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Regional Differences in Central Cholinergic Activity in Aggressive and Non-Aggressive Mice

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REGIONAL DIFFERENCES IN CENTRAL CHOLINERGIC ACTIVITY IN AGGRESSIVE AND NON-AGGRESSIVE MICE

By

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VITA

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TABLE OF CONTENTS

LIST OF TABLES

Page

Page

LIST OF FIGURES

INTRODUCTION AND REVIEW OF LITERATURE

I. DEFINITION OF AGGRESSION

A. EARLY AGGRESSION THEORIES

Aggression has been defined by Dollard (1939) and also by Flynn et al. (1970), as behavior which leads to the damage or destruction of a goal entity. There can be no disagreement that behavior directed toward causing physical injury to another individual of the same or a different species must clearly be labelled as aggressive. However, a difficulty is apparent almost at once because an attack or another individual usually involves the risk of injury for the attacker and concomitant defensive behaviors. Attack, therefore, is often associated with elements of self-protective and withdrawal responses. This was especially apparent in a study of threatening postures (Tinbergen, 1959) which included a variety of elements of attack, defense and withdrawal behavior. Because of this close association between various phases of aggression, attack, threat, fleeing, and submissive behavior are often lumped together as agonistic, aggressive behavior, even though some types of behavior within this category are clearly aggressive and others are not.

As a behavior directed toward causing physical injury, aggressive behavior often results in determining access to an object or space (Sheard, 1973). For example, a bird showing signs of territorial dominance and displaying an intent to injure any and all intruders will, in all likelihood, have access to the surrounding space and establish a boundary around this space (Kauffman, 1965). Also, much of the aggression seen in nature can be regarded as a consequence of the proximity between two individuals (Karczmar et al., 1973 and 1978); yet, proximity-induced aggression may also bring about control of needed space.

Freud (1922), in his earlier writings and MacDougall (1923), laid emphasis on frustration as a cause of aggression, and the frustration-aggression hypothesis was later espoused by Dollard et al. (1939). Although this hypothesis has been applied to aggression in man, the induction of aggression by frustration has been also demonstrated in animals, in the laboratory. For example, a pigeon trained to peck a key for a food reward will attack another pigeon confined nearby during extinction trials (Whittaker, 1975) in the course of which "frustration" may be present. The incidence of attacks has been shown to increase with factors thought to increase frustration.

In general, behavioral psychologists have tended to distinguish between several classes of aggression on the basis of the stimuli evoking the response. Moyer (1968)

divided aggression into eight categories: 1)Predatory aggression, 2) Intermale aggression, 3) Fear-induced aggression, 4) Irritable aggression, 5) Territorial defense, 6) Maternal aggression, 7) Instrumental aggression, and 8) Spontaneous aggression. In the laboratory the most common model for irritable aggression is shock-induced aggression (see also below, Section IC). This behavior pattern was first described by O'Kelley and Steckle (1939). Briefly, if two or more rats are confined within a limited area and exposed to an aversine stimulus such as electric footshock, they will attack each other in species-specific patterns (Eichelman, 1971). The parameters of this behavior are related to cage size, shock intensity, age, strain, and the opportunity to escape the aversine stimulus. This behavior appears to be innate and has been observed in various species such as mice (Tedeschi et al., 1959; Bryson, 1975), snakes (Ayrin and Hutchinson, 1965), opposoms (Ayrin, 1967), cats (Ulrich et al., 1974), monkeys (Ayrin et al., 1967); Stedecker, 1975), and rats (Eichelman and Barchas, 1975; Jacobs and Cohen, 1976). Electrolytic lesions of the ventromedial hypothalamus (Eichelman, 1971) and septal nuclei (Trafton, 1967; cf. Paxinos, 1975) have been shown to increase shock-induced aggression in rats. (Other methods for inducing irritable as well as other forms of aggression in the laboratory are discussed in Section IC, below).

According to Moyer (1968) spontaneous aggression (see above) is the least understood form of aggressive behavior since it appears to occur without any ongoing, artificial stimulus. This behavior has been observed in rats that were regrouped after isolation (Hatch et al., 1965). As in other forms of aggression (i.e., isolation-induced, footshock) spontaneous aggression has been associated with sparring, biting, vocalization, side-to-side and submissive posturing.

A question of practical importance concerns the spontaneity of aggression. Spontaneity is not an easy concept, but it is meant to refer to a change in the output of a system without a corresponding change in input. For example, two rats left alone may fight intermittently and not continuously. The fighting could be called spontaneous since each animal is continuously exposed to what appears to be constant levels of stimulation emanating from the other animal, yet, the two animals exhibit aggression only some of the time; thus, the tendency to fight appears to fluctuate with time. Such fluctuations are presumably due to short-term waning in responsiveness which may not depend upon the severity of the stimulus, encounter time, and post-fight recovery.

In a similar context, Lorenz (1950) addressed himself to the question of whether aggressive behavior is an inevitable behavioral expression that must be exhibited by any living being. This model, or hypothesis, emphasizes the role

of an internal drive for aggression which inevitably finds expression. In this light, aggressive behavior is considered to be 'part of an animal'. If it is shown that the probability of aggressive behavior increases with time, it must also be shown that this increase continues to the point where objects that are sources of subthreshold stimuli are attacked. Then and only then can it be concluded that aggressive behavior inevitably finds expression.

B. AFFECTIVE AND PREDATORY AGGRESSION

Although Moyer (1968) has identified several classes of aggression based on the stimulus evoking the response (see above, Section IA), aggressive behavior may also be classified by the type of response elicited. It has been postulated that there are two neurologically distinct patterns underlying the expression of aggressive behavior. The two behaviors may be termed affective aggression and predatory aggression (Flynn et al., 1970).

Affective aggression has been one of the most intensively studied types of aggression. In the cat the prototype of this behavior has been called sham rage (Bard, 1928), or affective attack behavior (Scot, 1966). Affective aggression has been evoked in various species by brain lesions (Reis et al., 1967), electrical stimulation of the brain (Delgado et al., 1954), painful stimuli (Ulrich, 1966), or as a response to threatened attack (Brody et al., 1969). Affective

aggression is the mode of aggressive display seen in aggressive states characterized as irritable, intermale, territorial, or maternal (see above, Section IA). It is probably an ubiquitous mode of aggressive behavior in vertebrates (Reis, 1974), and includes an intense activation of the autonomic nervous system. Furthermore, in the course of affective aggression animals may assume threatening and defensive postures such as snarling, baring of the teeth and claws, and exhibit threatening vocalizations (Fernandez de Molina, 1962). The external stimulus may be somatic or exteroceptive. The attack may be intra- as well as interspecific, and the threshold for aggression is often very low. Affective aggression is not related to feeding or predation and its expression is probably hormonally controlled (Reis, 1974).

Predatory aggression, on the other hand, is that type of aggression which leads to the destruction of a natural prey, usually for food. This behavior is usually triggered by exteroceptive stimuli, usually visual, characterized by little autonomic arousal, and not associated with increased irritability; there may be little or no vocalization. The attack is quite specifically defined as including lethal blows to the back of the neck and little mutilation by the claws (Hernandez-Peon, 1956).

These two models of aggression are probably subserved by different neural pathways. The neural pathway mediating affective aggression is widely distributed in the brain and

spinal cord. This pathway includes a series of interlinked neuronal structures running from the amygdala caudally through its two main projections, the ventral amygdalofugal pathway and the stria terminalis, through the lateral hypothalamus and the periaqueductal gray matter (Kaada, 1967; Fernandez de Molina, 1962; see also below, Section IC). This neuronal network is closely related to the spinothalamic tract and to limbic structures. The responses for predatory aggression are anatomically less well-characterized but it is known that the hypothalamic nuclei that project into the ventral midbrain tegmentum are involved (Chi and Flynn, 1971).

C. LABORATORY MODELS OF INDUCED AGGRESSION

1. Isolation-Induced Aggression

Isolation-induced aggression has been used widely as a model for studying aggressive behavior. Although mice (Valzelli, 1969; Cairns and Nakelski, 1970; Da Vanzo et al., 1966), have been used frequently, monkeys (Harlow et al., 1975), rats (Johnson et al., 1970), and guinea pigs (Bunnell, 1967), have also been proven to provide acceptable models of isolate aggression. Usually, isolate aggression is considered to be a form of affective aggression (Reis, 1974).

In a typical experiment, animals are housed individually in cages for varying periods of time. Rats, for example, require a longer isolation period (7-9 weeks; Banerjee, 1974), than mice (3-4 weeks, Banerjee, 1971), to exhibit significant

aggression. In order to minimize external stimuli, animals should be isolated without manipulation by the experimenter (as in the present study; Banerjee, 1971). However, Da Vanzo et al. (1966) employed tail-pinching and prodding during the isolation period and reported a higher incidence of aggressive animals when compared to animals that were isolated without being thus stimulated (the incidence of aggression of isolated and tail-pinched versus isolated animals was 94 and 74%, respectively).

After the isolation period, animals are placed in cages containing animals of the same or different species for aggression testing. During the time period when two experimental animals are paired, parameters such as fighting latency, duration of each bout, number of attacks, and escape behavior are recorded; initiator of the attack is identified. According to previously determined scoring systems (Banerjee, 1971), these parameters are graded and used to define the observed behavior as being aggressive or defensive (non-aggressive), (see Section II, Materials and Methods, for aggression test parameters recorded in the present study).

The aggressive behavior patterns observed after isolation are quite complex and depend, in part, on the reaction of the other animal (Charpentier, 1969). Thus, the pattern may depend on whether the animal responds to the situation by being the attacker (winner) or the defender (loser). This

is important with respect to the consequences of pairing. This is particularly relevant in the present study where it has been determined that the result of the pairing session (i.e., winning or losing) can be correlated with changes in choline levels and acetylcholine turnover values (see Section IV, Discussion).

In addition to inducing aggression, isolation engenders numerous behavioral, physiological, and biochemical abnormalities that are collectively referred to as 'isolation syndrome' (Valzelli, 1973). For example, isolation has been shown to cause increased spontaneous activity and reactivity to pain (Valzelli, 1971), tremor (Sofia, 1969), and heightened muscle tonus (Krysiak et al., 1976). Until these and related responses are defined and explained, a clear picture of isolation-induced aggression cannot be realized.

2. Foot-Shock-Induced Aggression

Aggressive behavior can also be induced by painful stimuli such as electric footshock. This aggression is usually considered to correspond to either affective or irritable aggression (see above, Section IA). The most commonly used animals are rats (Eichelman and Barchas, 1975) although snakes (Ulrich and Ayrin, 1962) and monkeys (Ulrich and Ayrin, 1962) also show this phenomenon; in fact, electric footshock has been shown to induce aggression in most species tested. Aggression elicited by painful stimulation was initially described by O'Kelley and Steckle (1939). The usual

procedure is to apply electrical footshocks to the paws of the experimental animals in a no-escape environment. After or concurrently with (Tedeschi and Tedeschi, 1957) a series of footshock episodes, the animals are paired with members of the same or different species for aggression-test pairing sessions (see above Section I.C.1.). Shock-induced aggression is not completely a reflexive response since shocked animals that are given a choice between escape and attack, prefer to escape from the shock rather than attack (Ayrin et al., 1967).

As in the case of other forms of aggression, shock-induced aggression is affected by drugs. For instance, facilitation of attack occurred with several doses (0.125-1.000 mg/kg) of d,l-amphetamine (Sheard, 1967), while footshock aggression was decreased (Eichelman et al., 1973) when rats were subjected to a 5-week lithium pretreatment (see also Section III).

Recently, it has been reported that isolation and footshock that preceded pairing synergized in the induction of fighting of three strains of mice (Karczmar and Kindel, 1980). In this case, isolation or footshock alone caused fighting to occur at a longer latency and at a lesser frequency as compared to those recorded for isolated and footshocked mice.

3. Electrical Stimulation and Lesioning

Both brain stimulation and lesioning of various neural areas, especially limbic forebrain structures, have been utilized to investigate aggressive behavior. For example, electrical stimulation of the cat hypothalamus has been shown to evoke aggressive displays (Siegel and Flynn, 1968). More recent work has shown that this effect can be facilitated by concomitant electrical stimulation of the amygdala, pyriform cortex (Albert et al., 1978), and midline thalamic region (Paxinos, 1976; Albert and Richmond, 1976).

Electrical stimulation of the lateral septum has been shown to suppress aggressive behavior in the rat (Albert et al., 1978). However, lesions ventral to the anterior aspect of the septum attenuated this suppression of aggressivity (cf. Albert et al., 1978). Although aberrant behaviors such as hyperactivity and convulsions (Panksepp, 1971) are sometimes elicited by aggression-inducing stimulation, the data have been considered pertinent as to anatomical localization of aggression since electrically-induced aggression could be elicited repeatedly and differentially (Albert and Richmond, 1976; Brayley et al., 1977). Altogether, investigations involving electrical stimulation and lesioning of specific brain areas contributed to the knowledge of the neuronal pathways subserving aggressive behavior. Species differences must be, however, considered in this context. For example, the septo-hippocampal pathway has been implicated as an important medi-

ator of aggressive behavior in the rat as septal lesions inhibited aggressive behavior in the rat (Karli, 1960); on the other hand, aggressive behavior was enhanced in mice after septal lesioning (Montague et al., 1979). On the whole, the septum has been implicated in the mediation of aggressive displays in a number of species (cf. Myers, 1977; see also Section II.C.2.).

4. Predatory (Muricidal) Aggression

The predatory model of aggressive behavior concerns the behavior of various species toward their natural prey: rat with regard to a mouse; mouse with regard to a cricket; and rat with regard to a frog. Unlike affective or irritable types of aggression (isolation-induced; pain-induced) predatory aggression has been associated with little autonomic arousal and purposeful attacks (Barber, 1972, 1977; see also above Section IB). As mentioned earlier, predatory and affective forms of aggression are probably mediated by separate, independent neural pathways (Moyer, 1968). Evidence has been accumulated which indicates that these different categories of aggressive behavior have different genetic, neurophysiological, and neurochemical correlates, and are differentially affected by drugs (Moyer, 1968; Chi and Flynn, 1971; Eichelman, 1973; Mandel et al., 1977).

