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**THE INDUCTION OF BRAIN BIOCHEMICAL
CHANGES IN THE RAT BY ENRICHMENT**

by

George A. S. Park

A thesis submitted to the Faculty
of Graduate Studies and Research
in partial fulfillment of the
requirements for the degree of

Master of Science

Department of Psychology
Carleton University
Ottawa, Ontario
June, 1993.

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**THE INDUCTION OF BRAIN BIOCHEMICAL
CHANGES IN THE RAT BY ENRICHMENT**

submitted by George A. S. Park in
partial fulfillment of the requirements
for the degree of Master of Science

Thesis Supervisor

Chair, Department of Psychology

Carleton University
June, 1993.

ABSTRACT

The present thesis investigated the combined effect of learning and enriched rearing on brain choline acetyltransferase (ChAT) activity. In the two experiments reported here, 30 day-old male Long-Evans rats were reared in either enrichment (ENR) or impoverishment (IMP) for 50 days. Following rearing, rats were tested for one week in the Morris water maze task and then allowed to rest one further week. Naive rats, both ENR- and IMP-reared, served as controls.

The first experiment investigated the effect of learning and ENR on ChAT activity. It was found that in the caudate, ENR rats showed elevated ChAT activity as compared to IMP-naive rats. IMP-tested rats also showed elevated ChAT activity as compared to IMP-naive rats and had intermediate ChAT activity as compared to ENR rats. It was also found that ENR-tested rats showed a significant environment by testing interaction and a near-significant elevation of ChAT activity as compared to all other groups, suggesting a priming effect of ENR rearing on training.

The second experiment investigated the possibility that non-associative stress effects induced the observed changes. The design was identical to experiment one, except for the addition of a yoked control group, which could not escape from the Morris water maze. The results of this experiment

replicated the caudate findings of the first experiment except that the IMP-tested rats had ChAT activity elevation comparable to that of ENR rats. Findings in the frontal cortex revealed that the interaction between environment and testing failed to reach significance.

In summary, the combination of ENR and training in the Morris water maze induced persistent (at least one week) elevations in ChAT activity in the caudate, and an increase in frontal cortical ChAT activity. This increase in ChAT activity may indicate priming in the cholinergic system in response to a learning experience.

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The Induction of Brain Biochemical Changes by Enrichment

INTRODUCTION

One of the central issues of biological psychology has been the demonstration of a persistent change in the anatomy of the brain or of its chemistry in response to learning. As early as 1917, the neurophysiologist Karl Lashley began a systematic search for the "engram", the hypothesized biochemical structure that contained memories. While Lashley's attempts to locate the engram ultimately failed, the idea that memory is stored in some biochemical or anatomical structure persists even today and is the working theory for the study of learning and memory.

Work on Lashley's idea of the engram was continued by Donald Hebb in the mid 1940's. Hebb was interested in maze learning by rats. He found that rats raised in what he called "free environments" (essentially a large cage filled with many junk objects, where rats were raised together in groups of 10-12), were superior learners as compared to the standard laboratory conditions (Hebb, 1947).

This single observation has led to a great deal of study on the effects of "enrichment" on the brain and on learning. In their major review of this area, Renner and Rosenzweig (1987, p. 4) have defined the enrichment phenomenon (ENR or EC) as: "... Rats raised in a complex, challenging environment (designated as 'enriched condition' or EC) develop reliable differences in some brain measures and some aspects of behaviour from littermates placed in a relatively less

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stimulating environment (designated as 'impoverished condition' or IC).."

The earliest modern work connecting ENR-rearing to brain physiology and anatomy dates back to the 1960's when Krech, Rosenzweig, Bennett, and Diamond, working at the University of California at Berkeley, demonstrated that ENR increased an animal's cholinesterase (ChE) activity in the brain as compared to IMP controls (Krech, Rosenzweig, and Bennett, 1960). This group also reported that the ratio of cerebral acetylcholine (ACh) concentration to the activity of the enzyme acetylcholinesterase (AChE) is positively related to problem solving in rats--that is, the higher the ratio, the better the learning scores of the rat (Rosenzweig, Krech, and Bennett, 1960). In a subsequent study, this result was replicated and clarified: ENR rats have lower ChE activity in the sensory cortex and higher ChE activity in the subcortex (basically, the remaining cortex). It was also found that the sensory cortex of ENR rats was 4-5% heavier than that of IMP rats (Rosenzweig et al., 1962). AChE activity was also significantly increased in ventral and dorsal (excluding somaesthetic) cortex in ENR rats (Bennett et al., 1964). These authors reported no brain differences between ENR and IMP rats for total protein, hexokinase, serotonin, and adrenal weight.

The possibility that these differences were caused by isolation stress in the IMP rats was raised by Geller and co-workers (1965).

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They replicated the experiments of Rosenzweig and colleagues, and found that norepinephrine (NE) concentration and total brain NE were significantly higher in IMP rats. They also found that both liver and adrenal weight of ENR rats were lower than IMP rats, when corrected for body weight. It was found that IMP rats had elevated liver tyrosine transaminase and tryptophan, an indication of earlier activation of the adrenal system in response to stress. This study is confounded by the fact that the rats used in the study were weaned at 19 days of age, which, in of itself, may have contributed to the stress. They also reported that ENR rats were heavier than IMP rats, a finding contrary to that of Bennett et al. (1964).

Subsequently, it was found that rats housed in ENR and later tested in behavioural tasks had 6.2% greater occipital cortical thickness and lower neuron density than IMP-reared rats (Diamond, Krech, and Rosenzweig, 1964). This result has recently been replicated in cats (Beaulieu and Colonnier, 1985). Cortical thickness changes have also been reported in motor and somaesthetic cortex (Rosenzweig, Bennett, and Diamond, 1972a). There is also an increased glial cell count in ENR rats, particularly of the oligodendrocytes, and thus a reduction of the neuron/glia ratio in ENR rats (Diamond et al., 1966). Additionally, there have been reports of a smaller, later increase in astrocytes in ENR rats (Szelingo, 1971; Szelingo and Leblond, 1977). These data imply that there is higher cortical neuron activity in ENR rats as the increased glia concentrations are indicative of an

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increased supportive cell network for the neurons.

Preliminary examinations of fibre tracts in differentially reared rats showed the corpus callosum underlying the occipital cortex in ENR rats was thicker (Szelingo and Leblond, 1977), and had greater cross-sectional area (Juraska and Meyer, 1986). As well, ENR-reared rats have a significantly lower density of unmyelinated axons in the corpus callosum (Kopcik, Juraska, and Washburne, 1986).

The changes observed in neuron density mainly reflect increased dendritic ramifications. ENR rats have consistently more higher-order dendritic branching than IMP rats. In cortical pyramidal neurons (layers II, IV, and V), and stellate neurons (layer IV), dendritic branching was significantly increased in ENR rats as compared to IMP rats (Volkmar and Greenough, 1972). These ENR/IMP differences became more pronounced as the order of dendritic branching increased (Greenough, Volkmar, and Juraska, 1973). Closer examination of the dendritic branching revealed that the density of dendritic spines is increased in ENR rats as compared to IMP rats (Globus et al., 1973). This increased ramification of the dendritic tree has the effect of greatly increasing the number of synapses per neuron, or at the least, increases the total synaptic surface area. Electron microscopic work has confirmed this observation. ENR rats have been shown to have higher synapse/neuron ratios (Bhide and Bedi, 1984), and the number of synapses per neuron in layers I-IV of the occipital cortex is higher in

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ENR rats (Turner and Greenough, 1983; 1985). The numerical density of axon boutons is also greater in ENR rats as compared to IMP rats (Sirevaag and Greenough, 1988).

A recent study by Kolb and Gibb (1991) examined the effect of differential rearing on rats receiving either large unilateral or large bilateral cortical lesions. Behavioural consequences of these lesions typically included chronic reduction in body weight, reduced food hoarding behaviour, decreased tongue extension, and impairment in the Morris water maze (Kolb and Gibb, 1991). ENR was found to attenuate many of these changes, but it was found that it had similar effects on both normal and lesioned rats. Examination of brain weight and cortical dendritic branching revealed that enrichment induced the usual changes in both normal and lesioned rats, and that ENR rats with no lesions showed increased dendritic branching in parietal cortex (Kolb and Gibb, 1991).

