

Wii = within groups deviations

Tii = combined groups deviations

* $p \leq .10$

** $p \leq .05$

*** $p \leq .01$

S_1s than the S_3s in all cases (Table 16). This suggests that there are enzyme components independent of weight that are higher in the S_1s than in the S_3s.

Three further discriminant functions were computed. One was composed of VC and H weights and the corresponding total activities of ChE, AChE, DOPAD and MAO. Another included VC and H tissue weights and the corresponding total activities of GAD. Neither of these two discriminant functions were statistically significant. The third discriminant was composed of body weight, DC and SC II weights and the corresponding total activities of GAD. The test of this discriminant resulted in an $F_{5,6} = 7.79$ ($p \leq .01$). After body weight and tissue weights were partialled out significant discriminating power remained ($F_{2,9} = 5.29$; $p \leq .05$). This was largely due to the difference between adjusted means in the SC II section (pairwise $F_{1,9} = 10.52$; $p \leq .01$).

Comparison of S_1 and S_3 strains - enzyme specific activity

Table 17 shows the means and standard deviations for the S_1 and S_3 strains with respect to specific activities of the five enzymes in the four brain sections. In all but three comparisons the S_1s had higher enzyme specific activities than the S_3s. Only two of these comparisons were statistically significant. AChE was significantly higher in the S_1s than in the S_3s in the H and DC sections ($p \leq .05$), whereas MAO was significantly lower in the S_1s than in the S_3s in the H section ($p \leq .01$). The discriminant function composed of body weight and ChE, AChE, DOPAD and MAO specific activities in the DC and SC II sections was statistically significant ($F_{10,9} = 3.73$; $p \leq .05$), as was the discriminant function composed of ChE, AChE, DOPAD and MAO specific activities in the VC and H sections ($F_{10,9} = 3.69$; $p \leq .05$). Most of the discriminanting power of

Table 16

Adjusted Means of S_1, S_3, RDH, RDL, OMB and OMD Strains with Respect to Total Activities of ChE, AChE, DOPAD, MAO and GAD in DC and SC II Brain Sections After Body Weight and Tissue Weights were Partialled Out

		Strain					
Enzyme	Section	S_1	S_3	RDH	RDL	OMB	OMD
ChE	DC	1171	1162	1164	1070	1191	1233
	SC II	3411	3138	3348	3144	3507	3555
AChE	DC	2192	2124	2100	1934	2397	2425
	SC II	1232	1193	1147	1068	1281	1264
DOPAD	RDC	71	69	67	66	69	66
	SC II	637	598	580	568	678**	566
MAO	DC	1865*	1770	1941	1636	2031	2022
	SC II	3817	3727	3724	3672	4200	4172
GAD	DC	412	428	454	524	473	444
	SC II	870*	663	637*	789	855	851

* $p \leq .05$ for pairwise comparison of adjusted mean differences.

** $p \leq .01$ " "

Table 17

Means and Standard Deviations of S_1 and S_3 Strains with Respect to Specific Activities of ChE, AChE, DOPAD, MAO and GAD in VC, H, DC and SC II Brain Sections

Enzyme Section		S_1		S_3		$F_{1,N-2}$
		$\bar{x}$	SD	$\bar{x}$	SD	
ChE Specific	VC	2.82	0.28	2.73	0.41	.09
Activity	H	6.09	0.49	5.76	0.48	.22
(M x 10^{10} min/	DC	2.97	0.17	2.83	0.21	2.41
mg) n_i = 10	SC II	5.27	0.59	5.15	0.56	2.71
AChE Specific	VC	118.5	27.9	102.4	24.2	.23
Activity	H	94.2	4.8	87.7	4.6	1.93
(M x 10^{10} min/	DC	55.9	3.8	50.8	4.4	9.35***
mg) n_i = 10	SC II	195.4	10.8	189.9	8.3	7.60**
DOPAD Specific	VC	380.6	133.2	333.9	145.5	1.67
Activity	H	942.3	93.2	966.0	104.1	.75
(μg/2 hr/g)	RDC	262.2	40.3	242.1	61.6	.29
n_i = 10	SC II	993.9	51.2	967.1	65.9	.29
MAO Specific	VC	5.18	0.32	5.14	0.19	1.01
Activity	H	7.20	0.26	7.67	0.33	.15
(per cent/hr/	DC	4.57	0.09	4.50	0.14	12.87***
g) n_i = 10	SC II	6.06	0.41	5.95	0.15	2.23
GAD Specific	VC	796	76.7	695	103.9	.61
Activity	H	1021	118.9	1038	65.9	3.74*
(μg/hr/g)	DC	1077	32.4	1000	107.7	.20
n_i = 6	SC II	1233	144.6	1109	161.6	2.79

* $p \leq .10$
** $p \leq .05$
*** $p \leq .01$

these two functions can be attributed to those variables showing significant univariate comparisons. A third discriminant function composed of GAD specific activities resulted in an $F_{4,7} = 6.44$ ($p \leq .05$). The third discriminant function illustrates the increased power often gained using multivariate techniques since none of the univariate comparisons were significantly different between the two strains.

Comparison of RDH and RDL strains - enzyme total activities

Table 18 shows the means and standard deviations of the RDH and RDL strains with respect to VC, H, DC and SC II tissue weights and the corresponding total activities of the five enzymes. Body weight and the four brain section tissue weights were significantly higher in the RDLs than in the RDHs. The difference in SC II tissue weights (34 per cent) corresponded closely to the difference in body weights between the two strains (38 per cent).

Due to the large tissue weight differences between the RDHs and RDLs, all but two of the comparisons of enzyme total activities were in favor of the RDLs. ChE and AChE total activities did not differ significantly between the strains, however, even though the strains were selectively bred for cortical AChE specific activity. DOPAD, GAD and MAO activities were significantly higher in the RDLs than in the RDHs in all but three comparisons.

The discriminant function composed of body weight, DC and SC II tissue weights and the corresponding total activities of ChE, AChE, DOPAD and MAO resulted in an $F_{11,8} = 3.42$ ($p \leq .05$). When body weight and brain weights were partialled out, the $F_{8,18}$ dropped to 0.66 ($p > .05$), supporting the interpretation that the higher total activities in the RDLs were primarily dependent upon tissue weight.

Table 18

Means and Standard Deviations of RDH and RDL Strains with Respect to VC, H, DC and SC II Tissue Weights and Corresponding Enzyme Total Activities

Variable	Section	RDH		RDL		$F_{1,N-2}$
		$\bar{x}$	SD	$\bar{x}$	SD	
Brain Weights (mg) $n_i = 10$	VC	305	40.3	346	21.9	8.15**
	H	54	5.0	63	7.4	9.23***
	DC	375	23.5	426	11.6	39.49***
	SC II	571	58.6	645	25.5	13.30***
ChE Total Activity ($M \times 10^8$/min) $n_i = 10$	VC	1177	736	863	204	1.69
	H	305	71	365	64	4.00*
	DC	1113	116	1125	105	.05
	SC II	3183	253	3307	365	.77
AChE Total Activity ($M \times 10^8$/min) $n_i = 10$	VC ($\times 10^{-1}$)	2677	733	2925	465	.81
	H	5011	631	5635	734	4.18*
	DC ($\times 10^{-1}$)	2055	98	1986	152	1.46
	SC II ($\times 10^{-2}$)	1099	95	1117	101	.18
DOPAD Total Activity (μg/2 hr) $n_i = 10$	VC	86	29.4	126	36.6	7.22**
	H	48.0	10.6	59.4	6.7	8.21**
	RDC	57	16.1	75	18.1	5.02**
	SC II	536	69.0	612	55.9	7.27**
MAO Total Activity (per cent/hr) $n_i = 10$	VC	1755	304	1803	129	.22
	H	401	41	480	65	10.58***
	DC	1763	137	1825	441	.18
	SC II	3494	529	3903	274	4.73**
GAD Total Activity (μg/hr) $n_i = 6$	VC	219	41.5	249	28.6	2.08
	H	57.4	4.5	69.3	5.7	16.04***
	DC	375	18.0	442	31.7	20.40***
	SC II	640	25.7	751	30.1	47.47***

* $p \leq .10$
** $p \leq .05$
*** $p \leq .01$

Differences between strains in GAD total activities cannot be attributed entirely to differences in tissue weight. The discriminant function composed of body weight, tissue weights and the corresponding GAD total activities resulted in an $F_{5,6} = 44.40$ ($p \leq .01$). When tissue weights were partialled out, the $F_{2,9} = 4.83$ was still significant beyond the .05 level of confidence, due largely to the contribution of GAD total activity in the SC II section (c.f. Table 16).

Comparison of RDH and RDL strains - enzyme specific activities

Table 19 shows the means and standard deviations for the RDH and RDL strains with respect to specific activities of the five enzymes in the four brain sections. The RDHs had higher ChE (except in H) and AChE specific activities than the RDLs. This difference amounted to 12 and 9 per cent in the DC and SC II sections respectively for ChE, and 18 and 11 per cent in the same sections for AChE.

DOPAD and GAD specific activities were higher in all four brain sections in the RDLs than in the RDHs, but in only one comparison (GAD in H) did the difference reach an acceptable level of significance ($p \leq .05$).

The discriminant function composed of body weight and ChE, AChE, DOPAD, and MAO specific activities in the DC and SC II sections was not significant ($F_{1,18} = 2.48$; $p > .05$), in spite of the highly significant univariate weight differences. A second discriminant function was computed excluding DOPAD and MAO total activities. The $F_{5,14} = 6.22$ was significant beyond the .01 level of confidence, supporting the interpretation that the failure of the first discriminant to be significant was due to the large variance contributed by total DOPAD and MAO specific activities.

Table 19

Means and Standard Deviations of RDH and RDL Strains with Respect to Specific Activities of ChE, AChE, DOPAD, GAD and MAO in VC, H, DC and SC II Brain Sections

Enzyme	Section	RDH		RDL		$F_{1,N-2}$
		$\bar{x}$	SD	$\bar{x}$	SD	
ChE Specific	VC	2.77	0.46	2.48	0.30	2.74
Activity	H	5.65	0.79	5.83	0.48	.35
($Mx10^{10}$/min/mg)	DC	2.98	0.29	2.65	0.24	7.75**
n_i = 10	SC II	5.60	0.40	5.12	0.61	4.22*
AChE Specific	VC	87.6	13.9	83.8	10.9	.48
Activity	H	93.0	6.6	90.1	4.1	1.38
($Mx10^{10}$/min/mg)	DC	55.1	4.3	46.7	3.2	25.25***
n_i = 10	SC II	192.9	11.7	173.1	11.1	15.08***
DOPAD Specific	VC	279.1	68.7	360.8	92.7	.75
Activity,	H	899.4	153.3	947.9	115.6	.61
(μg/2 hr/g)	RDC	220.6	55.3	252.0	61.8	1.46
n_i = 10	SC II	939.1	61.9	950.0	72.5	.22
MAO Specific	VC	5.36	0.24	4.67	1.65	1.67
Activity	H	7.44	0.51	7.68	0.42	1.21
(per cent/hr/g)	DC	4.70	0.19	4.13	1.47	1.46
n_i = 10	SC II	6.09	0.27	6.05	0.30	.13
GAD Specific	VC	725	81.9	740	52.6	.15
Activity	H	1163	70.9	1317	65.4	15.44***
(μg/hr/g)	DC	987	54.1	1021	73.8	.82
n_i = 6	SC II	1109	70.7	1144	49.4	1.11

* $p \leq .10$
** $p \leq .05$
*** $p \leq .01$

The discriminant function composed of ChE, AChE, DOPAD and MAO specific activities in the VC and H sections was not significant ($F_{10,9} = 2.66$; $p > .05$). The discriminant function composed of GAD specific activities in the four brain sections also fell short of an acceptable level of significance ($F_{4,7} = 3.48$; $.05 \leq p \leq .10$).

Comparison of OMB and OMD strains - enzyme total activities

Table 20 shows the means and standard deviations for the OMB and OMD strains with respect to VC, H, DC and SC II tissue weights and the corresponding total activities of the five enzymes. As was pointed out in the Methods section (p.112), five OMB animals of the V line were paired with five OMD animals of the II line. The means and standard deviations in Table 20 were computed ignoring this fact. While the procedure was methodologically indefensible, a comparison of the mean differences between the OMB and OMD strains with and without the five sets of animals from different lines included failed to reveal any gross discrepancies. That is, the mean differences between the OMB and OMD strains were about the same in either case. Therefore, further statistics were computed ignoring this methodological error. Extreme caution must be exercised, however, in interpreting these differences. The OMBs had significantly heavier VC and SC II tissue weights than the OMDs. The same direction of differences observed for the DC and H sections, but the differences did not reach an acceptable level of significance ($p > .05$).

The means for the two strains were almost identical with respect to ChE total activity, but the OMBs were higher than the OMDs in mean total activities for the other four enzymes in all four sections. Differences in cortical (DC) enzyme total activities were not statistically significant except for GAD ($p \leq .01$).

Table 20

Means and Standard Deviations of OMB and OMD Strains with Respect to Body Weight, VC, H, DC and SC II Tissue Weights and Corresponding Enzyme Total Activities

Variable	Section	OMB		OMD		$F_{1,N-2}$
		$\bar{x}$	SD	$\bar{x}$	SD	
Brain Weights	VC	368	18.7	348	20.2	5.44**
(mg)	H	63	6.4	59	6.6	1.87
n_i = 10	DC	446	21.6	429	26.6	2.39
	SC II	696	26.5	658	44.1	5.47**
ChE Total	VC	1040	179	1076	401	.07
Activity	H	326	60	324	70	.02
($Mx10^8$/min)	DC	1203	107	1221	97	.14
n_i = 10	SC II	3544	308	3516	380	.04
AChE Total	VC($x10^{-1}$)	3328	422	3084	323	2.11
Activity	H	5563	636	5269	705	.97
($Mx10^8$/min)	DC($x10^{-1}$)	2444	168	2374	169	.85
n_i = 10	SC II ($x10^{-2}$)	1323	43	1222	130	5.38**
DOPAD Total	RDC	70	8.9	65	11.3	1.53
Activity	VC	112	20.5	92	12.4	6.97**
(μg/2 hr)	H	59.8	7.4	53.5	9.8	2.59
n_i = 10	SC II	678	46.8	568	66.4	18.22***
MAO Total	VC	1928	156	1782	59	7.60**
Activity	H	460	53	425	40	2.68
(per cent/hr)	DC	2050	109	1998	130	.94
n_i = 10	SC II	4302	221	4071	201	6.03**
GAD Total	VC	278	31.7	259	24.2	1.36
Activity	H	69.0	7.0	58.5	7.3	6.37**
(μg/hr)	DC	505	35.8	425	48.6	10.37***
n_i = 6	SC II	947	119.9	799	113.0	4.79*

* $p \leq .10$
** $p \leq .05$
*** $p \leq .01$

Differences between the OMBs and OMDs in subcortical (SC II) activities were significantly different for AChE, DOPAD and MAO ($p \leq .05$) and approached an acceptable level of significance for GAD ($F_{1,10} = 4.79$; $.05 \leq p \leq .10$).

The discriminant function composed of body weight, DC and SC II tissue weights and the corresponding total activities of ChE, AChE, DOPAD and GAD resulted in an $F_{11,8} = 5.59$ ($p \leq .025$). When body weight and tissue weights were partialled out, the $F_{8,11} = 4.55$ was still sginificant ($p \leq .05$), due largely to the contribution of DOPAD in the subcortex (pairwise $F_{1,16} = 14.60$; $p \leq .01$).

ChE, AChE, DOPAD and MAO taken together in the VC and H sections were not able to differentiate the two strains ($F_{10,9} = 1.80$; $p > .05$) even though the OMBs were significantly higher than the OMDs with respect to DOPAD and MAO total activities in the VC section when tested separately.

GAD total activity was higher in the OMBs than in the OMDs in all four brain sections. The discriminant function composed of body weight, DC and SC II tissue weights and the corresponding GAD total activities approached statistical significance ($F_{5,6} = 3.80$; $.05 \leq p \leq .10$), but the test of the discriminant was reduced to $F_{2,9} = 2.46$ ($p > .10$) when body weight and tissue weights were partialled out.

Comparison of OMB and OMD strains - enzyme specific activities

Table 21 shows the means and standard deviations of the OMB and OMD strains with respect to specific activities of the five enzymes in the four brain sections. None of the comparisons between the OMBs and OMDs with respect to ChE, AChE or MAO specific activities reached an acceptable level of significance ($p > .05$). The means for the OMDs were slightly higher in ChE specific activities than the OMBs, whereas

Table 21

Means and Standard Deviations of OMB and OMD Strains with Respect to Specific Activities of ChE, AChE, DOPAD, GAD and MAO in VC, H, DC and SC II Brain Section

Enzyme	Section	OMB		OMD		$F_{1,N-2}$
		$\bar{x}$	SD	$\bar{x}$	SD	
ChE Specific	VC	2.83	0.23	3.09	0.98	.67
Activity	H	5.23	0.63	5.30	0.71	.65
($Mx10^{10}$/min/mg)	DC	2.73	0.26	2.86	0.26	1.15
n_i = 10	SC II	5.02	0.62	5.35	0.30	2.27
AChE Specific	VC	90.2	11.2	89.0	13.9	.09
Activity	H	88.0	2.9	89.7	5.7	.79
($Mx10^{10}$/min/mg)	DC	55.0	2.9	55.4	2.73	.16
n_i = 10	SC II	190.4	6.5	185.9	17.0	.59
DOPAD Specific	VC	303.4	57.5	263.9	48.2	.95
Activity	H	964.8	132.3	905.1	117.1	1.15
(μg/2 hr/g)	RDC	227.3	40.1	216.1	38.7	.40
n_i = 10	SC II	981.9	81.2	864.5	116.5	16.86***
MAO Specific	VC	5.21	0.22	5.11	0.16	1.11
Activity	H	7.29	0.59	7.19	0.42	.15
(per cent/hr/g)	DC	4.59	0.10	4.57	0.16	.56
n_i = 10	SC II	6.21	0.14	6.29	0.27	.65
GAD Specific	VC	752	63.0	741	44.2	.11
Activity	H	1208	99.7	1120	53.8	3.48*
(μg/hr/g)	DC	1087	46.9	972	66.0	12.10***
n_i = 6	SC II	1311	110.8	1182	124.7	3.59*

* $p \leq .10$
** $p \leq .05$
*** $p \leq .01$

the means for AChE specific activities were almost identical for the two strains.

DOPAD specific activities were higher in all four brain sections in the OMBs than in the OMDs, but the difference was significant only in the subcortex ($F_{1,18} = 16.86$; $p \leq .01$). GAD specific activities were also higher in all four sections in the OMBs than in the OMDs. In the cortex (DC) the difference was significant ($F_{1,10} = 12.10$; $p \leq .01$), and in the subcortex (H and SC II) the differences approached acceptable levels of significance ($F_{1,10} = 3.48$; $.05 \leq p \leq .10$; $F_{1,10} = 3.59$; $.05 \leq p \leq .10$).

The discriminant function composed of GAD specific activities in the four brain sections approached an acceptable level of significance ($F_{4,7} = 3.04$; $.05 \leq p \leq .10$).

Comparison of high and low maze performance strains - enzyme total activities

The S_1, RDL and OMB strains have been shown to be superior to the S_3, RDH and OMD strains, respectively, with respect to maze performance (c.f. Chapter I). In the hope of finding morphological and/or biochemical factors common to the high and low performance strains the animals in this experiment were dichotomized on this dimension, combined and the two groups compared with respect to body weight, VC, H, DC and SC II brain tissue weights and the corresponding total and specific activities of ChE, AChE, DOPAD, GAD and MAO.

Table 22 shows the combined means and standard deviations of the High Performance strains (HP; animals from the S_1, RDL and OMB strains) and the Low Performance strains (LP; animals from the S_3, RDH and OMD strains) with respect to body weight, VC, H, DC and SC II brain tissue weights and the corresponding total activities of the five enzymes.

The HP animals had a mean body weight of 333 g, whereas the LP animals had a mean body weight of 281 g. The difference (18.5 per cent) was signi-

Table 22

Means and Standard Deviations of HP and LP Combined Strains with Respect to VC, H, DC and SC II Tissue Weights and Corresponding Total Activities of ChE, AChE, DOPAD, GAD and MAO

Variable	Section	HP		LP		$F_{1,N-2}$
		$\bar{x}$	SD	$\bar{x}$	SD	
Brain Weights	VC	345	30.4	331	36.9	2.54
(mg)	H	62	6.4	58	6.8	5.04**
n_i = 30	DC	418	32.4	409	33.3	1.18
	SC II	648	52.7	629	63.7	1.36
ChE Total	VC	938	196.8	1060	490.5	1.24
Activity	H	352	59.8	327	63.5	2.77
($Mx10^8$/min)	DC	1156	98.6	1176	105.3	.58
n_i = 30	SC II	3348	389.7	3351	329.2	.06
AChE Total	VC ($x10^{-1}$)	3342	702.7	3069	717.5	1.55
Activity	H	5604	638.6	5215	719.7	4.84**
($Mx10^8$/min)	DC ($x10^{-1}$)	2190	238.2	2203	190.4	.12
n_i = 30	SC II ($x10^{-2}$)	1206	125.9	1189	124.4	.29
DOPAD Total	VC	120	34.1	96	32.0	7.76***
Activity	H	58.0	6.2	53.1	10.0	5.18**
(μg/2hr)	RDC	72	12.6	64	15.9	3.83*
n_i = 30	SC II	630	63.8	580	80.5	7.25***
MAO Total	VC	1797	182.1	1759	200.3	.29
Activity	H	457	53.7	431	52.4	3.58*
(per cent/hr)	DC	1877	290.7	1800	168.1	.06
n_i = 30	SC II	3954	386.2	3815	434.4	1.73
GAD Total	VC	255	33.0	240	32.7	1.94
Activity	H	65.4	8.3	58.2	6.0	8.67***
(μg/hr)	DC	455	46.6	409	43.0	9.41***
n_i = 18	SC II	816	134.9	732	121.1	3.78*

* $p \leq .10$
** $p \leq .05$
*** $p \leq .01$

ficant beyond the .001 level of confidence ($F_{1,58}$ = 18.51). It should be pointed out that the RDHs lowered the mean of the LPs considerably and the OMBs raised the mean of the HPs considerably. The strains showing the most consistent behavioral differences, namely, the S_1 and S_3 strains, did not differ in body weight in this experiment.

The HPs also had slightly heavier brain tissue weights than the LPs, although not significantly so ($p \geq .05$). This finding is probably not in itself important in maze performance, since the S_3s show consistently heavier brain weights than the S_1s.

ChE, AChE and MAO total activities did not differentiate the HPs from the LPs except in the H section. The HPs had significantly higher AChE and MAO total activities than the LPs in the H section ($p \leq .05$), and the difference approached significance for ChE ($.05 \leq p \leq .10$).

The HPs had higher DOPAD and GAD total activities in all four brain sections than the LPs. The differences were significant for DOPAD total activities in the VC ($p \leq .01$), H ($p \leq .05$) and SC II ($p \leq .01$) sections. The differences for GAD total activity were significant in the H ($p \leq .01$) and DC ($p \leq .01$) sections and approached significance for the SC II section ($F_{1,34}$ = 3.78; $.05 \leq p \leq .10$).

The discriminant function composed of body weight, brain tissue weights and the corresponding total activities of ChE, AChE, DOPAD and MAO resulted in an $F_{21,38}$ = 1.93 ($p \leq .05$). When body weight and tissue weights were partialled out the F dropped below one and none of the individual comparisons was statistically significant.