The induction of predatory (muricidal) aggression usually can be affected by introduction of a suitable attack object (MacDonnell and Flynn, 1964), or natural prey, to the

predator (see above). Frog-killing behavior in the rat was employed by Bandler (1970; 1971) as a model for predatory aggression. It was shown that direct intrahypothalamic and intrathalamic carbachol injections facilitated this behavior; however, subsequent intrahypothalamic and intrathalamic atropine injections failed to inhibit this carbachol-facilitated expression of predatory aggression (see also Section II.C.2. for further description of the cholinergicity of predatory aggression).

Due to the limited involvement of the autonomic nervous system (see also Section IB) in predatory aggression, this behavior has been referred to as "quiet biting attack" by Wasman and Flynn (1962). It has been reported that the hypothalamic sites from which this quiet biting attack may be elicited are those where self-stimulation behavior may be induced (Panksepp, 1971; Yamamoto, 1977). This suggests the possibility that this type of aggressive behavior may be self-reinforcing and thus be a useful model for nonaffective aggression in man.

II. ACETYLCHOLINE AND CNS FUNCTION

A. ACETYLCHOLINE BIOSYNTHESIS AND HYDROLYSIS

The substrates for the biosynthesis of acetylcholine (ACh) are acetylcoenzyme A (acetyl-CoA) and choline (Ch). ACh is synthesized in a reaction catalyzed by choline acetyltransferase (ChAc) in the following manner:

$$\text{ACETYL CoA} + \text{CH} \xrightarrow{(\text{ChAc})} \text{ACH} + \text{CoA}$$

In order to synthesize ACh, three substances are required: ChAc, Ch, and acetyl-CoA. Only one of these substances, acetyl-CoA, is produced directly in the nerve ending. Choline is probably obtained from the extracellular fluid and ChAc is supplied from the cell bodies of cholinergic neurons by the mechanism of axonal transport (cf. Tucek, 1978).

1. Choline Acetylase (Choline Acetyltransferase)

Choline acetylase was first obtained in cell-free extracts of the electric organ of *Electrophorus electricus* (Nachmansohn and Machado, 1943). The final step in the synthesis of acetylcholine is in the ChAc-mediated transfer of an acetyl group from acetyl-CoA to Ch. The prior step, the acetylation of CoA, is catalyzed by an acetyl kinase; the immediate source of the acetyl group is adenylacetate, formed by the reaction of ATP with acetate. This sequence of events is known as the acetate-activating reaction (cf. Koelle, 1963).

Large variations in choline acetylase activity occur in mammalian nervous tissue. High concentrations have been reported for peripheral efferent cholinergic nerves that exhibit activity mounting to 5,000 μg ACh/g/hr for ventral spinal roots; lower concentrations have been reported for afferent nerves which are capable of generating up to 2000 μg ACh/g/hr for dorsal spinal roots (cf. Koelle,1963). Similar differences have been found by ultramicrodetermination of the concentrations of ChAc in single cholinergic and non-cholinergic neurons of autonomic ganglia (Buckley et al., 1967). In the central nervous system activity values range from 0-6 μg ACh/g/hr in the cerebellar cortex to 3000-4000 μg ACh/g/hr in the caudate nucleus (Hebb, 1963).

Just like other protein constituents of the neuron, choline acetylase is synthesized within the granular endoplasmic reticulum of the perikaryon, then transported within the agranular reticulum, either by growth or by axoplasmic streaming, along the length of the axon to its terminal end (cf. Koelle, 1963; Tucek, 1978). The axon terminals synthesis and storage of ACh takes place sequentially following the transport of choline into the nerve terminal, leading to accumulation of ACh in synaptic vesicles. According to this scheme, ACh that is bound to the synaptic vesicles can be viewed as the precursor to free ACh. However, the means by which synaptic vesicles obtain their stores of ACh is not known at this time.

2. Choline

Since cholinergic nerve terminals are dependent on an extracellular source of free choline (Ch) to support the synthesis of ACh, it is important to determine the source of this Ch. Although one possible source is the de novo synthesis of Ch by nerve tissue, this route does not appear to exist in brain (Browning and Schulman, 1968; cf. Tucek, 1978; cf. Browning, 1976). The other possible source is plasma (Collier and MacIntosh, 1969; cf. Tucek, 1978; Spanner et al., 1976). It has been shown that plasma free choline is the source of choline for the synthesis of ACh in the cat superior cervical ganglia (Collier and MacIntosh, 1969). In brain, this tissue is somewhat more complex. Because brain tissue does not synthesize choline de novo to any extent, nor does it operate on a negative choline balance, it is obvious that extra-brain sources of choline must be available for brain ACh synthesis (Ansell and Spanner, 1975).

The uptake of free choline in vivo can be simulated in vitro with brain slices, and it appears that the brain receives its supply of choline by an uptake process (Haga, 1971; Yamamura and Snyder, 1973; Haubrich et al., 1975). The lipid-bound choline formed in the liver (cf. Ansell and Spanner, 1971, 1972; Freeman and Jenden, 1976) is released by hydrolytic procedures in a water-soluble free form into the bloodstream and taken up by the brain (cf. Ansell and Spanner, 1975). There are some indications, however, that

choline is transported to the brain in the lipid-bound forms, lysophosphatidylcholine and glycerylphosphorylcholine (Bjornstad and Bremer, 1966; Mann, 1976); both of these substances are substrates in the phosphatidylserine-choline synthetic pathway (Bremer and Greenberg, 1960; 1961). Further evidence supporting lipid-bound transport of choline to the brain is evidenced by the presence, in brain, of phospholipase D (Saito and Kanfer, 1973) and lysophospholipase D (Wykle and Schremmer, 1974), enzymes that catalyse the direct release of choline from phosphatidylcholine and lysophosphatidylcholine, respectively.

Data presented by Friesen et al. (1971), and Kewitz and Pleul (1976) suggest that cholinergic neurons may store appreciable quantities of free choline for the biosynthesis of ACh. In one group of experiments, the septo-hippocampal pathway (a well-known cholinergic pathway; cf. Atweh and Kuhar, 1976, and below, Section II.C.1.) was lesioned at the septum and ACh and Ch levels were determined for hippocampus; hippocampal ACh levels decreased 70% while hippocampal Ch levels decreased only 32%. A second group of experiments yielded similar results; here, chronic denervation of the superior cervical ganglia led to a 92% reduction of ACh levels and a 32% reduction of Ch levels. It has been suggested that the relatively high choline levels reported by the above experiments may be due to high concentrations of esterified choline (Ansell and Spanner, 1975).

3. Acetyl-CoA

In addition to choline, acetyl-CoA is also essential for the synthesis of ACh. Its three main precursor candidates are pyruvate, acetate, and citrate. Tucek and Chang (1970) and Tucek (1978) showed that in the rat and guinea pig brain pyruvate, and to a lesser extent acetate, label brain ACh. Citrate was found to yield only traces of labelling of ACh in these experiments. The ratio of the specific activities of citrate and ACh after labelling with acetate and pyruvate differed, and this has been interpreted by Nakamura et al. (1970) to indicate that citrate and ACh belong to different pools of acetyl-CoA. On the other hand, Sollenberg and Sorbo (1972) concluded that the acetyl group passed through citrate. There is, therefore, little certainty in the current understanding of the origin and metabolism of acetyl groups in the brain as related to the biosynthesis of ACh.

It is known, however, that the ultimate source of acetyl units for ACh synthesis is glucose. It has been determined that isotopically labelled glucose contributes carbon atoms to the acetyl unit of ACh in slices of cerebral cortex (Browning and Schulman, 1968) and striatum (Lefresne et al., 1973) of the rat. Also, it has been shown that injection of ^{14}C-glucose into the cerebral ventricles of the cat leads to considerable labelling of ACh (Tucek and Cheng, 1974). Glucose leads to ACh via pyruvate, as Tucek and

Chang (1974) identified pyruvate to be the most proximate precursor of the acetyl unit of ACh concerning the transfer of labelled carbon atoms in the glycolytic pathway.

The conversion of pyruvate to acetyl-CoA is normally catalyzed by the pyruvate dehydrogenase enzyme complex (Peterson et al., 1973;Reed et al,1972).Pyruvate dehydrogenase occurs in the mitochondrial matrix space of mammalian cells along with the enzymes of the citric acid cycle (Lefresne et al.1975). The mitochondrial location of the pyruvate dehydrogenase complex poses a problem for the pathway of carbon flow to the acetyl unit of ACh since coenzyme-A compounds do not readily pass the mitochondrial membrane (Lowenstein, 1968). Because ACh synthesis occurs in the cytosol (see above, Section II.A.1.), a transport mechanism must transfer the acetyl units across the mitochondrial membrane for ACh synthesis. Citrate functions as the acetyl carrier for the egress of acetyl units from mitochondria. Citrate is synthesized intramitochondrially. The citrate then migrates out of the mitochondria and is reconverted to acetyl-CoA and oxaloacetate (cf. Tucek, 1967). Experiments that have examined the conversion of glucose to citrate and ACh have yielded results consistent with citrate being a carrier for acetyl units (Sollenberg and Sorbo, 1970).

In summary, synthesis of the acetyl unit of ACh from glucose involves conversion of glucose to pyruvate. Carbon atoms (acetate) are then transferred from pyruvate to an

acetyl-carrier via the pyruvate dehydrogenase complex. The acetyl carrier then transports acetate from the mitochondria to the cytosol where ACh synthesis occurs.

4. Acetylcholinesterase

In addition to the synthesis of acetylcholine, its degradation to choline and acetic acid must also be discussed. This is accomplished by the hydrolytic cholinesterase enzymes. The important member of this family of enzymes is acetylcholinesterase, also known as specific or true cholinesterase, which is present in central and ganglionic neurons and at the neuromuscular junction (Barnard et al., 1971). Butyrylcholinesterase, also known as nonspecific or pseudo-cholinesterase, is found in glial cells of the central and peripheral nervous systems, parasympathetic effector organs, as well as the plasma and liver (Heilbronn, 1958; cf. Wilson, 1960). Both types of cholinesterases are able to hydrolyze ACh along with other aliphatic esters. However, a distinction must be made between the two enzyme types. First of all, acetylcholinesterase hydrolyzes ACh at a higher V_{max} than butyrylcholine, propionylcholine, or tributyrin; the reverse is true with butyrylcholinesterase. Concerning substrate concentration as related to enzyme activity, acetylcholinesterase is inhibited by high concentrations of ACh, butyrylcholine, and propionylcholine. In contrast, butyrocholinesterase is not inhibited by high concentrations of substrate so that the usual Michaelis-Menten type of sub-

strate concentration curve can be observed. This is primarily due to the fact that there is at least a two-point attachment of the substrate to acetylcholinesterase whereas with butyrylcholinesterase the substrate is attached to the enzyme only at one site (cf. Main, 1976).

In general, neural tissue contains acetylcholinesterase while nonneuronal tissue usually contains butyrylcholinesterase. This, however, is a generalization; neural tissue such as autonomic ganglia contains both esterases (Changeux, 1966). In the blood of man and most but not all vertebrates, erythrocytes contain only acetylcholinesterase, while plasma contains butyrylcholinesterase (cf. Dudai et al., 1973).

Information concerning acetylcholinesterase has been derived not only from kinetic studies but also from studies of inhibitors known as anticholinesterase agents. The anticholinesterases are classified as reversible and irreversible inhibitors. Both types of inhibitors acylate (bonding of the carboxylic ester group of acetylcholinesterase with the hydroxyl group of the enzyme inhibitor forming an acyl enzyme, see Main, 1976) the enzyme at the esteratic site. Although nerve gas agents, the organophosphorous anticholinesterase, are classified as irreversible inhibitors of cholinesterase, these compounds actually detach themselves from the enzyme, although very slowly. Wilson (1960) observed that hydroxylamine speeded up this dissociation and regenerated the enzyme. Based on these observations Wilson de-

signed nucleophilic agents that would fit the active site of acetylcholinesterase; the prototype of these agents is 2-pyridine aldoxime methiodide (2-PAM).

B. HOMEOSTASIS AND THE CHOLINERGIC SYSTEM

Early evidence indicated that the central regulation of respiration (Gesell and Hansen, 1945; Metz, 1971) and cardiovascular mechanisms (Chang et al., 1937; Brezenoff, 1972) might be mediated by the cholinergic system. A cholinergic role has also been implicated in other behaviors that may be understood to preserve homeostasis such as 1) Sleep, 2) Appetitive behavior, 3) Drinking behavior, 4) Memory and learning, and 5) Temperature regulation.

1. Sleep

Animal and human sleep has several phases (Jouvet, 1975; Hobson, 1976; Pappenheimer et al., 1975). The main divisions of sleep are the Rapid Eye Movement (REM) or paradoxical sleep and the slow-wave sleep; the cholinergic system is involved especially in REM sleep (Jasper and Tessier, 1971; Gadea-Ciria, et al., 1973; Haranath and Venkatakrishna-Bhatt, 1973), as numerous studies have suggested a cholinergic role in the induction and maintenance of REM sleep (Jouvet, 1975; Hobson et al., 1976; Toyoda et al., 1966). For example, REM sleep has been induced by administration of ACh-bromide and carbachol into the brainstem of cats (Hobson et al., 1976). Also, induction of REM sleep may be facilitated

by physostigmine administered intravenously in intact cats (cf. Jouvet, 1975). In this case, physostigmine may exert its effects by amplifying the spontaneous activity of cholinergic neurons. This interpretation is consistent with the data reported by Hobson et al. (1976). To summarize, Hobson and his associates have hypothesized that REM sleep is controlled by a dipole consisting of cholinergic and catecholaminergic centers of the midbrain and pons. Since physostigmine was unable to induce REM sleep early (5 min) after sleep onset (physostigmine-induced REM sleep occurred 35 min after sleep onset) it was proposed that inhibitory, catecholaminergic influences were active early in the sleep cycle. Later on, catecholaminergic neurons were found to be less active, upon which physostigmine-induced REM sleep occurred.