The effects of ENR are not restricted to the cortex. There is a report showing increased hippocampal thickness in ENR rats (Walsh et al., 1969), but only weak evidence of weight changes in hippocampus (Rosenzweig and Bennett, 1978; Jones and Smith, 1980a; Jones and Smith, 1980b). The Jones and Smith work, however, is not directly comparable to the bulk of ENR work as their ENR condition was not particularly enriching and their IMP condition was merely social isolation. [One other type of environmental manipulation should be mentioned here. In

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this condition, called Social Condition or SC), rats are housed in groups as in ENR, but with no toys or junk objects.] Katz and Davies (1983) reported no significant differences in hippocampal cross-sectional thickness, but the total area of the cross-sections was found to be significantly increased in ENR rats as compared to IMP rats. ENR rats have a significantly higher number of granular cells in the dentate gyrus (Susser and Wallace, 1982), and increased synaptic density in CA3 (Altschuler, 1979). In juvenile (but not adult) rats, dendritic branching and overall size of the dendritic field of the dentate gyrus was higher in ENR rats (Fiala, Joyce, and Greenough, 1978).

Initial research showed that only a brief exposure to enrichment (as little as two hours per day) can induce the observed changes in brain weight and AChE activity (Rosenzweig, Love, and Bennett, 1968). It was also recently reported that a mere 40 minutes of ENR experience for 4 days was capable of significantly increasing both cortex weight and cerebral RNA content of 30-40 day-old ENR-reared rats (Ferchmin and Eterovic, 1986).

NEUROCHEMICAL EFFECTS OF ENRICHMENT

As detailed above, biochemical changes induced by differential rearing are well documented. ENR-reared rats have been found to have enhanced cortical cholinesterase (ChE) activity as compared to IMP rats (Rosenzweig et al., 1962), even when corrected for brain weight

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differences (Rosenzweig, Bennett, and Diamond, 1972b). Initial reports revealed that in ENR rats, acetylcholinesterase (AChE) activity in the cortex and subcortex was greater than in IMP rats (Bennett et al., 1964). Later replication of this work revealed a more complex picture--the pattern observed in ENR rats was a decrease in AChE activity per unit weight in the cortex and increased ChE activity throughout the brain; the ChE/AChE ratio was increased in the cortex (Rosenzweig et al., 1972a). There is also a general trend towards higher total brain AChE in ENR rats (Rosenzweig et al., 1972b). These differences are influenced by certain drugs: ENR rats given methamphetamine or d-amphetamine showed enhanced ENR effects. For example, the weight of the cortex in methamphetamine-ENR rats was significantly higher than saline-ENR rats, and the ChE/AChE ratio of methamphetamine-ENR rats in the occipital cortex was higher than saline-ENR rats but this result just failed to reach significance. These effects were probably due to the drugs' influencing the animals' reactions to the environment (Bennett, Rosenzweig, and Wu, 1973). Phenobarbital administration depressed the usual brain weight changes, but enhanced the AChE/ChE changes (Bennett, Rosenzweig, and Wu, 1973).

Changes in other neurotransmitter systems have also been investigated in connection with the observed ENR/IMP phenomenon. Whole brain norepinephrine (NE) was increased in IMP rats (Geller et al., 1965), and NE was found to be increased in the cortex of ENR rats, yet NE was found to be decreased in the hypothalamus and caudate of ENR

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rats (Riege and Morimoto, 1970). A study by Walker (1971) reported that IMP (as compared to SC) rats showed a decrease in NE in the caudate and cerebellum. This work has been disputed by recent reports--no environmental differences were found in cortical NE levels (Renner et al., 1986), nor in alpha and beta-NE receptor binding sites (Por et al., 1982).

Interest in NE was increased following the landmark studies by Kasamatsu and Pettigrew (1976, 1979), which showed that ocular dominance shifts in monocularly deprived kittens failed to occur in kittens receiving 6-hydroxydopamine (6-OHDA), a neurotoxin which causes central NE depletion. This work led to a series of studies which investigated the role of NE in brain plasticity. The ENR phenomenon seemed like a likely candidate for such investigations. In a study by Ellison and Bryan (1976), rats were given intraventricular 6-hydroxydopamine and raised in differential environments. IMP-reared rats were hyper-irritable, showed greater rearing in open field testing, and showed hyper-responsivity to d-amphetamine. The ENR rats showed none of these deficits, and showed some recovery of the behavioural deficits exhibited by the IMP-reared rats (Ellison and Bryan, 1976). In a similar study, it was reported that ENR rats showed progressive improvement in behaviour (less frequent fighting, running wheel activity patterns, order of passage through a narrow tube to the feeding area, and less fighting over food with other rats) as compared to SC rats (Díaz, Ellison, and Masuoka, 1978). In this study, it was

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paradoxically noted that SC rats recovered NE uptake more quickly than ENR rats.

In a study by O'Shea and colleagues (1983), rats received neonatal 6-OHDA lesions and were later reared in ENR and IMP environments. Rats receiving the NE depletion and raised in ENR were indistinguishable from NE-depleted IMP-reared rats or vehicle-IMP-reared rats, as assessed by behavioural testing in the Lashley-III maze, total cortical weight, hypothalamic weight, and dopamine content in posterior cortex and hypothalamus. These authors conclude that NE plays a permissive role in the behavioural, biochemical, and neuroanatomical changes induced by differential rearing (O'Shea et al., 1983). A subsequent study also revealed that neonatal NE lesions eliminated the behavioural effects of ENR on Lashley III and Hebb-Williams maze learning (Pappas et al., 1987). In contrast however, a subsequent experiment found no effect of NE depletion on ENR in a subsequent study (Murtha, Pappas, and Raman, 1990). Recent work by Kolb and Sutherland (1992) has examined the role of NE in recovery from cortical lesions. Rats which receive cortical aspiration lesions on post-natal day 7 normally show recovery of spatial localization, but if rats are depleted of cortical NE by the neurotoxin 6-OHDA failed to show the normal behavioural recovery (Kolb and Sutherland, 1992). Thus, it seems that NE is implicated in this response. The exact influences of NE on enrichment remains unresolved.

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The changes induced by enrichment on the dopamine (DA) system have been less well studied. The levels of DA were found to be increased in the caudate in IMP rats (Walker, 1971). Isolation rearing was found to reduce levels of DA metabolites in frontal mesocortical areas (Blanc et al., 1980, Holson, Ali, and Scallet, 1985). These experiments did not look at the effects of enrichment, however. A recent report indicated that DA content in the occipital cortex was increased in ENR rats (Renner et al., 1986), but no differences in DA-receptor binding have been detected (Por et al., 1982). Long-term isolation has been shown to induce enhanced DA sensitivity in the striatum (after three months) and in both striatum and frontal cortex (after 12 months) (Oehler, Jahkel, and Schmidt, 1987). The control group in this experiment was SC and not ENR, however. Elevated DA activity in the hypothalamus and posterior cortex of ENR rats has been reported (O'Shea et al., 1983). This result is in contrast with that of Riege and Morimoto (1970), who found a significant 6% decrease in the combined caudate/hypothalamus after ENR rearing. Clearly, further work in this area is needed to resolve the issue.

Selective dopamine lesions in neonates can be induced by using 6-OHDA in conjunction with desmethylinipramine (DMI), a NE re-uptake blocker. Rats given a selective DA lesion were later raised in ENR and IMP. It was shown that DA depletion by 6-OHDA had no effect on cortical thickness or on the increased cortical thickness caused by ENR rearing (Pappas et al., 1992). It was also found that ENR eliminated

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the spatial learning and memory deficits induced by the lesion (Pappas et al., 1992).