The discriminant function composed of body weight, brain tissue weights and the corresponding GAD total activities resulted in an $F_{9,26}$ = 3.50 ($p \leq .01$). When body weight and brain tissue weights were partialled out of this discriminant, the $F_{4,30}$ = 11.59 was still highly significant ($p \leq .01$).

Comparison of HP and HL animals - enzyme specific activities

Table 23 shows the means and standard deviations of the HP and LP animals with respect to specific activities of the five enzymes in the four brain sections. ChE, AChE or MAO specific activities did not differentiate the HPs from the LPs in any of the four brain sections. DOPAD specific activities were higher in all four brain sections in the HP than in the LP animals, but the difference reached an acceptable level of significance only in the H section ($F_{1,58} = 5.97$; $p \leq .01$). GAD specific activities were also higher in the HP than in the LP animals in all four brain sections. The differences were statistically significant in the DC ($F_{1,34} = 11.15$; $p \leq .01$) and SC II ($F_{1,34} = 5.53$; $p \leq .05$) sections and approached an acceptable level of significance in the VC ($F_{1,34} = 3.08$; $.05 \leq p \leq .10$) and H ($F_{1,34} = 3.28$; $.05 \leq p \leq .10$) sections.

For easy reference the means with respect to DOPAD and GAD specific activities for the six strains that constituted the HP and LP combined groups are shown together in Table 24. Each strain categorized in the HP group was higher in DOPAD and GAD specific activities than the corresponding strain categorized in the LP group in all brain sections but H.

The mean concentration of serotonin in TB I of the HPs was 781 ng/g compared to 769 ng/g in the LPs. The $F_{1,58} = 4.70$ was statistically significant ($p \leq .05$), due largely to the difference between S_1s and S_3s.

Relative regional specific activities of ChE, AChE, DOPAD, GAD and MAO in rat brain

Table 25 shows the relative regional specific activities of ChE, AChE, DOPAD, GAD and MAO in the eight brain sections defined in the Methods section of this experiment. Each value is expressed as a percentage of the specific activity found in the RSB section and is based

Table 23

Means and Standard Deviations of HP and LP Combined Strains with Respect to Specific Activities of ChE, AChE, DOPAD, GAD and MAO in VC, H, DC, and SC II Brain Sections

Enzyme	Section	HP		LP		$F_{1,N-2}$
		$\bar{x}$	SD	$\bar{x}$	SD	
ChE Specific	VC	2.71	0.31	2.86	0.66	1.28
Activity	H	5.72	0.63	5.57	0.68	.75
($Mx10^{10}$/min/hr)	DC	2.78	0.26	2.89	0.26	2.67
n_i = 30	SC II	5.14	0.60	5.37	0.46	2.73
AChE Specific	VC	97.5	23.5	93.0	18.6	.70
Activity	H	90.8	4.7	90.2	5.9	.17
($Mx10^{10}$/min/hr)	DC	52.5	5.3	53.8	4.3	1.04
n_i = 30	SC II	186.3	13.5	189.6	12.7	.87
DOPAD Specific	VC	348.3	101.5	292.3	98.4	.81
Activity	H	951.7	111.2	923.6	125.9	5.97**
(μg/2 hr/g)	RDC	247.2	49.1	226.3	52.2	2.55
n_i = 30	SC II	975.4	69.6	923.6	93.2	.99
MAO Specific	VC	5.02	0.98	5.20	0.22	.17
Activity	H	7.39	0.48	7.44	0.46	.99
(per cent/hr/g)	DC	4.43	0.85	4.59	0.18	.00
n_i = 30	SC II	6.11	0.30	6.11	0.27	.58
GAD Specific	VC	763	65.5	720	78.2	3.08*
Activity	H	1182	155.7	1107	80.2	3.28*
(μg/hr/g)	DC	1062	58.9	986	75.4	11.15***
n_i = 18	SC II	1229	124.1	1133	122.5	5.53**

* $p \leq .10$
** $p \leq .05$
*** $p \leq .01$

Table 24

Means with Respect to DOPAD and GAD Specific Activities for the Six Strains Constituting the HP and LP Combined Strains

Enzyme	Section	HP			LP		
		S_1	RDL	OH	S_3	RDH	OL
DOPAD	RDC	262	252	227	242	221	216
	VC	381	361	303	334	279	264
	H	942	948	965	966	899	905
	SC II	994	950	982	967	939	864
GAD	VC	796	740	752	695	725	741
	H	1021	1317	1208	1038	1163	1120
	DC	1077	1021	1087	1000	987	972
	SC II	1233	1144	1311	1109	1109	1182

Table 25

Relative Regional Specific Activities* of ChE, AChE, DOPAD, GAD and MAO in Rat Brain

Section	Enzyme				
	ChE	AChE	DOPAD	MAO	GAD
V	62	21	**	72	60
S	61	25	**	70	67
RDC	60	25	25	74	79
VC	59	45	34	83	54
H	120	43	100	118	83
M+P	101	47	60	58	48
RSB	100	100	100	100	100
Ce	83	16	**	67	64

* Expressed as per cent specific activity found in RSB.
** Insufficient tissue and/or activity for reliable assay.

on the mean computed over all six strains of rats for each section and enzyme.

All five enzyme specific activities tended to be higher in subcortical regions than in cortical regions. AChE and DOPAD were the most unevenly distributed of the five enzymes in their relative regional specific activities. Both enzymes had about four-fold higher specific activities in the subcortex than in the cortex. Older cortical areas (VC) had higher AChE and DOPAD specific activities than neocortical areas (V, S, RDC). The activities of both enzymes were quite low in the cerebellum. The activity of DOPAD was too low to measure reliably in the V and S sections in this experiment. The distribution of AChE differed from DOPAD in brain stem structure. AChE had only 43 and 47 per cent as high specific activity in the hypothalamus (H) and brain stem (M+P), respectively, as in the RSB. DOPAD, on the other hand, had as high specific activity in the hypothalamus as in the RSB and 60 per cent as high specific activity in the brain stem as in the RSB.

ChE had a fairly even distribution in both the dorsal and ventral cortex. These sections had about 60 per cent as high specific activity found in the RSB. Unlike AChE and DOPAD, ChE had a relatively high specific activity in the cerebellum (83 per cent of the specific activity found in RSB). The highest ChE specific activity was found in the hypothalamus (120 per cent of the specific activity found in RSB), being quite different from AChE in this respect. Also, unlike AChE, ChE had a relatively high specific activity in the brain stem (101 per cent of the specific activity found in RSB).

MAO specific activity was lowest in the brain stem (58 per cent of the specific activity found in RSB) and highest in the hypothalamus (118 per cent of the specific activity found in RSB). Older cortical structures (VC) had slightly higher MAO specific activity than neocortical structures

(DC), whereas the cerebellum had slightly lower activity.

DISCUSSION

The results of this experiment and Experiments I and II can be summarized as follows:

(1) The S_1 strain had a higher concentration of brain serotonin and higher ChE, AChE, DOPAD and GAD specific activities throughout most of the brain than the S_3 strain. The results of the three experiments with respect to serotonin are shown in Table 26 for easy reference.

(2) The S_1 strain was lighter in brain tissue weights and had lower DOPAD and MAO total activities than the S_3 strain, but the reverse was true for GAD total activities throughout most of the brain.

(3) No differences were apparent between the S_1 and S_3 strains with respect to ChE and AChE total activities.

(4) The RDH strain was lighter in body weight and brain tissue weights, had lower DOPAD, GAD and MAO total activities and lower DOPAD and GAD specific activities than the RDL strain.

(5) The RDH strain had higher ChE and AChE specific activities than the RDL strain, but there were no significant differences between the strains with respect to ChE or AChE total activities, MAO specific activity or serotonin concentration.

(6) The OMB strain was heavier in body weight and brain tissue weights, and had higher ChE, AChE, DOPAD, MAO and GAD total activities than the OMD strain.

(7) The OMB strain had higher DOPAD and GAD specific activities than the OMB strain, but there were no significant differences between the two strains with respect to ChE, AChE or MAO specific activities or serotonin concentration.

(8) The high performance strains tended to have heavier body weights and brain weights, and higher DOPAD and GAD total and specific activities

Table 26

Comparison of Means between S_1 and S_3 Strains for Four Replications with Respect to Total Serotonin and Serotonin Concentration

			Strain					
			S_1		S_3		Per cent Difference	
Experiment	Age	Section	Total Serotonin (ng)	Serotonin Conc. (ng/g)	Total Serotonin (ng)	Serotonin Conc. (ng/g)	Total Serotonin	Serotonin Conc.
I	40	DC	123	329	123	306	0	8
		VC	88	321	90	310	2	4
		SC I	352	704	377	100	7	1
	110	TB I	820	594**	735	478	11	24
II	114	TB II	851	651**	789	551	8	18
III	88	TB I	1147	829**	1157	752	1	10

** $p \leq .02$

than the low performance strains.

(9) The specific activities of all five enzymes were higher in subcortical than cortical brain regions, but the enzymes differed considerably from each other in their respective patterns of distribution apart from this.

Table 27 shows these results in an ordinal fashion, together with results from several other experiments. The other experiments include comparisons of one or more of the pairs of strains with respect to maze performance, locomotor activity, seizure threshold, per cent brain protein, LDH specific activity and ACh concentration.

The purpose of the experiments reported in this chapter was to relate observed morphological and biochemical differences between strains to known behavioral differences or, more specifically, to known differences in maze performance. The six strains in this experiment were chosen because one strain in each pair was superior to the other in maze performance, but the differences in maze performance were not consistently related to differences in the ACh-AChE system. The results of this experiment showed that the choice of strains was adequate with respect to AChE specific activity - namely, that the S_1s had higher AChE specific activity than the S_3s, the RDHs had higher AChE specific activity than the RDLs and the OMBs did <u>not</u> have significantly higher AChE specific activity than the OMDs*. ACh concentration and maze performance were not measured in this experiment, so that it had to be assumed that the animals in this experiment were similar in these respects to animals of the same strains on whom these variables had been measured in earlier generations.

The only variables in Table 26 that are consistently related to maze performance between strains are DOPAD and GAD total and specific activities. Both are enzymes that catalyze the synthesis of possible neurohumors. DOPAD

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* This result must be accepted only with caution due to the inclusion of comparisons between OMBs from the V line with OMDs from the II line, as discussed earlier (p. 131).

Table 27

Ordinal Comparison of S_1-S_3, RDH-RDL and OMB-OMD Strains with Respect to a Number of Biochemical, Morphological and Behavioral Measures

Variable	Strain		
	S_1-S_3	RDH-RDL	OMB-OMD
Body Weight	?*	<	>
Brain Weight (TB I)	<	<	>
ACh Concentration[1] (TB I)	>	≃	≤
AChE Total Activity (TB I)	≃	≃	>
AChE Specific Activity (TB I)	>	>	≤
ChE Total Activity (TB I)	<	<	≃
ChE Specific Activity (TB I)	>	>	≃
DOPAD Total Activity (TB II)	<	<	>
DOPAD Specific Activity (TB II)	>	<	>
Serotonin Concentration (TB I)	>	≃	≃
MAO Total Activity (TB I)	<	<	>
MAO Specific Activity (TB I)	≃	≃	≃
GAD Total Activity (TB I)	≃	<	>
GAD Specific Activity (TB I)	>	<	>
LDH Specific Activity[2] (V,S,TB II)	≃		
Percent Protein[3]	≃		
Maze Performance[4,5]	>	<	>
Locomotor Activity	<		
Seizure Threshold[6]	<	<	

*Comparison uncertain due to conflicting results.

[1]Bennett *et al.* (1960).
[2]Bennett *et al.* (1958).
[3]Bennett *et al.* (1961).
[4]Rosenzweig *et al.* (1961).
[5]R. Olson, personal communication
[6]Woolley, Timiras, Rosenzweig, Krech and Bennett (1963).

decarboxylates 5-HTP to serotonin and DOPA to 3,4-dihydroxyphenylethylamine (which is further converted to noradrenaline), and GAD decarboxylates L-glutamic acid to GABA. This suggests a greater capacity of the HP animals to synthesize neurohumors which in turn may be related to better neural efficiency. Comparable information is not available for ChA so that the capacity to synthesize ACh in these six strains is not known directly.

The capacity to synthesize and metabolize a possible neurohumor is known only for the DOPAD-serotonin-MAO system in these six strains. If the ratio of DOPAD to MAO is taken as an index of the functional efficiency of the system, each of the high maze-performance strains has a higher ratio than the corresponding low maze-performance strains. Assuming this ratio to be the only factor regulating the levels of the neurohumor, then the strains with the higher ratio should have had higher serotonin concentrations. For the S_1 and S_3 strains this was the case. The S_1s had more serotonin per gram tissue and higher DOPAD specific activity than the S_3s but did not differ in MAO activity. A similar result was not evident for the other two pairs of strains. Serotonin concentration did not conform in these strains to the prediction made on the basis of the ratio of DOPAD to MAO activity.

The capacity to form and degrade serotonin may be more important in terms of functional significance than the actual gross amounts of serotonin present. Since serotonin exists in two forms, free and bound, and since the technique used to measure serotonin does not differentiate the two, it is not known whether the strains differed with respect to binding capacity. The extent to which the serotonin system was being activated just prior to sacrifice in the six strains was also unknown. If there were a differential release of serotonin just prior to sacrifice, the amount of serotonin measured after sacrifice would reflect the net amount of serotonin synthesized, released and destroyed.

The S_1 and S_3 strains are relatively less excitable than the RDH, RDL, OMB and OMD strains. If it is assumed that serotonin is involved in parasympathetic control, then serotonin measured in the S_1 and S_3 strains might reflect primarily the bound form, and would therefore correspond to the synthetic capacity of the system. The other four strains, being more excitable, might exhibit a lower ratio of bound to free serotonin and, consequently, would not reflect very closely the synthetic capacity of the system, i.e., the system would be in a greater state of flux in these four strains than in the S_1s and S_3s.

Information from the experiments reported in this chapter for the ACh and GABA systems involve only one enzyme in each case. Consequently, an analysis involving the ratio of synthetic capacity to metabolic capacity cannot be made for these systems. On the basis of available data some spectulations are, however, possible. AChE did not consistently differentiate the high maze-performance strains from the low maze-performance strains. If the assumption is maintained that maze performance is positively related to neural efficiency, and that neural efficiency is a function of the ratio of synthetic capacity to metabolic capacity, then it would be predicted that the ratio of ChA to AChE would be higher in the high maze-performance than in the low maze-performance strains. ACh is higher in the S_1 strain than in the S_3 strain, and, using the same argument as for serotonin, it can be assumed that this reflects the activity of ChA. The ratio of ChA to AChE would thus also be higher in the S_1 strains than in the S_3 strain. For the other four strains ACh may not reflect the activity of ChA, and, consequently, no prediction as to the ratio of ChA to AChE would be possible on the basis of ACh concentration alone. On the basis of maze performance, however, the prediction would be made that RDLs have as high or higher ChA activity than the RDHs and that the OMBs have higher ChA activity than the OMDs.

GAD total and specific activity was higher in the high maze-performance

strains than in the low maze-performance strains. Therefore, the prediction would be made that the activity of the enzyme responsible for the degradation of GABA (GABA-α-ketoglutarate transaminase) would be equal or lower in the high maze-performance strains as compared to the low maze-performance strains.

An important consideration in the search for relations between brain biochemistry and behavior is what constitutes a functionally more efficient system. The suggestion was just made that the ratio of synthesis to metabolism might be taken as an index of efficiency. Measurement of the relevant enzymes, however, are made with respect to a relatively large mass of neural tissue, whereas the nervous system is composed of discrete units, and, presumably, functions in terms of discrete units. The measurement of enzyme activities in these discrete units is prohibited except on a very limited sampling basis. In order to overcome the problem of sampling, enzyme activity has been integrated over thousands of such units with the hope that such an integration might reveal relations that would be lost in the exploration of discrete units (c.f. Rosenzweig et al., 1959).

Associated with the loss of information concerning enzymatic activity in discrete neural units, the measurement of enzyme activities or substrate levels in masses of tissue does not take into consideration what contributes to the weight of the tissue. It is not at all apparent whether the results should be expressed in terms of total activity or as activity per unit tissue (i.e., specific activity).

The approach taken by Krech, Rosenzweig and Bennett has been to present both total and specific activities. This approach was very useful in explaining the results of some otherwise puzzling results (c.f. Chapter I). A similar approach was taken here, and , in addition, the attempt was made to partial out the tissue weight parameter statistically.

The conclusions that can be drawn from the results of experiments reported in this chapter and presented at the beginning of the discussion depend on

which measures of enzyme activities are used. It is not possible, however, on the basis of presently available information, to decide which of the three approaches is most satisfactory, i.e., describes the functional activity of enzymes in brain.

Clearly, the conclusions to be drawn may be different in many cases depending on the measure selected. For example, the RDHs had higher AChE specific activity in all four brain sections than the RDLs, but the reverse was true for AChE total activity, except in the cortex. The RDHs and RDLs were selectively bred for differential cortical AChE specific activity with no control for tissue weight or AChE total activity. In fact, Roderick (1960) found the concomitant selection response of body weight and brain weight to be opposite that of AChE specific activity. The high AChE lines had lower body weights and brain weights than the low AChE lines as was true of the descendents of these strains used in this experiment. The response to selection for AChE specific activity could have occurred: (1) as a result of changes in AChE total activity, (2) as a result of changes in tissue weight with no change in AChE total activity or (3) as a result of a combination of both.

Until more is known about what constitutes functional activity of enzymes in neural tissue, a measure that takes into account the actual correlations of tissue weights and total enzyme activities may be useful. The statistical techniques used in this chapter are tentatively suggested for this purpose, i.e., enzyme total activity with tissue weight partialled out. The adjusted means for enzyme total activities with tissue weights partialled out may be called "independent total activity". Results of this experiment with respect to independent total activity may be summarized as follows:

(1) Independent ChE, AChE, DOPAD, GAD and MAO total activities tended to be higher in the S_1s than in the S_3s.

(2) Independent ChE, AChE, DOPAD and MAO total activities tended to be higher in the RDHs than in the RSLs, but the reverse was true for GAD.

(3) No clear differences were apparent between the OMBs and OMDs with respect to independent ChE, AChE or MAO total activities, but the OMBs had higher independent DOPAD and GAD total activities than the OMDs.

(4) The HP combined strains did not differ significantly from the LP combined strains with respect to independent ChE, AChE or MAO total activities, but the HP combined strains had higher independent DOPAD and GAD total activities than the LP combined strains.

These results suggest that the three pairs of strains differ from each other with respect to enzyme total activities that are independent (at least in a statistical sense) of tissue weight. If it is assumed that this measure represents functional enzyme activity, then it is tentatively concluded that maze performance is positively related to independent DOPAD and GAD total activities. In the next chapter, correlations between a number of behavioral measures, serotonin concentration and enzyme activities will be examined in animals within the S_1 and S_3 strains.

Chapter IV

Within-Strain Correlations Between Brain Morphology, Biochemistry and Behavior

Differences in brain serotonin concentration between the S_1 and S_3 strains were reported for three experiments in Chapter III. In addition, the brain enzymes ChE, AChE, DOPAD, MAO and GAD were measured in these and four other strains known to differ in maze performance. Only DOPAD and GAD consistently differentiated the high performance from the low performance strains. It was suggested that the ratio of the capacity to form and destroy a neurohumor be used as an index of the efficiency of the system. Using this index, the serotonin system was found to be positively related to maze performance between strains.

In this chapter experiments will be reported in which the attempt was made to find within-strain correlations between brain serotonin and a number of behavioral tests. In addition ChE and AChE activities were measured in samples from the sensory cortex and served as additional biochemical variables, together with the respective tissue weights and body weight. The behavioral variables included an open-field defecation and urination test of emotionality, three measures of exploratory and/or locomotor activity and a spatial discrimination-reversal test.

Results from several experiments suggested that within-strain correlations might be expected. Krech *et al.* (1962) found that the cortical to subcortical ratio of AChE specific activity was significantly correlated with mean errors per reversal problem in the Krech Hypothesis Apparatus within the S_1 strain of rats ($r = .81$; $p \leq .01$). These animals had been raised for 30 days from the time of weaning in an enriched environment.

Littermates of these animals raised under isolated conditions did not show significant correlations between brain biochemistry and behavior. The investigators attributed the lack of correlation among the previously isolated animals to the state of flux that the ACh - AChE system was undergoing during the training procedures.

Halevy & Stone (1962) reported that serotonin concentration in the posterior hypothalamus of Long-Evans rats was negatively correlated with responses in a non-discriminated avoidance learning task. In that experiment rats were required to press a lever to avoid being shocked.

In Chapter III (pp. 67-68) a negative correlation was reported between the mean ranks of six inbred strains of mice with respect to brain serotonin and rated emotionality. A study in progress in this laboratory investigating these variables in a four-way cross of mice has tended to confirm this inverse relation between brain serotonin and emotionality (Calhoun, Schresinger, & Pryor, unpublished observation).

The results reported in Chapter III also encouraged a search for within-strain correlations between behavior and chemical measures. While the substrate, serotonin, was not consistently related to maze performance, the ratio of DOPAD to MAO was. Furthermore, the concentration of serotonin reflected this DOPAD/MAO ratio in the S_1 and S_3 strains. Therefore, in these two strains serotonin levels in brain might be expected to be correlated with overt responses that are under the control, or partial control, of the serotonin system.

The evidence presented in Chapter II suggests that serotonin is involved in the regulation of behavior in which a large emotional component is present. For this reason some of the behavioral tests were chosen in such a way as to maximize emotional components. For example, the open-field defecation test might be expected to have a high loading

on an emotionality factor. Also, the discrimination-reversal problems were motivated by escape or avoidance of an electric shock, which presumeably involves emotional responses.

Others tests were chosen that could be considered relatively free of emotional involvement. These included locomotor activity in a revolving wheel and exploratory behavior in a standard colony cage. Also, the discrimination-reversal problems could be considered to have non-emotional components, but separation of emotional and non-emotional components in this apparatus might be difficult. Another test, exploratory activity in the Dashiell maze, probably also involves emotional as well as non-emotional components.

Although these tests by no means exhaust a rat's behavioral repertoire, they do sample a fairly large number of responses. It was hoped that these tests sampled enough resonses to maximize the chances of finding significant within-strain correlations with brain serotonin, if they do indeed exist. Tasks involving deprivation of any kind were avoided, since the effects of deprivation on brain serotonin are largely unknown.

A problem encountered in investigating correlations between behavioral and physiological variables is that the physiology may be altered in the process of measuring the behavior. In an attempt to avoid this problem, littermates were used. One littermate was tested behaviorally, and the other served as a control for possible effects of the testing procedures on brain serotonin. Both animals were then sacrificed, their brains analyzed for serotonin and these values correlated with the behavioral measures of the tested littermate.

In order for this procedure to be valid the assumption must be made that the correlation between littermates under naive conditions

is very high. Unfortunately, this assumption is untested for naive animals, and the results of the experiments in this chapter were ambiguous in this respect between tested and untested animals. Therefore, this approach was abandoned and only those correlations obtained from the same animals will be reported.

METHODS

Subjects

Subjects were run in two groups about two months apart. The first group consisted of ten littermate pairs of male S_1 rats. They were selected from ten sets of quadruplets at the time of weaning on the basis of matching body weights. The other pair from each litter was used in an experiment testing the effects of an enriched *versus* an impoverished environment on brain serotonin to be reported in Chapter V. The second group consisted of ten littermate pairs of male S_1 rats and thirteen littermate pairs of male S_3 rats.