In other investigations (Hazra, 1970; see also Karczmar, 1979) cholinergic mechanisms have been implicated in a number of sleep-related processes in man, including the induction of REM sleep and arousal, the initiation of dreaming in REM sleep, and the release of growth hormone during sleep (Gillin et al., 1978; Sitaram et al., 1977). The complete extent of the cholinergic contribution to the regulation of these processes has not yet been determined.

While the well-known mechanism underlying slow-wave sleep depends on serotonergic sites originating in the Raphe nuclei (Jouvet, 1975), there is some evidence that indicates

that the cholinergic system may contribute to slow sleep as well (Hernandez-Peon,et al.1963; Karczmar et al., 1970). Altogether, sleep and its phases depend on a complete interaction of cholinergic, catecholaminergic, and serotonergic systems centered on tegmental Raphe and locus coeruleus nuclei.

The complete homeostatic system that concerns an organism's states of consciousness included sleep and wakefulness. Again, the cholinergic system is involved in the latter. This is understandable in view of the presence of cholinergic synapses in the reticular formation (see also Section II.C.), and the evidence indicating cholinergic activation of the reticulo-mesencephalic system as well as reticular phenomena such as EEG arousal, is convincing and extensive; this role for the cholinergic system was proposed early by Rinaldi and Himrich (1955) and Bradley and Elkes (1953), and similar investigations continue being carried out at present (see Karczmar, 1979).

2. Appetitive Behavior

Appetitive behavior is now regarded as a much more complex phenomena than previously imagined, since the effects of centrally-applied agents (cholinergic as well as adrenergic) have differed markedly with regard to the species studied. For example, in rabbits intra-hypothalamic injections of carbachol elicited feeding behavior (Sommer et al., 1967), while in cats cholinergic stimulation had

no effect on appetite (Myers, 1964). In monkeys, injections of noradrenaline into the diencephalon elicited both feeding and drinking when animals were satiated, while cholinergic agents elicited under these circumstances feeding but not drinking (cf. Myers, 1964). Also, there may be a difference with respect to feeding between cholinergic receptors, as muscarinic compounds have been shown in several species to decrease feeding while nicotinic drugs were facilitatory with respect to hypothalamic hunger-satiety centers (cf. Karczmar, 1976; 1979; 1980).

3. Drinking Behavior

Studies involving rats have shown that drinking behavior and the cholinergic system are closely related. Kribstone and Levitt (1970) demonstrated drinking behavior in satiated rats after carbachol application to septal, thalamic, and hypothalamic sites. Drinking responses have also been elicited by implantation of crystalline carbachol (Grossman, 1964), or by injection, intracerebrally, of carbachol in solution (Levitt, 1970). In other experiments (Winson and Miller, 1975) carbachol-induced drinking was blocked by central and peripheral injections of atropine.

Experimentation with higher mammals (other than rats) revealed a more complicated picture. Sharpe (1972; cf. Myers, 1974) found that neither cholinergic nor adrenergic stimulation of the cat's lateral or medial hypothalamus elicited drinking; and carbachol injections into the pre-

optic area of monkeys did not produce drinking behavior. It remains unclear, therefore, whether the data obtained clearly indicate the existence of a cholinergic drinking center (cf. Myers, 1974).

4. Memory and Learning

It has been postulated that learning experience initiates a neuronal process whereby excitability at cholinergic synapses increases over a period of several days and persists for several days thereafter (Deutsch, 1971). These changes at the cholinergic synapses are probably due to an increase in the sensitivity of the postsynaptic membrane. Additional evidence for the cholinergic involvement in memory was published by Rosenzweig et al. (1967). It was reported that rats selected for the trait of superior learning ability had higher brain ACh and AChE levels than rats selected for the trait of poor learning ability (for criticism, see Karczmar, 1969). More recent evidence has shown that the selective loss of cholinergic neurons in the cortex and hippocampus is associated in man with Alzheimer's-type senile dementia and amnesia (Davies and Maloney, 1977; Reis, 1971).

Since ACh has been implicated in the memory and learning process, investigators have attempted to supply in man additional amounts of the precursor, choline, to the cholinergic neuron to observe its pharmacological and behavioral effects and to remedy old age ACh deficit. Phos-

phatidylcholine (the main source of choline in the brain, see also Section II.A.) was administered orally to a group of relatively "slow learners" (Sitaram et al.,1978). Marginal improvement in performance of a serial learning task was reported. Evidence obtained in animal studies have shown a somewhat more positive effect. For example, Staves and Brown (1976) reported that physostigmine (cholinesterase inhibitor) improved maze performance by rats with poor initial learning ability, while it caused little or no improvement in performances of fast learners. Also, it was reported by McGauh (1973) that physostigmine increased retention time in monkeys.

Although these investigations have implicated learning and memory to be related to cholinergic activity in the brain, conclusive evidence for a positive effect of ACh precursors has not yet been obtained in man. It has been suggested (Sitaram,1978) that phosphatidylcholine may eventually prove to be the most efficacious memory enhancing agent.

5. Temperature Regulation

Central thermoregulatory centers are located in the posterior hypothalamus of the brain and are regulated by Na+ - Ca++ ratio (Myers, 1974). Hyperthermia can be induced by localized injection of cholinomimetics into the medial preoptic area of the hypothalamus, or into the cerebral ventricles (Myers, 1974). Norepinephrine has the opposite effect (Avery, 1971). In fact, Hensel et al.

(1973) have hypothesized that there may be a balance between the two transmitters, and perhaps serotonin as well (Lomax, 1966; Myers, 1964). Avery and Penn (1973) have also shown that cholinomimetics induce behavior leading to early escape from environmental heat, while late escape is seen with norepinephrine. It appears also that cholinergic hypothalamic pathways regulate not only the thermostat but also heat dissipation mechanism (cf. Myers, 1964).

C. ACETYLCHOLINE AND AGGRESSION AND THEIR ANATOMICAL CORRELATES

1. Pathways

a) Cholinergic Limbic System

Since this dissertation deals with acetylcholine, the cholinergic system, and aggression, a brief discussion of cholinergic pathways is necessary; particular attention will be focused upon the brain parts assayed in these experiments, septum, striatum, thalamus, and hippocampus. Some of these brain parts have been considered to be part of the limbic system which has been described (Papez, 1973; pp. 37) as follows:

> It is proposed that the hypothalamus, the anterior thalamic nuclei, the gyrus cinguli, the hippocampus and their interconnections constitute an harmonious mechanism which may elaborate the functions of central emotion as well as participate in emotional expression.

The limbic system is particularly relevant for this dissertation as it is concerned with aggression, particularly of affective type (see above, Section I), and as it is rich

in cholinergic synapses as known since researches of Lewis and Shute (1967).

Thus, it is important to discuss the cholinergic limbic system in some detail. While the earlier investigators (cf. Koelle, 1963; Lewis and Shute, 1967) based their description of cholinergic pathways, including the cholinergic limbic system, on histochemical localization of acetylcholinesterase, using this localization as a marker of cholinergic pathways, newer methods of determining these pathways are based on, additionally, localizing acetylcholine and cholineacetylase as well as cholinoceptive binding sites (Yamamura and Snyder, 1974) in the central nervous system; some of the methods in question are remarkably sensitive and precise (Kuhar, 1976).

The cholinergic limbic system can be described, briefly, as follows: one group of afferent fibers to the hippocampal formation originates in the medial septal nuclei and the nucleus of the diagonal band of Broca. A second group of fibers flows out from the anterior dorsal and ventral nuclei of the thalamus to the retro-splenial and cingular areas. The initial outflow from the hippocampus is non-cholinergic, but it impinges upon nuclei rich in acetylcholinesterase-containing fibers (Green, 1954); these sites are also rich in acetylcholine and cholinoceptive binding sites (Kuhar, 1976). The dorsal and ventral tegmental cholinergic pathways receive fibers from the hippocampal fornix projection system.

It is known that these acetylcholinesterase-containing pathways do not form compact bundles but rather comprise a relatively diffuse network of very fine fibers related to the limbic brain.

Among these sites the septo-hippocampal pathway is particularly well established as cholinergic (cf. Pepeu et al., 1973). Lesions of the septo-hippocampal pathway or the septal area itself have resulted in large decreases of acetylcholinesterase and choline acetylase on the side of the lesion in the hippocampus (Lewis et al., 1967; Fonnum, 1970). Also, it has been shown that acetylcholine can be released from the hippocampus after septal stimulation (Smith, 1972).

Nordberg and Sundwall (1975) have contributed additional evidence of cholinergic involvement in limbic structures. For example, it was shown that endogenous acetylcholine levels and specific radioactivity of acetylcholine (determined following ^{3}H-choline injection) were very high in the thalamus (ACh level of 20.7 nM/g) and hippocampus (ACh level of 17.0 nM/g; endogenous level) as compared to the levels present in other brain structures. It must be noted, however, that striatal acetylcholine levels 60.0 nM/100 g protein, were among the highest for the various brain parts investigated. Septal acetylcholine levels were found by Bisco and Straugham (1966) to be intermediary (13.1 nM/g) when compared to other brain areas.

b) Other Cholinergic Pathways

Although the dorsal and ventral tegmental pathways are not considered to be the cholinergic limbic system, they are closely coupled with the latter and should be discussed at this time.

The dorsal and ventral tegmental pathways have been described by Lewis and Shute (1967), as being concerned with cholinergic outflow to the limbic pathways. The dorsal tegmental pathway originates from the nucleus cuneiformis in the midbrain and extends from the pons to the pretectal area. It has been shown to receive cholinesterase-containing fibers from the reticular formation. After lesioning of the nucleus cuneiformis, decreased cholinesterase staining of the inferior and superior colliculi was reported (Butcher, 1977; Nordberg and Sundwall, 1975).

The ventral tegmental pathway is also an acetylcholinesterase and acetylcholine containing nerve and axon system (Fonnum, 1975) which originates from the pars compacta of the substantia nigra. This pathway enters the yona incerta, the supra mammilary region and the lateral hypothalamic area, and ascends rostrally to the basal area of the forebrain where it links with acetylcholinesterase-containing cells in the globus pallidus, caudate, putamen, and lateral preoptic area.

Additional evidence for cholinergic involvement in the ventral tegmental efferent pathway and its outflow to the basal ganglia has been provided by Lynch et al. (1972)

with regard to the striatum. Acetylcholinesterase-containing fibers in the caudate putamen have been visualized. Also, the striatum has been shown to contain some of the highest levels of acetylcholine (60 μM/100 mg protein), choline acetyltransferase (10.7 μM ACh/100 mg protein/hr), and acetylcholinesterase (281.0 μM ACh/100 mg protein/min), of any major structure in the central nervous system (McGeer and McGeer, 1976).

The majority of striatal neurons exhibiting these high levels of acetylcholine, choline acetylase, and acetylcholinesterase appear to be small Golgi type II interneurons. Additional evidence indicating that these are, indeed, interneurons is as follows. The striatum receives a massive neuronal input from the cerebral cortex and the midline thalamic nuclear group; lesioning of these known afferents has failed to bring about any major reduction in acetylcholine, choline acetylase, or acetylcholinesterase of the striatum. Furthermore, lesioning of striatal efferents has failed to bring about a reduction of cholinergic levels in globus pallidus or substantia nigra (Hattori, 1976). Accordingly, the small neurons staining for acetylcholinesterase and choline acetylase in the striatum must be interneurons.

The habenula-interpeduncular tract is another well-known cholinergic pathway in the brainstem. The medial habenula has been shown to exhibit high choline acetylase activity (Katooka, 1973). The interpeduncular nucleus exhibits the highest choline acetylase activity (26.6 μM/100 mg

protein), in the brain (McGeer and McGeer, 1976). Habenular lesions have dramatically lowered this enzyme activity (Katooka, 1976).

Central cholinergic systems, as well as other transmitter systems (e.g., noradrenaline, dopamine, glutamate; cf. De Feudis, 1974), appear to be involved in cortical arousal, which is mediated by the reticular-activating-system. This is a diffuse system with many peripheral inputs, including those from ascending spinal tracts, from the brainstem and from specific sensory pathways (i.e., via the spinal and cranial nerves; DeFeudis, 1974). The reticular-activating-system emanates from the brainstem reticular formation (i.e., mesencephalon, pons) and projects, via the thalamic and hypothalamic nuclei, to the cerebral cortex and hippocampus (Moruzzi and Magoun, 1949; cf. DeFeudis, 1974). Hippocampal activation seems to be generated predominantly by pathways emanating from hypothalamic structures, while cortical activation seems to occur by pathways which traverse the mesencephalic reticular formation (i.e., reticulo-mesencephalic-cortical ascending system: Domino, 1966; 1968).

Histochemical findings, based on acetylcholinesterase staining, have supported the concept of a cholinergically-mediated arousal system. A continuous system of cholinergic fibers appears to project to the hypothalamus, basal ganglia, and cerebral cortex from the mesencephalon (Shute and Lewis,

1963; 1967; Krnjevic and Silver, 1965). The acetylcholinesterase content of pyramidal cells in layer V of the cortex was found to be high (Krnjevic and Silver, 1965); acetylcholinesterase staining indicated that cells of the corpus striatum and septum project to the cerebral cortex (cf. DeFeudis, 1974; Syerb, 1967).

The above evidence, along with that provided by studies in which it was shown that cortical acetylcholine release was increased during cortical activation (Celesia and Jasper, 1966; Syerb, 1967), supported further the idea that the reticular-activating-system is activated, at least in part, by cholinergic mechanisms.