Other reports of biochemical changes induced by enrichment are somewhat sparse. No differences in whole brain serotonin (5-HT) levels were observed in initial investigations (Bennett et al., 1964; Geller et al., 1965). This work has subsequently been confirmed by recent reports (Por et al., 1982; Renner et al., 1986). 5-HT turnover was found to be increased in the hippocampus of IMP-reared rats (Renner et al., 1986). Isolation rearing has also been reported to decrease opiate receptor binding in the rat brain (Schenk et al., 1982). ENR rats have been found to have elevated glutamate receptor binding in cortex, caudate, and hippocampus as evaluated by quantitative receptor autoradiography (Park, 1989).

The total RNA/DNA ratio in ENR rats has been found to be higher than that of IMP rats (Ferchmin, Eterovic, and Caputto, 1970). Following 30 days of enrichment, cortical RNA showed a greater diversity in ENR-reared rats as compared to IMP-reared rats, while liver RNA from the same subjects showed no such differences (Grouse et al., 1978).

Protein synthesis has also been implicated in the enrichment phenomenon. Although initial studies revealed no brain differences in protein content (Bennett et al., 1964), subsequent research has

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revealed that inhibition of putrescine synthesis, which results in an inhibition of cellular proliferation, has no effect on ENR/IMP effects on cortical weight (Ferchmin and Eterovic, 1987). Thus, cellular multiplication is not a factor in the cortical weight gain. This strongly suggests protein synthesis must be implicated in the ENR phenomenon. Polyamines have been implicated in RNA, DNA, and protein synthesis (Marton and Morris, 1987). Rats given a polyamine synthesis inhibitor were exposed to 7 hours of ENR for three days. It was found that ENR was still able to induce the weight and spermidine content of the frontal cortex, and yet putrescine content of the occipital cortex was inhibited. Non-cortical areas were not affected by the drug (Ferchmin and Eterovic, 1990). This result suggests that protein synthesis still occurs in the rats. The influence of enrichment on protein synthesis still remains unclear.

The effect of enrichment on nerve growth factor (NGF) has recently been investigated. Both adult (3 month-old) and juvenile (2 month-old) rats were exposed to differential housing for 30 days. Some of these rats were then given testing in the Morris water maze. Rats were then sacrificed for assay of NGF levels. It was found that exposure to testing caused small but significant increases in NGF in the hippocampus and septum in IMP rats. Rats which did not receive behavioral testing did not show any differences (Mohammed et al., 1990). In a follow-up study, these researchers reported that exposure to Morris water maze testing in rats receiving differential housing

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induced an increase in brain-derived neurotrophic factor (BDNF) mRNA expression in the hippocampus. ENR-tested animals had significantly higher BDNF mRNA than any of ENR-untested, IMP-tested, and IMP-untested rats (Falkenberg et al., 1992). IMP-tested rats had a similar, but non-significant, increase in BDNF mRNA in the hippocampus. This result suggests that the ENR had a priming effect on the hippocampus, since the hippocampus responded to training with an increase in BDNF. Given this result, coupled with the observed biochemical changes caused by ENR, the combined effects of learning and environment bears further investigation.

ACETYLCHOLINE AND LEARNING

As has been outlined earlier, the cholinergic system may be implicated in the ENR phenomenon. Acetylcholine (ACh) is also believed to be implicated with learning and memory in the rat. A landmark paper by Bartus and co-workers (1982) introduced the so-called "Cholinergic Hypothesis of Memory Dysfunction". This theory states that one of the major factors in age-related central nervous system dysfunction and cognitive loss is the degeneration of the cholinergic system in the brain (Bartus et al., 1982). The type of learning and memory deficits present in patients with Alzheimer's Disease (AD) has been strongly correlated with the degree of cholinergic deficit, and with decreased ChAT activity specifically (Bowen et al., 1976; Davies and Maloney, 1976; Perry et al., 1978). These AD deficits are associated with cell loss in the nucleus basalis magnocellularis (NBM) and plaquelike

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lesions in many different brain areas (Arendt et al., 1985; Jacobs et al., 1985).

DRUG EFFECTS

If ACh is implicated in cognitive decline in AD, it is reasonable to propose that ACh is implicated in learning and memory. Considerable evidence in support of this hypothesis exists. As early as 1960, it was demonstrated that brain ChE in rats trained on more difficult learning tasks was higher than in quiet controls or in rats trained on simple shuttle box or T-maze tasks (Rosenzweig, Krech, and Bennett, 1960). Historically, investigations of the cholinergic system have been conducted in one of two ways: lesion studies, or the administration of cholinomimetic drugs (either agonists or antagonists). Both methods will be briefly reviewed below.

Pharmacological studies using the cholinergic antagonists scopolamine and atropine have strongly supported the implication of ACh in learning and memory processes. Cholinergic antagonists given prior to training impairs performance at subsequent retention trials in rats trained in passive avoidance tasks (Buresova et al., 1964; Meyers, 1965; Feigley, 1974; Blozovski and Hennocq, 1981). Pre-training injections of atropine have been found to severely impair the acquisition of discrimination learning of rats in the T-maze (Whitehouse, 1964). This impairment seems to be correlated with the complexity of the learning environment, as atropine-induced impairment

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was not seen when a single visual, auditory, or tactile cue was present, but was observed when there were multiple or irrelevant cues (Whitehouse, 1967). It is reasonable to predict that rats with experience with complex environments, such as ENR, would not show this deficit. Large doses of atropine cause impairments of both a spatial and visual (but non-spatial) discrimination task (Hagan et al., 1986).

Cholinergic antagonists also have effects on active avoidance tasks, with the effect being dependent on the specific task. For example, one-way avoidance in a shuttle box is impaired with scopolamine (Suits and Isaacson, 1968), but is improved in a 2-way shuttle box avoidance learning task (Suits and Isaacson, 1968; Worsham and Hamilton, 1973). The exact reasons for this discrepancy is not known. Cholinergic antagonists also disrupt spontaneous alternation on the T-maze (Meyers and Domino, 1964), reducing it to chance levels (Squire, 1969; Leaton and Utell, 1970; Kokkinidis and Anisman, 1976). Heise et al. (1976) trained rats to respond to either right or left levers on alternate trials to receive a food reward. Scopolamine-treated rats showed a dose-dependent reduction in response accuracy. Radial arm maze learning is also impaired in rats receiving pre-training scopolamine injections during task acquisition (Stevens, 1981; Watts et al., 1981; Hiraga and Iwasaki, 1984). Rats pre-trained with atropine sulphide (but not atropine methyl nitrate) failed to learn the location of the hidden platform in the Morris water maze (Sutherland et al., 1982). It was also found that selective blockade of muscarinic

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m₁-ACh receptors caused a profound impairment in place navigation learning (Hagan et al., 1987).

One major potential confound in drug studies is that the drug may cause performance deficits independent of any actual effects on memory itself. One study by Miller and co-workers (1971) showed that either scopolamine or physostigmine (a cholinergic agonist which inhibits AChE), administered immediately after T-maze training, reduced the rat's subsequent performance to chance levels. They did report on many occasions that these trained rats failed to eat the food pellets on rewarded trials. It is thus possible that the post-trial injections of the drugs, but not specifically the pharmacological effects of the drugs, induced a gradually increasing food aversion. This possibility was confirmed in a study by Preston et al. (1984), who demonstrated conditioned taste aversion using atropine injections as the unconditioned stimulus.

There is also the possibility that cholinergic systems are involved in sensory and attentional mechanisms. In experiments where changes in learning rate are studied using cholinomimetics, a sensory/attentional deficit may result, providing an alternate hypothesis to the learning and memory hypothesis (Hagan and Morris, 1988). An important factor may be whether or not the rat is drug-free at the time of the retention testing. Overton (1966) showed that a drug-induced state may be an important stimulus during training. An

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apparent retention deficit under drug-free testing may thus reflect state-dependent learning rather than anterograde amnesia.