Littermates were assigned to either a Social Testing (ST) group or a Social Control (SC) group. Assignment to groups was semi-random with the restriction that mean body weights be as nearly equal for the two groups as possible. Littermates were housed together, two per cage from weaning until sacrifice with food and water available *ad lib*. The light-dark cycle was controlled artificially with 12 hours light and 12 hours dark.

Testing Apparatus

Open-field

The open-field consisted of a 30" x 30" wooden enclosure supported on four legs. The walls were 3 1/2 inches high, and the device was covered by a 1/4 inch thick piece of clear plastic. The floor of the

enclosure was painted yellow and divided into 5 inch squares by thin black lines. Four 25 watt lights, diffused through a 15" x 15" x 1/4" frosted plastic cover and suspended 18 inches above the top of the apparatus illuminated the open-field.

Revolving wheels

Ten Wahmann-type LC-34 revolving wheels were used to measure locomotor activity. The wheels were 14 inches in diameter. Each revolution of the wheel operated an attached digital counter.

Dashiell checkerboard maze

This apparatus has been described by Dashiell (1925). The version used here consisted of a 36" x 36" x 4" wooden enclosure covered by a 1/4 inch thick piece of clear plastic. The floor of the enclosure was painted white and divided into 4 inch squares by thin black lines. Every other square was covered by a 4" x 4" x 4" wooden barrier painted a flat black. Four 25 watt lights, diffused through a 15" x 15" x 1/4" frosted plastic cover and suspended 18 inches above the top of the apparatus illuminated the maze.

Colony-cage activity

Colony-cage activity was sampled in an 8" x 13" x 8" animal cage similar in all respects to those in which the animals were normally housed. A metal tray covered with wood shavings served as a floor. The sides and top were constructed of 1/10 inch diameter bars spaced 2/5 inch apart.

A photocell was positioned so that the beam from a seven watt frosted white globe struck it 5/8 inch above the lip of the metal tray and bisected the long dimension of the cage. The photocell was protected from extraneous light by a wooden housing. Light from the

exciting source was directed onto the photocell by a one and one-half inch length of 3/4 inch tubing. Breaking the beam caused an electric digital counter to be advanced.

Ten of these units were constructed and connected to a bank of ten counters that was enclosed in a 3/4 inch thick plywood container. The units were placed on a metal rack in the center of a room illuminated by overhead fluorescent fixtures. The counters were located in the same room and were quite audible whenever any of the ten units were activated.

ATLAS

This apparatus is an automated adaptation of the Krech Hypothesis Apparatus. It consists of four compartments, two at each end of a rectangular enclosure joined by a central passageway that provides a choice point (see Figure 11). For a given trial an electrically operated door opens allowing the subject access to the passageway, while at the same time sealing off the other compartment at that end. At the other end the door goes to a center position allowing the animal access to either of the compartments at that end. The floor of the apparatus consists of bars that can be electrified by a silent scrambler. It is wired so that all but one compartment can be electrified on a given trial. A buzzer is located at either end and can act as a conditioned stimulus. The cues provided by the apparatus are spatial (i.e., right or left compartment correct) or visual (i.e., light or dark compartment correct) and these can be programmed independently by the operator. Photocells are located in positions to record the animal's initial choice and to terminate a trial after the animal enters the correct compartment, after which the door closes behind the animal. After completion of a

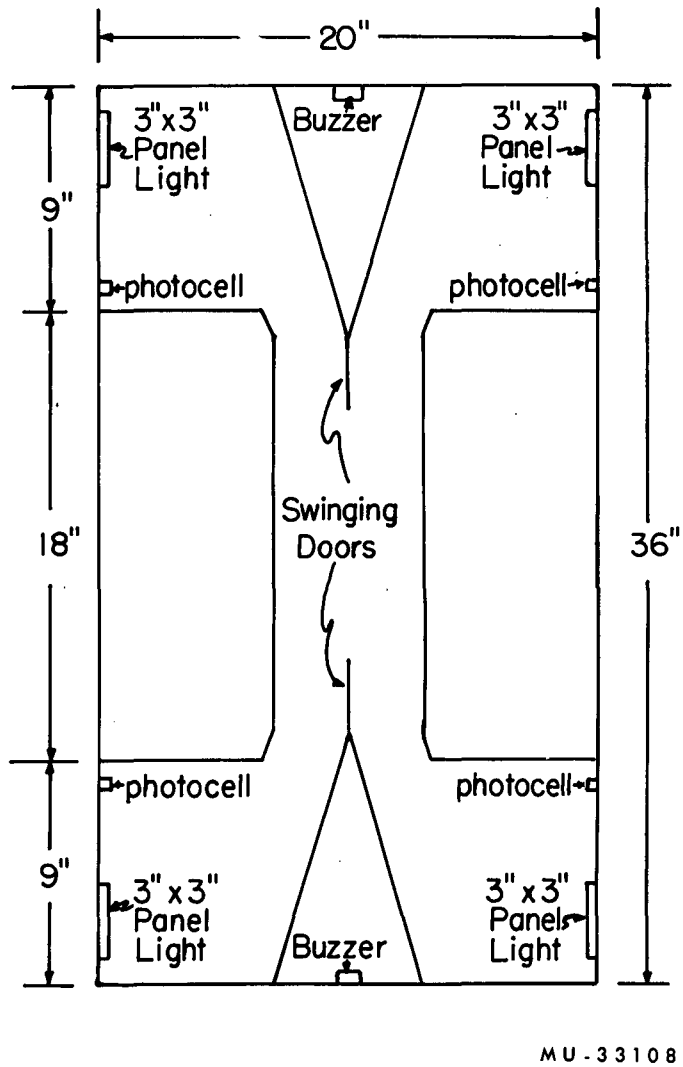

Fig. 11. Diagram of floor plan of ATLAS.

trial the opposite end becomes the choice point. Correct choices and cumulative latencies are recorded automatically on a control panel located in an adjoining room.

The parameters that can be programmed are: (1) the correct compartment, (2) whether the correct compartment is lighted or dark, (3) the time interval from the beginning of a trial to the onset of the conditioned stimulus (buzzer) and to the onset of the unconditioned stimulus (shock), (4) the intertrial interval and (5) the intensity of shock. Forty trials can be programmed in a single sequence.

Testing procedure

Behavioral testing began when the animals in the first group were approximately 95 days of age and when the animals in the second group were approximately 103 days of age. A slightly different testing schedule was used for each of the two groups. The reasons for the different schedules were: (1) the apparatuses for measuring colony-cage activity were not available at the time of testing the first group, and (2) the number of animals in the second group required that they be tested in subgroups due to the limited number of measuring devices. The testing schedules for the two groups will be described separately.

Testing schedule for animals in first group

Days 1 and 2:

A. At approximately 9:00 AM each animal was placed singly in an open-field. After five minutes the animal was removed, and the number of fecal boluses was counted and the presence or absence of urine noted. The apparatus was cleaned between each test with a wet sponge and dry paper towels.

B. At approximately 11:00 AM, each animal was placed in a revolving wheel. Assignment to the wheels was random. After two hours the animal was removed and returned to his home cage. The total number of revolutions made during this period was recorded.

C. Same as B beginning at approximately 8:00 PM during the dark period.

Day 3:

A. Beginning at approximately 9:00 AM each animal was placed singly in the Dashiell checkerboard maze. Activity was recorded by tracing the animal's movements in the maze on a scoring sheet that was a diagram of the floor plan of the apparatus. After five minutes the animal was removed and returned to his home cage. Lines crossed during each one-minute period and the sum over the five minute period were scored.

Days 4 and 5:

Same as Days 1 and 2.

Day 6:

Each animal was tested on a left correct spatial discrimination problem in the ATLAS. The animal was initially placed in a neutral compartment. After 30 seconds the first trial was begun. Time parameters were: (1) a 15 second intertrial interval, (2) five seconds at the beginning of the trial after the doors were opened and before the conditioned stimulus was turned on, and (3) another five second interval during which the conditioned stimulus was on before the unconditioned stimulus was initiated.

Trials were continued until a criterion of ten consecutive correct responses was reached irrespective of whether the animal avoided or escaped the shock. If this required more than the maximum of 40 trials that could be programmed at one time, the animal was left in the apparatus,

the program reset and the same sequence of 40 trials was begun again.

Correct responses, latencies on each trial, cumulative latencies and the number of avoidance responses were recorded. The lighted compartment coincided with the correct, left compartment on half of the trials. The order of appearance together of the correct and the lighted compartment was a semi-random sequence. The same sequence was used for all animals and for all problems.

Day 7:

Same as Day 6 but with right compartment correct.

Day 8:

Same as Day 6.

Day 9:

Same as Day 6 but with right compartment correct.

After the last day of testing the animals remained in their home cages until they were sacrificed three days later.

Testing schedule for animals in second group

Days 1 and 2:

A. Same as for first group. At approximately 9:00 AM each animal was placed singly into an open-field. After five minutes the animal was removed, and the number of fecal boluses was counted and the presence or absence of urine noted. The apparatus was cleaned between each test with a wet sponge and dry paper towels.

B. At approximately 11:00 AM about one-third of the animals (including S_1s and S_3s) were placed in revolving wheels. Assignment to the wheels was semi-random with the restriction that two animals from the same strain did not occupy adjacent wheels. Another one-third of the animals was placed in colony-cage activity devices located in

a separate room. Assignment to the cages was semi-random with the restriction that two animals from the same strain did not occupy adjacent cages.

A cumulative record of activity in the two types of apparatus was recorded every 15 minutes for one hour. After one hour all animals were removed from their respective activity devices. The group in the revolving wheels was placed in the colony-cages, and the group in the colony-cages was returned to their home cages. The remaining one-third of the animals was placed in the revolving wheels.

After one hour the same procedure was repeated so that all animals were measured for one hour each in the revolving wheels and the colony-cages. The order in which the animals were tested was counterbalanced over testing sessions.

C. Same as B beginning approximately 8:00 PM during the dark period.

Day 3:

Same as for the first group. Beginning at approximately 9:00 AM each animal was placed singly in the Dashiell checkerboard maze. Activity was recorded by tracing the animal's movements in the maze on a scoring sheet that was a diagram of the floor plan of the apparatus. After five minutes the animal was removed and returned to his home cage. Lines crossed during each one-minute period and the sum over the five minute period were scored.

Days 4 and 5:

Same as Days 1 and 2.

Day 6:

Same as for first group. Each animal was tested on a left-correct spatial discrimination problem in the ATLAS. Animals from the S_1 and S_3 strains were run alternately.

Day 7:

Same as Day 6 but with right compartment correct.

Day 8:

Same as Day 6.

Day 9:

Same as Day 6 but with right compartment correct.

After the last day of testing the animals remained in their home cages until they were sacrificed three days later.

Sacrifice and dissection of brain

All animals were assigned code numbers on the day of sacrifice so that the experimental group to which each animal belonged was not known to the persons performing the dissections and analyses. Littermates were sacrificed and analyzed in blocks, the order being randomly determined within blocks.

After sacrifice by decapitation the brain was exposed and a sample from each hemisphere was removed from the visual (V) and somesthetic (S) areas of the cortex. These samples were weighed to the nearest 0.1 mg, frozen on dry ice and stored at -22°C until they were assayed for ChE and AChE activities. The cerebellum was removed, weighed and used to carry tissue standards through the serotonin procedures. The rest of the brain including the remaining dorsal cortex (TB II) was weighed and extracted immediately for serotonin in the first group of animals. In the second group of animals the TB II sections from the SC animals were weighed, frozen on dry ice and stored between two blocks of dry ice until the next day when they were assayed for ChE, AChE, DOPAD, MAO and GAD - the results from these latter SC animals were presented in Experiment II, Chapter III, pp. 80-109.

Chemical analyses

Serotonin was extracted and analyzed using the procedures described in Chapter III.

ChE and AChE activities were determined by the photometric techniques described in Chapter III. Tissues were homogenized in 0.1 M sodium phosphate buffer, pH 8.0, to a concentration of 3 mg/ml. Assays were run in the same buffer using 9.0 mg of tissue for ChE and 1.8 mg of tissue for AChE determinations.

RESULTS

For convenience the results of this experiment will be divided into several sections. First, a comparison of littermates from the two groups of S_1s and the one group of S_3s will be made with respect to morphological and biochemical characteristics. These comparisons will be made in order to determine whether the testing procedures had any affect on these variables, and to examine the correlations between littermates with respect to the same variables. In addition, a comparison of the two groups of S_1s will be made which will show that they cannot be considered comparable on an absolute basis. Second, the groups will be compared with respect to the behavioral tests. This will include both a comparison of the two groups of S_1s, which will show that they also differed behaviorally as well as morphologically and biochemically, and a comparison of the two strains on these behavioral tests. Third, the intercorrelations between morphological and biochemical variables will be examined for the three groups, and this will be followed by the intercorrelations of the behavioral variables. Finally, the correlations of the morphological and biochemical variables with the behavioral variables will be presented to see how these two sets of variables might be related.

Comparison of littermates with respect to morphological and biochemical variables

Table 28 shows the means and standard deviations for littermates (ST and SC) of the first group of ten S_1 rats with respect to body weight, brain tissue weights, serotonin levels in the TB II section, and ChE and AChE activities in the V and S sections. Also, the percentage of pairs of littermates in which the ST value was greater than the SC values is indicated.

Most of the differences between means were in a direction that would be expected from the experiments of Krech *et al.* (c.f. Chapter I) investigating the effects of environmental complexity on brain morphology and biochemistry. The SC animals tended to be heavier but had lighter brains, less serotonin and lower ChE and AChE total activities, especially in the visual cortex, than their tested littermates. These differences were apparent in spite of the fact that the testing program lasted only nine days (c.f. Zolman, 1963).

Table 29 shows the means, standard deviations and the percentage of pairs of littermates in which the ST value was greater than the SC value for the second group of ten S_1s with respect to body weight, brain tissue weights, and ChE and AChE activities in the V and S sections. Serotonin was measured in only one of the littermates and has, therefore, been omitted from this table. In all comparisons except body weight and AChE specific activity in the V and S sections the tested animals had a higher mean value than their untested littermates. Only one of the comparisons was, however, statistically significant (ChE total activity in the V section; $p \leq .01$).

Both groups of S_1s showed similar patterns of differences between littermates. If the two groups were combined and the differences between littermates tested by means of the relatively unpowerful

Table 28

Means and Standard Deviations for SC and ST Littermates of First Group of S_1s with Respect to Body Weight, Brain Tissue Weights, Serotonin Levels, and ChE and AChE Activities in the V and S Sections

Variable	Section	Social Control $\bar{x}$	Social Control SD	Social Testing $\bar{x}$	Social Testing SD	Per Cent of Littermates in which ST > SC (n = 10)
Body Weight (g)		299	16.3	285	21.7	30
Brain Weights (mg)	V	66.6*	4.38	69.8	4.75	70
	S	57.1	2.48	56.6	2.96	50
	TB II	1258	43.5	1280	34.7	50
	Ce	232	12.5	231	7.4	40
Total Serotonin (ng)	TB II	881	67.3	903	50.0	70
Serotonin Concentration (ng/g)	TB II	708	59.3	706	38.7	40
ChE Total Activity ($M \times 10^8$/min)	V	204*	18.0	221	18.0	80
	S	179	14.5	183	15.1	50
AChE Total Activity ($M \times 10^8$/min)	V	3810	342	3928	366	60
	S	3590	245	3610	255	40
ChE Specific Activity ($M \times 10^{10}$/minmg)	V	3.07	0.18	3.16	0.17	60
	S	3.14	0.20	3.23	0.19	60
AChE Specific Activity ($M \times 10^{10}$/min/mg)	V	57.1	2.12	56.2	2.95	30
	S	62.9	3.91	63.8	3.60	60

* $p \leq .05$

Table 29

Means and Standard Deviations for SC and ST Littermates of Second Group of S_1s with Respect to Body Weight, Brain Tissue Weights, and ChE and AChE Activities in the V and S Sections

Variable	Section	Social Control $\bar{x}$	Social Control SD	Social Testing $\bar{x}$	Social Testing SD	Per Cent of Littermates in which ST > SC (n = 10)
Body Weight (g)		338	39.9	336	31.1	20
Brain Weights (mg)	V	73.5	4.81	76.7	4.48	70
	S	56.6	4.55	57.9	3.55	70
	TB II	1285	69.6	1308	77.7	80
	Ce	245	10.2	248	12.9	60
ChE Total Activity ($Mx10^8$/min)	V	241**	15.2	256	18.7	90
	S	195	18.1	202	18.7	50
AChE Total Activity ($Mx10^8$/min)	V	4522	312	4698	326	60
	S	4047	383	3962	292	50
ChE Specific Activity ($Mx10^{10}$/min/mg)	V	3.28	0.11	3.34	0.17	70
	S	3.45	0.25	3.49	0.22	40
AChE Specific Activity ($Mx10^{10}$/min/mg)	V	61.5	1.48	61.3	3.05	40
	S	71.6	4.85	68.5	3.43	20

* $p \leq .05$
** $p \leq .01$

sign-test (Siegel, 1956), then a number of the comparisons were statistically significant. Body weight was higher for the SC than for the ST animals ($p \leq .02$), whereas the reverse was true of V ($p \leq .02$), S ($p \leq .13$) and TB II ($p \leq .06$) tissue weights. Total activities of ChE ($p \leq .001$) and AChE ($p \leq .13$) were higher in the ST than in the SC animals in the V section. ChE specific activity in the V section was also higher in the tested animals than in the untested animals ($p \leq .06$), but AChE specific activity was lower ($p \leq .13$).

While the pattern of differences between littermates was very similar for the two groups of S_1s, it was nevertheless true that the two groups differed from each other on an absolute basis. The second group of S_1s was heavier in all respects than the first group of S_1s except in the S section. It will be pointed out later that the two groups also differed with respect to the behavior tests. The second group was eight days older than the first group throughout the experiment. Body weights of the S_1s in Group I eight days after the start of the experiment were very comparable to the body weights of the S_1s in Group II at the start of the experiment. This suggests that differences in age may have been responsible for the observed differences in morphology, biochemistry and behavior.

Table 30 shows the means, standard deviations and the percentage of pairs of littermates in which the ST value was greater than the SC value for the 13 S_3s with respect to body weight, brain tissue weights, and ChE and AChE activities in the V and S sections. Differences in brain tissue weights were in the direction expected from the results obtained for the S_1s. In the TB II section 85 percent of the ST animals had heavier tissue weights than their SC littermates ($p \leq .05$). None of the other differences in morphology or biochemistry was statistically significant.

Table 30

Means and Standard Deviations for SC and ST Littermates of the S_3 Strain with Respect to Body Weight, Brain Tissue Weights, and ChE and AChE activities in the V and S Sections

Variable	Section	Social Control $\bar{x}$	Social Control SD	Social Testing $\bar{x}$	Social Testing SD	Per cent of Littermates in which ST > SC (n = 13)
Body Weight (g)		294	33.4	298	30.3	46
Brain Weights (mg)	V	78.4	4.99	79.2	4.84	62
	S	58.1	4.61	59.4	3.30	62
	TB II	1397*	55.2	1435	67.0	85
	Ce	271	13.9	276	13.7	69
ChE Total Activity ($Mx10^8$/min)	V	246	23.1	243	17.7	38
	S	189	15.5	188	10.1	46
AChE Total Activity ($Mx10^8$/min)	V	4000	417	4184	397	46
	S	3604	301	3704	304	69
ChE Specific Activity ($Mx10^{10}$/min/mg)	V	3.14	0.16	3.06	0.13	46
	S	3.25	0.27	3.17	0.17	31
AChE Specific Activity ($Mx10^{10}$/min/mg)	V	52.3	3.80	52.9	4.91	46
	S	62.1	4.75	62.5	4.59	62

* $p \leq .05$
** $p \leq .01$

Differences between S_1s and S_3s with respect to morphology and brain biochemistry were reported and discussed in Experiment II, Chapter III for the SC animals. Similar differences between the strains were apparent for the ST animals - namely, the S_3s had heavier brain tissue weights but lower serotonin levels, and ChE and AChE total and specific activities than the S_1s.

Correlations between littermates with respect to morphological and biochemical variables.

Table 31 shows the correlations between SC and ST littermates for the two groups of S_1s and the one group of S_3s with respect to body weight, brain tissue weights and ChE and AChE activities in the V and S sections. Also included are the average correlations combined by using Fisher's z-transformation (c.f. Guilford, 1956) for the two groups of S_1s and the three groups disregarding strain.

The average correlation for the two groups of S_1s with respect to body weight was .56 ($p \leq .05$). For TB II and Ce tissue weights the average correlations were .65 and .76, respectively ($p \leq .01$). Somewhat lower correlations were found for weights of the V ($r = .41$) and S ($r = .19$) sections. Combining the three groups, disregarding strain, resulted in similar average correlations for body weight ($r = .62$), TB II weight ($r = .66$), Ce weight ($r = .71$), and V and S weights ($r = .37$ and $r = .17$, respectively).

For the two groups of S_1s the average correlations between littermates for ChE and AChE total activities in the V section were .65 ($p \leq .01$) and .45, respectively. The corresponding values in the S section were .25 and .11, neither of which was statistically significant. ChE specific activity was correlated .50 ($p \leq .05$) in the V section and .36 in the S section between littermates. AChE specific

Table 31

Correlations Between SC and ST Littermates for the Two Groups of S_1s and the one Group of S_3s

Variable	Section	First Group of S_1s	Second Group of S_1s	Group of S_3s	Average of S_1s	Average of S_1s and S_3s
Body Weight		.30	.74*	.72*	.56*	.62**
Brain Weights	V	.59	.20	.28	.41	.37
	S	.54	-.21	.11	.19	.17
	TB II	.29	.85**	.69*	.65**	.66**
	Ce	.73*	.78**	.60	.76**	.71**
Total Serotonin	TB II	.62	-	-	-	-
Serotonin Concentration	TB II	.58	-	-	-	-
ChE Total Activity	V	.51	.75*	.17	.65*	.51*
	S	.45	.03	-.43	.25	.02
AChE Total Activity	V	.73*	.05	.09	.45	.34
	S	.10	.12	.45	.11	.23
ChE Specific Activity	V	.51	.48	.25	.50*	.42*
	S	.55	.14	.51	.36	.41*
AChE Specific Activity	V	.69*	-.39	.26	.21	.23
	S	.41	-.01	.63*	.22	.38*

* $p \leq .05$
** $p \leq .01$

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activity was correlated .21 and .22 in the V and S sections, respectively, between littermates.

Combining the three groups, disregarding strains, resulted in correlations of .51 ($p \leq .01$) and .34 for ChE and AChE total activities, respectively, in the V section. Corresponding values for the S section were .02 and .23. For ChE specific activity in the V and S sections the correlations were .42 ($p \leq .05$) and .41 ($p \leq .05$), respectively, and .23 and .38 ($p \leq .05$) for AChE specific activity.

The interpretation of correlations between littermates in this experiment is somewhat ambiguous due to the different treatments administered to each littermate. If the observed correlations can be assumed to reflect the relations between naive littermates, then it must be concluded that the reliability of littermates on the variables measured in this experiment are only moderate to low. Body weight and brain tissue weights were more reliable in this respect than enzyme activities. If the experimental treatments attenuated the correlations between littermates then the observed correlations may represent a lower bound, but the upper bound is unknown.