Another line of evidence which supports the hypothesis that cholinergic mechanisms may be involved in reticular formation-mediated arousal stems from behavioral experiments performed with intracerebral injection techniques. Injection of acetylcholine or carbachol directly into the mesencephalon or thalamic reticular formation of the rat produced reversible behavioral changes in both appetitive and aversine test situations (Grossman, 1968; Grossman and Peters, 1966). These workers concluded that cholinergic stimulation increased markedly the animal's responsiveness to sensory stimulation. These results indicated that cholinergic reticular mechanisms might be involved in behavioral arousal. In a rather complex way, these data are in accord with the EEG effects of cholinergic agonists (see below Section II.C.2. and Karczmar, 1979).

The great majority of cholinergic pathways have not yet been described in detail. Although the highest levels of acetylcholine in the brain have been reported for striatum (53.0 nM/g; Cheney et al.,1975), a definite cholinergic pathway to or from the striatum has not yet been described. As already mentioned, cholinoceptive interneurons and intrinsic cholinergic pathways appear to be present in the striatum. Additional efforts to map the cholinergic system in the brain are necessary to elucidate an understanding of the role of acetylcholine and of cholinergic synapses in central nervous system functions.

2. Acetylcholine and Aggression

a) Introduction

Aggressive behavior has been shown to be influenced to a large degree by the central cholinergic system, especially as it is present in the limbic areas (Bandler, 1971; Beleslin and Samardzic, 1979; Karczmar et al., 1973; 1980). Cholinergic agonists and antagonists exert enhanced and depressed aggressive effects, respectively, when applied directly to limbic and related structures such as septum (antagonists inhibit aggression; Albert and Richmond,1976), hypothalamus (agonists facilitate aggression; Bragley and Albert, 1977), and thalamus (agonists facilitate aggression; Bandler, 1969; Baxter, 1966). In general, both affective and predatory types of aggression (see section IB for definitions; Moyer, 1968) have been elicited by localized

application of cholinergic, particularly muscarinic, agonists and anticholinesterase agents (Pradham, 1975; cf. Karczmar, 1978). Cholinergic agonists such as acetylcholine and carbachol have been shown to facilitate muricidal behavior in rats (McCarthy, 1966; Avis, 1974) and isolation-induced aggression in mice (Karczmar and Scudder, 1969; Karczmar et al., 1973).

Conversely, several types of aggression have been blocked by localized as well as systemic application of anticholinergic drugs, generally of atropinic rather than curaremimetic type (Romanjeck et al., 1973; Yoshimura and Ueki, 1977). Anticholinergic drugs, such as atropine and scopolamine, have been shown to block isolation-induced aggression in mice (Janssen et al., 1960). Also, atropine administered via intrahypothalamic injection inhibited muricidal aggression in rats (Bandler, 1971), as well as several forms of electrically-elicited aggression (Slotnick and McMullen, 1972).

b) Predatory Aggression

Predatory aggression (e.g. frog- or mouse-killing by rats; rat-killing by cats) has been among the most widely studied type of aggressive behavior. The most compelling evidence for the participation of central cholinergic networks in aggression is the demonstration of its role in the regulation of predatory killing described in the elegant investigations of Bandler (1969, 1970, 1971, 1972). Using

as a model predatory frog-killing behavior in the rat (Bandler and Moyer, 1970). Bandler demonstrated (Bandler, 1970; 1971), that a lethal attack on a frog could be facilitated by intracerebral injection of carbachol or acetylcholine (in conjunction with a cholinesterase inhibitor) at specific hypothalamic sites, medial or midline thalamic nuclei, and ventral midbrain tegmental areas. The cholinergic effect seemed to be related to the evocation of predatory killing since the killing response was of stereotyped nature characterized by the straight-forward, well-aimed attack and lack of increased irritability (cf. Section IB for description of predatory attack). Significantly, it was also shown in these experiments that local injection of the cholinesterase inhibitor neostigmine at active hypothalamic sites facilitated the aggressive behavior and that systemic injection of atropine depressed it. Although several other neurotransmitter systems may play an active role in mediating this muricidal behavior at thalamic and hypothalamic sites in the brain regions (cf. also below, Section III), these data support the hypothesis that the cholinergic system may play a facilitatory role in mediating predatory aggression.

Additional evidence for the cholinergic mediation of predatory aggression has been provided by Smith et al. (1970). These investigators described the production of mouse-killing behavior in rats, which were not constitutionally killers, by the intracerebral injection of carbachol into the lateral

hypothalamus given intracerebrally. The anticholinesterase agent, neostigmine was shown to produce killing in non-killer animals, whereas atropine was shown to block predatory killing in killer rats.

Neurochemically, killer and non-killer rats differed. For example, killer rats exhibited higher acetylcholine levels in the diencephalon than non-killer rats; no differences in acetylcholine levels were found with cortex, striatum, or amygdala, however (Yoshimura and Ueki, 1976). So, the act of aggressing-upon a natural prey has been shown to be related to brain levels of acetylcholine -- an example of behavior affecting a neurochemical change. In other words, it has been shown that a) The cholinergic system affects predatory aggression converting "non-killers" into "killers" (Bandler, 1970, 1971), and b) Predatory aggression affects cholinergic neurochemistry as elevated diencephalic acetylcholine levels seem to characterize "killers" (Yoshimura and Ueki, 1977).

c) Affective Aggression

There is considerable pharmacological evidence that central cholinergic mechanisms participate in the neural organization of affective aggression (cf. Reis, 1974; Valzelli, 1973). Affective aggression has been described as the mode of aggressive display which can be seen in aggressive states characterized as irritable, intermale, territorial, maternal, or pain-induced (see Section IA; Moyer, 1968;

Avis, 1974). When introduced into the third or lateral ventricles of the cat, acetylcholine or its congenor carbachol have been shown to evoke fully developed displays of aggressive behavior and affective attack (Baxter,1966; Beleslin et al., 1974; Smith et al., 1970). It has been postulated that this evoked, affective behavioral phenomena observed after application of cholinomimetics is mediated by drug action on central muscarinic cholinoceptive sites (Beleslin et al., 1974; Beleslin and Samardzic,1977, 1979). In some investigations (Baxter, 1967; Smith et al., 1970) in which central application of cholinergic agents was employed, the evoked affective aggression has been attributed to the development of seizure discharges in the hippocampus and the amygdala. However, the local injection of cholinergic agents into specific intracranial sites has produced affective display in the cat and rat without the appearance of seizure activity (Senault,1971; Paxinos,1976).

Isolating experimental animals for varying lengths of time has been a popular method of inducing affective-type aggressive displays (Banerjee,1971; Malick, 1978). Experimenters have been almost uniformly unable to find any effect of isolation on brain acetylcholine levels (Garattini et al., 1969; Consolo and Valzelli, 1970) or acetylcholine turnover (cf. Avis, 1974). In addition, Karczmar and Kindel (1980), demonstrated decreased acetylcholine turnover values for the CF-1 strain mice found to be aggressive after isolation.

There has been a paucity of relevant data in the current literature concerning this problem; this then, motivated this research. In the present work it was shown that septal and thalamic acetylcholine turnover values were elevated in aggressive CF-1 mice when compared to turnover values from non-aggressive mice (see Results, Section III). It should be noted, however, that the data presented in this dissertation were obtained with isolated mice that were paired with aggregated mice (cf. Results).

Anti-cholinergic drugs have been considered to be reliable blockers of isolation-induced aggression (Janssen et al., 1960). Nevertheless, it has been demonstrated that low doses of anticholinergic drugs (as well as low doses of cholinergic agonist) may increase isolation-induced aggression (Karczmar and Scudder, 1969). Possibly, there may exist in the brain a cholinergic system which inhibits and another which facilitates aggression.

d) Brain Lesions and Aggression

Numerous brain regions have implicated the cholinergic system to be a mediator of aggressive behavior; some of these include septo-hippocampal pathway (Atweh and Kuhar, 1976), thalamus (Bandler, 1970, 1971), hypothalamus (Bandler, 1971; Smith et al., 1970), amygdaloid nuclei (Fonberg, (1973) thalamic-hypothalamic-medial forebrain bundle network (Nauta, 1968), and the amygdalo-fugal fiber system.

The well-defined anatomical organization of the septo-hippocampal cholinergic pathway has made it a target of pharmacological and behavioral studies of central cholinergic mechanisms. Acute interruption of impulse flow by placing an electrolytic lesion in the medial septum results in elevation of acetylcholine content in the hippocampus as well as elicitation of aggressive behavior in the rat (Atweh and Kuhar, 1976; cf. Albert et al., 1978). Similarly, the septo-hippocampal pathway appears to be inhibitory with regard to aggression in the rat. It has been postulated by Atweh and Kuhar that additional afferent fiber tracts innervating the hippocampus are inhibitory for aggressive behavior since the aggressive displays observed in the septal-lesioned rats were neither intense or long-lasting.

Lesions of the septum and the ventromedial nucleus of the hypothalamus facilitated shock-induced fighting, while lesions of the amygdala and hippocampus inhibited this form of aggression (Blananchard et al., 1968; Albert and Richmond, 1976). In man, Spiegel et al. (1947) reported that destruction of the dorsomedial thalamus reduced aggression, temporarily. The data are, however, controversial, as in the monkey, cat, and rat, lesions of the dorsomedial thalamus increased (Schreiner et al., 1969), elicited (Eclancher and Karli, 1968; MacDonnell, 1968), or did not affect (Neto and Nunes, 1973), as well as reduce (Routtenberg, 1967; Atrens et al., 1974) aggressive behavior.

III. NEUROTRANSMITTERS (OTHER THAN ACH) AND AGGRESSION

This section concerns the relevance of four putative neurotransmitters, GABA, dopamine, serotonin, and norepinephrine, to aggressive behavior.

A. GABA AND AGGRESSION

Of all the putative neurotransmitters in the limbic system, the strongest evidence for neurotransmitter function pertains perhaps to GABA. Glutamic acid decarboxylase (GAD), the enzyme catalyzing the formation of GABA, appears to be restricted to GABAergic neurons and is therefore a suitable marker for such neurons. In the hippocampus, GAD is concentrated between the perikarya of the pyramidal and granular cells and in the most superficial layers of the hippocampal cortex (Roberts et al., 1976). The hippocampal formation projects bilaterally to the septum and mamillary bodies, and according to McLennan and Miller (1974) these projections are excitatory but probably not GABAergic. There is also evidence (Johnson,1972) that cells in the medial septum projecting to the hippocampus and lateral septum are controlled by short-axoned inhibitory neurons which likely use GABA as their transmitter.

GABA appears to be involved in the development of

aggressive behavior. It has been reported (Brody et al., 1969; Earley and Leonard, 1977), that GABA inhibited attack responses and increased the threshold of electrically induced attacks in cats with hypothalamic implants. Further evidence that GABA exerts an inhibitory influence on aggressive behavior has been reported by DeFeudis et al. (1976), as they reported that the binding capacity of GABA was decreased in isolated aggressive mice and increased in aggregated non-aggressive mice.

On the other hand, GABA level determinations in specific brain areas of aggressive and non-aggressive mice present a varied picture (Early and Leonard, 1977). Here, high GABA levels in the septum and striatum were found in non-aggressive mice, while high GABA levels were present in the hippocampus and amygdala of aggressive mice. Clearly then, further studies involving endogenous GABA levels and turnover values in select portions of the brain are needed to further elucidate the role of GABA in aggression.

B. DOPAMINE AND AGGRESSION

Cell bodies of dopaminergic neurons reside within the substantia nigra, hypothalamus and the interpeduncular nucleus (Ungerstedt, 1971), and give rise to the nigrostriatal, tuberoinfundibular, and mesolimbic-mesocortical pathways, respectively. There are, additionally, dopaminergic interneurons in the brainstem, superior cervical ganglia, retina,

olfactory bulb, and carotid body (Iversen and Glowinski, 1966).

The nigrostriatal dopamine system is now one of the most widely studied pathways in the brain. Dendrites from dopaminergic neurons in the substantia nigra extend rostrally in the lateral hypothalamus just dorsolateral to the medial forebrain bundle. They enter the crus cerebri at the mid-hypothalamic level and fan out through the globus pallidus to enter the caudate and putamen (Ungerstedt, 1971).

The next most prominent dopaminergic tract is the mesolimbic-mesocortical pathway. The axons ascend together with axons of the nigrostriatal dopamine system past the crus cerebri and innervate limbic structures such as the nucleus accumbens, olfactory tubercle, and possibly the amygdala (Iversen and Glowinski, 1966). Cortical innervation of dopaminergic neurons is an extension of this complex.

The tuberoinfundibular dopamine system has cell bodies located within the arcuate nucleus of the hypothalamus. These cells innervate the external layer of the median eminence. It has been suggested that interstitial neurons in the hypothalamus might give rise to dopaminergic axons ascending into the thalamus (Ungerstedt, 1971)

Although the role of dopamine in aggressive behavior is unclear at this time, recent evidence suggests that dopaminergic neurons facilitate the expression of affective aggression (cf. Reis, 1974). For example, it has been demonstrated that rats treated with the dopaminergic agonist, apo-

morphine, exhibit increased intraspecific affective aggression (McKenzie, 1971). This behavior can only be demonstrated in the male of this species and it is facilitated by pain and isolation (McKenzie, 1971;Senault, 1971). Also, aggressive behavior has been induced in rats and cats by the administration of the dopamine precursor, L-DOPA; however, it has been suggested that the noradrenergic system may also play a significant role in this type of drug-induced aggressive behavior (Scheel-Kruger and Randrup, 1967).

Along these same lines, Lycke et al. (1969) have demonstrated that mice infected with herpes simplex encephalitis which exhibited increased synthesis of brain dopamine were exceptionally aggressive and fought intensively when paired with each other. Since the aggressivity of these mice was reduced by inhibition of dopamine synthesis with α-methyl-paratyrosine (αMPT), it seems likely that the behavior in question is related to the increased availability of dopamine.