One major problem with the drug studies described above is that the drugs were administered peripherally. Thus, possible peripheral side effects tend to confound interpretation of the results. More recent studies have used intra- cranial or intraventricular drug injections, which give much less ambiguous results. In one such study, Flood and co-workers (1981) used mice which were trained to avoid shock preceded by a buzzer in a T-maze task. Immediately after training, the mice were anaesthetized and given intraventricular drug or saline injections, and retrained to criterion. Post-training inhibition of ACh synthesis by hemicholinium-3, blockade of muscarinic ACh receptors, or blockade of nicotinic ACh receptors in the brain all exerted a dose-dependent impairment of retention. Facilitation of cholinergic activity with agonist drugs enhanced performance at intermediate, but not high, doses (Flood et al., 1981).

Using a similar technique, some researchers have chronically implanted cholinomimetic drug crystals in the brain of rats. Placement of crystalline scopolamine in the dorsal caudate (a brain region densely populated with ACh interneurons) impaired two-way active avoidance, but if the crystals were placed in the ventral caudate, learning was improved (Neill and Grossman, 1970). Placement of carbachol crystals (a cholinergic receptor agonist) in the caudate

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virtually eliminated avoidance retention in rats trained to drink from a water spout (Deadwyler et al., 1972). In a follow-up study, Haycock et al. (1973) injected scopolamine intracranially immediately following avoidance training in rats having carbachol implants. They found that scopolamine impaired retention in unoperated controls, but not in the carbachol-implanted controls. Physostigmine slightly (but not significantly) improved performance in these rats.

In rats trained to lever-press for milk reward, post-training injection of atropine into the anterior caudate impaired retention of inhibitory avoidance, but neither pre-training nor post-training atropine injections in the posterior caudate influenced retention (Prado-Alcala et al., 1984a). To be effective, the post-training injections must be conducted very quickly after training (less than seven and a half minutes) (Prado-Alcala et al., 1981). The avoidance deficit is seen only if the retention test is delayed for 24 hours. If the animals are tested 30 minutes after drug administration, no effect is seen (Prado-Alcala et al., 1984b). These findings suggest that ACh neurons in the anterior caudate may be important for the consolidation of new information into a learning/memory trace.

LESION EFFECTS

The literature on the effects of cholinergic lesions on learning and memory is fairly extensive, but most of the work has come in the last decade. NBM lesions with the excitotoxin ibotinic acid disrupt

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the acquisition of place navigation in the Morris water maze task (Whishaw et al., 1985; Dunnett et al., 1985). Rats trained pre-operatively on a mixed working memory and reference memory T-maze task who received either electrolytic or ibotenic acid NBM lesions were impaired on only the working memory component of the task (Hepler et al., 1985). NBM lesions were also studied in an experiment by Murray and Fibiger (1985). The lesion caused a loss of reference memory in the radial arm maze task. Over 6 weeks of post-operative testing, correct arm entries rose by 86% in the controls but by only 62% in the NBM-lesioned rats. Furthermore, physostigmine, injected prior to a training session, improved performance in the NBM-lesioned rats. There was also a persistent decrease in reference memory errors in the control, but not the NBM-lesioned, rats (Murray and Fibiger, 1985). This data seems to support a role for the NBM in reference memory deficits. There is also a report of the effect of hippocampal lesions on cholinergic function; fimbria-fornix lesions in the rat selectively disrupt working memory (Olton and Papas, 1979).

There are major problems with lesion studies, however. One cannot help damaging other brain structures when doing either aspiration or electrolytic lesions; or if one uses excitotoxic drugs, one cannot rule out the possibility of non-specific damage. There is also the problem of neuron sprouting or outgrowth in the case of excitotoxic lesions. One major problem with studying the cholinergic system is the lack of a specific cholinergic neurotoxin. This makes it difficult to

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conclusively demonstrate that the observed deficits are solely due to the destruction of cholinergic neurons.

A recent review by Dunnett and co-workers (1991) had addressed the issue of excitotoxic lesions and the cholinergic hypothesis of learning and memory. Dunnett and colleagues particularly emphasize a recent study by Page et al. (1991), where the neurotoxin AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) was injected into the NBM. ChAT activity in the cortex was reduced by more than 70% while sparing the dorsal palladium and other non-cholinergic basal forebrain neurons. Despite this dramatic cholinergic cell loss, there were no effects on acquisition or retention in the Morris water maze task. Clearly, the lesion work, due to its lack of specificity, is not very useful in answering the question of the involvement of acetylcholine in learning and memory.

LEARNING AND CHOLINERGIC FUNCTION

Recent studies have looked at correlations between ChAT activity and learning, particularly in aged animals. In a study by Hellweg and colleagues (1990), levels of nerve growth factor (NGF) and ChAT activity were measured in young and aged rats exposed to the Morris water maze task. It was observed that there was no correlation between NGF levels and impairment, but it was noted that there was decreased ChAT activity in the striatum and brainstem of aged impaired rats. Curiously, these rats had elevated ChAT activity in the cerebellum

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(Hellweg et al., 1990). A study by Gallagher et al. (1990) further studied this phenomenon. In this study, both aged and young rats were trained in place learning in the Morris water maze. Aged rats with no behavioural impairments were found to be indistinguishable from young controls, but aged impaired rats had decreased ChAT activity in the basal forebrain, striatum, and frontal cortex. A cluster analysis revealed that decreased ChAT activity in the basal forebrain and striatum appeared to be the best predictors of spatial learning impairments in these rats (Gallagher et al., 1990).

One other method of assessing the impact of ACh on learning and memory is the observation of changes in the cholinergic system following training on learning and memory. The changes in brain ChE and AChE have been mentioned above in the context of the enrichment phenomenon. These findings are extended by the work of Luine and Hearn (1990), who found decreased ChAT activity in the vertical diagonal band, dentate gyrus, and striatum of rats trained in the radial arm maze task.

An experiment by Nakamura and Ishihara (1989) looked at the effect of maze training on ChAT and AChE activity in young and aged rats. In this study, rats were trained in both a two-way shuttle box active avoidance task and swimming in a water-filled T-maze. Aged rats were significantly impaired in these tasks, yet they showed significantly

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elevated ChAT activity in the frontal cortex after five days of training on the shuttle-box task, and they also showed significant elevation of hippocampal ChAT activity after 6 days of training in the water-filled T-maze. None of these changes were observed in the young rats after either 2 or 5 days of active avoidance, or in the adult rats after 10 days of training (at which point their performance had matched that of the young animals). These authors also reported significantly lower frontal cortical and hippocampal AChE activity in naive aged rats as compared to naive or tested young control rats. Following the training described above, the AChE activities of aged and young controls were indistinguishable.

It is believed that choline uptake is the rate limiting step in ACh synthesis (Raaijmakers, 1982). To study choline uptake, one must look at high-affinity uptake (HACU) in synaptosomes. Research in HACU dates back to the late 1970's, where Raaijmakers (1978) reported that two-way active avoidance learning or passive avoidance learning did not influence hippocampal HACU, but discrimination learning in a go/no-go avoidance discrimination task led to a significant increase in hippocampal HACU immediately following training. Comparing rats which received training to naive rats, Wenk et al. (1984) observed that HACU was significantly elevated in the hippocampus of rats receiving either radial arm maze or T-maze training. They also observed a slight HACU drop in radial maze-tested rats in the frontal cortex. The significant elevation of hippocampal HACU persisted for 20 days and returned to

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control levels after 40 days. As only the hippocampal neurons showed elevated HACU, Wenk and colleagues argued that the medial septal cholinergic neurons (which innervate the hippocampus) were selectively activated by the training, while the NBM neurons (which innervate the cortex) were unaffected (Wenk et al., 1984).

A study by Toumane et al. (1987) revealed that initial acquisition of the radial arm maze task induced immediate increases in cortical and hippocampal HACU in mice, but the effect only lasted for three hours. Following acquisition, the mice were tested for reversal, retention, and extinction. Results similar to the initial acquisition were obtained (Toumane et al., 1987). These results were later confirmed in a separate study (Toumane et al., 1989).