Comparison of S_1 and S_3 strains with respect to behavioral tests

Comparisons of the two strains with respect to the behavioral tests will be made on two levels. First, the groups will be compared on total scores obtained on each behavioral test and tests of significance made. Second, with the aid of graphs, the two strains will be compared on separate components of the behavioral tests.

Table 32 shows the means and standard deviations for the two groups of S_1s and the one group of S_3s with respect to total scores

Table 32

Means and Standard Deviations for S_1 and S_3 Strains with Respect to a Number of Behavioral Tests

Variable	S_1 Strain				S_3 Strain	
	First Group (n = 10)		Second Group (n = 10)		(n = 13)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Open Field Test of Emotionality (Total Boluses + urinations)	8.0**#	5.8	15.0**##	6.9	7.2	8.1
Revolving Wheel Activity (Total Revolutions)	468	209	356**	249	1333	911
Colony-Cage Activity (Total Beam Interruptions)	-	-	382	94	397	111
Dashiell Activity (Total Lines Crossed)	155.5**	26.8	98.2**	60.2	159.5	34.6
ATLAS Total Errors to Criterion	87*	19.0	109	30.0	114	32.1
ATLAS Total Trials to Criterion	18	10.6	32	13.6	32	15.9
ATLAS Total Cumulative Latency to Criterion	2147**	925	3767	1740	3894	2009

* $p \leq .10$
** $p \leq .05$
*** $p \leq .01$

\# For comparison of two groups of S_1s
\#\# For comparison of S_1s and S_3s in second group

in the open-field test of emotionality, total revolutions over four days in the colony-cage activity devices, total line crosses during five minutes in the Dashiell maze and total scores over four spatial discrimination-reversal problems given in the ATLAS.

Comparing the two groups of S_1s, it is seen that the first group made lower scores in the open-field test ($p \leq .05$) and was, in general, superior in terms of the spatial reversal-discrimination problems to the second group. The first group made fewer errors ($p \leq .10$), took fewer trials and had shorter latencies ($p < .05$) to reach a criterion of ten out of ten correct responses in the ATLAS than the second group. In addition the first group was more active than the second group in the revolving wheels, although not significantly so, and in the Dashiell maze ($p \leq .05$). All tests of significance were made using the Mann-Whitney U-statistic (c.f. Siegel, 1956).

Comparing the two strains in the second group, the S_1s made significantly higher scores in the open-field test than the S_3s ($p \leq .05$). The S_1s were significantly less active in the revolving wheels ($p \leq .05$) and Dashiell maze ($p \leq .05$) than the S_3s, but there were no differences in colony-cage activity between the two strains. Also, there were no differences in total scores in the ATLAS between the two strains. It should be pointed out, however, that several S_3s failed to reach a criterion performance in a reasonable number of trials or time and training was discontinued for that problem, whereas all S_1s reached the preestablished criterion. Conversely, several S_3s performed much better than the average S_1 so that the means did not differ between strains. This large difference in variance displayed by the two strains in the ATLAS has been observed by others in this laboratory (H. Markowitz, personal communication).

Figure 12 shows the mean number of defecations made in the open-field during each five minute period on four days for the two groups of S_1s and the one group of S_3s. The first group of S_1s had a pattern similar to the S_3s which tended to decrease slightly as a function of successive exposures to the situation. The second group of S_1s (the appropriate reference group for strain comparisons) did not show any indication of adapting to the open-field. The behavior of the second group of S_1s was decidedly different in the open-field test from the first group of S_1s and the S_3s.

Figure 13 shows the median number of revolutions made during successive 15 minute periods by the second group of S_1s and the S_3s on the second day of testing. Both strains reached a very low level of activity by the end of one hour. The S_3s maintained a relatively high degree of activity for the first 45 minutes during the morning testing period, but in the evening activity was reduced to almost zero after only 15 minutes. The S_1s showed little difference between day and night activity and were essentially inactive after 15 minutes in either case.

Figure 14 shows the mean number of revolutions made by the two groups of S_1s and the one group of S_3s during each successive morning and evening test over four days. Although the first group of S_1s spent two hours in the wheels compared to the one hour spent by animals in the second group, they are shown on the same graph since, as was just pointed out, little, if any, activity took place after 45 minutes. The only difference between the two groups of S_1s to be seen from this graph, is the more pronounced day-night cycle in the first group. Both groups tended to maintain a fairly constant low number of revolutions over successive tests compared to the S_3s who had a very pronounced day-night cycle, with more revolutions occuring during the morning than in the evening. Further,

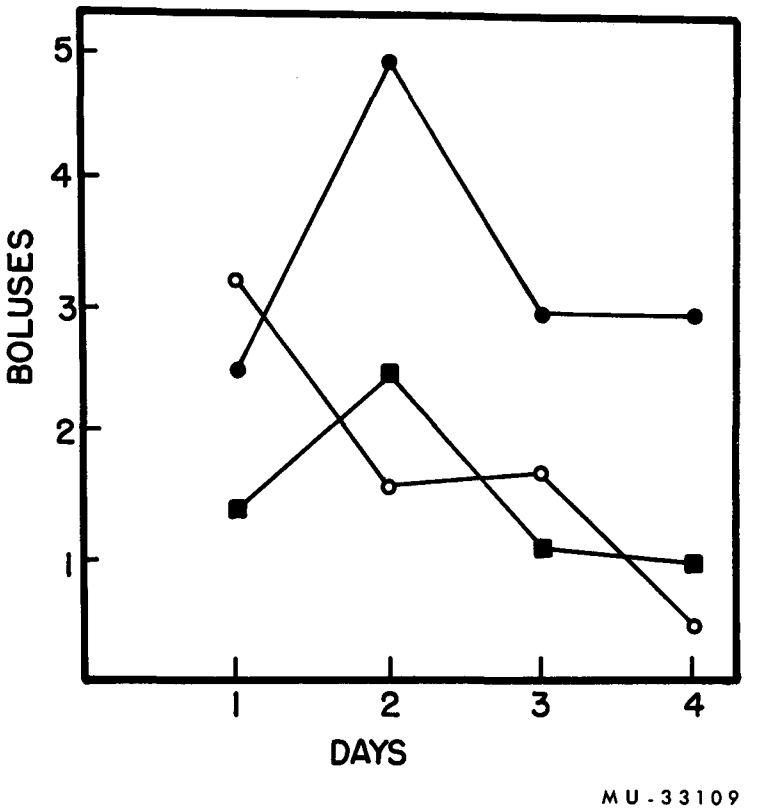

Fig. 12. Mean number fecal boluses in an open-field during a five minute period on each of four days. ● First group of S_1s; o Second group of S_1s; ■ S_3s.

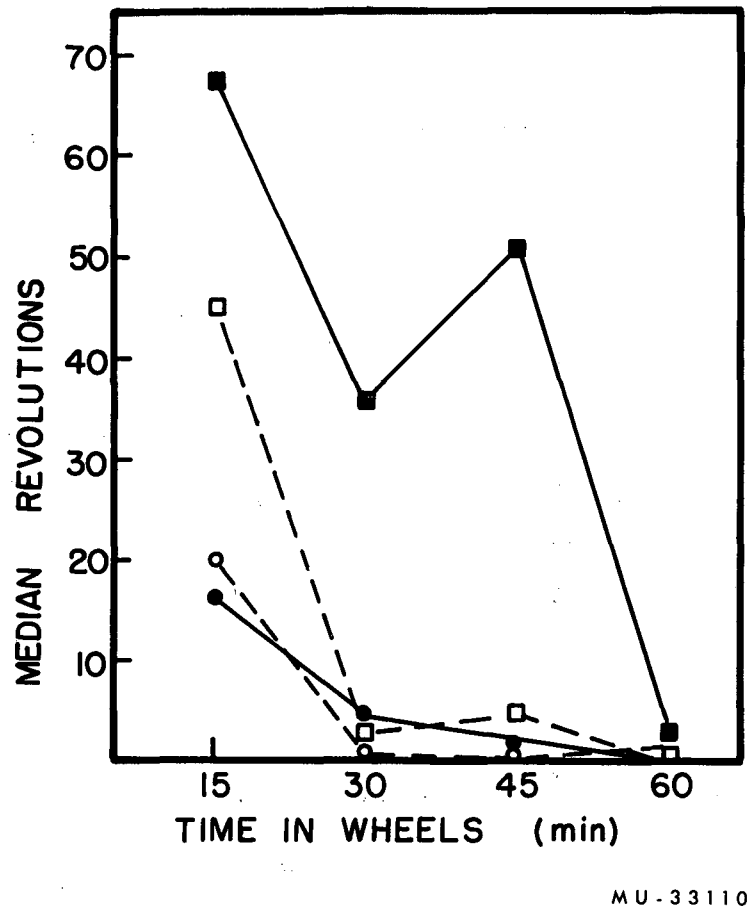

Fig. 13. Median number of revolutions made during successive 15 minute intervals on second day of testing.

●——● Second group of S_1s, morning test.

o---o Second group of S_1s, evening test.

■——■ S_3s, morning test.

□---□ S_3s, evening test.

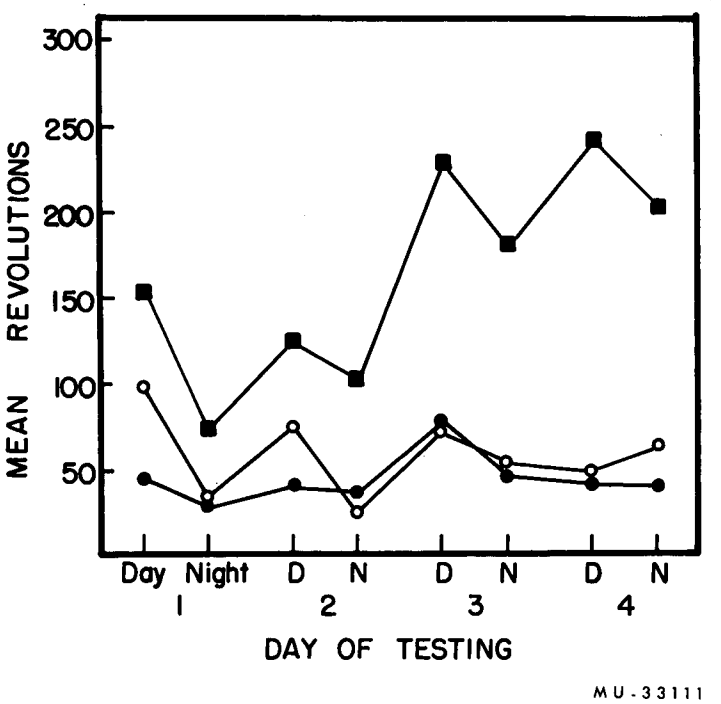

Fig. 14. Mean number of revolutions made during successive morning and evening tests, over four days.

- ● First group of S_1s.
- o Second group of S_1s.
- ■ S_3s.

in contrast to the S_1s, the S_3s showed a definite increase in mean revolutions over successive days.

The results of this experiment with respect to differences in revolving wheel activity between the S_1s and S_3s confirm and extend earlier results. In a series of three experiments involving a total of 21 S_1s and 22 S_3s, the S_3s consistently made more revolutions than the S_1s regardless of whether the animals were locked in the wheels or had free access to the wheels. In these experiments activity was measured over several days and the animals were exposed to the wheels continuously over each 24 hour period. The mean number of revolutions made per day over all four groups by the S_1s was 60 as compared to 427 made per day by the S_3s (Pryor, unpublished observation).

Figure 15 shows the median number of beam interruptions in the colony-cage activity devices over successive 15 minute periods on the second day of testing for the second group of S_1s and S_3s. Both strains showed more beam interruptions in the first 15 minutes than any other period. The S_1s maintained a low level of beam interruptions in successive 15 minute periods, whereas the S_3s reached an asymptote near zero after only 15 minutes. The S_3s made more beam interruptions than the S_1s in the first 15 minute period, and both strains had higher scores in the morning than in the evening.

Figure 16 shows the mean number of beam interruptions made by the two strains on successive one hour tests over four days. First, it is apparent that the two strains performed in quite similar fashions when one hour was taken as the time unit. Second, both strains showed a very marked day-night cycle, with more beam interruptions being made in the morning than in the evening. Third, both strains showed a linear decrease in beam interruptions over the four days. This was in striking contrast to their activity in the revolving wheels where the S_1s maintained a

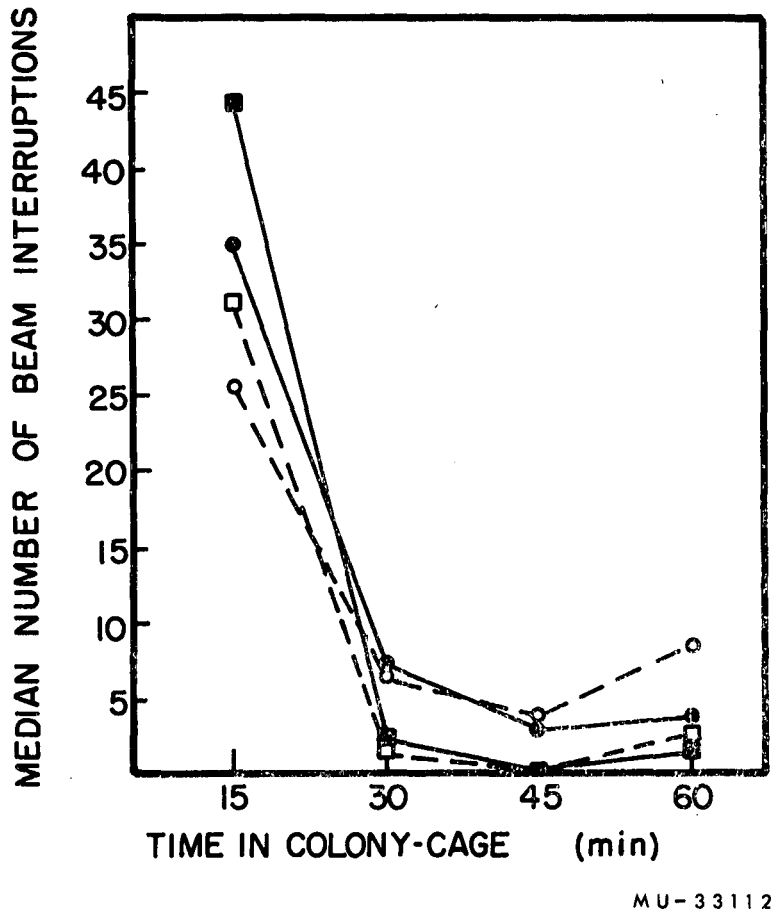

Fig. 15. Median number of beam interruptions made in colony-cage activity apparatus during successive 15 minute intervals on second day of testing.

●——● Second group of S_1s, morning test.

o---o Second group of S_1s, evening test.

■——■ S_3s, morning test.

□---□ S_3s, evening test.

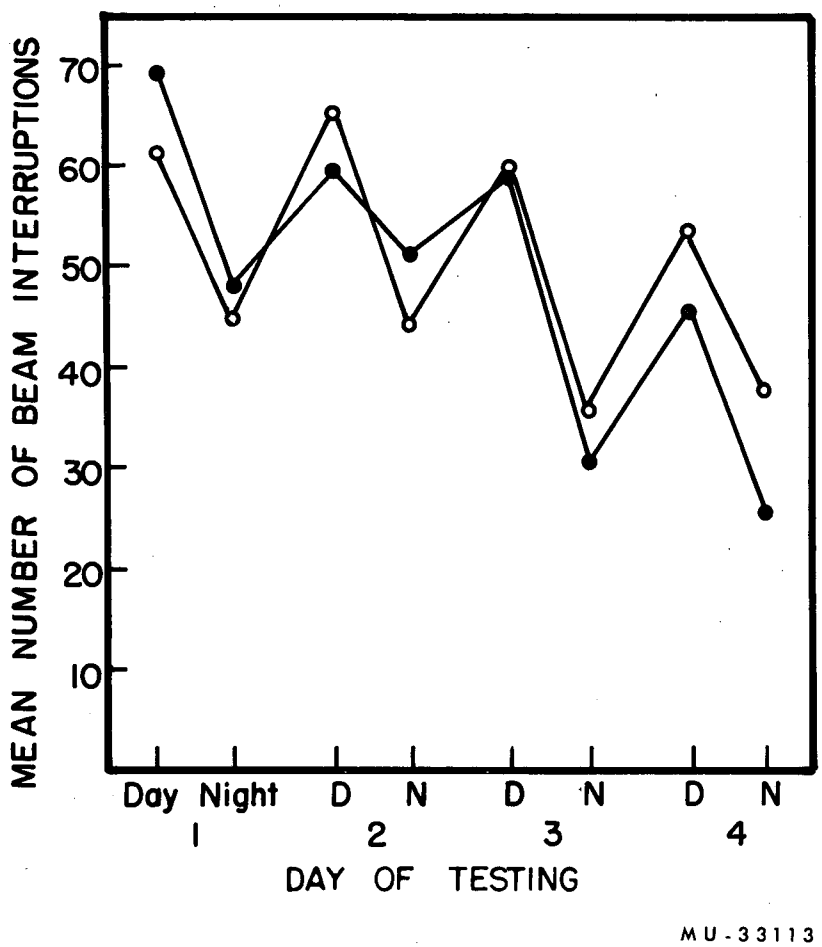

Fig. 16. Mean number of beam interruptions made during successive morning and evening tests over four days.

● Second group of S_1s.

o S_3s.

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constant low number of revolutions over days, and the S_3s increased the number of revolutions made on successive days in a linear fashion.

Figure 17 shows the mean number of lines crossed in successive one-minute intervals in the Dashiell maze by the two groups of S_1s and the one group of S_3s. While both groups of S_1s tended to increase their locomotion through the maze after the first minute, the S_3s first increased and then decreased over the last three minutes. The difference in activity between the second group of S_1s and the S_3s was apparent during each one-minute interval.

Figure 18 shows the performance of the two groups of S_1s and the one group of S_3s on successive spatial discrimination-reversal problems in the ATLAS using trials to reach a criterion of ten out of ten consecutive correct choices as a measure. Errors to criterion and cumulative latencies to criterion showed very similar patterns. The superior performance of the first group of S_1s compared to the second group of S_1s and the S_3s was apparent on all four problems. All groups found the first reversal most difficult and then improved on the two succeeding reversals. The S_1s were superior to the S_3s on the first two problems, but this superiority was reversed on the last two problems.

Intercorrelations of morphological and biochemical variables

Two sets of intercorrelations were obtained from each group of animals (i.e., two groups of ten pairs of S_1s and one group of thirteen pairs of S_3s). Since each group was small (10 or 13), the four sets of correlations from the S_1s were averaged using Fisher's z-transformation as were the two sets of correlations from the S_3s. The separate correlation matrices can be found in Appendices E_1 to E_3.

Table 33 shows the average intercorrelations for each of the two strains with respect to body weight, brain tissue weights, total serotonin in the TB II section, and ChE and AChE total activities in the V and S

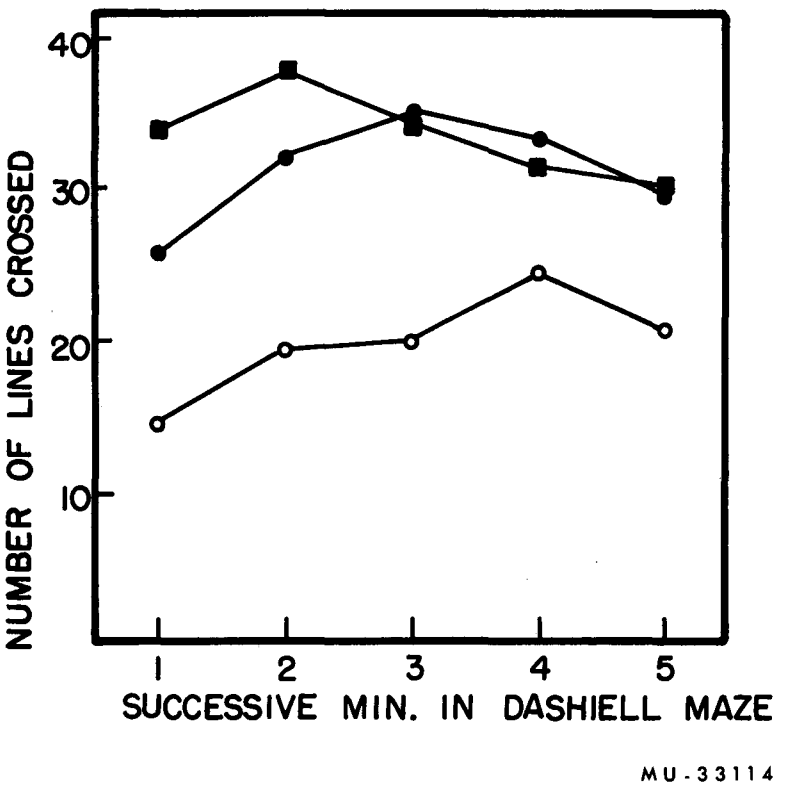

Fig. 17. Mean number of lines crossed during successive one minute intervals in the Dashiell maze.

- ● First group of S_1s.
- o Second group of S_1s.
- ■ S_3s.

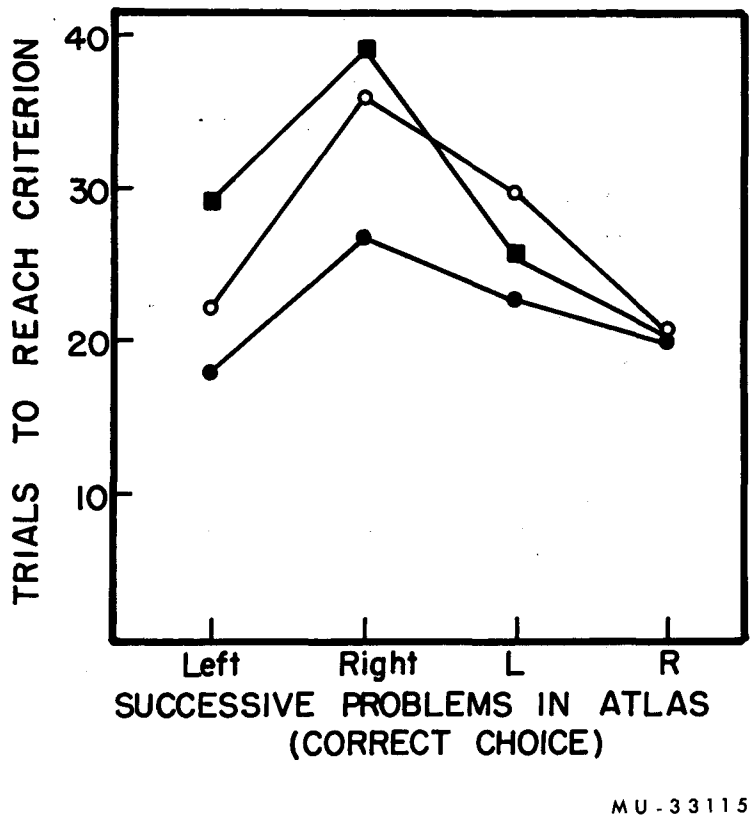

Fig. 18. Performance on successive spatial discrimination-reversal problems in the ATLAS.

- ● First group of S_1s.
- o Second group of S_1s.
- ■ S_3s.