Altogether, dopamine seems to exert facilitatory influences with regard to affective aggression. Predatory aggression, on the other hand, may be inhibited by dopaminergic agents since several drugs that enhance the availability of dopamine such as amphetamine, L-DOPA, and monoamine oxidase (MAO) inhibitors, block predatory aggression (Salama and Goldberg, 1970; cf. Reis, 1974). Undoubtedly a clearer picture will be realized when data concerning the role of other putative neurotransmitters (norepinephrine, acetylcholine,

and serotonin) in aggression is correlated with the results of experimentation on the dopaminergic system; this type of work is only beginning (cf. Karczmar, 1978).

C. SEROTONIN AND AGGRESSION

As was the case with dopamine, the neural systems synthesizing and releasing the neurotransmitter serotonin (5-HT) are relatively restricted; the cell bodies of these neurons are localized in the Raphe nuclei and reticular systems of the brainstem and send axon projections to the hippocampus, subiculum, and cingulate bundle (Ungerstedt, 1971). The more caudal serotonergic neurons give rise to descending pathways to the spinal cord, whereas the more rostral ones give rise to ascending pathways coursing to the diencephalon and telencephalon.

Three main ascending serotonergic pathways to the brain have been described by Ungerstedt (1971). The most medial ascending pathway into the forebrain innervates the hypothalamic, preoptic, and septal areas. The next pathway, slightly more lateral, innervates the cerebral cortex. It runs through the medial forebrain bundle, sweeps dorsally along the cingulate gyrus and curves laterally into the hippocampus. Along the way, branches are given off to all cortical areas. The third system primarily innervates the corpus striatum. This pathway is located in the region lateral to the medial forebrain bundle.

Serotonergic mechanisms have been implicated in inhibition of aggressive behavior in male rats and mice, and in female mice (Blanchard and Blanchard, 1968). It has been observed that when 5-HT neurons are lesioned selectively with 5,7-dihydroxytryptamine (5,7-DHT) injected stereotactically, into 5-HT pathways, mouse killing behavior in rats is increased (Breese, 1975), and biting attacks on males by female rats occur (Neckers et al., 1975). This work supports the assumption, therefore, that central 5-HT neurons block aggressive behavior. Similarly, it was shown that aggression-inducing paradigms such as isolation cause a decrease of the turnover of brain serotonin (cf. Karczmar and Scudder, 1969, and Valzelli, 1973).

Further evidence for serotonin's influence on aggression was presented by Sheard (1969; 1970) who employed PCPA to inhibit the serotonin synthesizing enzyme, tryptophan hydroxylase. The inhibiting effect of 5-HT on aggression was abolished by PCPA pretreatment. It was also shown that shock-induced fighting and predatory killing in the rat (Sheard, 1970), and rat killing in the cat (Ferguson et al., 1970) were increased after PCPA pretreatment. Present evidence suggests, therefore, that serotonin is preponderantly inhibitory to aggressive behavior (Hodge and Butcher, 1974; Hole et al., 1977; Valzelli, 1973).

D. NOREPINEPHRINE AND AGGRESSION

The cell bodies of noradrenergic neurons reside in the mesencephalon, pons, and medulla and send widely ramified axonal processes throughout the brain and spinal cord. On the basis of lesion studies, Ungerstedt (1971) has defined two principal pathways - a dorsal and ventral noradrenergic system. The processes of these cells are widely ramified with their processes innervating cerebellum,neocortex, hippocampus, amygdala, and also the hypothalamus.

There is considerable evidence that the neuronal release of norepinephrine facilitates or possibly initiates affective aggression (Reis, 1974; 1972). This evidence includes the demonstration of increased turnover of norepinephrine in the brains of animals made aggressive by isolation (Welsh, 1975) or electric footshock (Janoy et al., 1972). In general, drugs or environmental changes which increase central norepinephrine levels increase affective aggression (cf. Sheard, 1977; Eichelman and Thao, 1971). For example, rubidium, which increases norepinephrine turnover (Eichelman et al., 1973), increases aggressive behavior, as do tricyclic antidepressants and MAO inhibitors (Eichelman and Barchas, 1975; cf. Sheard, 1977).

Predatory aggression, however, is inhibited by noradrenergic agents (Thoa et al., 1975; see also Section III.B.).

E. CONCLUSIONS

In conclusion, when a correlation has been proposed relating, for instance, norepinephrine with facilitation of aggressive behavior, or 5-HT with inhibition of affective aggression, this evidence must be viewed with a degree of skepticism, as the data are on the whole, controversial, and as results obtained depend very much on the species used and on aggression-inducing paradigm. A true understanding of neurotransmitter function in aggressive behavior, or any behavior for that matter, can only begin to be realized when multiple-transmitter studies are performed. Studies that concern individual transmitters shed a limited amount of light on this complex puzzle (cf. Karczmar, 1978).

IV. REVIEW OF NEUROTRANSMITTER TURNOVER

A. DEVELOPMENT OF TURNOVER METHODOLOGY

Various approaches have been used to achieve the goal of establishing a workable isotopic method for the analysis of brain acetylcholine turnover rate *in vivo*. For years it was maintained that the capacity of brain to synthesize choline is very limited (Ansell and Spanner, 1971). The brain, therefore, was believed to be dependent to a major extent on circulating choline supplied from the periphery for the synthesis of acetylcholine. This concept was reinforced by the demonstration that radiolabelled choline can be transported into the brain after either its intravenous or parenteral injection in animals (Diamond, 1971). The fact that acetylcholine in the central nervous system can be labelled with a radioactive precursor, such as choline, injected into an animal peripherally contributed to the development of the isotopic approach to the study of brain ACh turnover in mammals *in vivo*. This approach was first described by Dross and Kewity (1966) and was later expanded by Schuberth et al. (1969; 1970), Haubrich et al. (1974), Sparf (1973), and Jenden et al. (1974).

In Schuberth's work, tritium-labelled Me-^{3}H-choline was injected into mice (0.5 μM/mouse, I.V.) and incorpora-

tion of radioactivity into brain ACh was measured for one time point (30 sec) following the injection. This is an example of a "one-point" turnover analysis (see below for discussion of "multiple-point" turnover analysis; Jenden, 1974). Calculations were based on the following equation: ACH formed (nM)=^{3}H-ACH/^{3}H-CH X ENDOGENOUS CH (nM).

Haubrich (1974) also employed this equation and the "one-point" turnover analysis in his calculation of ACh turnover. Tritiated choline was injected and the animals were sacrificed at one specific time point (30 sec) after the injection. All turnover calculations were based on this time point. According to Haubrich (1974) one-point analysis is adequate as compared to analysis based on data obtained at multiple time points since the uptake of choline into the brain and its subsequent conversion into ACh are extremely rapid; thus, the "one-point" analysis may be considered to be an "instantaneous" evaluation of ACh synthesis. Care must be taken to employ a time point soon after ^{3}H-choline injection. Possible data perturbation may result from increased ^{3}H-choline levels which may be present as a result of ACh hydrolysis or excessive choline levels (see Materials and Methods Section; in the present study the time point chosen for analysis was 30 sec).

Jenden et al. (1974) employed gas chromatography-mass spectrometry to estimate the ACh turnover rate of mouse brain <u>in vivo</u>. A deuterium-labelled variant of choline was

introduced intravenously by constant infusion and its rate of incorporation into brain ACh was measured by a "multiple-point" turnover analysis. Values for choline and ACh specific activity were recorded several times following the initiation of labelled-choline infusion (20 sec, 40 sec, 60 sec, 80 sec, 120 sec, 240 sec, 480 sec). This evaluation involves computerized analysis of the precursor-product relationship for an 8 minute time period. This "multiple-point" analysis for this dissertation was employed initially, in this research; however, due to inadequacies in computer programming this method was abandoned and the "one-point" analysis was employed. It should be added that it was shown recently, that "one point" (Schuberth et al., 1970) and "multiple-point" (Jenden, 1974) analysis yield well-nigh identical turnover values (Karczmar and Kindel, 1980).

The turnover rate of ACh as determined by means of "multiple-point" analysis may be expressed as follows:

$$\frac{dy^*}{dt} = V \times \frac{x^*}{x} - \frac{y^*}{y}$$, where x and y

represent total choline and ACh concentrations, respectively, the asterisk represents deuterium-labelled variants of these compounds, and V is the ACh turnover rate.

B. DISCUSSION OF ACETYLCHOLINE TURNOVER

Acetylcholine turnover has been defined as the rate at which ACh is synthesized (Hanin and Costa, 1976). The turnover rate of acetylcholine has been commonly expressed

(Sparf, 1973) in nanomoles of acetylcholine formed per hour per gram of tissue. ^{3}H-choline has been used as the radiolabelled precursor of ACh; thus, specific activity ratios (^{3}H-ACh/^{3}H-choline; Schuberth et al., 1970), have been employed in turnover calculations. The turnover rate of acetylcholine is a function of the ^{3}H-ACh/^{3}H-choline ratio. Radiolabelled ACh represents the incorporation of ^{3}H-choline into newely-synthesized ^{3}H-ACh (Hanin and Schuberth, 1974).

Perturbation of the choline and acetylcholine steady-state has been regarded as an inherent danger in ACh turnover methodology (Hanin and Costa, 1976; Hanin and Schuberth, 1974). Steady state conditions exist when, hypothetically, equimolar ratios of precursor and product are present in the system; that is, one mole of acetate and one mole of choline are present to combine to form one mole of acetylcholine. If the quantity of choline, for example, was increased to a value greater than one molar (relative to acetylcholine), the choline pool would be overloaded and steady-state conditions would no longer exist (Hanin and Costa, 1976).

The Km for choline is 250 μM (Singh and McGeer, 1977). Since the amount of free choline in brain is only 25-45 μM (cf. McGeer and McGeer, 1976), an overabundance of choline would raise acetylcholine synthesis to a new plateau thereby, disturbing the steady-state. A five-fold increase in choline levels, for example, would not fully saturate the synthesizing enzyme for acetylcholine, choline acetylase;

thus, high choline levels would result, proportionately, in higher than normal acetylcholine levels and acetylcholine turnover values.

Racagni et al. (1974) proposed several assumptions regarding choline and acetylcholine biochemistry; these assumptions are pertinent for the concept and measurement of turnover: 1) Plasma choline is rapidly transferred to a pool of free brain choline which equilibrates with the various metabolic pools of brain choline at a rate proportional to their intrinsic metabolic rates. 2) In various brain areas the conversion of labelled choline into acetylcholine proceeds at a rate which is approximately equal to the rate of disappearance of radioactive choline from each brain area. 3) The pool of free choline which is re-utilized in the synthesis of acetylcholine is much smaller than the total metabolic pool of brain choline; thus, because of the method of labelling, the feedback effect of radioactive choline formed from the hydrolysis of ACh is insignificant.

Regarding assumption #1, the choline pool used for ACh synthesis must be in equilibrium with all choline pools. This would be true if the choline pools were not overloaded with an oversupply of ^{3}H-choline (as discussed above). Other experimenters (Haubrich et al., 1975; Karczmar and Kindel, 1980), and myself have employed low concentrations (5.66 nM - ^{3}H-choline; present study) of the precursor choline to preserve steady-state. The preservation of steady-state is

also alluded to in assumption #2, which states that the rates of acetylcholine synthesis and hydrolysis must be equal in the steady-state conditions. Again, the prevention of precursor-overload is necessary.

Assumption #3 states that the reuptake of ^{3}H-choline by the nerve terminal after ^{3}H-ACh hydrolysis is insignificant. If significant levels of ^{3}H-choline were taken up by the high-affinity reuptake system and rapidly converted into ^{3}H-ACh, specific activity values for ^{3}H-ACh would be elevated; and ACh turnover would be increased according to the formula:

$$ACh_{TR} = \frac{\text{ACh SPECIFIC ACTIVITY}}{\text{Ch SPECIFIC ACTIVITY}} \times Ch \quad \text{, where}$$

ACH_{TR} = ACh turnover rate,
ACh = acetylcholine,
Ch = choline,
Ch = endogenous choline levels.

Elevated ACh turnover values, therefore, would reflect elevated ^{3}H-choline reuptake and not augmented ACh synthesis. It has been suggested by Schuberth et al. (1970), and Haubrich et al. (1975), that when determinations of ^{3}H-choline and ^{3}H-ACh specific activity are made as soon as possible after injection of ^{3}H-choline, ^{3}H-choline reuptake is minimized. In this case, ACh turnover values would reflect ^{3}H-ACh synthesis from injected (plasma) ^{3}H-choline: less time would be available for ^{3}H-choline reuptake to become a significant factor (Sparf, 1973).

MATERIALS AND METHODS

The section on Materials and Methods will include 1) Description of experimental animals and general conditions of their maintenance, as well as a description of the behavioral test employed, 2) Discussion of the brain part dissection technique, and 3) Description of the biochemical assay and measurement of ACh turnover.

I. ANIMAL MAINTENANCE AND BEHAVIORAL TESTING

Thirty-six day old male Mus musculus CF-1 mice obtained from the Carworth Farms Distributors were used in this study. The average weight of these mice was 30.2 g + 1.2 (S.E.M.).

In general, rodents can be inbred for the selection of various behavioral (Green, 1968; Karczmar et al., 1973; 1978) and neurochemical parameters (Mandel et al., 1977; Everett, 1973). It is well known that inbreeding techniques may be used to produce animals that exhibit increased aggreasion (Craig et al., 1965; Lagerspety and Lagerspety, 1971). The Mus musculus CF-1 strain was selected for this study because of the highly aggressive nature of this strain (Tinbergen, 1953; Karczmar and Scudder, 1969; Karczmar et al., 1978). In preliminary studies, the mice of the CF-1 strain

employed by us were compared to the C57Bl/6J strain and the former were found to be more aggressive.

Animals were housed in 24 cm X 18 cm X 48 cm plastic cages containing 2 cm of wood shavings for bedding; they were kept on a 12 hr light-dark cycle. Mice were given water and Purina mouse chow pellets ad libitum. Isolated animals were housed one per cage while aggregated mice were housed twelve per cage. In order that external stimuli be kept at a minimum, litter in all cages was changed weekly rather than daily (see also Introduction and Review of Literature, Section I.C.1.). Isolated and aggregated animals were housed for 21 day periods prior to experimentation.