Rats trained in the Morris water maze showed different results, however. In an experiment by Decker et al. (1988), rats were trained for 4 days on the hidden platform task. Hippocampal and parietal cortical HACU were significantly decreased relative to rats trained on the visible platform task, even when cue- and place-trained rats were yoked for equal swimming time. The same experiment revealed that the decrease in hippocampal (but not parietal cortical) HACU only occurred during the acquisition phase of learning and was related to the rate of acquisition. Aged rats showed a diminished response in the septo-hippocampal system to the training (Decker et al., 1988). A subsequent study by Gallagher and Pelleymounter (1988) confirmed the result that

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place learning in the Morris water maze significantly reduced hippocampal HACU in young rats and in a sub-population of aged rats which were capable of learning the task rapidly. Aged rats which were impaired on the task did not show any effects on HACU. The literature seems to be divided on the effect of learning on HACU. In general, there seems to be an increase in hippocampal HACU in response to learning, but it remains to be proven whether this increase is transient. It may well be that HACU changes are task-dependent.

In summary, the above literature suggests that acetylcholine is strongly implicated in learning and memory. Brain changes in response to learning include increased BDNF synthesis, increased mRNA diversity, increased overall protein synthesis, and increased activity in cholinergic neurons as demonstrated by changes in HACU, AChE activity, and ChAT activity. These increases are found in many brain areas, but are particularly concentrated to the cortex, caudate, and hippocampus.

A more precise determination of the effect of learning on cholinergic systems could be derived from the highly overtrained subject, for whom presumably, there are elevations in enzymic activity, protein synthesis, and NGF and BDNF-associated phenomena. The ENR paradigm provides such a model. The possibility that changes in the cholinergic system cause the improved performance of ENR-reared animals is also of theoretical interest. To my knowledge, there are no published studies dealing with the interactive effect of enrichment and

The Induction of Brain Biochemical Changes by Enrichment behavioural training on brain cholinergic function. I propose to examine this issue in the following experiments.

EXPERIMENT 1: EFFECT OF ENRICHED REARING ON ChAT ACTIVITY

The effect of ENR and learning on ChAT activity was investigated in a two by two design, with rearing condition being one independent variable, and exposure to behavioural testing being the second variable. The behavioural test used was the Morris water maze task (Morris, 1980). This task is preferred because it provides a unique motor task as a potential control treatment, as well as testing both spatial reference memory (in the hidden platform task) and cued memory task (in the cued or black platform task).

Based on literature results, it was expected that the ENR rats would perform better than IMP-rats. As far as the ChAT activity is concerned, it was expected, based on the above literature review, that ChAT activity would be elevated in the caudate of trained rats as compared to untrained rats, as suggested by the research of Prado-Alcala and co-workers. One would also expect ENR rats would show a greater change due to their greater exposure to learning experiences.

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MATERIALS AND METHODS

Subjects: Fifty-six male Long-Evans rats bred in our colony were used in this study. Rats were weaned at 28 days of age and randomly assigned to either enrichment (ENR) or impoverishment (IMP). ENR consisted of 8 to 10 rats housed in a large (72 x 72 x 45 cm) stainless steel wire cage that contained assorted items such as tunnels, running wheels, loose toys, spinners, etc. Each group of rats were moved to a new cage with a different assortment of toys every three days. IMP consisted of rats housed two per hanging cage (20 x 24 x 18 cm). These rats were handled every three days to equate their handling to the ENR rats. The rats were differentially housed for 50 days. All rats were on a reversed light cycle, with lights on at 20:00 and off at 08:00.

Behavioural Testing: Following 50 days of differential rearing, all rats were transferred to single housing in wire mesh hanging cages to facilitate individual identification. Ten rats from each of ENR and IMP were not tested and were sacrificed for ChAT assay one week after the end of differential rearing. (This delay in assaying was to allow for all rats to complete maze testing as the experiment was run in approximately equal size replications staggered across three weeks as only eight animals per day could be assayed for ChAT activity.

On the same day that the rats were transferred to single housing, they began training in the Morris water maze (Morris, 1980). The Morris water maze consisted of a circular pool 1.5 m in diameter, and

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was filled to a height of 40 cm with 21°C water. The water was made opaque by the addition of about 400 g of skim milk powder. Rats were given four trials per day for five consecutive days to locate a hidden platform, which was concealed 1 cm below the surface of the water in the SW quadrant. Each individual trial lasted a maximum of 90 seconds. If the rat had not located the platform in that time, he was guided to the platform. Following platform location, the rat was allowed to rest for 30 seconds before the next trial was begun. Following the fourth trial, the rat was removed from the maze, towelled dry, and placed under a heat lamp for five minutes before being returned to his cage.

On the sixth day of testing, the platform was removed from the pool and a spatial probe test was conducted. This consisted of allowing the rat to swim freely for 60 seconds in the platformless pool. His pattern of swimming and time spent in searching for the platform was videotaped for later analysis. On the seventh day of testing, a black visible platform was placed in the NE quadrant, and the rat was given 4 trials as described above.

Data from the hidden platform and cued platform tasks were analyzed in terms of the time it took the rat to find the platform (platform location latency). Data from the probe trial were analyzed in two ways. The time spent in the quadrant which previously contained the platform was compared to the time spent swimming in other quadrants to determine a ratio. A rat which has learned the task will spend

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significantly more time in the quadrant which had previously contained the hidden platform. The total path length and path length the rats swam in the various quadrants were also measured by means of tracing the rat's swimming path on a piece of paper and measuring the distance with the aid of a curvimeter, a drafting device for measuring a curved line.

ChAT Assay: Seven to eleven days following training, the animals were sacrificed by decapitation and their brains were rapidly removed and dissected on ice into anterior cortex (rostral to the rhinal fissure), posterior cortex, hippocampus, and caudate. These tissues were then assayed for ChAT activity by the method of Fonnum (1975). Each replication of the assay contained at least one rat from each group.

Statistical Analysis: The behavioural data was statistically analyzed by using a 2 x 2 ANOVA, with environmental condition and training days as the variables. Biochemical results were analyzed with a 2 x 2 ANOVA, with environmental condition and training conditions as the variables. The analyses were performed with SPSS-PC+, a statistics package for the IBM-PC.

RESULTS:

Morris Water Maze:

Results for the hidden platform task for the Morris water maze are shown in figure 1 below. The ANOVA revealed that the ENR rats located

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Figure 1: The mean ($\pm$ standard error of the mean) daily latency (in seconds) to locate the hidden platform is shown for enriched (ENR) and impoverished (IMP) groups.

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the hidden platform more quickly than IMP rats across the five test days (main effect for environment, $F(1,324) = 18.57$, $p < .0001$). There was also a significant main effect for training days [$F(5,324) = 85.10$, $p < .0001$], indicating that the rats' performance improved over days. There was no significant environment by testing day interaction ($F < 1$). No differences between ENR and IMP were observed for the probe test or on latencies to locate the black platform (F 's < 1).

ChAT Assays:

Results for the ChAT assay are presented in figure 2 below. As shown in figure 2a, there were no significant effects in the hippocampus. A trend towards a main effect for testing was observed, [$F(1,52) = 2.60$, ns], but high variance in one of the groups eliminated this effect from achieving statistical significance. No main effect for environment or environment by testing interactions were noted (F 's < 1).

The ANOVA of the ChAT activity of the caudate revealed a main effect for environment [$F(1,52) = 16.69$, $p < .001$], as shown in figure 2b. The main effect for testing and the testing by environment interaction were not significant [$F(1,52) = 1.63$ and 1.83 , $p > .05$, respectively]. Post-hoc analysis using Scheffe's test revealed that IMP-naïve animals had lower ChAT activity than all other groups ($p < .01$), and that ENR-reared rats, regardless of training condition, had significantly higher ChAT activity than IMP-tested ($p < .05$) or

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Figure 2. ChAT activity (in nmol/hr/mg protein) for: (a) hippocampus, (b) caudate, (c) frontal cortex, and (d) posterior cortex. Each panel shows the mean ChAT activity ($\pm$ standard error of the mean) for the maze-trained rats (open bars) and naive rats (hatched bars), who were raised in either enriched (ENR) or impoverished (IMP) conditions.