Table 33

Average Intercorrelations of Biochemical and Morphological Variables for SC and ST Groups of S_1 and S_3 Strains

Variable	Strain	Body Weight	V Weight	S Weight	TB II Weight	Total Serotonin TB II	ChE Total Activity V	ChE Total Activity S	AChE Total Activity V
V Weight	S_1	.35*							
	S_3	.27							
S Weight	S_1	.13	.24						
	S_3	-.08	.34						
TB II Weight	S_1	.76**	.42**	.21					
	S_3	.69**	.32	.27					
Total Serotonin TB II	S_1	.14	.19	.03	.27				
	S_3	-.01	.32	-.04	.06				
ChE Total Activity V	S_1	.36*	.83**	.06	.40*	.28			
	S_3	.23	.85	.38	.11	-.31			
ChE Total Activity S	S_1	.08	.33*	.67**	.24	-.02	.21		
	S_3	.02	.31	.50*	.08	.34	.25		
AChE Total Activity V	S_1	.28	.88**	.08	.29	-.02	.66**	.14	
	S_3	.26	.58**	.02	-.04	-.50*	.40	-.19	
AChE Total Activity S	S_1	.08	.14	.63**	.12	-.40*	-.10	.37*	.18
	S_3	.48*	.22	.34	.56**	-.36	0.07	-.17	.60**

* $p \leq .05$
** $p \leq .01$

sections. The patterns are fairly similar for both strains. Body weight and TB II weight were correlated .76 for the S_1s and .69 for the S_3s ($p \leq .01$). The smaller brain tissue sections were only slightly correlated with body weight, or TB II weight, or among themselves.

The correlations between ChE total activity and tissue weights were .83 and .85 for the S_1s and S_3s, respectively, in the V section, and .67 and .50 for the S_1s and S_3s, respectively, in the S section. All four correlations were statistically significant at, or beyond, the .05 level of confidence. For AChE total activity the corresponding correlations were .88 and .58 for the S_1s and S_3s, respectively, in the V section, and .63 and .34 for the S_1s and S_3s, respectively, in the S section. All were significant (except for the S_3s in the S section) at, or beyond, the .05 level of confidence.

Low to moderate intercorrelations were found for ChE and AChE total activities in the V and S sections. ChE total activity in the V section correlated .66 and .40 for the S_1s and S_3s, respectively, with AChE total activity in the same section. For the S section these correlations were .37 and -.17 for the S_1s and S_3s respectively.

Only two significant correlations were found between total serotonin in the TB II section and the other variables. For the S_1s the correlation between total serotonin and AChE total activity in the S section was -.40 ($p \leq .05$). For the S_3s this correlation was -.36 which was not statistically significant. The correlations between AChE total activity in the V section were -.50 ($p \leq .05$) for the S_3s but only -.02 for the S_1s.

Intercorrelations of behavioral variables

One set of intercorrelations was obtained for each group of S_1s and for the one group of S_3s on the behavioral variables. Since the samples were small, the two correlation matrices obtained from the S_1s were

averaged using Fisher's z-transformation. This procedure may not be fully justified since the two groups differed in absolute values on some of the variables. It was felt, however, that combining the groups would be more desirable than presenting each separate correlation even though some of the correlations were quite different for the two groups. Conclusions based on correlations obtained from only one small group but not replicated in the other would be without value until they could be replicated again. Averaging the groups would partially avoid this problem since very divergent correlations (especially if the signs were different in the two groups) would be reduced whereas replicated correlations would be retained. The separate correlation matrices can be found in Appendix F.

Table 34 shows the average correlations obtained from the two groups of S_1s and the one set of correlations obtained from the S_3s with respect to the behavioral variables. The two measures taken in the open-field (defecations and urinations) were intercorrelated .52 ($p \leq .05$) for the S_1s and .78 ($p \leq .01$) for the S_3s. Revolving wheel activity in the morning was significantly correlated with the same activity measured in the evening. For the S_1s the correlation was .53 ($p \leq .05$), and for the S_3s the correlation was .65 ($p \leq .01$) All three measures of performance in the ATLAS were highly intercorrelated for both strains. Errors to criterion were correlated .93 ($p \leq .01$) and .92 ($p \leq .01$) with trials to criterion for the S_1s and S_3s, respectively. Errors to criterion and cumulative latencies to criterion were correlated .90 ($p \leq .01$) and .79 ($p \leq .01$) for the S_1s and S_3s respectively. Trials to criterion and cumulative latencies to criterion were correlated .85 ($p \leq .01$) and .84 ($p \leq .01$) for the S_1s and S_3s respectively.

Colony-cage activity measured during the morning was not significantly

related to the same activity measured during the evening for the S_1s ($r = .18$), but for the S_3s this correlation was .54 ($p \leq .05$). Colony-cage activity measured in the morning was not significantly correlated with revolving wheel activity measured in the morning or evening for either strain. There was, however, a moderate positive correlation between colony-cage activity measured in the morning and evening. For the S_1s the respective correlations were .44 and .48, and for the S_3s they were .58 ($p \leq .05$) and .27.

Dashiell activity was not significantly correlated with any of the other measures of activity or with the open-field test of emotionally for the S_1s. For the S_3s this measure was negatively correlated with defecations ($r = -.41$; $p \leq .10$) and positively correlated with morning ($r = .66$; $p \leq .01$) and evening ($r - .48$; $p \leq .05$) activity in the revolving wheels, and morning colony-cage activity ($r = .40$; $p \leq .10$).

No significant correlations were found between the open-field defecation test and the activity measures for the S_1s. For the S_3s a negative correlation was found between this test and both revolving wheel activity and colony-cage activity. The latter measures were positively intercorrelated for the S_3s.

Only one correlation out of the possible 42 between the three measures of performance in the ATLAS and the other seven behavioral measures was significant beyond the .05 level of confidence. This correlation can probably be discounted as being due to sampling error.

Correlations between morphological and biochemical variables and behavioral variables

For the purpose of obtaining correlations between these two sets of variables the following behavioral measures were combined. Morning activity in the revolving wheels was added to evening activity in the revolving wheels to give one measure, and the same was done for morning and evening

Table 34

Average Intercorrelations of Behavioral Variables for ST Groups of S_1 and S_3 Strains

Variable	Strain	Open-Field Defecations	Open-Field Urinations	Revolving Wheel Activity, Days	Revolving Wheel Activity, Nights	Colony-Cage Activity, Days	Colony-Cage Activity Nights	Dashiell Activity	ATLAS Total Errors to Criterion	ATLAS Total Trials to Criterion
Open-Field Urinations	S_1	.52*								
	S_3	.78**								
Revolving Wheel Activity, Days	S_1	-.33	-.14							
	S_3	-.43	-.48*							
Revolving Wheel Activity, Nights	S_1	.06	-.35	.53*						
	S_3	-.47*	-.63**	.65**						
Colony-Cage Activity, Days	S_1	.09	.45	-.26	-.42					
	S_3	-.21	-.28	.40	.02					
Colony-Cage Activity, Nights	S_1	.11	-.20	.44	.48	.18				
	S_3	-.47*	-.57*	.58*	.27	.54*				
Dashiell Activity	S_1	-.07	.03	-.06	-.04	-.32	-.25			
	S_3	-.41	-.12	.66**	.48*	.40	.10			
ATLAS Total Errors to Criterion	S_1	.22	.09	-.32	-.18	-.46*	.03	-.20		
	S_3	.01	-.44	.09	.25	.08	.24	-.30		
ATLAS Total Trials to Criterion	S_1	.07	-.12	-.21	-.06	-.36	.21	-.28	.93**	
	S_3	.06	-.38	.24	.17	.19	.28	-.24	.92**	
ATLAS Total Cumulative Latencies to Criterion	S_1	.20	.10	-.03	-.18	-.34	.18	-.23	.90**	.85**
	S_3	.04	-.21	-.03	-.15	.10	.31	-.44	.79**	.84**

* $p \leq .05$
** $p \leq .01$

colony-cage activity. Two sets of correlations were obtained from the S_1 strain, corresponding to the two groups of S_1 animals. The two correlation matrices were averaged using Fisher's z-transformation for the same reasons given in the previous section. The separate correlation matrices can be found in Appendix G.

Table 35 shows the correlations of the morphological and biochemical variables with the behavioral variables for the S_1 and S_3 strains. For the S_1s body weight was positively correlated with urinations and defecations in the open-field ($r = .52$; $p \leq .05$) and negatively correlated with revolving wheel activity ($r = -.44$; $p \leq .05$). A correlation of .42 (not significant) was found between body weight and colony-cage activity for the S_1s. Other correlations with body weight were low and not statistically significant. For the S_3s there were no significant correlations with body weight.

Brain weight of the V section was not significantly correlated with any of the behavioral tests for either strain with one exception. For the S_1s tissue weight of the V section was negatively correlated with revolving wheel activity ($r = -.41$; $p \leq .05$).

The S section presented a different picture than the V section. Correlations between tissue weight of the S section and the measures of emotionality or activity were not significant for either strain. For the S_1s, however, tissue weight of the S section was positively correlated with all three measures of performance in the ATLAS (a spatial discrimination-reversal test). For errors to criterion the average correlation was .72 ($p \leq .01$); for trials to criterion the average correlation was .79 ($p \leq .01$); and for cumulative latencies to criterion the average correlation was .64 ($p \leq .01$). Further, in the two separate groups of S_1s these corresponding correlations were high and statistically significant in all

Table 35

Average Correlations of Morphological and Biochemical Variables with Behavioral Variables for ST Groups of S_1 and S_3 Strains

Variable	Strain	Open-Field Defecations	Revolving Wheel Activity, Days + Nights	Colony-Cage Activity, Days + Nights	Dashiell Activity	ATLAS Total Errors to Criterion	ATLAS Total Trials to Criterion	ATLAS Total Cumulative Latencies to Criterion
Body Weight	S_1	.52*	-.44*	.42	-.05	.02	-.23	-.18
	S_3	-.06	.16	.21	-.12	.17	.19	.02
Brain V Weights	S_1	-.14	-.41*	-.47	-.05	.24	.10	.24
	S_3	-.14	.22	.37	.07	.34	.02	.31
S	S_1	-.04	-.24	-.18	-.31	.72**	.79**	.64**
	S_3	-.26	.08	.46	-.13	.20	-.01	.44
TB II	S_1	.50*	-.06	.38	-.20	.00	-.26	-.13
	S_3	-.16	.22	.31	-.38	.25	.29	.22
Total Serotonin	S_1	.05	.38	.34	-.42*	.23	.15	.17
	S_3	-.71**	.03	-.15	-.04	-.33	-.12	-.17
Serotonin Concentration	S_1	-.26	.47*	.10	-.33	.24	.33	.29
	S_3	-.64**	-.06	-.26	.03	-.38	-.19	-.21
ChE V Total Activity	S_1	-.02	-.26	-.46	-.28	-.01	-.13	-.10
	S_3	-.17	.28	.21	.12	.17	-.04	.16
S	S_1	-.14	-.19	-.15	-.53*	.62**	.58**	.62**
	S_3	-.30	-.36	.10	-.09	-.12	-.09	.31
AChE V Total Activity	S_1	.11	-.36	-.07	-.21	.07	-.12	.14
	S_3	.34	.07	.43	.14	.08	-.15	-.20
S	S_1	.24	-.24	.07	-.18	.53*	.54*	.41*
	S_3	.18	.23	.61*	-.01	.26	.09	.04
ChE V Specific Activity	S_1	-.18	-.02	-.10	-.40*	-.30	-.31	-.41
	S_3	-.09	.16	-.18	.12	-.22	-.09	-.19
S	S_1	-.16	-.05	-.05	-.46	.19	-.09	.31
	S_3	-.04	-.45	-.36	.06	-.34	-.09	-.16
AChE V Specific Activity	S_1	.03	-.09	.45	-.30	-.16	-.31	-.07
	S_3	.58*	-.61*	-.20	-.02	-.19	-.21	-.24

Table 35 (cont.)

S	S_1	.31	-.03	.32	.12	-.06	-.11	-.15
	S_3	.09	-.31	.04	-.50*	.56*	.70**	.62*

* $p \leq .05$
** $p \leq .01$

but one case, indicating the reproducibility of the correlations in spite of absolute differences between the two groups. For the S_3s none of these correlations with S tissue weight were statistically significant suggesting a strain difference in this respect.

TB II weight was significantly correlated with the open-field test of emotionality for the S_1s ($r = .50$; $p \leq .05$) but not for the S_3s ($r = -.16$). None of the other correlations with TB II tissue weight was statistically significant for either strain.

A high negative correlation was obtained between both total serotonin ($r = -.71$; $p \leq .01$) and serotonin concentration ($r = -.64$; $p \leq .01$) in TB II, and the open-field test of emotionality for the S_3 strain. For the S_1 strain the respective average correlations were low and not statistically significant. Total serotonin in TB II was negatively correlated with Dashiell activity ($r = -.42$; $p \leq .05$) for the S_1s, but no relation was observed for the S_3s. Other correlations with total serotonin in TB II were low and not statistically significant in either strain. Serotonin concentration in TB II was positively related to revolving wheel activity ($r = .47$; $p \leq .05$) for the S_1s but no relation was observed for the S_3s. Other correlations with serotonin concentration in TB II were low and not statistically significant in either strain.

No significant correlations were found between ChE total activity in the V section and any of the behavioral tests for either strain. ChE total activity in the S section was significantly positively correlated with performance in the ATLAS for the S_1s but not for the S_3s. The same was true for AChE total activity in the S section for the S_1s reflecting the high positive correlations between ChE total activity and tissue weight, and AChE total activity and tissue weight.

AChE total activity was also positively correlated with colony-

cage activity in the V ($r = .43$; $p \leq .05$) and S ($r = .61$; $p \leq .05$) sections for the S_3s.

ChE specific activity in the V section was negatively correlated with Dashiell activity ($r = -.40$; $p \leq .05$) and performance in the ATLAS for the S_1s. In the latter case only the correlation with cumulative latencies to criterion was statistically significant ($r = -.41$; $p \leq .05$). A similar pattern was found for AChE specific activity in the S section for the S_1s, but none of the correlations reached statistical significance.

For the S_3s ChE specific activities in the V and S sections were not significantly correlated with any of the behavioral tests. AChE specific activity in the V section was positively related to the open-field test of emotionality ($r = .58$; $p \leq .05$) and negatively related to revolving wheel activity ($r = -.61$; $p \leq .01$). AChE specific activity in the S section was negatively related to Dashiel activity ($r = -.50$; $p \leq .05$) and positively related to performance in the ATLAS (errors to criterion, $r = .56$; $p \leq .05$; trials to criterion, $r = .70$; $p \leq .01$: latencies to criterion, $r = .62$; $p \leq .05$).

DISCUSSION

The purpose of this experiment was to investigate relations between morphological and biochemical characteristics and behavioral responses within the S_1 and S_3 strains of rats. This was done with the intent of discovering physiological correlates of overt behavior. More specifically, it was hypothesized that the serotonin system was negatively related to behavioral responses in which a substantial emotional component was present.

The hypothesis of a negative relation between brain serotonin and emotionality was originally based on the assumption that emotionality is partly an overt manifestation of the reciprocal actions of the sympathetic

and parasympathetic divisions of the autonomic nervous system, and that serotonin is involved in the central excitatory control of the parasympathetic system. On this basis it should be possible to classify animals with respect to the relative dominance of the two components of the autonomic system. Animals in which the sympathetic system is dominant by virtue of genetic and/or environmental factors should display more (or more intense) emotional responses than animals in which the parasympathetic system was dominant. If individual differences in brain levels of serotonin are a reflection of individual differences in parasympathetic control, then in lieu of additional information concerning the sympathetic system it would be predicted that animals having a high level of brain serotonin would display fewer (or less intense) emotional responses than animals having a low level of brain serotonin.

The possible inconsistency in this argument is revealed when the commonly used open-field test is taken as an index of an animal's emotional reactivity. The emotional animal urinates and defecates more in an open-field than the non-emotional animal. Urination and defecation are, however, primarily under peripheral control of the parasympathetic system. This being the case, the emotional animal should urinate and defecate less than the non-emotional animal.

This contradiction requires a more careful analysis than was previously attempted. It may be that the equation of emotionality with the autonomic nervous system is inadequate. If this were the case, then it is still possible to hypothesize that serotonin is involved in the control of emotional behavior, but the more specific hypothesis that serotonin is involved in emotional behavior by way of the parasympathetic system might have to be abandoned.

A second interpretation that would retain the more specific hypothesis can be made by recourse to a more detailed, albeit ad hoc, analysis of

the events that occur when an animal makes an emotional response. The initial response made by an animal in an emotion-provoking situation is a massive sympathetic discharge, i.e., the "fight-or-flight" reaction. This results in pupil dilation, accelerated heart rate, inhibited peristalsis, etc. The mechanisms involved in homeostasis require that this response be attenuated or at least modified to eventually bring the system back to a neutral state. To do this, reflex feedback mechanisms cause reciprocal discharge of the parasympathetic system. Since the bladder and rectum have little, if any, sympathetic innervation, the result is elimination even though this is normally a non-sympathetic reaction. A weakness in this argument is the fact that parasympathetic discharge is usually specific. Whether this reflex mechanism can account for the observed responses of an animal in an emotional situation or not is an empirical question deserving further investigation.

The results of the open-field test of emotionality were encouraging, with respect to the hypothesis of an inverse relation between brain serotonin and emotionality. As predicted the S_3 strain showed a high, statistically significant, negative correlation between urination and defecations in the open-field and brain serotonin. In the two groups of S_1s the results were ambiguous. The first group of ten S_1s conformed to the hypothesis. Total brain serotonin and the open-field test were correlated -.38 (not significant). Serotonin concentration and the open-field test were correlated -.70 ($p \leq .05$). The second group of ten S_1s, however, did not replicate these findings, and in fact, the corresponding correlations were positive (.47 and .31, respectively), although not significantly so. The average correlations for the two groups of S_1s were consequently low (.05 and -.26, respectively) and not statistically significant.

It is possible that the observed correlations between brain serotonin and emotionality were linked through respective correlations with other variables. For example, in the first group of S_1s there was also a high positive correlation between body weight and the open-field test ($r = .78$). This was not replicated in the second group of S_1s ($r = .10$) or in the S_3s ($r = -.06$). Furthermore, brain serotonin was moderately correlated with body weight in the second group of S_1s only. Therefore, it is not possible to conclude that body weight *per se* was responsible for the observed correlations. The same argument can be made for tissue weight of TB II which was highly correlated with body weight.

A somewhat puzzling set of correlations was found between brain serotonin and locomotor and exploratory activity. First, there was a positive relation between brain serotonin and revolving wheel activity for both groups of S_1s. Second, there was a negative relation between brain serotonin and Dashiell activity for both groups of S_1s. Third, there was no relation between revolving wheel activity and Dashiell activity for the S_3s, but the two measures were themselves highly correlated in this strain.

Any simple interpretation of this set of correlations is prohibited and, perhaps, should be postponed until they have been more thoroughly verified. The following suggestions may, however, have some heuristic value in this respect. Activity, as measured in these various devices, is probably the net result of a number of factors. Some of these factors may be labeled "locomotor activity," "exploratory activity," "curiosity," "emotionality," etc. There is no reason to assume that the factors are all orthogonal to each other, and, in fact, they may be related to a large extent in any given situation. Presumably, all of the factors are under some degree of genetic control via biochemical and neurological mechanisms as well as environmental control acting through the central

nervous system. If this is true, then individual differences in any or all of these factors should be correlated with individual differences in the underlying biochemical or neurophysiological mechanisms.

The argument was made that the serotonin system is involved in the control of emotional behavior. It is also possible, of course, that this system is involved in the control of other kinds of behavior as well, but this possibility will be discounted for the moment. If it is assumed that the most dominant factor determining activity in the revolving wheels and Dashiell maze is a "locomotor activity" factor (i.e., a factor expressed as movement through space or exercise of the appropriate sets of muscles for such movement), then the S_3 strain can be assumed to be higher on this factor than the S_1 strain. Because of this, the S_3s are more active in both apparatuses than the S_1s. Furthermore, since this factor is dominant in the S_3s, there is little room for other factors to exhibit any control over activity in this strain. Therefore, for the S_3s activity in the revolving wheels and activity in the Dashiell maze are correlated since they are expressions of the same factor, and neither is correlated with brain serotonin since other biochemical systems underly this activity factor. It is interesting to note in this respect that for the S_3s AChE specific activities in the V and S sections were negatively correlated with both revolving wheel and Dashiell activity. For the S_1s the "locomotor activity" factor can be assumed to be low and, therefore, other factors may have more influence over the activity exhibited by this strain. It is still not clear, however, why serotonin should be positively related to one measure of activity and negatively related to another in the S_1s if serotonin is related to emotionality. Several speculations are possible. (1) The observed correlations may be the result of sampling error. This is possible, especially in view of the large number of variables measured.

But, the correlations were replicated in two independent groups of animals thus weakening this interpretation. (2) Serotonin may also be involved in the control of non-emotional behavior, such as "curiosity" or "exploratory behavior." This would mean that two different factors were operative for the S_1s in the two activity devices, and that both were correlated with brain serotonin but in opposite directions. (3) The "emotionality" factor may interact differently with the other factors determining activity, depending upon the specific situation.

Brain serotonin was not significantly related to any of the measures of performance in the ATLAS within either strain. Brain tissue weight of the somesthetic cortex was, however, highly correlated with performance in the ATLAS in both replications within the S_1 strain. This finding is potentially of considerable importance in that discrimination problems in the ATLAS were spatial. It has been assumed that the visual cortex mediates to a large extent problems that are visual in content, and that the somesthetic cortex has this role in spatial learning. Krechevsky (1935) showed that removal of the visual cortex in rats caused a shift to spatial hypotheses in the Krech Hypothesis Apparatus, and that destruction of the somesthetic cortex caused a shift to visual hypotheses. The apparatus used here is an adaptation of the Krech Hypothesis Apparatus, and it is very tempting to veiw the results of the present experiments in terms of the results obtained by Krechevsky. Further, Krech et al. (1963) have shown that enucleation of rats at weaning results in an increase in the weight of the somesthetic cortex, suggesting some sort of compensatory mechanism that operates when visual cues have been surgically removed.

The correlations between S tissue weight and performance in the ATLAS were positive. In other words, animals with heavier somesthetic

cortices made more errors and took more trials to learn the correct response than animals with lighter somesthetic cortices. The heavier S section was also associated with higher ChE and AChE total activities.

Two interpretations of these results are possible. First, tissue weight and the associated enzyme activities could be considered causal in producing poorer performance in the ATLAS. This interpretation is not intuitively appealing, although there is no a priori reason for supposing this not to be the case. Krech et al. (1962) have shown that EC animals have heavier cortices and are superior learners in visual discrimination-reversal problems in the Krech Hypothesis Apparatus than IC animals. Preliminary results indicate, however, that EC animals do poorer on visual problems in the ATLAS than IC animals (Rosenzweig, personal communication). Also, S_3s are superior to S_1s on visual problems in the ATLAS (Markowitz, personal communication). It appears that the ATLAS gives diametrically opposed results to those obtained in the Krech Hypothesis Apparatus. Differences in apparatus and/or motivation (hunger versus escape or avoidance of shock) may interact in some way with the effects of environmental stimulation and genetic factors to produce these rather puzzling results. In any case, this interpretation cannot be discounted without further experimentation.

Second, the direction of causality might be reversed. Performance in the ATLAS might differentially affect tissue weight and enzyme activities in the same way that ECT affects brain morphology and biochemistry. It is a fact that animals are differentially stimulated in the ATLAS depending upon how long it takes them to solve the problem. And, the stimulation is of a rather severe sort, i.e., shock to the feet. This would account for the positive correlations observed. In other words, animals that take longer to solve a problem are stimulated more than animals who solve the problem right away. Further, it is just that

area of the cortex (S) that receives afferent projections from the receptors being stimulated by shock to the feet that is affected.