Animals were tested for aggression in the following manner, which is an adaptation of the method used by Banerjee (1971); animals were selected at random to form pairs for the behavioral testing session; cages were numberd to insure that each animal was paired with a different opponent for each one of the the three pairing sessions, one session per day. Mice were marked with red and blue ink to distinquish individuals during the pairing session. After each animal was marked they were transferred to a "waiting cage" for 2 minutes before being placed in the "fighting chamber". The "fighting chamber" was divided in half by a cardboard partition and individual mice were placed on either side. At time zero, the partition was lifted and the following information was obtained during the 10 min. period: 1) La-

tency to attack was measured, 2) The attacking animal and the animal being attacked were identified, 3) The number of attacks per minute and the initiator of each attack were recorded, 4) The duration of each attack was measured, and 5) Escape behavior, if present, was noted and the escaping mouse identified. An attack was defined (Banerjee, 1971) as biting contact that lasted for at least one second. Animals displaying escape behavior sometimes leaped out of the cages; when this occurred the session was terminated. Also, the appearance of blood on any animal at any time during the pairing session resulted in termination of the session.

Animals received scores of 0-4 for each pairing session based on the following scheme.

SCORE	OBSERVED BEHAVIOR
4	a) Initiation of first attack $\leq$ 90 sec, b) The mouse was the initiator of every attack during the session, c) The mouse participated in $\geq$ 3 bouts/min for at least 3 consecutive minutes, and each bout was $\geq$3 sec duration.
3	a) Initiation of first attack $\leq$180 sec., b) The mouse was the initiator of every attack during the session, c) The mouse participated in $\geq$3 bouts/min for at least 3 consecutive minutes, and each bout was $\geq$3 sec. duration.
2	a) Initiation of not more than 2 attacks $\leq$180 sec, and/or b) Initiation of some attacks, passive in others.
1	a) Passive in initial and subsequent bouts, b) The mouse must, however, participate in $\geq$3 bouts, c) The mouse assumes defensive postures.
0	a) Escape behavior - the mouse did not participate in any bouts during the session.

Scores for each of the 3 paired sessions were added and animals were scored as follows:

Score 11-12:	AGGRESSIVE	
Score 5-10:	SPLIT-BEHAVIOR:	Not clearly aggressive or non-aggressive.
Score 0-4:	NON-AGGRESSIVE	

Animals scoring a "split" behavior rating were not used for subsequent neurochemical tests, as they may have yielded ambiquous data. These animals are referred to in the Results Section as eliminated.

II. BRAIN PART DISSECTION

Mice were sacrificed in a Sears, Model A1200 commercial microwave oven by placing each animal in a 24 cm X 18 cm X 48 cm plastic cage, free from bedding, and exposing the cage and the animal to 15 sec of microwave irradiation. Butcher and Butcher (1974) employed 6,000 watt-sec. fixed-beam microwave irradiation to inactivate the acetylcholinesterase activities. Animals in the present study were exposed to 18,000 Wat-sec of microwave irradiation (1200 Watts for 15 sec: 1200 Watt X 15 sec = 18,000 Wat-sec). After sacrifice the animals were beheaded and the heads were cooled at 5°C for 30 min to allow for easy dissection. A midline, caudal-frontal incision was made in the skin (head) and iris scissors were used to remove the rostral and superior aspects of the cranium. Whole brains were scooped out with a spatula and placed on glass over ice. A 2-3 mm coronal cut was made posterior to the frontal pole, and this section of frontal cortex was discarded. Four coronal cuts, 2-3 mm apart, were made consecutively while moving in a caudal direction until the whole mouse brain had been sliced. The rostral-most slice was placed on a #10 scapel blade and held under a 10X dissecting hand-lens. A #20 scalpel blade was used to dissect out septal nuclei, caudate-putamen, thalamus (without

hypothalamus), and hippocampus. The #10 scalpel blade was wiped clean after every slice was dissected, and a new blade was used for each mouse brain. This same procedure applied to the #20 scalpel blade. Sterile gloves were worn by the experimenter at all times.

After each brain part was dissected out it was placed in a scintillation vial and immersed in liquid nitrogen until assayed. Approximately 6 min was needed to completely dissect out all four brain parts from a single mouse brain. Immediately after each dissecting session, the brain parts were homogenized and assayed as described below. Brain parts were pooled since significant results could only be obtained by using a minimum of 10 mg of brain matter; parts from 6-12 brains were routinely pooled for each determination. The average weight of the four brain parts dissected out are listed below:

SEPTUM:	6.7 mg $\pm$ 1.0 (S.E.M.)
STRIATUM:	15.7 mg $\pm$ 3.5 (S.E.M.)
THALAMUS:	17.6 mg $\pm$ 4.0 (S.E.M.
HIPPOCAMPUS:	18.9 mg $\pm$ 3.7 (S.E.M.)

III. BIOCHEMICAL ASSAY

Endogenous choline and ACh levels were determined according to Haubrich and Reid (1974). ACh turnover values were determined according to a combination of the methods of Haubrich and Reid (1974) and Schuberth et al. (1969; 1970). The radioisotope employed for the determination of endogenous choline and ACh levels was ^{32}P-ATP, and the radioisotope employed for ACh turnover determinations was ^{3}H-choline (as explained below).

The Haubrich and Reid (1974) method was chosen because the sensitivity of this assay (40 pM) was high and comparable to other well-known procedures (Goldberg and McCaman, 1973; McCaman et al., 1971).

An important feature of this assay (Haubrich and Reid, 1974) is the wide variety of possible applications. For example, in addition to measuring the concentration of choline and ACh, this method has been used successfully to estimate changes in the rate of synthesis of ACh (ACh turnover) using radio-labelled choline as the precursor (Haubrich et al., 1975; Schuberth et al., 1969; 1970; the present study). Such studies are possible because the specific activities of ACh and its precursor, choline, can be measured simultaneously along with the concentrations of the

endogenous amines. The aforementionted simultaneous determination technique was employed in this dissertation.

A. REAGENT PREPARATION

1. Preparation of adenosine 5'-[γ -^{32}P] Triphosphate, Triethylammonium Salt

^{32}P-ATP was purchased from the Amersham Corp. It exhibited specific activity of 2600 Ci/mmol and a radioactive concentration of 1.0 mCi/ml.

For the chemical assay of choline and acetylcholine, a tracer amount of ATP - γ -^{32}P was added to the enzyme-ATP-NaCl-NaF preincubation solution to yield activity of 1 x 10^5 to 3 x 10^5 cpm in 0.1 ml 2 x 10^5 cpm in 0.1 ml constituted optimal activity since each incubation tube contained 200 nM of unlabeled ATP. When 2 x 10^5 cpm of ATP-γ -^{32}P were added to each tube, a typical sample containing 1 nM of choline yielded 100 cpm (200,000 cpm/ 200 nM ATP).

2. Preparation of ME-^{3}H-Choline Chloride in Ethanol Solution

^{3}H-choline was purchased from the Amersham Corp. It exhibited specific activity of 13 Ci/mmol and a radioactive concentration of 5 mCi/ml.

In preparation for tail-vein injection, 1 ml of ^{3}H-choline was placed in a scintillation vial and the ethanol

was evaporated off in a 50°C oven for 6 hrs. 17.0 ml distilled H_2O was added to the dry vial yielding a final concentration of 312.5 μCi/ml. Each animal was given 0.25 ml of this solution in the tail vein injection. Each animal, therefore, received 73.5 μCi of ^{3}H-choline chloride.

3. Preparation of Non-Isotopic Reagents

Non-isotopic reagents used for this assay were prepared in the following manner:

Electrophoresis buffer: 100 ml of pyridine and 100 ml of 88% formic acid were diluted to 8 liters with distilled water.

Tris buffer: 4.8 g of Tris (hydroxymethyl) aminomethane and 4.8 g of anhydrous $MgSO_4$ were dissolved in 4 liters of distilled water the pH was adjusted to 10 with 1 N NaOH.

ATP solution: 27.4 mg of adenosine-5'-triphosphate (disodium salt) was dissolved in 9.0 ml of Tris buffer. The pH was adjusted to 9 with 1 N NaOH and the volume was brought to 10.0 ml with Tris buffer. This solution was stored frozen for one day. New ATP solution was made for each assay.

NaCl - NaF solution: 105.0 mg of NaF and 1.45 g of NaCl was dissolved in 100 ml of Tris buffer.

15% formic acid plus acetone: 15.0 ml of 1 N formic acid was mixed with 85.0 ml of acetone.

10% formic acid plus acetone: 10.0 ml of 1 N formic acid was mixed with 90.0 ml of acetone.

Water-saturated ether: Anhydrous ether was shaken with an equal volume of distilled water in a separatory funnel. When the phases separated the water (bottom) layer was discarded.

Dowex columns: Dowex AX-8 anion-exchange resin (chloride form, 8% cross-linked, dry mesh 200-400) was washed twice with an equal volume of distilled water, and then washed twice with equal volumes of Tris buffer. While mixing, 2.0 ml of this mixture was added to disposable columns (4.0 mm i.d.). Each column was then washed with 5.0 ml of Tris buffer containing $MgSO_4$.

Choline-acetylcholine marker: 165.7 mg of choline iodide was dissolved in 5.0 ml of dissolved water with 100.0 mg of acetylcholine iodide.

Choline standard: 23.2 mg of choline chloride was dissolved in 100.0 ml of 0.1 mM HCl, and was stored at -20°C in 1.0-ml aliguots. Just before use, a 1:10 dilution of this standard was made with distilled water.

Acetylcholine standard: 27.3 mg of acetylcholine iodide was dissolved in 100.0 ml of 0.1 mM HCl and stored at -20°C in 1.0-ml aliquots. Just before use, a 1:10 dilution of this standard was made with distilled water to yield final concentration of 2.73 mg ACh/ml.

B. TISSUE PREPARATION

1.0 g of tissue was homogenized for 30 seconds in 2.50 ml of cold (0-5°C) 15% formic acid plus acetone using

a Polytron homogenizer. This homogenate (I) was incubated on ice for 20.0 minutes. The homogenate (I) was centrifuged at 0-5°C for 30 min at 12,000 xg. The supernatant (I) fluid was decanted into a 15 ml conical glass-stoppered centrifuge tube and stored on ice. The pellet was resuspended in cold 10% formic acid plus acetone (2.0 ml for each gram of tissue). The sample was incubated for 20 min on ice and again centrifuged at 0-5°C for 30 min at 12,000 xg. The supernatant fluid from this centrifugation (II) was combined with supernatant I and an equal volume of water-saturated ether was added. The vessel containing this solution (supernatant I & II & water-saturated ether) was inverted several times to extract the acetone into the ether. After the phases had separated (15 min), the top organic layer was aspirated from each tube and discarded. This layer contains ether, acetone, and dissolved lipids. The organic solvent remaining in the formic acid phase was evaporated by placing the sample on ice under a stream of nitrogen. The samples were then centrifuged for 5 min at 1,000 xg and the supernatant was transferred to a 12.0 ml conical centrifuge tube. The samples were then freeze dried for 12 hrs.

C. ISOLATION OF CHOLINE AND ACETYLCHOLINE

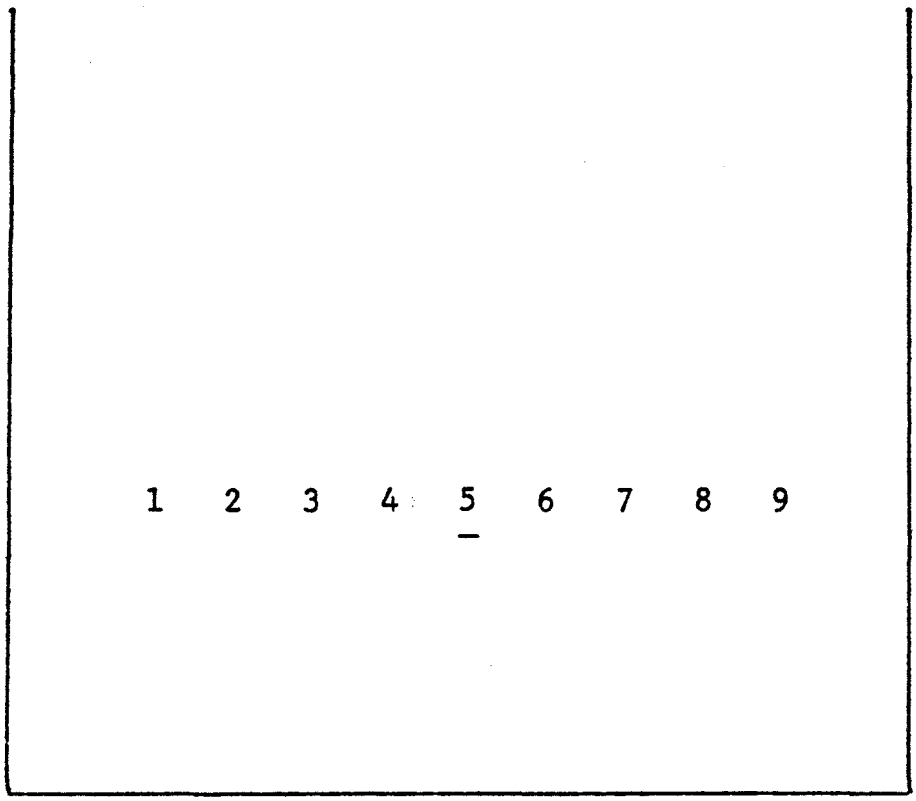
10 μL of distilled water was added to the free-dried sample containing choline and acetylcholine and the tube was then centrifuged at 1,000 xg for 5 min. Isolation and separation of choline and acetylcholine was accomplished

with high-voltage paper electrophoresis. The first step involves applying samples to sheets of electrophoresis paper according to the diagram on the following page.