Figure 2(a)

Figure 2(b)

Figure 2(c)

Figure 2(d)

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IMP-non-tested ($p < .01$). There was no statistical difference between the ENR-trained or ENR-non-trained groups ($p > .1$). It was also found that IMP-tested rats had significantly elevated ChAT activity as compared to IMP-non-tested rats ($p < .05$).

These data reveal that ENR rats had higher ChAT activity than IMP rats (main effect for environment), and that the ENR-tested rats had higher ChAT activity than IMP-tested, who in turn, had higher ChAT activity than IMP-non-tested rats. Thus, ENR-rearing induces a "ceiling effect" on ChAT activity, as no differences due to testing were revealed. However, IMP-tested rats show an elevation in ChAT activity, having an intermediate ChAT activity level between ENR rats and IMP-non-tested rats (see also figure 2b).

The results for the frontal cortex, shown in figure 2c below, revealed a near-significant main effect for environment [$F(1,52) = 3.98$, $p < .06$] and a significant environment by testing interaction [$F(1,52) = 6.85$, $p < .025$]. Simple effect analysis revealed significant simple effects for environment at tested [$F(1,52) = 10.61$, $p < .01$] and testing at ENR [$F(1,52) = 4.56$, $p < .05$]. The significant interaction suggests a priming effect of maze training on ChAT activity. From figure 2c, we can see that the ENR-trained rats show significant elevation of ChAT activity. We can thus hypothesize that the training in combination with ENR rearing was responsible for the ChAT activity elevation, as there was no such elevation in the ENR-non-

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tested rats. The simple effect of environment by testing is due to ENR-tested ChAT activity being higher than IMP-tested, and the simple effect of testing at ENR is due to $ENR > IMP$ differences in ChAT activity.

The results for posterior cortex are shown in figure 2d below. A near-significant main effect for environment was noted [$F(1,52) = 3.82$, $p < .08$, ns]. No other main effects, interactions, or simple effects were noted. The interpretation of this data is similar to the hippocampal results, indicating a trend towards ENR rats having higher ChAT activity than the IMP-rats.

DISCUSSION

In summary, in the frontal cortex of ENR-trained rats, there was a significant elevation of ChAT activity. ENR thus seemed to "prime" the cortex to respond to the learning experience. This result is of particular interest when compared to the research of Falkenberg and co-workers (1992). These researchers found that ENR-reared rats showed an increase in brain-derived neurotrophic factor (BDNF) m-RNA expression in the hippocampus. IMP-tested rats showed a similar, but non-significant increase in BDNF m-RNA in the hippocampus (Falkenberg et al., 1992). Considering the fact that these two radically different experimental methodologies produced very similar results, it is reasonable to suggest that some aspect of the water maze task induces these changes.

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A trend towards increased ChAT activity after ENR-rearing was noted in the hippocampus and posterior cortex, but these data failed to reach significance. In the caudate however, ENR caused a significant elevation of ChAT activity, as training did not further increase ChAT activity levels of the two ENR groups. As the caudate is implicated in sensory-motor processes, it seems reasonable that ENR could cause an elevation in ChAT activity which persists for at least a week. The extensive degree of sensory and motoric learning opportunities experienced by ENR rats probably caused this increase in caudate ChAT activity. Further research is required to ascertain if this elevation was transient, long-lasting, or permanent.

Interestingly, the IMP rats who were exposed to sensory-motor learning in the water maze also showed an increase in caudate ChAT activity. Thus, caudate ChAT activity is responsive to even relatively brief experiences and this change persists for at least seven to eleven days. For ENR-reared rats, training in the water maze appears to have no further effect on ChAT activity, probably due to the fact that the ENR environment, with its multitude of sensory-motor learning opportunities, has caused ChAT activity to be chronically elevated for at least seven days. It remains to be determined if this elevation is permanent.

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One major confound must be resolved in this experiment however. It is possible that the observed ChAT activity increases could be due to non-associative consequences of water immersion such as stress.

EFFECTS OF STRESS ON THE CHOLINERGIC SYSTEM

The effects of stress on cholinergic systems and how this relates to early environment is not well studied. There are perhaps a dozen published reports dealing with this aspect of the cholinergic system and with the related issue of the effects of stress on learning. Initial reports suggested that the isolation experienced by IMP-reared rats was stressful (Hatch et al., 1963). Direct investigation of this phenomenon with differentially reared rats suggested that this was not the case, however. No evidence of stressful effects such as increased adrenal weight or increased blood corticosterone levels were detected (Bennett et al., 1964; Rosenzweig, 1968). The effects of chronic stress and differential rearing was investigated by Riege and Morimoto (1970). They studied rats who received 4 to 10 minutes of inescapable tumbling in a drum. Specific AChE activity in the occipital cortex of non-stressed rats revealed that IMP-reared rats had significantly increased values as compared to non-stressed ENR rats. No difference was observed in stressed rats. Total ChE activity in somesthetic cortex revealed that in non-stressed rats, ENR rats had higher activity than IMP rats. Again, no differences were observed in stressed rats. Further, stress had no effect on the typical weight differences observed in differential rearing experiments (Riege and Morimoto,

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1970). Thus, it appears that stress eliminates the differences in cortical ChE activity between ENR and IMP rats.

One study by Gottesfeld and co-workers (1978) found that 150 minutes of daily forced immobilization stress for 7 or 42 days in the Sprague-Dawley rat reduced caudate ChAT activity by 33%. A subsequent study indicated that ChAT activity in Wistar-Kyoto or Brown-Norway rats was unaffected by two hours of either acute or chronic restraint stress (Gilad, Rabey, and Shenkman, 1983). In Wistar rats, after 24 hours of cold stress, ChAT activity was significantly increased in the ventral hippocampus. In the hypothalamus, AChE activity was significantly decreased (Fatranska et al., 1987).

In summary, changes in ChAT activity seem to occur only in response to severe stress. These changes also seem to vary between strains, with Sprague-Dawley rats showing decreased ChAT activity in response to stress, and Wistar- strain rats showing conflicting results, depending on the task. To my knowledge, no studies examining the effects of stress on cholinergic function in Long-Evans rats have ever been published.

Rats given short-term restraint show no differences in ACh turnover rate in cortex, hippocampus, striatum, or hypothalamus. The same negative results are seen if the rat is immobilized for 1, 4, or 24 hours (Costa et al., 1980). If this restraint stress is aggravated

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by also introducing cold stress, after one hour decreased ACh turnover is observed, but this may be related to decreased enzyme activity at the lower body temperature (Costa et al., 1980). In Wistar rats, 1 or 24 hours of cold stress caused a significant decrease in ACh concentration in hypothalamus and hippocampus (Fatranska et al., 1987).

It has been reported that immobilization stress resulted in decreased choline uptake and may lead to increased muscarinic acetylcholine (m-ACh) receptor binding (Gilad, Rabey, and Shenkman, 1983). In Sprague-Dawley rats, chronic stress (2 hours of restraint per day for five days) resulted in a significant increase in muscarinic m-ACh receptor binding (Finkelstein et al., 1985). Subsequent work has revealed that with Sprague-Dawley rats, immobilization stress produces an increase in m-ACh receptor binding in septum, striatum, hippocampus and pons + medulla via increased affinity of the m-ACh receptors (Takayama et al., 1987). These workers propose that the stress induces supersensitivity of the post-synaptic m-ACh receptors due to decreased pre-synaptic activity (Takayama et al., 1987). Recently, Mizukawa and co-workers (1989) have followed up this work and have looked at hippocampal sub-fields. They looked at the effects of 3 hours of immobilization stress on m-ACh receptor binding in CA1 + CA2 and in dentate gyrus in Sprague-Dawley rats. They found that the stress causes a significant increase in m-ACh binding sites in both CA1 + CA2 and in the dentate gyrus. After 3 hours, only the dentate gyrus still showed a significant increase in binding sites. These authors proposed

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that the immobilization stress induced hypersensitivity of the septo-hippocampal system and induced an up-regulation of post-synaptic m-ACh receptors, particularly in the hippocampus (Mizukawa et al., 1989).