Chapter V

Effects of an Enriched Versus an Impoverished Environment on Brain Serotonin

In this chapter results of experiments testing the effects of an enriched versus an impoverished environment on brain serotonin in S_1 and S_3 rats will be reported. Much of the work done by Krech, Rosenzweig and Bennett over the past six years has been directed toward this end with respect to the ACh-AChE system. In Chapter I their experimental findings and theoretical notions were discussed. For convenience their assumptions and findings will be briefly reviewed.

They assumed that: (1) the amount and patterns of neural activity maintained by an animal were partly a function of the amount of environmental stimulation the animal received, and that animals housed communally with opportunities for exploration and training in mazes (ECT condition) would receive more environmental stimulation than animals housed singly in small cages with little opportunities for visual, auditory or tactual experience (IC condition); (2) the amount of ACh synthesized and released in brain was partly a function of the amount and patterns of chronic neural activity; (3) the amount of AChE produced was partly a function of the amount of ACh released, i.e., increasing levels of ACh induces greater synthesis of AChE; and (4) increases in environmental stimulation and, hence, neural activity associated with the ECT conditions would result in more or better neural connections (in some sense) and, therefore, ECT animals should benefit from the experience in terms of having a greater capacity to learn or adapt to new situations than IC animals.

The results of experiments testing these assumptions indicated

that: (1) ECT animals had heavier cortical weights and higher cortical and subcortical AChE total activities than IC littermates; (2) the difference in cortical weight was greater than the difference in AChE total activity, so that specific activity of AChE in the cortex was lower in ECT than IC animals, but since there was little or no change in subcortical brain weight, AChE specific activity was higher in this section for ECT than IC animals; (3) the changes in brain tissue weight and enzyme activity associated with the ECT-IC conditions were not a function of (a) differential handling, (b) differential locomotor activity, (c) changes in more general metabolic enzymes or percent protein, or (d) changes in the non-specific cholinergic enzyme, ChE; (4) there was no period beyond weaning when these morphological and biochemical changes could not be induced by differential environmental exposure; (5) visual experience, per se, was not necessary to produce the observed changes resulting from the ECT and IC conditions, but visual experience did add to the other forms of experience to produce greater changes; and (6) the EC condition did produce animals who were superior to animals in the IC condition with respect to performance on a series of visual discrimination-reversal problems.

One of the questions that might be asked with reference to the results just summarized is whether non-cholinergic neurohumors are modified by differential environmental stimulation in the same way as the ACh-AChE system. It was the purpose of the experiments to be reported in this chapter to partially answer this question with respect to the proposed neurohumor serotonin.

In considering the results of the ECT-IC experiments Krech, Rosenzweig and Bennett have maintained that environmental stimulation results in an "intellectually" superior animal, and that the changes

observed in brain tissue weight and AChE activity are the underlying substrates of this superiority. In other words they have assumed, at least implicitly, that an ainmal's performance in a maze is predominantly a function of a factor psychologists have called intelligence, and that this factor is at least partly dependent upon the participation of the ACh-AChE system and its role in neural transmission. They have also assumed that this intellectual factor is under genetic and environmental control via cholinergic mechanisms.

It has become increasingly more evident in human psychometrics that intelligence is not unitary, but, rather, a composite of a number of factors, some of which are interrelated and others of which are apparently mutually orthogonal. This is probably also true of an animal's performance in so-called learning tasks. It is clear, for example, that an animal's performance in a maze or discrimination apparatus is partly a function of motivation, emotionality, perception, etc. And, as was pointed out in Chapter III, any of these factors might determine an animal's final score. Presumably, all of these factors interact with each other to result in the formation of 'memory traces', and it is the ease with which the 'memory traces' are formed that is the basis for any purely intellectual factor. It is certainly possible that the ease with which 'memory traces' are formed is related to the ACh-AChE system, and to this extent a relation between the ACh-AChE system and performance or vice versa may be expected. But, it is also possible (and therefore must be carefully considered) that other factors and their physiological substrates are responsible for observed differences in performance. This being the case, changes in brain morphology and biochemistry as a function of environmental stimulation may be the result of other, non-intellectual, factors.

It was with the above argument in mind that the experiments to be reported here were designed. The proposal that the serotonin system in brain participates in the control of emotional behavior has been

maintained throughout this dissertation. One of the differences casually observed between ECT and IC animals has been the relative ease with which they could be handled. The ECT animals are very tame, having been handled daily over many days, whereas the IC animals tend to be more jumpy. These observations suggest that the two groups differ in terms of an emotionality factor. This possible behavioral difference betweem ECT and IC animals has not been systematically tested.

If such differences in emotionality are present between ECT and IC animals and if brain serotonin levels are related to emotionality, then differences in brain serotonin should be observed between ECT and IC animals. Further, it would be predicted that ECT animals would have higher resting levels of brain serotonin than IC animals as a result of induction, since the serotonin system is presumably being activated more often and more intensely by the rigors of the ECT environment than by the relative quiet of the IC environment.

To test this hypothesis littermates from the S_1 and S_3 strains were placed in the various environmental conditions at weaning, and at the completion of the standard 80 day training program they were sacrificed and their brains analyzed for serotonin. Three groups of animals were run in semi-replications. The differences between replications were the strains used and the brain sections analysed for serotonin, ChE and AChE activities. Two groups of S_1s were run. Serotonin was measured in the RDC, VC and SC II sections for the first group and the TB II sections for the second group. ChE and AChE activities were determined in the V and S sections for both groups of S_1s. One group of S_3s was run in which serotonin was measured in the DC, VC and SC I sections. ChE and AChE activities were not determined in the S_3s.

METHODS

Subjects

Subjects in Group I were 12 sets of male littermate triplets from the S_1 strain. At weaning one animal from each set of triplets was assigned to one of the following three conditions: Experimental Complexity and Training (ECT); Social Control (SC); or Isolated Control (IC). Assignment was semi-random with the restriction that body weights be as nearly equal for all groups as possible.

Subjects in Group II were 10 sets of male littermate quadruplets from the S_1 strain. At weaning one animal from each set of quadruplets was assigned to one of the following four conditions: ECT; SC; Social Testing (ST); or IC. Assignment was semi-random with the same restrictions as for Group I. Results comparing SC and ST littermates were reported in Chapter IV. Only comparisons of ECT, SC and IC littermates will be considered in this chapter.

Subjects in Group III were 10 sets of male littermate pairs from the S_3 strain. At weaning one animal from each pair was assigned to either the ECT or IC condition. Assignment was semi-random with the same restriction as for Groups I and II.

Environmental conditions

The ECT, SC and IC conditions have been described by Krech *et al.* (1960). For easy reference and completeness they are repeated in full below. The only difference in environmental conditions was the building in which the experiments were conducted. The experiments reported here were conducted in Tolman Hall, whereas the earlier experiments were conducted in Life Sciences Building. The possible significance of this change is that a more complete differentiation of the ECT and IC conditions was possible in Tolman Hall. In Tolman Hall, ECT and SC cages were in a

large experimental room, whereas IC cages were in a separate, semi-soundproof room with reduced illumination. The light-dark cycle was controlled artificially for all conditions. In Life Sciences Building all groups were housed in the same room with much less control over lighting and auditory stimuli.

ECT Condition

"Each ECT group consisted of about 10 rats. When they were weaned, at about 25 days, they were housed together in a large cage (25 in. by 25 in. by 18 in.) whose walls were hardware cloth. There was a small wooden maze in the cage, and two different wooden "toys" from a set of seven were put in the cage each day. (This procedure was an adaptation of that of Forgays and Forgays, 1952). Chow pellets and water were available ad lib. on a platform 10 in. above the cage floor. For 30 min. each day the rats were allowed to explore, in groups of five, the Hebb-Williams maze where the pattern of barriers was changed daily. No reward was present. In both the home cage and the maze the young pups were frisky and playful. The animals were weighed every few days at the start, and then every two weeks.

"When the S_s were about 60 days old, pretraining was started for testing in the Lashley III maze. The rats were run for 13 days in the Lashley maze, one trial a day; during the last 3 days, the maze was reversed. Then followed 10 days in the Dashiell maze, one trial a day. Finally, the rats were run in the Krech hypothesis apparatus for 14 days, two trials a day. For 5 days the lighted alleys were correct, for the next 5 days the dark alleys were correct, and for the final 4 days the lighted alleys were again correct. The schedules of some groups varied by a day or two in the duration of some of the training procedures. On each trial of each problem a 50-mg glucose pellet was given as a reward." (Krech et al., 1960, p. 510).

IC condition

"At weaning a littermate of each ECT rat was assigned to the IC group. The ECT and IC groups were matched for weight. The IC rats were put in individual cages (11 in. by 8 in. by 8 in.) lined on three sides with sheet metal. They could never see or touch another rat. Chow pellets and water were available ad lib. When the ECT rats received glucose pellet rewards, glucose pellets were put in the IC cages. The individual cages had grid bottoms so that the IC rats did not have to be handled for cleaning. They were weighed on the saye days as their ECT littermates." (Krech et al., 1960, p. 510).

SC condition

"The rats in the SC groups were littermates of ECT and IC rats. At weaning they were housed, usually three in a cage, in regular laboratory colony cages (13 in. by 10 in. by 9 in.). They could see other rats, and their cages were changed at least once a week for cleaning, at which time they were handled by E. The SC rats had an environment whose complexity was intermediate between that of the ECT and IC groups, and they received no formal training. The SC groups had Chow pellets and water ad lib., and they were given glucose pellets when the ECT group received them. The SC rats were weighed on the same days as their ECT and IC littermates.

"The day after the ECT group finished its training, they and their littermates were sacrificed for chemical analysis. All rats were between 100 and 110 days of age at sacrifice. Thus, each rat had spent about 80 days in one of the three experimental conditions." (Krech et al., 1960, p. 510).

Sacrifice and dissection

All animals were sacrificed by decapitation under code numbers, so that the experimental conditions to which each animal belonged was unknown

to the persons performing the sacrifice, dissection, weighing and chemical analyses. Dissection of the brains for each of the three groups was different, so that each will be described separately.

Dissection of brains of animals in Group I (S_1s)

After exposure of the cortex, samples of tissue were removed from the somesthetic (S) and visual (V) areas of both hemispheres with the aid of a small, plastic T-square. These sections were weighed, frozen on dry ice, and stored at -22°C until they were assayed for ChE and AChE activities. The remaining dorsal cortex (RDC) was peeled off down to the temporal ridge. The ventral cortex (VC) comprised all the remaining cortical and contiguous tissue, including the hippocampus, amygdala and corpus callosum. The cerebellum was removed by severing its peduncles, weighed, and used to carry standards through the serotonin procedures. Remaining structures, including olfactory bulbs and brain stem, constituted the section labeled Subcortex I (SC I). The three sections (RDC, VC and SC I) were weighed and immediately extracted and analysed for serotonin.

Dissection of brains of animals in Group II (S_1s)

After exposure of the cortex, samples of tissue were removed from the somesthetic (S) and visual (V) areas of both hemispheres with the aid of a small, plastic T-square. These sections were weighed, frozen on dry ice, and stored at -22°C until they were assayed for ChE and AChE activities. The cerebellum was removed by severing its peduncles, weighed, and used to carry standards through the serotonin procedures. The remaining brain, including RDC, constituted the section labeled Total Brain II (TB II). The TB II section was weighed and immediately extracted and analysed for serotonin.

Dissection of brains of animals in Group III (S_3s)

After exposure of the cortex the dorsal cortex (DC) was peeled off

down to the temporal ridge (V and S sections were not removed for enzyme analyses in this group). The ventral cortex (VC) comprised all remaining cortical and contiguous tissue, including the hippocampus, amygdala and corpus callosum. The cerebellum was removed by severing its peduncles, weighed and used to carry standards through the serotonin procedures. Remaining structures including olfactory bulbs and brain stem constituted the section labeled Subcortex I (SC I). The three sections (DC, VC and SC I) were weighed and immediately extracted and analysed for serotonin.

Chemical analyses

Serotonin was extracted and analysed using the procedures described in Chapter III.

ChE and AChE activities were determined by the photometric techniques described in Chapter III. Tissues were homogenized in 0.1 M sodium phosphate buffer, pH 8.0, to a concentration of 3 mg/ml. Assays were run in the same buffer using 9.0 mg of tissue for ChE and 1.8 mg of tissue for AChE determination.

RESULTS

Results obtained from the three groups in this experiment will be reported separately, since different morphological and/or biochemical measurements were taken on each group.

S_1 ECT, SC and IC animals in Group I

Table 36 shows the means and standard deviations of the S_1 animals in Group I from the ECT, SC and IC conditions with respect to body weight, brain tissue weights, total serotonin in the RDC, VC and SC I sections, and ChE and AChE total activities in the V and S sections. Also included are the percentages of littermates in which the values of ECT>SC, ECT>IC and SC > IC (n = 12 in each condition).

Body weights decreased significantly as a monotonic function of increasing environmental stimulation ($F_{2,22} = 7.18$; $p \leq .01$). That is, the ECT animals were lighter than the SC and IC animals, and the SC animals were slightly lighter than the IC animals.

As has been found previously,(Rosenzweig et al., 1962) the effects of environmental stimulation were not consistent with respect to the various brain tissue weights. The weight of the V section increased significantly as a monotonic function of environmental stimulation ($F_{2,22} = 9.09$; $p \leq .01$), but the weight of the S section was not significantly affected by the environmental conditions. The weight of the RDC section was significantly different among animals of the three conditions ($F_{2,22} = 4.97$; $p \leq .01$), but the ordering of the mean tissue weights was SC (289 mg) > ECT (284 mg) > IC (274 mg). Neither the VC nor SC I sections differed significantly among animals from the three conditions in terms of wet weight. The means weights for the total reconstituted brain were 1451 mg, 1469 mg and 1421 mg for the ECT, SC and IC animals, respectively.

Total serotonin did not differ significantly among animals from the three conditions in any of the three brain sections (RCD, VC and SC I). The ECT animals had higher means with respect to total serotonin than their IC littermates in all three brain sections, but the differences did not reach an acceptable level of significance. The means of the SC animals were intermediate in the VC and SC I sections and were lowest in the RDC sections.

Combining the three sections (RDC + VC + SC I) resulted in an ordering of mean total serotonin for the three conditions that was in keeping with the predictions made with respect to the effects of environmental stimulation on brain serotonin, i.e., ECT (1420 mg) > SC (1381 mg) > IC (1337 mg). However, the differences among means were not statistically

Table 36

Means and Standard Deviations of ECT, SC and IC Animals (S_1s - Group I#) with Respect to Body Weight, Brain Tissue Weights, Total Serotonin and ChE and AChE Total Activities

Variable	Section	Experimental Conditions: ECT $\bar{x}$	ECT SD	SC $\bar{x}$	SC SD	IC $\bar{x}$	IC SD	Percentage of Littermates in which ECT > SC	Percentage of Littermates in which ECT > IC	Percentage of Littermates in which SC > IC
Body Weight (g)		303**	18.3	325	18.8	331	27.9	17	8	50
Brain Weights (mg)	V	76.52**	4.37	73.60	5.04	69.90	5.51	75	92	83
	S	54.80	2.63	56.34	3.02	54.97	3.46	42	50	50
	RDC	284.0**	9.8	288.6	15.4	273.8	23.7	42	75	75
	VC	305.9	16.6	307.7	30.2	292.2	30.7	58	75	75
	SC I	728.7	14.9	741.8	27.8	729.6	37.1	25	50	75
Total Serotonin (ng)	RDC	228	26.9	198	53.2	208	67.5	67	58	25
	VC	241	56.2	240	122.2	204	64.7	75	67	42
	SC I	951	64.4	943	72.8	925	51.8	58	67	50
ChE Total Activity ($Mx10^8$/min)	V	348**	31.7	291	38.7	276	46.2	83	83	58
	S	275	29.1	290	44.2	276	27.5	33	58	67
AChE Total Activity ($Mx10^8$/min)	V	4634	418	4590	538	4283	471	58	75	75
	S	4361	311	4410	381	4321	464	33	50	50

* $p \leq .05$ for F-test
** $p \leq .01$ for F-test

n = 12 for each condition

significant.

ChE total activity in the V section increased significantly as a monotonic function of environmental stimulation ($F_{2,22} = 9.91$; $p \leq .01$). Ten out of twelve ECT animals had higher ChE total activity than their SC and IC littermates. Seven out of twelve SC animals had higher ChE total activity than their IC littermates. The mean ChE total activity for the ECT animals (348 M x 10^8/min) was 20 per cent higher than the SC animals (291 M x 10^8/min) and 26 per cent higher than the IC animals (276 M x 10^8/min). In the S section, however, there were no significant differences among the three environmental conditions in ChE total activity.

While the mean AChE total activity in the V section of the ECT animals (4636 M x 10^8/min) was higher than that of the SC (4590 M x 10^8/min) and IC animals (4283 M x 10^8/min), the differences were not statistically significant. Nor were the differences significant between the three conditions with respect to AChE total activity in the S section.

Table 37 shows the means and standard deviations of the S_1 animals in Group I from the ECT, SC and IC conditions with respect to serotonin concentration in the RDC, VC and SC I sections, and ChE and AChE specific activities in the V and S sections. Also included are the percentages of littermates in which the values of ECT > SC, ECT > IC and SC > IC (n = 12 in each condition).

Mean concentration of serotonin increased as a monotonic function of environmental stimulation. The differences among means were not, however, statistically significant in any of the three sections (RDC, VC or SC I) or combinations of the three sections.

ChE specific activity in the V section was significantly higher in the ECT animals (4.55 M x 10^{10}/min/mg) than in the SC (3.95 M x 10^{10}/min/mg) and IC (3.95 M x 10^{10}/min) animals ($F_{2,22} = 5.88$; $p \leq .01$). No significant

Table 37

Means and Standard Deviations of ECT, SC and IC Animals (S_1s - Group I#) with Respect to Serotonin Concentration and ChE and AChE Specific Activities

Variable	Section	Experimental Conditions						Percentage of Littermates in which ECT > SC	Percentage of Littermates in which ECT > IC	Percentage of Littermates in which SC > IC
		ECT		SC		IC				
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD			
Serotonin Concentration	RDC	804	104	689	194	751	247	75	58	17
	VC	788	176	779	398	706	236	67	67	33
	SC I	1306	83	1276	110	1267	63	50	75	50
ChE Specific Activity ($Mx10^{10}$/min/mg)	V	4.55**	0.26	3.95	0.51	3.95	0.56	83	67	42
	S	5.02	0.46	5.13	0.61	4.90	0.47	50	75	75
AChE Specific Activity ($Mx10^{10}$/min/mg)	V	60.7	5.86	62.2	4.77	61.2	4.26	50	42	50
	S	79.6	4.76	78.7	3.76	78.8	8.32	42	58	50

* $p \leq .05$ for F-test
** $p \leq .01$ for F-test

n = 12 for each condition

differences were found among animals from the three conditions with respect to ChE specific activity in the S sections, although the means for the ECT (5.02 M x 10^{10}/min/mg) and SC (5.13 M x 10^{10}/min/mg) animals were slightly higher than the means for the IC animals (4.90 M x 10^{10}/min/mg).

No significant differences were found with respect to AChE specific activity in the V or S sections among animals from the three conditions.

S_1 ECT, SC and IC animals in Group II

Table 38 shows the means and standard deviations of the S_1 animals in Group II from the ECT, SC and IC conditions with respect to body weight, brain tissue weights, total serotonin in the TB II section, and ChE and AChE total activities in the V and S sections. Also included are the percentages of littermates in which the values of ECT > SC, ECT > IC and SC > IC (n = 10 in each condition).

As was true of the S_1s in Group I, body weights decreased significantly as a monotonic function of increasing environmental stimulation ($F_{2,18}$ = 15.62; $p \leq .01$). The mean body weight of the ECT animals (288g) was 4 per cent lower than the SC animals (299g) and 14 per cent lower than the IC animals (327g).

Again, as was true of the S_1s in Group I, the tissue weight of the V section increased significantly as a monotonic function of increasing environmental stimulation ($F_{2,18}$ = 3.85; $p \leq .05$). The mean tissue weight of the V section for the ECT animals (41.11 mg) was 7 per cent higher than for the SC (66.61 mg) and IC animals (66.37 mg), but the difference between SC and IC animals was less than 1 per cent. Neither the weight of the S section nor the weight of the TB II section differed significantly among animals from the three environmental conditions.

No significant differences were found among ECT, SC and IC animals with respect to total serotonin in the TB II section. These negative

Table 38

Means and Standard Deivations of ECT, SC and IC Animals (S_1s - Group II#) with Respect to Body Weight, Brain Tissue Weights, Total Serotonin and ChE and AChE Activities

Variable	Section	Experimental Conditions						Percentage of Littermates in which ECT > SC	Percentage of Littermates in which ECT > IC	Percentage of Littermates in which SC > IC
		ECT		SC		IC				
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD			
Body Weight (g)		288***	18.8	299	16.3	327	28.3	10	0	20
Brain Weights (mg)	V	71.11*	5.48	66.61	4.38	66.37	3.10	70	70	40
	S	56.30	1.79	57.14	2.48	55.04	2.57	30	70	70
	TB II	1273	31.0	1257	43.5	1278	34.7	60	40	30
Total Serotonin (ng)	TB II	888	58.9	881	67.3	914	54.5	70	30	20
ChE Total Activity ($Mx10^8$/min)	V	227**	26.6	204	18.0	198	8.6	80	90	60
	S	177*	5.7	179	14.5	168	12.1	40	80	70
AChE Total Activity ($Mx10^8$/min)	V	3882	301	3810	342	3741	238	40	70	70
	S	3565	161	3590	245	3524	135	50	50	30

* $p \leq .05$ for F-test
** $p \leq .01$ for F-test
*** $p \leq .001$ for F-test

n = 10 for each condition

results were also found for the S_1s in Group I. The direction of mean differences was reversed in some of the comparisons from those found in Group I. The mean for the ECT animals (888 ng) was almost identical to that of the SC animals (881 ng) and was lower than the IC animals (914 ng). For the reconstructed TB II section of animals in Group I the respective means were 1420 ng, 1380 ng and 1334 ng. The differences in means between Groups I and Group II reflect the difficulty in obtaining comparable absolute values from experiments done at different times, using different chemical reagents, and slight differences in procedure (e.g., duration of shaking tissue standards).

The results with respect to ChE total activity in the V section qualitatively replicated the results found for Group I. The mean ChE total activity for the ECT animals (227 M x 10^8/min) was 11 per cent higher than that for the SC animals (204 M x 10^8/min) and 15 per cent higher than that for the IC animals (198 M x 10^8/min). The $F_{2,218} = 9.03$ for the test of the differences among these three means was statistically significant ($p \leq .01$). For ChE total activity in the S section the differences among the three means was also significant ($F_{2,18} = 4.30$; $p \leq .05$). Both the ECT (177 M x 10^8/min) and SC (179 M x 10^8/min) animals had higher mean ChE total activities than the IC animals (168 M x 10^8/min).

None of the differences among the three environmental conditions were significant with respect to AChE total activity in the V and S sections. Mean AChE total activity in the V section was, however, slightly higher in the ECT animals (3882 M x 10^8/min) than the SC animals (3810 M x 10^8/min), and the latter was higher than in the IC animals (3741 M x 10^8/min).