Whatman 1 mm chromatography paper was used for this assay. A pencil line was drawn 10 cm from the bottom of the sheet and a series of 9 areas were marked with a small pencil mark to indicate where samples (tissue extracts of choline and acetylcholine) and a marker (choline-acetylcholine marker) were to be applied. These 9 pencil dots were centered and spaced 3 cm apart. The sheet of electrophoresis paper was cut down to measure 38 cm by 72 cm. The paper was dipped in pyridine buffer and blotted to semi dryness.

After the tissue samples containing choline and acetylcholine have been already freeze-dried and resuspended in 10 ml distilled water, they were ready to be applied to the electrophoresis paper. Each one of the experiments included 8 brain samples; 5 µL aliquots of each sample were applied onto the area on the paper marked with pencil dots (#1 to #9 , **see Figure 1**). The central dot was used for marking the area where a 5 µL aliquot of choline-acetylcholine marker was applied (#5, **see Figure 1**). 5 µL aliquots of choline-acetylcholine markers were also applied onto the pencil-dot areas where the tissue samples have been applied so that the areas of subsequent migration of choline and acetylcholine could be visualized on the paper following the high voltage run.

FIGURE 1



Electrophoresis paper preparation for sample and marker spots. Sample spots: #1-4; 6-9. Marker spot: #5.

After preparing the paper by pencilling in the areas where samples would be headed, dipping the paper in pyridine buffer and placing the sheet on the electrophoresis plate, the tissue samples and choline-acetylcholine markers were applied. The high voltage electrophoresis run was carried out for 2 hrs at 1500 v. During this time, choline and acetylcholine from the tissue samples migrated to the areas indicated on the diagram.

After the 2 hr run, the paper was removed from the electrophoresis plate and dried in air under a hood. Tissue choline and acetylcholine migrations were identified by placing the sheet in an iodine tank for 60-90 sec. Areas where choline and acetylcholine have migrated were indicated by brown circles resulting from exposure to the iodine vapor. These spots were circled with a pencil and outlined as rectangles by using a 20 X 25 mm template. These rectangular areas were cut out, rolled, and placed at the bottom of a 12 x 75 mm test tube. The paper rectangles were handled with a clean forceps to avoid contamination. 2.0 ml of distilled water was added to each test tube containing electrophoresis paper and the amines were eluted by shaking the tubes gently for 1 sec in a Vortex-type mixer. The samples were then incubated at room temperature for 10 min and two aliquots (0.4 ml and 1.0 ml) were transferred to disposable test tubes. The 0.4 ml aliquot was used for turnover determination; it was combined with 10 ml of prepared cocktail D

counted in the tritium channel (no minimum or maximum limits of an Amerssham Mark Number III scintillation counter for 10 min. The 0.75 ml aliquot was used for quantifying endogenous acetylcholine and choline; it was combined with 10 µL of ammonium hydroxide and heated in boiling water for 20 min to hydrolyze the acetylcholine. External standards were added to the test tubes just prior to the hydrolysis of acetylcholine with ammonium hydroxide. The test tubes containing the samples were then heated to dryness at 60°C in an oven. After 1 hr, the dry tubes were removed and placed in a dry area while the assay for determining endogenous choline and acetylcholine levels was taken to its final step.

D. CHEMICAL ASSAY OF ACETYLCHOLINE

An enzyme-ATP solution was prepared by mixing the following stock reagents just before use:

4 ml 2 mM ATP,

5 ml 0.5 M NaCl-NaF,

1 ml of 1 unit choline kinase per ml distilled water.

This mixture was preincubated for 30 min at 37°C to phosphorylate any residual choline present within the enxyme. For determinations a tracer amount (described earlier) of ATP-γ-^{32}P was added to this enzyme-ATP solution to yield 1×10^5 to 3×10^5epon in 0.1 ml.

0.1 ml of the enzyme-ATP-γ-^{32}P solution was added to the tubes containing the eluate from the electrophoresis paper and the contents were mixed vigorously for 20 sec to

redissolve the choline from the sides of the tubes. The samples were then incubated at 37°C for 1-2 hrs to allow sufficient conversion of choline to phosphorylcholine. This step can be represented as:

$$\text{(CHOLINE)}\quad (CH_3)_3\overset{4}{N}\text{-}CH_2\text{-}OH + ATP\text{-}\ \text{-}^{32}P \xrightarrow[MGH]{\text{CHOLINE KINASE}}$$

$$\text{(PHOSPHORYLCHOLINE)}\quad (CH_3)_3\overset{4}{N}\text{-}CH_2\text{-}CH_2\text{-}O\text{-}\overset{OH}{\overset{|}{\overset{32}{P}}}\text{-}OH \quad (O)$$

Next, Dowex columns were prepared (as described above) and rinsed with 4.5 ml Tris buffer before adding the contents of each tube to the columns. After samples were placed in the column, 4.0 ml of Tris buffer was added and the total effluent and eluates (7.0 ml) were collected in a counting vial. Samples were counted in the liquid scintillation counter on the ^3H-channel.

IV. CALCULATIONS

A. CALCULATION FOR ENDOGENOUS CHOLINE AND ACETYLCHOLINE LEVELS

Endogenous choline and ACh levels were calculated from the following equation:

$$\text{nM/g of } \underline{\text{CH}} \text{ or } \underline{\text{ACH}} = \frac{\text{CPM}_{\text{SAMPLE}} - \text{CPM}_{\text{REAGENT BLANK}}}{\text{CPM}_{\text{INTERNAL STANDARD}} - \text{CPM}_{\text{REAGENT BLANK}}} \quad \text{GRAMS OF TISSUE}$$

where CPM = counts per min.

B. CALCULATION FOR SPECIFIC ACTIVITY

Specific activities for ACh turnover determinations were calculated according to the following equation:

$$\text{DPM/nM} = \frac{\text{(DPM/g)}}{\text{ENDOGENOUS CH OR ACH LEVEL (nM/g)}}$$

where DPM = dissintegrations per minute.

C. CALCULATION FOR ACETYLCHOLINE TURNOVER

Acetylcholine turnover was calculated according to the following equation:

$$\text{nM/g/min} = \frac{SA_{ACH}}{SA_{CH}} \times \text{endogenous CH} \quad \frac{1}{\text{minute}}$$

where SA_{ACH} = specific activity of acetylcholine

SA_{CH} = specific activity of choline

RESULTS

EXPLANATION OF BEHAVIORAL AND EXPERIMENTAL GROUPS

Behavior: The term "NO PAIR" is employed in this section to emphasize the fact that unpaired, aggregated and unpaired, isolated mice were not paired in the aggression test.

Experimental: Only intragroup comparisons for isolated mice paired with each other (ISO-ISO, immediate and 7-day sacrifice) are valid, with regard to biochemical data. 'Control groups' (unpaired, aggregated; unpaired, isolated; and paired, aggregated) were not conducted concomitantly with ISO-ISO (immediate and 7-day sacrifice) groups.

RESULTS

I. BEHAVIORAL RESULTS FOR AGGRESSION TEST AND BIOCHEMICAL RESULTS FOR STEADY-STATE AND PRECURSOR-PRODUCT VERIFICATION

A. BEHAVIORAL TESTING FOR AGGRESSION

The parameters for the aggression test have been described previously (see Materials and Methods). The behavioral results for the 3-day, paired, aggression tests are listed in Table 1 and are described as follows:

BEHAVIORAL GROUP

1) No Fight-Unpaired, Aggregated

DEFINITION: Mice that have had no fight experience; they are not paired in the aggression test. Mice were housed in aggregate conditions (12 per cage).

BEHAVIORAL RESULTS: None

2) No Fight-Unpaired, Isolated

DEFINITION: Mice that have had no fight experience; they are not paired in the aggression test. Mice were housed individually (1 per cage).

BEHAVIORAL RESULTS: None

3) Paired, Aggregated Non-Aggressive

DEFINITION: Mice that have been subjected to behavioral test; and considered non-aggressive. Some animals had fight experience,

TABLE 1

BEHAVIORAL RESULTS FROM 3-DAY, MATCHED-PAIR FIGHTING SESSIONS; PERCENT AGGRESSIVE, NON-AGGRESSIVE, ELIMINATED

	Group	Aggressive	Non-aggressive	Eliminated
1.	Unpaired, aggregated	NO FIGHT	NO FIGHT	NO FIGHT
2.	Unpaired, isolated	NO FIGHT	NO FIGHT	NO FIGHT
3.	Paired, aggregated	0.0 (0)	98.4 (60)	1.6 (1)
4.	Isolated (Winners) vs Aggregated (Losers)	96.0 (105) 0.0 (0)	2.5 (3) 97.9 (115)	1.5 (2) 2.1 (4)
5.	Isolated (Winners) vs Isolated (Losers)	38.0 (237)	32.3 (202)	29.7 (185)

( )=number of animals

BEHAVIORAL GROUP

others did not fight. Mice were housed in aggregate conditions (12 per cage).

BEHAVIORAL RESULTS: 0.0% animals were aggressive; 98.4% of the animals tested were non-aggressive; 1.6% of the animals tested could not be defined, behaviorally, and were not evaluated biochemically.

4) Winners (ISO-AGG)

DEFINITION: Mice that have had fighting experience; these animals were aggressive and they won the fight. Mice were housed individually (1 per cage) and were paired with a mouse housed in aggregate conditions (12 per cage).

BEHAVIORAL RESULTS: When paired with an aggregated mouse, 96.0% of these mice were aggressive; 2.5% were non-aggressive; 1.5% were not defined, behaviorally, and were not evaluated biochemically.

5) Losers (ISO-AGG)

DEFINITION: Mice that have had fighting experience; these animals were non-aggressive and they lost the fight. Mice were housed in aggregate conditions (12 per cage) and were paired with a mouse housed individually (1 per cage).

BEHAVIORAL RESULTS: When paired with an isolated animal, 0.0% of these mice were aggressive, 97.9% of these mice were non-aggressive; 2.1% were not defined, behaviorally, and were not evaluated biochemically.

6) Winners (ISO-ISO)

DEFINITION: Mice that have had fighting experience; these animals were aggressive and they won the fight. Mice were housed individually (1 per cage) and were paired with a mouse housed individually (1 per cage).

BEHAVIORAL RESULTS: When paired with another isolated animal, 38.0% of these mice were aggressive; 29.7% were not defined, behaviorally, and were not evaluated biochemically.

BEHAVIORAL GROUP

7) Losers (ISO-ISO)

DEFINITION: Mice that have had fighting experience; these animals were non-aggressive and they lost the fight. Mice were housed individually (1 per cage) and were paired with a mouse housed individually (1 per cage).

BEHAVIORAL RESULTS: When paired with another isolated animal, 32.3% of these mice were non-aggressive; 29.7% were not defined behaviorally, and were not evaluated biochemically.

B. BIOCHEMICAL VERIFICATION OF THE STEADY-STATE

According to Hanin and Costa (1976), it was necessary to determine whether the pulse injection of ^{3}H-choline (0.25 ml; 73.5 µCi ^{3}H-choline) employed in my acetylcholine turnover experiments perturbed choline and acetylcholine steady-state levels in the four brain areas studied (see Discussion). The following experiment was conducted; endogenous choline and acetylcholine levels were determined in the septum, striatum, thalamus, and hippocampus of winning (ISO-ISO) and losing (ISO-ISO) mice for a) "control" animals injected with 0.25 ml of 0.9% NaCl, and b) "experimental" animals injected with 0.25 ml of 73.5 µCi ^{3}H-choline. Choline and acetylcholine levels were determined at time points 30 sec, 45 sec, 60 sec, 120 sec, and 250 sec after the injection of

either saline or ^{3}H-choline pulse. Data was accumulated for these time points because the original experimental design for this dissertation involved the use of the acetylcholine turnover technique according to Racagni et al. (1975) where multiple data points were used to determine acetylcholine turnover values. Due to computer programming difficulties encountered in evaluating multiple-point acetylcholine turnover, this method had to be abandoned in favor of the one-point (30 sec, see Methods) turnover analysis of Schuberth et al (1970). However, the data accumulated for the "control" and "experimental" groups of mice mentioned above in multiple-point analysis proved to be valuable for the determination of steady-state conditions.

Tables 2A-B show choline and acetylcholine levels in the septum, striatum, thalamus, and hippocampus of winning (ISO-ISO) and losing (ISO-ISO) mice after a pulse injection 0.25 ml of 0.9% NaCl solution. Tables 3A-B and Tables 4A-B show choline and acetylcholine levels in the septum, striatum, thalamus, and hippocampus of winning (ISO-ISO) and losing (ISO-ISO) mice 30 sec, 45 sec, 60 sec, 120 sec, and 240 sec, after a pulse injection of 0.25 ml of 73.5μCi ^{3}H-choline. It can be seen from these data that the choline and acetylcholine levels in winning (ISO-ISO) and losing (ISO-ISO) mice in all four brain areas at all five time points were not significantly different when "control" (saline-injected) and "experimental"

TABLE 2A

ENDOGENOUS CHOLINE LEVELS IN FOUR BRAIN AREAS OF AGGRESSIVE (ISO-ISO) AND NON-AGGRESSIVE (ISO-ISO) MICE

BRAIN REGION CH LEVELS (nM/g + S.E.M.)

Brain Area	Aggressive	Non-aggressive
Septum	51.7 ± 5.2**[1] (5-72)	32.5 ± 2.9**[1] (5-68)
Striatum	27.5 ± 2.3 (6-85)	31.8 ± 1.1 (7-89)
Thalamus	31.4 ± 3.3 (5-82)	24.7 ± 2.2 (6-85)
Hippocampus	19.4 ± 2.6 **[2] (6-79)	29.1 ± 3.4**[2] (6-79)

(x-y): x = Number of data points; y = Number of animals

* = $p < 0.05$; ** = $p < 0.01$

Intergroup comparison: 1,2

TABLE 2B

ENDOGENOUS ACETYLCHOLINE LEVELS IN FOUR BRAIN AREAS OF AGGRESSIVE (ISO-ISO) AND NON-AGGRESSIVE (ISO-ISO) MICE

BRAIN REGION ACH LEVELS (nM/g + S.E.M.)