The effects of stress on high-affinity choline uptake (HACU) have also been examined. In rats receiving acute footshock as a stressor, Sprague-Dawley (but not Fischer-344) rats showed increased HACU in dorsal hippocampus (Schmidt, Cooper, and Barrett, 1980). This result has been correlated with motor suppression in Sprague-Dawley rats and suggests that the septo-hippocampal cholinergic system mediates a suppressive behavioural response to environmental stress (Schmidt, Cooper, and Barrett, 1980). In a study by Finkelstein et al. (1985), the effect of 10 minutes of acute immobilization stress on HACU in the Sprague-Dawley rat was studied. It was found that after 10 minutes of stress, HACU in hippocampal synaptosomes was significantly elevated, but the increase was transient, with HACU returning to baseline values after 3 hours. Chronic stress (2 hours of restraint per day for five days) resulted in a significant decrease in HACU (Finkelstein et al., 1985). In contrast to this work, a study by Lai, Zabawska, and Horita (1986) showed that in Sprague-Dawley rats, 45 minutes of restraint stress significantly decreased HACU in hippocampal and frontal cortical synaptosomes. This effect was blocked by pre-treatment with naltrexone, an endogenous opioid antagonist. Thus, it seems that opioids may be implicated in the stress response of hippocampal cholinergic terminals (Lai, Zabawska, and Horita, 1986).

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In summary, there is evidence that stress may play an important role in the modulation of the cholinergic system. The Riege and Morimoto (1970) study suggests that stress eliminates differential rearing effects, but there may be strain differences as the immobilization stress research revealed (see, for example, Gilad et al, 1983). The m-ACh receptor binding studies of Gilad et al. (1983), Takayama et al. (1987), and Mizukawa et al. (1989) suggest that stress can have significant (if short-lived) influences on the cholinergic system of hippocampus in particular. There are, however, many examples of different responses in various strains of rats. To my knowledge, no-one has looked at the Long-Evans strain with respect to stress effects on the cholinergic system. In addition, there are no published reports where the effects of the combination of learning and possible stress from swimming have been examined. In order to clarify the result from experiment one, a second experiment was called for.

EXPERIMENT 2: INVESTIGATION OF STRESS EFFECTS ON ChAT ACTIVITY

In the next experiment, I examined the effect of learning the Morris water maze and its possible stressful effects on ChAT activity in rats raised in ENR and IMP. I intended to test the hypothesis that the elevations in ChAT activity observed in the previous experiment were due to learning or training and not due to possible stressful effects of water immersion. In order to examine the stress effects of swimming the maze, the yoked control method of Decker and colleagues (1990) was used. In this procedure, each rat receiving the training

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was yoked to an animal in the "stressed" group. This stressed animal swam in the maze, with the platform removed, for exactly the same length of time as its paired experimental rat. It was otherwise handled identically to its matched rat.

MATERIALS AND METHODS

Subjects: The subjects were 90 male Long-Evans rats bred in our colony. These rats were raised and weaned as described above. Rats were randomly assigned to one of two environmental rearing conditions as described in experiment one. Following 50 days of differential rearing, the rats in each environmental rearing condition were randomly assigned to one of three training conditions: trained yoked control, and naïve ($n=15$ per group).

Behavioural Testing: Rats were tested in the Morris water maze as described in experiment one. Rats in the yoked control groups were paired with an appropriate ENR- or IMP-tested rat. Each yoked rat swam in the pool for the same length of time as its tested counterpart, but with no platform present. Following the prescribed swim time for each trial, the platform was replaced in the pool and the rat was allowed a 30 second rest period. Following the rest period, the platform was removed and the next trial was started as described above. All rats underwent probe testing on day 6 of training as described in experiment one.

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ChAT Assay: One week following the end of the training period, the rats were sacrificed by decapitation and assayed for ChAT activity as described in experiment one.

Statistical Analysis: Statistical analysis was conducted using the BMDP88 IBM PC package. The data was analyzed by Factorial ANOVA. The Morris water maze analysis was divided into two components: Acquisition, with the variables being environment and testing day; and the Probe test (with variables being environment and training condition (tested or yoked)).

RESULTS:

Morris water maze:

Results for the acquisition of the Morris water maze task are shown in figure 3 below. As in the previous experiment, the ENR rats performed better than the IMP rats, as expected. The ANOVA revealed significant main effects for environment [$F(1,141) = 15.11, p < .0002$] and testing day [$F(4,141) = 63.67, p < .0001$]. The environment by days interaction was non-significant ($F < 1$). Post-hoc analysis using Scheffe's test revealed that IMP rats had longer platform location latencies than did ENR rats on testing days 1 and 2 ($p < .01$) and day 3 ($p < .05$). The main effect for testing day indicates that performance improved over days. Unlike the first experiment, ENR and IMP rat's performance were not statistically different after the third day, as revealed by the Scheffe's test. There were no significant group

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Figure 3: The mean ($\pm$ standard error of the mean) daily latency (in seconds) to locate the hidden platform is shown for enriched (ENR) and impoverished (IMP) groups.

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differences observed on the black platform test ($F < 1$).

Results from the probe test are shown in figure 4 below. For the NW (probe) quadrant, ANOVA revealed significant main effects for environment [$F(1,56) = 4.95$, $p < .03$] and training condition [$F(1,56) = 32.44$, $p < .0001$]. There was no significant interaction [$F(1,56) = 1.43$, ns]. Post-hoc analysis using Scheffe's test revealed that ENR-tested rats spent significantly more time in the probe quadrant than did either ENR-yoked or IMP-yoked ($p < .01$), and that IMP-tested rats spent significantly more time in the probe quadrant than did IMP-yoked ($p < .05$). There was no significant difference between ENR-tested and IMP-tested rats. Thus, both ENR and IMP-tested rats learned the location of the platform, and the yoked rats failed to learn the location of the platform (see figure 4). For the NE quadrant, no significant main effects or interactions were noted.

For the SW quadrant, ANOVA revealed a near significant main effect for Environment [$F(1,56) = 3.71$, $p < .059$], and a significant main effect for training condition [$F(1,56) = 8.74$, $p < .005$]. There was no significant interaction ($F < 1$). Post-hoc analysis using Scheffe's test revealed that IMP-yoked rats spent significantly more time in the SW quadrant than did ENR-tested rats ($p < .05$). There were no other group differences. From figure 4, we can see that the yoked rats showed a preference for the SW quadrant as compared to tested rats.

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Figure 4: Results from the probe test, showing the mean time (in seconds, $\pm$ standard error of the mean) spent in the four quadrants of the pool. Maze tested rats are indicated by the hatched bars, and naive rats by the open bars. The training quadrant (site of the platform during acquisition) was the NW quadrant.

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For the SE quadrant, a significant main effect for training condition was found [$F(1,56) = 9.96, p < .003$]. Post-hoc analysis using Scheffe's test revealed that IMP-yoked rats spent significantly more time in the SE quadrant than did ENR-tested rats. From figure 4, we can see that the yoked rats show a preference for the SE quadrant as compared to the tested rats, as was also seen in the SW quadrant. Thus, it seems as if the IMP-yoked rats prefer the southern part of the pool.

ChAT Assay:

Results for the ChAT assay for experiment 2 are shown in figure 5 below. The hippocampal ChAT activity data, shown in figure 5a below, showed no significant main effects, interactions, or simple effects, with the only F value greater than one being the main effect for training condition [$F(2,78) = 2.20, ns$].

The results for the caudate are shown in figure 5b. ANOVA revealed that there were main effects for environment [$F(1,78) = 27.14, p < .0001$], training condition [$F(2,78) = 3.46, p < .025$], and an environment by training condition interaction [$F(2,78) = 4.45, p < .025$]. Analysis of simple effects revealed significant simple effects for training condition by IMP [$F(2,78) = 5.80, p < .001$], environment by tested [$F(1,78) = 9.48, p < .001$], and environment at non-tested [$F(1,78) = 25.83, p < .0001$]. From figure 5b, we can see that all groups showed elevations of ChAT activity relative to IMP-naïve. As in

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Figure 5: ChAT activity (in nmol/hr/mg protein) for :
(a) hippocampus, (b) caudate, (c) frontal cortex, and
(d) posterior cortex. Each panel shows the mean ChAT activity ($\pm$ standard error of the mean) for the maze-trained rats (T), naive rats (N), and yoked control rats (Y), who were raised in either enriched (ENR) or impoverished (IMP) conditions.