Table 39 shows the means and standard deviations of the S_1 animals in Group II from the ECT, SC and IC conditions with respect to serotonin concentration in the TB II section, and ChE and AChE activities in the V

Table 39

Means and Standard Deviations of ECT, SC and IC Animals (S_1s - Group II#) with Respect to Serotonin Concentration and ChE and AChE Specific Activities

Variable	Section	Experimental Conditions						Percentage of Litter-mates in which ECT > SC	Percentage of Litter-mates in which ECT > IC	Percentage of Littermates in which SC > IC
		ECT		SC		IC				
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD			
Serotonin Concentration (ng/g)	TB II	689	51.2	708	59.3	716	52.5	50	40	40
ChE Specific Activity ($Mx10^{10}$/min/mg)	V	3.19**	0.19	3.07	0.18	2.98	0.08	100	90	50
	S	3.14*	0.18	3.14	0.20	3.06	0.19	40	70	80
AChE Specific Activity ($Mx10^{10}$/min/mg)	V	54.6*	2.43	57.1	2.12	56.4	2.32	30	40	60
	S	63.3	2.71	62.9	3.91	64.1	3.29	60	40	30

* $p \leq .05$ for F-test
** $p \leq .01$ for F-test

n = 10 for each condition

and S sections. Also included are the percentages of littermates in which the values of ECT > SC, ECT > IC and SC > IC (n = 10 for each condition).

Serotonin concentration did not differ significantly among animals from the three environmental conditions. The means for the ECT, SC and IC animals were 689 ng/g, 708 ng/g and 716 ng/g, respectively.

Results with respect to ChE specific activities in the V and S sections were relatively comparable to those found for total activity. In the V section the mean ChE specific activity for the ECT animals (3.19 M x 10^{10}/min/mg) and 7 per cent higher than for the IC animals (2.98 M x 10^{10}/min/mg). In the S section the means for the ECT and IC animals were identical (3.14 M x 10^{10}/min/mg), and both were 4 per cent higher than the mean for the IC animals (3.06 M x 10^{10}/min/mg).

AChE specific activity in the V section was significantly different among the three environmental conditions ($F_{2,18} = 3.89$; $p \leq .05$). The mean AChE specific activity for the ECT animals (54.6 M x 10^{10}/min/mg) was lower than for the SC (57.1 M x 10^{10}/min/mg) and IC (56.4 M x 10^{10}/min/mg) animals. No significant differences in AChE specific activity in the S section were found among animals from the three conditions.

S_3 ECT and IC animals in Group III

Table 40 shows the means and standard deviations of the S_3 animals in Group III from the ECT and IC conditions with respect to body weight, brain tissue weights, total serotonin and serotonin concentrations in the DC, VC and SC I sections. Also included are the percentages of littermates in which the values of ECT > IC.

None of the mean differences between ECT and IC animals were statistically significant for the S_3s in Group III. Body weight was slightly lower for the ECT animals (295g) than for the IC animals (306g) as was found for both groups of S_1s. The DC and VC tissue weights were slightly higher for the ECT animals (420 mg and 364 mg) than for the

Table 40

Means and Standard Deviations of ECT and IC Animals (S_3s - Group III#) with Respect to Body Weight, Brain Tissue Weights, Total Serotonin and Serotonin Concentration

Variable	Section	Experimental Conditions				Percentage of Littermates in which ECT > IC
		ECT		IC		
		$\bar{x}$	SD	$\bar{x}$	SD	
Body Weight (g)		295	289	306	48.0	50
Brain Weights (mg)	DC	420	14.6	408	21.3	60
	VC	364	20.0	348	27.0	50
	SC I	757	25.5	769	39.0	40
Total Serotonin (ng)	DC	198	89.7	166	87.9	50
	VC	260	73.3	236	51.6	50
	SC I	1384	152	1422	124	40
Serotonin Concentration (ng/g)	DC	468	209	408	212	60
	VC	707	175	686	167	60
	SC I	1820	176	1855	204	30

* $p \leq .05$ for F-test

\# n = 10 for each condition

IC animals (408 mg and 348 mg), but the SC I tissue weights were slightly lower for the ECT animals (757 mg) than for the IC animals (767 mg). Mean total serotonin and mean serotonin concentration showed the same patterns between ECT and IC animals as the respective tissue weights.

DISCUSSION

The purpose of the experiments reported in this chapter was to investigate the effects of differential environmental stimulation on brain serotonin. The results indicated that, while an enriched versus an impoverished environment had measureable effects on body weight, brain morphology and brain ChE and AChE activities in the S_1 strain, there were no significant effects on the levels of brain serotonin.

It must, therefore, tentatively be concluded that brain serotonin is not affected by these environmental conditions. The question must be asked, however, whether the procedures followed in these experiments provided an adequate test of the hypothesis presented in the introduction to this chapter. Briefly, this hypothesis was that the different environmental conditions provided differential activation of the biochemical mechanisms underlying emotional behavior and, thus, ought to induce quantitative changes in the substrate-enzyme systems involved in these mechanisms.

Serotonin was assumed to be involved in the regulation of the neural substrates underlying emotional behavior. If this assumption were wrong, then the observed results with respect to serotonin would be irrelevant to the hypothesis. On the other hand, the assumption with respect to serotonin may be correct, but measured levels of brain serotonin may not have reflected the actual capacity of the system to respond in an emotional situation. More explicitly, conditions at the time the animals in these experiments were sacrificed may not have permitted an accurate picture of the normal resting serotonin system. Animals from the ECT

condition were brought from their communal living quarters and placed in small compartments one day before sacrifice. At the same time animals from the SC and IC conditions were similarly removed from their accustomed environments and placed in these small compartments. It may be that these abrupt changes in environment were sufficient to mask any differences in brain serotonin among animals from the three conditions. The hope was that by allowing the animals 24 hours in this new, confined environment, any transient changes that might have occured as a result would have dissipated by the time the animals were sacrificed. Two alternatives are, however, possible. First, 24 hours may not have been sufficient time for alterations in brain serotonin that resulted from the abrupt changes in environment to dissipate. Second, rather than just an initial response to the change in environment, there may have been a persisting response. That is, the animals may not have adapted to the new environment by the time of sacrifice. In either case, the serotonin system may have been in a state of flux. Measurement of brain serotonin under these conditions could not be expected to reveal the animals' capacity to respond emotionally.

It should also be pointed out that the magnitude of differences to be expected in the serotonin system as a result of environmental stimulation may be, as was the case with ChE and AChE, relatively small. Differences of the order of 4 to 10 per cent might have been masked in these small samples by the experimental error of the chemical procedures. If this were the case, then either further refinement of the techniques are called for (which may be difficult, since they were being pushed to the limits of their sensitivity as it was) or a large increase in the number of animals used may be necessary.

If the analysis just presented is correct, then a rigorous test of the serotonin-emotionality-environment hypothesis was not made. Two

suggestions are offered with respect to a more adequate test. First, measurement of DOPAD and MAO may provide more reliable information about the serotonin system in animals raised under differential environmental conditions. These enzymes are not as labile as serotonin and, therefore, would not be so apt to change measureably following any abrupt changes in environment just prior to sacrifice. Second, it may be more favorable to measure brain serotonin in animals from ECT, SC and IC conditions after a controlled exposure to an emotional situation. For example, if animals from the three conditions were exposed to, say, a bright light and open-field or, perhaps, a series of electric shocks to the feet exactly 30 minutes prior to sacrifice, then serotonin levels might reflect more closely the animals' capacity to respond in an emotion-provoking situation.

Chapter VI

Summary of Major Findings

The primary purpose of this dissertation was to investigate possible relations between non-cholinergic neurohumoral systems in brain and behavior. Three sets of experiments were performed with this in mind. First, strain differences in brain serotonin, DOPAD, GAD and MAO activities were investigated together with ChE and AChE activities, and the results were related to known behavioral differences between the strains. Second, the S_1 and S_3 strains were investigated more thoroughly with respect to within-strain correlations between brain serotonin and behavior. Third, the direction of study was reversed, and the effects of environmental stimulation on brain serotonin were investigated in the S_1 and S_3 strains.

In this chapter the major results of these three sets of experiments will be brought together. It should be pointed out that this investigation was exploratory in many respects, and, consequently, a larger than usual number of dependent variables was measured in relation to the number of subjects used.

Experiments II and III reported in Chapter III and the experiments in Chapter IV were designed with this multivariate approach in mind. The success of this approach is dependent upon reliable measurement of the variables involved and, assuming that to be satisfactory, the reliabilities of the resulting mean vectors and their associated variance-covariance matrices. In other words, assuming the measurements to be adequate, the estimates of means, standard deviations and especially correlations must be reliable in order for the results to have any general predictive or explanatory value.

The results reported in Chapters III and IV were based on relatively small samples. For this reason they must be considered only as suggestive

until they have been satisfactorily replicated. With these cautions in mind the major finding of this dissertation will be summarized.

In Experiment I (pp. 68-79) an exploratory attempt was made to find strain differences with respect to a non-cholinergic neurohumor in rats. The brains of young (40 days old) and adult (110 days old) S_1 and S_3 rats were analyzed for serotonin (c.f. Chapter II for discussion of serotonin as a neurohumor). In the young animals it was found that the mean serotonin concentration in the dorsal cortex (DC), ventral cortex (VC) and subcortex (SC I) was slightly (although not significantly), higher in the S_1s than in the S_3s. This strain difference was more evident in the adult animals where the S_1s had 24 per cent more serotonin per gram brain tissue (TB I) than the S_3s ($p \leq .01$). In spite of the fact that the S_3s had heavier brains (11 per cent), they also had less total serotonin (11 per cent) than the S_1s.

Brain serotonin was again measured in adult animals (114 days old) from the S_1 and S_3 strains in Experiment II (pp. 79-109). The results of Experiment I were successfully replicated on this sample. The S_1s had 18 per cent more serotonin per gram brain tissue (TB II) than the S_3s ($p \leq .01$). In this sample the S_1s also had slightly more total serotonin (7.6 per cent) than the S_3s even though the usual difference in tissue weight was found with the S_3s having the heavier brains.

In addition to serotonin, activities of the brain enzymes ChE, AChE, DOPAD (the enzyme responsible for synthesis of serotonin), MAO (the enzyme responsible for degradation of serotonin) and GAD (the enzyme responsible for synthesis of GABA) were determined in littermates of the animals on which serotonin was measured. In all comparisons the S_1s had higher enzyme specific activities than the S_3s, but when total activities were considered the differences decreased or even reversed, depending on the particular correlations between enzyme total activities and tissue weight. When tissue weight was partialled out statistically

(c.f. pp. 99-102 for a discussion of this technique), the S_1s tended to have higher ChE, AChE and GAD total activities but lower MAO total activity than the S_3s.

In Experiment III (pp. 109-143) the results of Fxperiments I and II were again replicated with respect to brain serotonin and the five brain enzymes measured in Experiment II. In addition, two other pairs of strains (RDH-RDL, OMB-OMD) were measured on these variables. The choice of the six strains in this experiment was such that the two strains within each pair differed with respect to maze performance and/or the ACh-AChE system in the brain. Compared to the S_3s, the S_1s have higher ACh concentrations and AChE activities and are superior on most mazes. The RDHs have higher cortical AChE activity than the RDLs by virtue of the fact that these strains were selectively bred on this variable, but when tested the RDHs were slightly inferior to the RDLs in maze performance. The OMBs were selected to be superior to the OMDs on the Lashley III maze, but in one of the lines the two strains did not differ in ACh concentration or AChE specific activity, and in the other line the OMDs were actually higher in ACh concentration and AChE specific activity than the OMBs.

The S_1s had 10 per cent more serotonin per gram tissue (TB I) than the S_3s, but there was no difference in this experiment in total serotonin. This replication constitutes the fourth group of animals in which a strain difference in brain serotonin between the S_1s and S_3s has been observed. In terms of the five enzymes the S_1s again exceeded the S_3s in mean ChE, AChE, DOPAD, MAO and GAD specific activities in the four brain sections considered (DC, VC, H and SC II; except for DOPAD, MAO and GAD in the H section). In terms of total activities the results were less consistent and depended on the particular enzyme and brain section, and the respective correlations between total activity and tissue weight.

The RDHs did not differ from the RDLs in terms of serotonin con-

centration, but were significantly lower in total serotonin than the RDLs. Specific activities of ChE and AChE were higher in the RDHs than in the RDLs in all four brain sections, but the reverse was true for mean specific activities of DOPAD and GAD. Mean specific activities of MAO were higher in the cortex (DC and VC) in the RDHs than in the RDLs, but no difference between the two strains was suggested in the subcortex (H and SC II). The RDLs tended to have higher mean enzyme total activities than the RDHs except for cortical AChE (in DC) and ChE (in VC).

The OMBs had slightly more total serotonin than the OMDs, but the reverse was true for serotonin concentrations; neither comparison was, however, significant. There were no apparent differences in ChE, AChE or MAO specific activities between these two strains, but DOPAD and GAD specific activities were higher in the OMBs than in the OMDs. Mean total activities of AChE, DOPAD, MAO and GAD were also higher for the OMBs than for the OMDs, partly by virtue of the heavier tissue weights of the OMBs. The conclusions based on these two strains must, however, be made with extreme caution due to methodological error and the resulting small sample sizes (c.f. pp. 112-113).

To determine whether those strains that were superior in maze performance had anything in common in terms of the morphological and biochemical variables measured in this experiment, the S_1s, RDLs and OMBs were combined and compared to the S_3s, RDHs and OMDs. The high maze performance strains tended to be heavier in body weight and to have higher brain weights than the low maze performance strains. The difference in brain weights, <u>per se</u>, was <u>not</u>, however, felt to be directly related to maze performance, since the S_1s have consistently lower brain weights than the S_3s but perform consistently better in most learning tasks. There were no consistent differences between the high and low performance strains with respect to total or specific activities of ChE, AChE and

MAO. The high performance strains, however, had higher mean DOPAD and GAD total and specific activities, and higher mean total serotonin and serotonin concentration than the low performance strains.

It was suggested that the relevant parameters in terms of maze performance were either the capacity to form neurohumors or the ratio of the capacity to form and destroy neurohumors (pp. 145-147).

By using covariance techniques in conjunction with discriminant function analysis, it was also possible to compare the strains with respect to the biochemical variables independent of tissue weight. This approach gave similar results to those obtained using specific activities, i.e., the high performance strains had higher DOPAD and GAD independent total activities than the low performance strains. The degrading enzymes, ChE, AChE and MAO were not consistently different between the high and low maze performance strains in terms of independent total activities.

In Chapter IV the S_1s and S_3s were investigated more thoroughly on a number of behavioral tests, and the scores were correlated with ChE and AChE activities in the visual (V) and somesthetic (S) cortex, and brain serotonin (TB II). It was found that the S_1s were more active in the Dashiell maze and revolving wheels, and defecated more in an open-field than the S_3s. There were no differences between the two strains in locomotor and/or exploratory activity measured by interruption of a light beam in a standard colony cage. Nor were there strain differences on a series of four spatial reversal-discrimination problems run in the ATLAS using escape or avoidance of shock as the relevant motivation.

In the activity wheels the S_1s maintained a constant low number of revolutions over successive testing sessions, whereas the S_3s increased the number of revolutions made in a linear fashion. Both strains were more active in the morning than in the evening. The two strains showed a linear decrease in beam interruptions in the colony-cages over

successive testing sessions.

The S_1s and S_3s showed a similar pattern of performance in the ATLAS. The first reversal was most difficult with the second and third reversals becoming increasingly easier. The S_3s were considerably more variable than the S_1s in their performance in the ATLAS, but there were no differences between the means on any of the four problems.

There was evidence of a negative relation between the open-field test of emotionality and brain serotonin. For the S_3s both total serotonin and serotonin concentration were significantly negatively correlated with defecations and urination in an open-field. In two replications using 10 S_1s in each group, the results were ambiguous. The first group showed moderate to high negative correlations, but in the second group the correlations were positive (although not significantly) so that the average correlations for the two groups were not significant.

For the S_1s, brain serotonin was positively correlated with revolving wheel activity but negatively correlated with Dashiell activity. These two measures of activity were not correlated for this strain. For the S_3s, however, revolving wheel activity was positively correlated with Dashiell activity, but neither measure of activity was correlated with brain serotonin.

Tissue weight of the S section was positively correlated with performance in the ATLAS for the S_1s, as were ChE and AChE total activities in this section. This relation was not found for the V section or for the S_3s in either section. An interpretation of this relation between brain tissue weight and performance in the ATLAS was that animals who took longer to solve the problems received more stimulation of the afferent projections to the somesthetic cortex by the unconditioned stimulus, i.e., shock to the feet. In other words it was considered to be a result similar to those obtained on brain morphology and bio-

chemistry as a consequence of an enriched versus an impoverished environment. The S_3s did not show this effect, but for this strain AChE specific activity in the S section was significantly related to performance in the ATLAS.

In Chapter V the effects of an enriched versus an impoverished environment on brain serotonin and ChE and AChE activities were investigated in the S_1 and S_3 strains. ECT and SC animals of the S_1 strain had lower body weights and higher visual cortex (V) weights than IC littermates, thus confirming earlier reports (c.f. Chapter I). Total activity of AChE in the V section was also higher (but not significantly so) in ECT than IC littermates in both replications with 10 sets of S_1s in each group. The reverse was true of AChE specific activity, since the increases in tissue weight with ECT was greater than the increase in AChE total activity.

Both total and specific activities of ChE in the V section were significantly higher in ECT and SC animals than in IC littermates in both replications. ChE specific activity in the S section was also higher in ECT and SC animals than in their IC littermates. These results confirm the preliminary report of Bennett et al. (1963).

Neither total serotonin nor serotonin concentration was significantly affected by the environmental conditions in either strain. It was pointed out in the discussion (pp. 219-221) that an adequate test may not have been made, since serotonin in brain is quite labile and may have been in a state of flux at the time of sacrifice.

Appendix A

Computational Steps for Computing Discriminant Function and Wilks' Lambda Test of the Significance of the Discriminant*#

$$\underset{N\times p}{\text{Let } X} = \begin{bmatrix} x_1 \\ \cdots \\ x_2 \\ \cdots \\ \\ \cdots \\ x_g \end{bmatrix}$$

be the $\underline{N}$ by $\underline{p}$ supermatrix of scores (measures) of $\underline{N}$ subjects on $\underline{p}$ variables partitioned into $\underline{g}$ subgroups of $\underline{n_i}$ subjects each. Then, $\underset{p\times p}{W} = \sum_{i=1}^{g} (X_i' X_i - \frac{1}{n_i} a_i' a_i)$, the pooled within-groups deviation score cross-products matrix where,

$a_i = 1' X_i$

1 = an $n_i \times 1$ unit vector

And, $\underset{p\times p}{T} = (X'X - \frac{1}{N} a'a)$, the total sample deviation score cross-products matrix.

And, $\underset{p\times p}{A} = T - W$, the among-groups cross products of deviations of groups from grand means weighted by group sizes, $\underline{n_i}$.

The multiple-discriminant functions (of which there are $\underline{g}$-1 mutually orthogonal) are computed as the vectors (eigenvectors) associated with the latent roots (eigenvalues) of the determinantal equation:

$$\left| W^{-1}A - \lambda I \right| = 0$$

where, I is an identity matrix.

Wilks' lambda criterion for the discriminating power of the $\underline{p}$ variables as a function of the latent roots of $W^{-1}A$ is :

$$\Lambda = \prod_{k=1}^{r} \frac{1}{(1-\lambda_k)}$$

Appendix A (contd.)

where $\underline{r}$ is the rank of $W^{-1}A$.

The associated $F_{p,N-p-1}$ $\frac{(N-p-1)(1-\Lambda)}{p\Lambda}$

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* c.f. Cooley & Lohnes, Multivariate procedures for the behavioral sciences. New York: Wiley & Sons, 1962.

\# Procedures used in Experiments II and III, Chapter III, pp. 79-199.

Appendix B

Computational Steps for Partialling Out q Variables from a p Variable Discriminant Function*#

Let $\underset{pxp}{W} = \left[\begin{array}{c|c} \underset{mxm}{W} & \underset{mxq}{W} \\ \hline \underset{qxm}{W} & \underset{qxq}{W} \end{array}\right]$

the within-groups deviation score cross-products matrix defined in Appendix A be partitioned such that the m variables to be retained are in the upper left and the q variables, the influence of which are to be removed by regression, are in the lower right.

And $\underset{pxp}{T}$, the total sample deviation score cross-products matrix defined in Appendix A be partitioned similarly.

Then, the three adjusted matrices are:

$$W_{m \cdot q} = W_{mm} - W_{mq} \, W_{qq}^{-1} \, W_{qm}$$

$$T_{m \cdot q} = T_{mm} - T_{mq} \, T_{qq}^{-1} \, T_{qm}$$

$$A_{m \cdot q} = T_{m \cdot q} - W_{m \cdot q}$$

And, the adjusted multiple-discriminant functions and tests of significance are computed as before (c.f. Appendix A) from the solution to the determinantal equation:

$$\left| W_{m \cdot q}^{-1} \, A_{m \cdot q} - \lambda I \right| = 0$$

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* c.f. Cooley & Lohnes, Multivariate procedures for the behavioral sciences. New York: Wiley & Sons, 1962.

\# Procedures used in Experiments II and III, Chapter III, pp. 79-199.

Appendix C

Computational Steps for Partialling Out q Variables from a p Variable Variance-Covariance Matrix and Obtaining the Corresponding Partial Correlation Matrix*#

Let $\underset{p \times p}{C} = \left(\frac{1}{N-1}\right) W$, the variance-covariance matrix obtained from N subjects measured on p variables where W is the within-groups deviation score cross-products matrix defined in Appendix A.

If $\underset{p \times p}{C} = \left[\begin{array}{c|c} C_{mm} & C_{mq} \\ \hline C_{qm} & C_{qq} \end{array}\right]$.

is partitioned such that the m variables to be retained is in the upper left and the q variables, the influence of which are to be removed by regression, is in the lower right,

then the adjusted variance-covariance matrix is:

$$C_{m \cdot q} = C_{mm} - C_{mq}\, C_{qq}^{-1}\, C_{qm}$$

and the corresponding partial correlation matrix is:

$$R_{m \cdot q} = D_{C_{m \cdot q}}^{-\frac{1}{2}}\, C_{m \cdot q}\, D_{C_{m \cdot q}}^{-\frac{1}{2}}$$

where $D_{C_{m \cdot q}}^{-\frac{1}{2}}$ is an m x m diagonal matrix composed of inverted square roots of the diagonal elements of $C_{m \cdot q}$.

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* c.f. Anderson, T. W. Introduction to multivariate statistical analysis. New York: Wiley & Sons, 1958.

\# Procedures used in Experiment II, Chapter III pp. 79-109.

Appendix D_1*

Means and Standard Deviations of S_1 and S_3 Strains with Respect to AChE Total and Specific Activities in Eight Brain Sections

Section**	Strain							
	S_1				S_3			
	AChE Total Activities ($Mx10^8$/min)		AChE Specific Activities ($Mx10^{10}$/min/mg)		AChE Total Activities ($Mx10^8$/min)		AChE Specific Activities ($Mx10^{10}$/min/mg)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	3,197	278	48.4	3.74	3,030	235	42.0	3.42
S	2,849	232	53.9	6.04	2,777	151	50.2	3.93
RDC	15,346	635	58.2	3.82	15,984	1,164	54.2	2.64
VC	37,740	8,460	118.5	26.4	34,450	7,880	102.4	22.9
H	5,613	575	94.2	4.60	5,366	793	97.7	4.38
M+P	18,310	1,250	109.0	2.80	19,580	1,410	99.2	4.69
RSB	93,170	9,570	214.5	5.82	104,740	9,100	217.8	6.77
Ce	8,458	375	35.7	2.57	8,929	474	33.7	1.78

* Note - Appendices D_1 to D_{15} contain means and standard deviations of S_1-S_3, RDH-RDL and OMB-OMD strains with respect to AChE, ChE, DOPAD, MAO and GAD total and specific activities. Pertinent information concerning these animals can be found in Chapter III, Experiment III, pp. 66-151.