FIGURE 5 (a)

FIGURE 5 (b)

FIGURE 5 (c)

FIGURE 5 (d)

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experiment one, the main effect for environment is due to higher ChAT activity in ENR rats. The fact that ChAT activity was also elevated in all rats receiving testing in the Morris water maze replicates the findings of the first experiment.

Results for the frontal cortex ChAT activity is shown in figure 5c above. The ANOVA revealed that there was a near-significant main effect for environment [$F(1,78) = 3.67, p < .059$]. Post-hoc analysis using Scheffe's test revealed no group differences ($p > .05$).

The results for the posterior cortex ChAT activity is shown in figure 5d above. No main effects, interactions, or simple effects were observed.

DISCUSSION:

The second experiment was attempted in order to determine if non-associative factors (such as stress) contributed to the observed elevation of ChAT activity in maze-trained ENR rats. From the results of the probe test for this experiment, it is apparent that this experiment was only partially successful in settling the issue. From figure 4, we can see that the IMP-yoked rats had a significant preference for the southern quadrants of the pool. This was the part of the room in which the experimenter sat whilst conducting the trials. While it is obvious from the data for the probe quadrant (figure 4a) that the yoked rats did NOT learn the hidden platform task, their

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preference for the southern part of the pool clearly indicated that they learned to stay near the part of the pool which afforded them the quickest exit from the pool—the experimenter's relative position in the room. Thus, while they failed to learn the platform location (as expected), they still did learn something. This confound, at least in part, limits the applicability of the yoked control group as a "non-learning" control for the stress of water immersion.

With this fact in mind, one can interpret the biochemical¹ data. Similar results to the first experiment were obtained in all brain areas. The caudate data (Figure 5b) revealed the same effects as in the first experiment: ENR-trained rats had the highest ChAT activity, and IMP rats receiving either training (IMP-trained) or maze exposure (IMP-yoked) showed intermediate ChAT activity levels, as compared to IMP-naive rats. From these observations, we can conclude that ENR elevates ChAT activity in the caudate, possibly due to the increased motoric activity inherent in the paradigm. This conclusion is borne out by the observation of an increase in ChAT activity for the IMP-trained and IMP-yoked rats.

The frontal cortex showed similar results to the first experiment. While not quite achieving statistical significance ($p < .059$), this result supports that of the first experiment, where ENR rats had higher cortical ChAT activity than IMP-reared rats.

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The priming effect of training on ChAT activity in frontal cortex seen in the first experiment failed to reach statistical significance in the second experiment, probably due to unusually high variance in the yoked groups. It is likely that these rats "learned" as discussed above, and that this may have confounded the data.

While not statistically significant, the data for the posterior cortex ChAT activity for the ENR-tested rats implies a trend towards the priming effect of maze training seen in frontal cortex in the first experiment.

If one speculates on the way that ACh is involved in the formation of a memory trace for, say, platform location, one could expect hippocampal ChAT activity to peak first, as a short-term memory trace is evoked by the learning experience, and there is evidence for a trend in the above two experiments. Since this trend failed to reach statistical significance, it is possible that such a change is transient, as in the above experiments, there was a one week delay between the last learning sessions and the biochemical assay. After a short-term memory of the platform location is formed, in order for the rat to locate it in future, a long-term memory must be formed. It seems likely that such a memory must have a cortical location, and this could cause increased cortical ChAT activity. This conclusion is indirectly supported by the decreased ChAT activity in dorsal hippocampus found in aged impaired rats in the radial arm maze (Luine

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and Hearn, 1990), and by the research of Toumane and co-workers (1988, 1989), who found that cortical and hippocampal high-affinity choline uptake (HACU) were significantly elevated following the initial acquisition of the radial arm maze task in mice. It is also interesting to note that Toumane reported cortical HACU elevation persisted longer than hippocampal HACU elevation (Toumane et al., 1988). These results are disputed by Decker et al. (1988), who found decreased hippocampal HACU in rats trained in the Morris water maze, but this result was obtained 15 minutes following training, whereas the Toumane result showed immediate elevation, which persisted for at least 3 hours.

ENR rearing enhanced the rat's ability to locate the platform and, in experiment one, the subsequent ChAT activity increase in frontal cortex for rats receiving maze training. Enrichment thus seems to benefit the rat, both in the ability to solve the task (as shown by the behavioural data), and in an enhanced ChAT activity level in the cortex.

The most consistent result of the two experiments was found in the caudate. Enrichment did not seem to "prime" ChAT activity in the caudate. Rather, ENR increased ChAT activity in the caudate, and no further effect of training was observed. As the caudate is implicated in motoric behaviours, and ENR itself provided substantial opportunities for motoric learning, no further increase was seen in

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caudate ChAT activity in ENR rats. IMP-tested or IMP-yoked rats, on the other hand, did show intermediate levels of ChAT activity increase in caudate, probably as a result of the rat having to perform a novel (for an IMP-reared rat, anyway) motor task. Thus, one can conclude that ChAT activity in the caudate is increased by motoric learning.

Changes in caudate HMCU have also been reported in mice. The results obtained in the two experiments described above are in agreement with those of Luine and Hearn (1990), who found that striatal ChAT activity was significantly decreased in aged, impaired rats tested in the radial arm maze. The research of Prado-Alcala and co-workers (1981, 1984a, 1984b) using atropine injections into the caudate immediately following training for lever-pressing behaviour, found that the atropine impaired inhibitory avoidance behaviour, suggesting an important role for caudate ACh in the consolidation of the memory trace.

CONCLUSIONS:

The purpose of these experiments was to determine the combined effects of differential rearing and maze learning on the behaviour and biochemistry of the rat. The first experiment demonstrated that exposure to differential rearing caused an improvement in maze learning for ENR-reared rats as compared to IMP-reared rats. In addition, cortical ChAT activity was significantly elevated by the combined exposure to training and ENR-rearing, as compared to non-tested control

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rats. This result suggests that ENR causes a priming effect on cortical ChAT activity.

ENR was also found to cause a long-lasting elevation in caudate ChAT activity, and exposure to maze training caused an elevation in caudate ChAT activity for IMP-reared rats. This result indicates the caudate is involved in motoric aspects of the task.

The second experiment essentially replicated the results of the first experiment with respect to caudate and frontal cortical ChAT activity. However, a potential confound caused by the unexpected learning of the yoked control groups prevents dissociation of any possible non-associative causes of the changes in ChAT activity from that of any purely behavioural or learning causes.

DIRECTIONS FOR FUTURE RESEARCH:

The major flaw with the second experiment was the completely unexpected learning of the yoked rats. This problem could have been avoided by having the experimenter sit in random locations of the room during trials. The rats would thus soon regard the experimenter as an irrelevant cue.

It would also be of interest to determine the exact time course of the brain biochemical changes in ChAT activity caused by the maze training/differential rearing paradigm used in these experiments. One

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series of experiments could look at ChAT activity each day following the completion of the learning task. In the above experiments, a one week delay was necessitated by the difficulty in performing the ChAT assay on so many rats. If one examined the ChAT activity on a daily basis following learning, one might catch a transient increase in hippocampal ChAT activity, followed by an increase in cortical ChAT activity (which was observed after one week).

It would also be interesting to see just how long these brain biochemical changes persist. It appears that for ENR rats, that caudate changes are relatively long-lasting, as they were elevated for a week following testing. It may well be that ENR-rearing permanently elevates caudate ChAT activity. Another series of experiments could use a longitudinal design, where one could monitor ChAT activity during ENR (but before testing) to see when ChAT activity changes occur.

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