**V-sample from visual cortex; S-sample from somesthetic cortex; RDC-remaining dorsal cortex; VC-ventral cortex including hippocampus, amygdala and corpus callosum; H-hypothalamus; M+P-medulla and pons; RSB-remaining subcortical brain including caudate n., thalamus, superior and inferior colliculi, and olfactory bulbs; Ce-cerebellum.

Appendix D_2

Means and Standard Deviations of S_1 and S_3 Strains with Respect to ChE Total and Specific Activities in Eight Brain Sections

Section	Strain							
	S_1				S_3			
	ChE Total Activities ($Mx10^8$/min)		ChE Specific Activities ($Mx10^{10}$/min/mg)		ChE Total Activities ($Mx10^8$/min)		ChE Specific Activities ($Mx10^{10}$/min/mg)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	203	26.0	3.06	0.19	217	14.9	3.03	0.29
S	160	19.9	3.03	0.37	158	14.0	2.83	0.17
RDC	776	64.7	2.94	0.21	819	65.2	2.79	0.22
VC	910	171	2.82	0.27	926	180	2.73	0.39
H	365	50.1	6.09	0.46	350	42.3	5.76	0.45
M+P	847	162	5.15	0.87	881	78.0	4.46	0.45
RSB	2,000	374	4.55	0.66	2,130	271	4.45	0.68
Ce	965	65.7	4.05	0.22	1,002	73.6	3.75	0.28

Appendix D_3

Means and Standard Deviations of S_1 and S_3 Strains with Respect to DOPAD Total and Specific Activities in Eight Brain Sections

Section	Strain							
	S_1				S_3			
	DOPAD Total Activities (μg/2hr)		DOPAD Specific Activities (ug/2hr/g)		DOPAD Total Activities (μg/2hr)		DOPAD Specific Activities (μg/2hr/g)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
RDC	69,140	8,694	262	38.2	71,230	17,076	242	58.5
VC	122,100	41,031	381	126	111,080	41,208	334	138
H	54,850	2,733	942	88.5	57,734	7,480	966	98.7
M+P	100,117	7,573	600	45.6	115,250	11,858	584	45.0
RSB	441,067	42,583	1,022	26.2	500,783	58,291	1,037	73.9

Appendix D_4

Means and Standard Deviations of S_1 and S_3 Strains with Respect to MAO Total and Specific Activities in Eight Brain Sections

Section	Strain							
	S_1				S_3			
	MAO Total Activities (%/hr)		MAO Specific Activities (%/hr/g)		MAO Total Activities (%/hr)		MAO Specific Activities (%/hr/g)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	291	27.9	4.38	0.16	317	31.9	4.26	0.32
S	228	13.5	4.32	0.27	239	14.3	4.30	0.22
RDC	1,238	74.2	4.68	0.13	1,358	77.7	4.60	0.14
VC	1,658	151	5.18	0.30	1,739	171	5.14	0.18
H	431	29.0	7.20	0.26	468	52.2	7.67	0.31
M+P	599	48.6	3.49	0.24	682	61.7	3.55	0.32
RSB	2,569	269	5.92	0.18	2,959	322	6.08	0.25
Ce	1,014	101	4.26	0.17	1,107	93.0	4.11	0.20

Appendix D_5

Means and Standard Deviations of S_1 and S_3 Strains with Respect to GAD Total and Specific Activities in Eight Brain Sections

Section	Strain							
	S_1				S_3			
	GAD Total Activities* (μg/hr)		GAD Specific Activities (μg/hr/g)		GAD Total Activities* (μg/hr)		GAD Specific Activities (μg/hr/g)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	662	78.8	979	78.1	601	77.5	808	87.2
S	543	28.4	999	28.9	528	49.1	939	53.0
RDC	2,976	83.6	1,116	31.3	3,141	337	1,091	95.5
VC	2,387	268	796	69.1	2,415	175	695	94.8
H	580	68.1	1,021	119	587	64.1	1,038	65.9
M+P	1,132	57.0	664	29.7	1,165	101.4	588	34.9
RSB	6,358	1,105	1,452	172	6,412	1,256	1,322	198
Ce	2,000	214	851	33.0	2,564	160	907	86.6

* $x10^{-2}$

Appendix D_6

Means and Standard Deviations of RDH and RDL Strains with Respect to AChE Total and Specific Activities in Eight Brain Sections

Section	Strain							
	RDH				RDL			
	AChE Total Activities ($Mx10^8$/min)		AChE Specific Activities ($Mx10^{10}$/min/mg)		AChE Total Activities ($Mx10^8$/min)		AChE Specific Activities ($Mx10^{10}$/min/mg)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	3,119	281	45.9	3.41	2,908	267	39.7	2.88
S	2,751	218	54.8	6.49	2,370	253	48.1	11.9
RDC	14,782	914	57.5	3.99	14,584	1,207	49.0	3.30
VC	26,774	6,951	87.6	13.2	29,250	4,415	83.8	10.4
H	5,011	598	93.0	6.30	5,635	696	90.1	3.86
M+P	16,023	1,318	100.9	1.56	17,852	501	92.0	3.15
RSB	91,832	7,625	219.8	14.3	91,482	6,526	198.3	9.43
Ce	8,274	599	35.4	2.82	8,864	511	32.9	1.46

Appendix D_7

Means and Standard Deviations of RDH and RDL Strains with Respect to ChE Total and Specific Activities in Eight Brain Sections

Section	Strain							
	RDH				RDL			
	ChE Total Activities ($Mx10^8$/min)		ChE Specific Activities ($Mx10^{10}$/min/mg)		ChE Total Activities ($Mx10^8$/min)		ChE Specific Activities ($Mx10^{10}$/min/mg)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	205	19.0	3.10	0.27	203	24.4	2.77	0.36
S	164	21.3	3.29	0.46	146	26.7	2.65	0.46
RDC	744	93.9	2.90	0.31	776	61.3	2.62	0.20
VC	1,177	698	2.77	0.43	863	194	2.48	0.28
H	305	67.2	5.65	0.75	365	60.6	5.83	0.45
M+P	805	86.4	5.05	0.42	900	139	4.65	0.54
RSB	2,051	122	4.91	0.23	2,234	245	4.86	0.63
Ce	1,019	94.3	4.35	0.37	1,051	97.5	3.90	0.38

Appendix D_8

Means and Standard Deviations of RDH and RDL Strains with Respect to DOPAD Total and Specific Activities in Eight Brain Sections

Section	Strain							
	RDH				RDL			
	DOPAD Total Activities (μg/2hr)		DOPAD Specific Activities (μg/2hr/g)		DOPAD Total Activities (μg/2hr)		DOPAD Specific Activities (μg/2hr/g)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
RDC	57,501	1,526	221	52.5	75,230	1,717	252	58.7
VC	85,995	27,924	278	65.2	125,860	34,700	361	87.9
H	48,034	1,003	899	145	59,425	6,393	948	110
M+P	93,400	9,310	596	97.2	108,983	16,034	561	84.7
RSB	418,350	48,055	1,001	108	443,967	33,805	965	66.5

Appendix D_9

Means and Standard Deviations of RDH and RDL Strains with Respect to MAO Total and Specific Activities in Eight Brain Sections

Section	Strain							
	RDH				RDL			
	MAO Total Activities (%/hr)		MAO Specific Activities (%/hr/g)		MAO Total Activities (%/hr)		MAO Specific Activities (%/hr/g)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	318	65.9	4.73	0.52	333	17.4	4.55	0.20
S	222	10.9	4.40	0.29	248	13.4	4.48	0.18
RDC	1,230	91.6	4.40	0.29	248	13.4	4.48	0.18
VC	1,755	288	5.36	0.23	1,775	93.2	5.19	0.19
H	406	37.2	7.44	0.51	482	64.6	7.68	0.42
M+P	621	36.7	3.83	0.22	741	48.9	3.86	0.23
RSB	2,610	182	6.25	0.33	3,017	215	6.54	0.30
Ce	1,024	85.3	4.34	0.25	1,184	60.5	4.39	0.19

Appendix D_{10}

Means and Standard Deviations of RDH and RDL Strains with Respect to GAD Total and Specific Activities in Eight Brain Sections

Section	Strain							
	RDH				RDL			
	GAD Total Activities* (μg/hr)		GAD Specific Activities (μg/hr/g)		GAD Total Activities* (μg/hr)		GAD Specific Activities (μg/hr/g)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	572	35.0	858	89.5	625	49.0	851	28.1
S	522	36.0	1,016	74.2	549	82.2	990	155
RDC	2,656	173	1,012	68.3	3,247	351	1,069	94.5
VC	2,193	378	725	74.8	2,490	262	740	48.0
H	574	41.1	1,163	70.9	693	51.8	1,317	65.4
M+P	1,077	73.3	670	29.7	1,340	101	692	51.3
RSB	5,317	293	1,275	91.8	6,168	285	1,336	62.1
Ce	2,186	367	887	37.6	2,436	291	874	40.3

* $x10^{-2}$

Appendix D_{11}

Means and Standard Deviations of OMB and OMD Strains with Respect to AChE Total and Specific Activities in Eight Brain Sections

Section	Strain							
	OMB				OMD			
	AChE Total Activities ($Mx10^8$/min)		AChE Specific Activities ($Mx10^{10}$/min/mg)		AChE Total Activities ($Mx10^8$/min)		AChE Specific Activities ($Mx10^{10}$/min/mg)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	3,350	370	44.4	3.51	3,375	319	46.5	2.62
S	3,107	361	55.2	4.23	3,020	243	53.9	1.97
RDC	17,984	1,140	57.5	2.69	14,351	1,263	57.9	2.85
VC	33,284	4,001	90.2	10.6	30,839	3,068	89.0	13.2
H	5,563	603	88.0	2.80	5,269	669	89.7	5.37
M+P	18,768	1,071	97.7	3.52	18,323	1,307	97.6	4.20
RSB	11,252	3,245	214.5	11.4	9,808	1,401	216.8	7.83
Ce	9,496	646	34.7	2.46	9,482	996	36.9	2.58

Appendix D_{12}

Means and Standard Deviations of OMB and OMD Strains with Respect to ChE Total and Specific Activities in Eight Brain Sections

Section	Strain							
	OMB				OMD			
	ChE Total Activities ($Mx10^8$/min)		ChE Specific Activities ($Mx10^{10}$/min/mg)		ChE Total Activities ($Mx10^8$/min)		ChE Specific Activities ($Mx10^{10}$/min/mg)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	200	29.2	2.67	0.38	208	29.0	2.85	0.35
S	147	36.0	2.63	0.60	152	31.0	2.71	0.45
RDC	857	118	2.75	0.34	861	66.5	2.89	0.30
VC	1,040	170	2.83	0.21	1,076	381	3.09	0.93
H	326	56.5	5.23	0.59	352	108	5.30	0.71
M+P	955	196	4.61	0.55	877	107	4.64	0.32
RSB	2,358	242	4.50	0.54	2,449	258	5.00	0.34
Ce	980	85.4	3.55	0.33	1,026	132	4.00	0.21

Appendix D_{13}

Means and Standard Deviations of OMB and OMD Strains with Respect to DOPAD Total and Specific Activities in Eight Brain Sections

Section	Strain							
	OMB				OMD			
	DOPAD Total Activities (μg/2hr)		DOPAD Specific Activities (μg/2hr/g)		DOPAD Total Activities (μg/2h4)		DOPAD Specific Activities (μg/2hr/g)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
RDC	40,450	8,488	227	38.0	64,829	10,756	216	36.7
VC	111,570	19,413	303	54.5	91,605	11,752	264	45.7
H	59,752	7,062	965	126	53,498	9,245	905	111
M+P	106,300	12,166	554	50.7	96,100	10,852	512	49.8
RSB	531,383	53,520	1,021	119	43,437	33,742	889	63.1

Appendix D_{14}

Means and Standard Deviations of OMB and OMD Strains with Respect to MAO Total and Specific Activities in Eight Brain Sections

Section	Strain							
	OMB				OMD			
	MAO Total Activities (%/hr)		MAO Specific Activities (%/hr/g)		MAO Total Activities (%/hr)		MAO Specific Activities (%/hr/g)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	346	35.1	4.58	0.26	321	25.1	4.43	0.14
S	250	21.0	4.41	0.11	247	18.2	4.39	0.22
RDC	1,456	72.3	4.63	0.13	1,394	73.2	4.64	0.21
VC	1,928	148	5.21	0.21	1,783	55.7	5.11	0.15
H	460	50.3	7.29	0.56	425	38.3	7.19	0.40
M-P	734	26.7	3.21	0.99	682	70.8	3.78	0.22
RSB	3,363	204	6.41	0.17	3,130	172	6.42	0.28
Ce	1,128	86.6	4.08	0.19	1,085	67.7	4.20	0.17

Appendix D_{15}

Means and Standard Deviations of OMB and OMD Strains with Respect to GAD Total and Specific Activities in Eight Brain Sections

Section	Strain							
	OMB				OMD			
	GAD Total Activities* (μg/hr)		GAD Specific Activities (μg/hr/g)		GAD Total Activities* (μg/hr)		GAD Specific Activities (μg/hr/g)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
V	578	55.5	760	97.4	525	75.0	720	77.7
S	450	26.3	809	62.6	452	95.3	791	120
RDC	4,010	363	1,216	79.5	3,275	313	1,067	58.4
VC	2,782	290	752	57.5	2,592	221	741	40.3
H	690	63.6	1,208	99.7	586	67.0	1,120	53.8
M+P	1,373	70.5	710	61.3	1,294	98.1	689	31.9
RSB	8,093	1,071	1,534	150	6,702	974	1,373	170
Ce	2,648	329	890	84.4	1,947	743	888	31.0

* $x10^{-2}$

Appendix E_1#

Intercorrelations of Biochemical and Morphological Variables for SC and ST Animals of First Group of S_1s

Variable	Group	Body Weight	V Weight	S Weight	TB II Weight	Total Serotonin TB II	ChE Total Activity-V	ChE Total Activity-S	AChE Total Activity-V
V Weight	SC	.66*							
	ST	-.30							
S Weight	SC	-.36	-.09						
	ST	-.22	-.27						
TB II Weight	SC	.79**	.74*	-.35					
	ST	.20	-.01	-.18					
Total Serotonin TB II	SC	.02	.39	.49	.27				
	ST	.08	.12	-.40	-.13				
ChE Total Activity-V	SC	.58	.74*	-.11	.57	.47			
	ST	-.21	.76**	-.48	.33	.29			
ChE Total Activity-S	SC	-.28	.09	.67*	-.37	.31	.18		
	ST	-.29	.15	.60	.30	-.21	.32		
AChE Total Activity-V	SC	.45	.83**	-.06	.44	.18	.66*	.31	
	ST	-.20	.94**	-.25	-.08	.06	.73*	.22	
AChE Total Activity-S	SC	-.38	-.07	.62	-.45	-.17	-.18	.58	.27
	ST	-.13	.03	.44	-.21	-.73*	-.17	.38	.27

* $p \leq .05$
** $p \leq .01$
\# Note-Appendices E_1 to E_3 contain separate intercorrelation matrices of biochemical and morphological variables for SC and ST animals of first and second groups of S_1s and group of S_3s. Pertinent information concerning these animals can be found in Chapter IV, pp. 152-199.

Appendix E_2

Intercorrelations of Biochemical and Morphological Variables for SC and ST Animals of Second Group of S_1s

Variable	Group	Body Weight	V Weight	S Weight	TB II Weight	Total Serotonin TB II	ChE Total Activity-V	ChE Total Activity-S	AChE Total Activity-V
V Weight	SC	.05							
	ST	.72*							
S Weight	SC	.21	.49						
	ST	.73*	.67*						
TB II Weight	SC	.85**	.23	.36					
	ST	.89**	.54	.77*					
Total Serotonin TB II	SC	.31	.04	-.02	.59				
	ST	--	--	--	--				
ChE Total Activity-V	SC	.23	.72*	.01	.19	.06			
	ST	.66*	.96**	.69*	.52	--			
ChE Total Activity-S	SC	-.03	.57	.75*	.25	-.17	.07		
	ST	.57	.44	.65*	.66*	--	.28		
AChE Total Activity-V	SC	.13	.71*	.06	.29	-.29	.50	.41	
	ST	.63*	.94**	.51	.48	--	.72*	.38	
AChE Total Activity-S	SC	.23	.07	.74*	.32	-.16	-.35	.21	-.31
	ST	.55	.48	.69*	.63*	--	.31	.29	.47

* $p \leq .05$
** $p \leq .01$

Appendix E_3

Intercorrelations of Biochemical and Morphological Variables for SC and ST Animals of Group of S_3s

Variable	Group	Body Weight	V Weight	S Weight	TB II Weight	Total Serotonin TB II	ChE Total Activity-V	ChE Total Activity-S	AChE Total Activity-V
V Weight	SC	.11							
	ST	.42							
S Weight	SC	-.16	.54*						
	ST	.01	.11						
TB II Weight	SC	.68**	.25	.16					
	ST	.69**	.08	.37					
Total Serotonin TB II	SC	-.01	-.32	-.04	.06				
	ST	--	--	--	--				
ChE Total Activity-V	SC	.03	.83**	.41	.35	-.31			
	ST	.42	.86**	-.03	-.14	--			
ChE Total Activity-S	SC	-.61*	-.02	.54*	-.35	.34	.01		
	ST	.64**	.58*	.45	.48*	--	.47		
AChE Total Activity-V	SC	.35	.39	-.07	.15	-.50*	.10	-.53*	
	ST	.16	.72**	.11	-.23	--	.63*	.21	
AChE Total Activity-S	SC	.50*	.38	.11	.43	-.36	.05	-.52*	.89**
	ST	.45	.05	.54*	.66**	--	.08	.22	-.04

* $p \leq .05$
** $p \leq .01$

Appendix F#

Intercorrelations of Behavioral Variables for ST Animals of First and Second Groups of S_1s

Variable	Group	Open-Field Defecations	Open-Field Urinations	Revolving Wheel Activity, Days	Revolving Wheel Activity, Nights	Colony-Cage Activity, Days	Colony-Cage Activity, Nights	Dashiell Activity	ATLAS Total Errors to Criterion
Open-Field Urinations	1st	.50							
	2nd	.54							
Revolving Wheel Activity, Days	1st	-.56*	-.30						
	2nd	.28	.02						
Revolving Wheel Activity, Nights	1st	-.13	-.47	.13					
	2nd	.24	-.22	.78**					
Colony-Cage Activity, Days	1st	--	--	--	--				
	2nd	.09	.45	-.26	-.42				
Colony-Cage Activity, Nights	1st	--	--	--	--	--			
	2nd	.11	-.20	.44	.48	.18			
Dashiell Activity	1st	.42	.25	.08	-.16	--	--		
	2nd	-.53	-.19	-.20	.08	-.32	-.25		
ATLAS Total Errors to Criterion	1st	.07	.43	-.49	-.30	--	--	-.08	
	2nd	.35	-.28	-.12	.05	-.46	.03	-.31	
ATLAS Total Trials to Criterion	1st	-.16	.24	-.35	-.15	--	--	-.05	.93**
	2nd	.30	-.45	-.06	.04	-.36	.21	-.48	.93**

Appendix F (Contd.)

Variable	Group	Open-Field Defecations	Open-Field Urinations	Revolving Wheel Activity-Days	Revolving Wheel Activity-Nights	Colony-Cage Activity-Days	Colony-Cage Activity-Nights	Dashiell Activity	ATLAS Total Errors to Criterion	ATLAS Total Trials to Criterion
ATLAS Total Cumulative Latencies to Criterion	1st	-.02	.33	-.13	-.29	--	--	.10	.85**	.80**
	2nd	.40	-.15	.08	-.07	-.34	.18	-.51	.93**	.88**

* $p \leq .05$
** $p \leq .01$
\# Note-Information concerning these animals can be found in Chapter IV, pp. 152-199.

Appendix G#

Correlations of Morphological and Biochemical Variables with Behavioral Variables for ST Animals of First and Second Groups of S_1s

Variable	Group	Open-Field Defecations	Revolving Wheel Activity, Days + Nights	Colony-Cage Activity, Days + Nights	Dashiell Activity	ATLAS Total Errors to Criterion	ATLAS Total Trials to Criterion	ATLAS Total Cumulative Latencies to Criterion
Body Weight	1st	.78**	-.63*	--	.32	.19	-.07	.08
	2nd	.10	-.21	.42	-.42	-.14	-.38	-.41
V Weight	1st	.33	-.36	--	-.09	.33	.01	.39
	2nd	-.06	-.45	-.47	-.02	.15	.18	.09
S Weight	1st	-.44	.04	--	-.21	.69*	.83*	.68*
	2nd	.37	-.48	-.18	-.41	.75**	.73**	.59*
TB II Weight	1st	.62	-.13	--	.22	-.08	-.34	-.07
	2nd	.36	.00	.38	-.55*	.08	-.19	-.18
Total Serotonin TB II	1st	-.38	.21	--	-.40	.31	.32	.36
	2nd	.47	.52	.34	-.45	.14	-.03	.03
Serotonin Concentration TB II	1st	-.70*	.27	--	-.51	.35	.50	.37
	2nd	.31	.63*	.10	-.11	.13	.14	.21
ChE Total Activity-V	1st	.16	-.49	--	-.50	.19	-.07	.05
	2nd	-.20	-.20	-.46	-.03	-.21	-.19	-.24
ChE Total Activity-S	1st	-.39	-.12	--	-.54	.48	.51	.53
	2nd	.15	-.26	-.15	-.52	.73**	.64*	.70*
AChE Total Activity-V	1st	.37	-.41	--	-.33	.42	.11	.45
	2nd	-.16	-.32	-.07	-.07	-.29	-.33	-.21
AChE Total Activity-S	1st	-.09	-.16	--	-.26	.59*	.58*	.53
	2nd	.52	-.32	.07	-.09	.47	.49	.27
ChE Specific Activity-V	1st	-.17	-.28	--	-.66*	-.10	-.11	-.39
	2nd	-.18	-.24	-.10	-.05	-.47	-.48	-.44

Appendix G (Contd.)

		Open-Field Defecations	Revolving Wheel Activity-Days + Nights	Colony-Cage Activity-Days + Nights	Dashiell Activity	ATLAS Total Errors to Criterion	ATLAS Total Trials to Criterion	ATLAS Total Cumulative Latencies to Criterion
ChE Specific Activity-S	1st	-.15	-.20	--	-.56*	.03	-.05	.11
	2nd	-.17	.10	-.05	-.36	.34	.23	.48
AChE Specific Activity-V	1st	.23	-.27	--	-.48	.31	.16	.27
	2nd	-.17	.09	.45	-.09	-.57*	-.67	-.40
AChE Specific Activity-S	1st	.27	-.20	--	-.12	.09	-.06	.02
	2nd	.35	.14	.32	.35	-.22	-.16	-.31

* $p \leq .05$
** $p \leq .01$
\# Note-Information concerning these animals can be found in Chapter IV, pp. 152-199.

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