Brain Area	Aggressive	Non-aggressive
Septum	14.1 ± 1.2 (7-94)	10.8 ± 1.2 (6-88)
Striatum	17.4 ± 1.2 (6-79)	17.9 ± 1.4 (6-80)
Thalamus	10.9 ± 0.7*[1] (7-86)	14.2 ± 1.3 *[1] (5-80)
Hippocampus	5.9 ± 0.8 (7-79)	8.5 ± 1.0 (7-79)

(x-y): x = Number of data points; y = Number of animals

* = $p < 0.05$; ** = $p < 0.01$

Intergroup comparison: 1

TABLE 3A

TEST FOR STEADY-STATE CONDITIONS; ENDOGENOUS CHOLINE LEVELS IN FOUR BRAIN AREAS OF AGGRESSIVE (ISO-ISO) MICE 30 SEC., 45 SEC., 60 SEC., 120 SEC., AND 240 SEC., AFTER INJECTION OF TRITIATED CHOLINE

BRAIN REGION CH LEVELS (nM/g + S.E.M.)

Brain Area	30 sec	45 sec	60 sec	120 sec	240 sec
Septum	52.7 ± 1.4 (5-47)	52.6 ± 1.5 (6-60)	53.9 ± 1.8 (5-60)	52.4 ± 1.2 (5-59)	52.0 ± 2.0 (4-40)
Striatum	27.2 ± 0.8 (5-52)	29.2 ± 0.7 (6-63)	26.9 ± 0.8 (5-59)	25.8 ± 0.4 (5-63)	29.1 ± 1.8 (4-40)
Thalamus	31.2 ± 1.0 (5-50)	33.5 ± 1.1 (6-60)	30.9 ± 1.3 (5-60)	33.0 ± 1.4 (5-60)	32.0 ± 1.8 (4-40)
Hippocampus	19.8 ± 0.5 (5-56)	21.5 ± 0.5 (6-64)	17.6 ± 0.7 (5-60)	17.7 ± 1.5 (5-62)	18.6 ± 0.5 (4-40)

(x-y): x = Number of data points; y = Number of animals

TABLE 3B

TEST FOR STEADY-STATE CONDITIONS; ENDOGENOUS ACETYLCHOLINE LEVELS IN FOUR BRAIN AREAS OF AGGRESSIVE (ISO-ISO) MICE 30 SEC., 45 SEC., 60 SEC., 120 SEC., AND 240 SEC., AFTER INJECTION OF TRITIATED CHOLINE

BRAIN REGION ACH LEVELS (nM/g + S.E.M.)

Brain Area	30 sec	45 sec	60 sec	120 sec	240 sec
Septum	14.6 ± 0.9 (5-47)	13.7 ± 0.4 (6-60)	15.1 ± 0.4 (5-60)	13.1 ± 0.8 (5-59)	13.1 ± 0.8 (4-40)
Striatum	17.5 ± 1.0 (5-52)	19.1 ± 0.9 (6-63)	18.2 ± 1.1 (5-59)	16.5 ± 0.4 (5-63)	15.2 ± 0.8 (4-40)
Thalamus	10.9 ± 0.9 (5-50)	12.0 ± 1.0 (6-60)	9.9 ± 0.6 (5-60)	11.5 ± 0.6 (5-60)	14.2 ± 1.9 (4-40)
Hippocampus	5.9 ± 0.5 (5-56)	6.3 ± 0.3 (6-64)	6.9 ± 0.5 (5-60)	5.2 ± 0.4 (5-62)	5.4 ± 0.4 (4-40)

(x-y): x = Number of data points; y = Number of animals

TABLE 4A

TEST FOR STEADY-STATE CONDITIONS; ENDOGENOUS CHOLINE LEVELS IN FOUR BRAIN AREAS OF NON-AGGRESSIVE (ISO-ISO) MICE 30 SEC., 45 SEC., 60 SEC., 120 SEC AND 240 SEC., AFTER INJECTION OF TRITIATED CHOLINE

BRAIN REGION CH LEVELS (nM/g + S.E.M.)

Brain Area	30 sec	45 sec	60 sec	120 sec	240 sec
Septum	31.9 ± 0.8 (5-45)	30.0 ± 0.5 (6-60)	30.5 ± 0.9 (5-62)	32.3 ± 1.0 (5-60)	34.0 ± 1.0 (4-40)
Striatum	31.8 ± 0.4 (5-60)	34.0 ± 0.6 (6-72)	32.3 ± 1.1 (5-58)	30.3 ± 0.3 (5-60)	29.6 ± 0.6 (4-48)
Thalamus	24.7 ± 0.5 (5-52)	26.3 ± 1.2 (6-61)	23.4 ± 1.5 (5-62)	25.9 ± 1.5 (5-60)	23.1 ± 1.2 (4-40)
Hippocampus	29.0 ± 1.6 (5-50)	31.6 ± 1.1 (6-60)	27.2 ± 1.4 (5-60)	31.1 ± 1.5 (5-58)	30.6 ± 1.6 (4-40)

(x-y): x = Number of data points; y = Number of animals

TABLE 4B

TEST FOR STEADY-STATE CONDITIONS; ENDOGENOUS ACETYLCHOLINE LEVELS IN FOUR BRAIN AREAS OF NON-AGGRESSIVE (ISO-ISO) MICE 30 SEC., 45 SEC., 60 SEC., 120 SEC., AND 240 SEC., AFTER INJECTION OF TRITIATED CHOLINE

BRAIN REGION ACH LEVELS (nM/g + S.E.M.)

Brain Area	30 sec	45 sec	60 sec	120 sec	240 sec
Septum	10.2 ± 0.5 (5-45)	12.0 ± 0.4 (6-60)	9.6 ± 0.6 (5-62)	10.8 ± 0.5 (5-60)	12.0 ± 0.8 (4-40)
Striatum	17.9 ± 1.3 (5-60)	18.8 ± 0.5 (6-72)	16.9 ± 0.6 (5-58)	19.5 ± 1.1 (5-60)	20.0 ± 0.5 (4-48)
Thalamus	14.1 ± 0.7 (5-52)	16.1 ± 0.5 (6-61)	13.7 ± 0.8 (5-62)	14.7 ± 0.9 (5-60)	14.1 ± 0.6 (4-40)
Hippocampus	8.9 ± 0.4 (5-50)	7.6 ± 0.4 (6-60)	8.4 ± 0.6 (5-60)	8.6 ± 0.6 (5-58)	10.1 ± 0.6 (4-40)

(x-y): x = Number of data points; y = Number of animals

(^{3}H-choline-injected) levels were compared (see Tables 2A-B, 3A-B, 4A-B). Therefore, it can be stated that the pulse-injection dose of ^{3}H-choline used 73.5 µCi ^{3}H-choline) for the following determinations does not perturb choline and acetylcholine steady-state levels.

C. PRECURSOR-PRODUCT VERIFICATION

Choline and acetylcholine specific activity curves plotted versus time have been known to exhibit characteristic conformations indicative of typical precursor-product relationships (Hanin and Costa, 1976; Haubrich et al., 1975). It is necessary to demonstrate that such a graphic representation of specific activity is exhibited in turnover evaluation since data are expressed in specific activity ratios of product (acetylcholine) to precursor (choline) (Schuberth et al., 1970, 1971; Hanin and Costa, 1974; see Discussion).

Tables 5A-D show choline and acetylcholine specific activity values for winning (ISO-ISO) and losing (ISO-ISO) mice in the septum, striatum, thalamus, and hippocampus. Specific activity values were determined 30 sec, 45 sec, 60 sec, 120 sec and 240 sec after ^{3}H-choline pulse injection. Graphs 5A-D show choline and acetylcholine specific activity values plotted versus time. Graphs 5A-D resemble similar choline and acetylcholine specific activity plots determined by Jenden (1974), and Hanin and Costa (1976). It can be

TABLE 5A

TEST FOR STEADY-STATE CONDITIONS; CHOLINE AND ACETYLCHOLINE SPECIFIC ACTIVITY LEVELS IN SEPTUM OF AGGRESSIVE (ISO-ISO) AND NON-AGGRESSIVE (ISO-ISO) MICE 30 SEC., 45 SEC., 60 SEC., 120 SEC., AND 240 SEC., AFTER INJECTION OF TRITIATED CHOLINE

SEPTAL CH AND ACH SPECIFIC ACTIVITY LEVELS (DPM/nM) X 10^3

Septum Behavioral Group	CH;ACH	30 sec	45 sec	60 sec	120 sec	240 sec
Aggressive	CH	3864 ± 202 (5-47)	4819 ± 551 (6-60)	3599 ± 124 (5-60)	2835 ± 112 (5-59)	1400 ± 73 (4-40)
Aggressive	ACH	1497 ± 123 (5-47)	2809 ± 329 (6-60)	3199 ± 137 (5-60)	3121 ± 93 (5-59)	2400 ± 64 (4-40)
Non-aggressive	CH	5927 ± 209 (5-45)	5471 ± 90 (6-60)	4439 ± 143 (5-62)	2000 ± 175 (5-60)	1810 ± 71 (4-40)
Non-aggressive	ACH	1592 ± 209 (5-45)	2809 ± 106 (6-60)	3200 ± 136 (5-62)	2874 ± 150 (5-60)	2230 ± 64 (4-40)

(x-y): x = Number of data points; y = Number of animals

FIGURE 5A

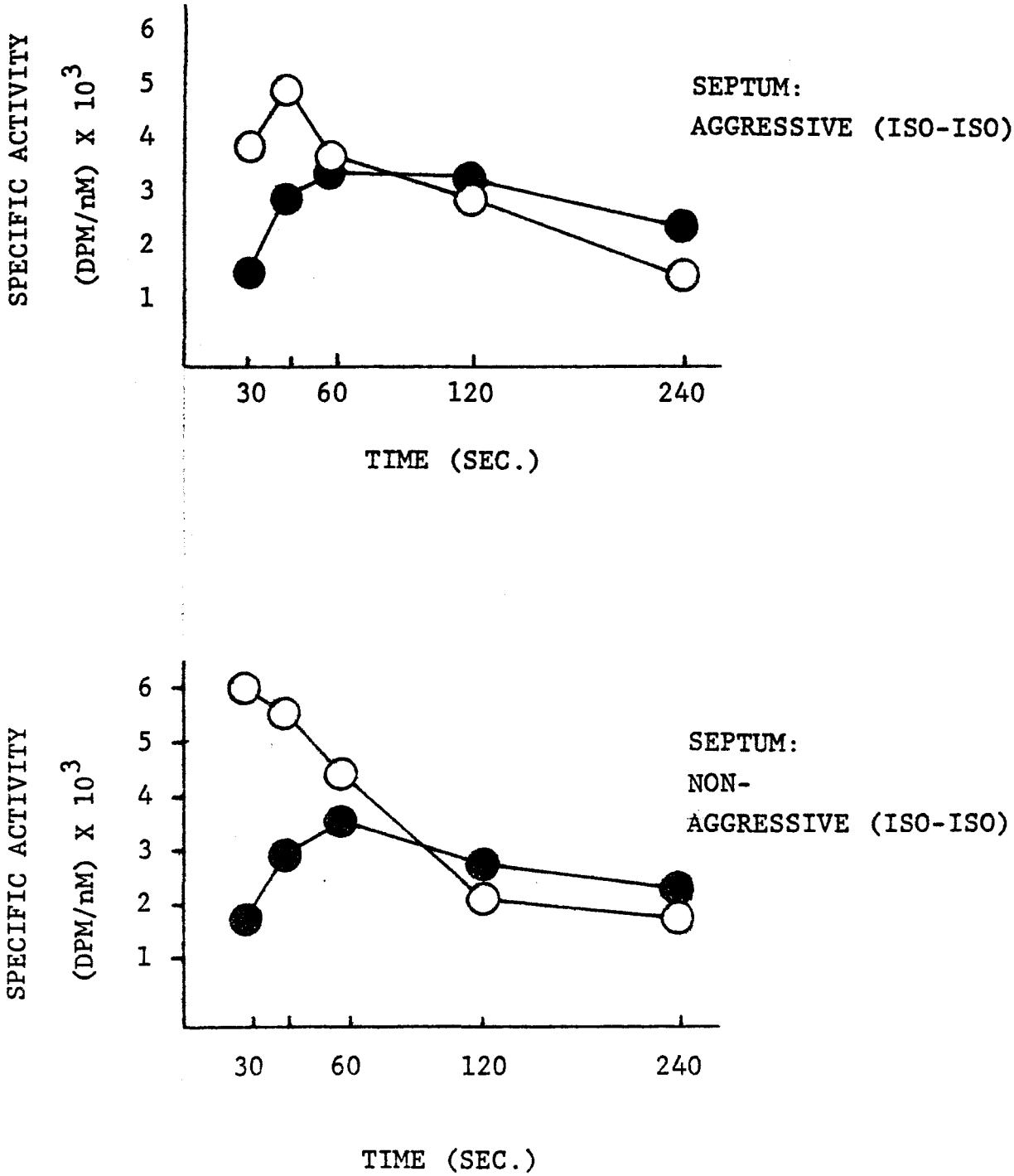

SPECIFIC ACTIVITY
(DPM/nM) X 10^3
6
5
4
3
2
1
30
60
120
240
TIME (SEC.)
SEPTUM:
AGGRESSIVE (ISO-ISO)
SPECIFIC ACTIVITY
(DPM/nM) X 10^3
6
5
4
3
2
1
30
60
120
240
TIME (SEC.)
SEPTUM:
NON-
AGGRESSIVE (ISO-ISO)

TABLE 5B

TEST FOR STEADY-STATE CONDITIONS; CHOLINE AND ACETYLCHOLINE SPECIFIC ACTIVITY LEVELS IN STRIATUM OF AGGRESSIVE (ISO-ISO) AND NON-AGGRESSIVE (ISO-ISO) MICE 30 SEC., 45 SEC., 60 SEC., 120 SEC., AND 240 SEC., AFTER INJECTION OF TRITIATED CHOLINE

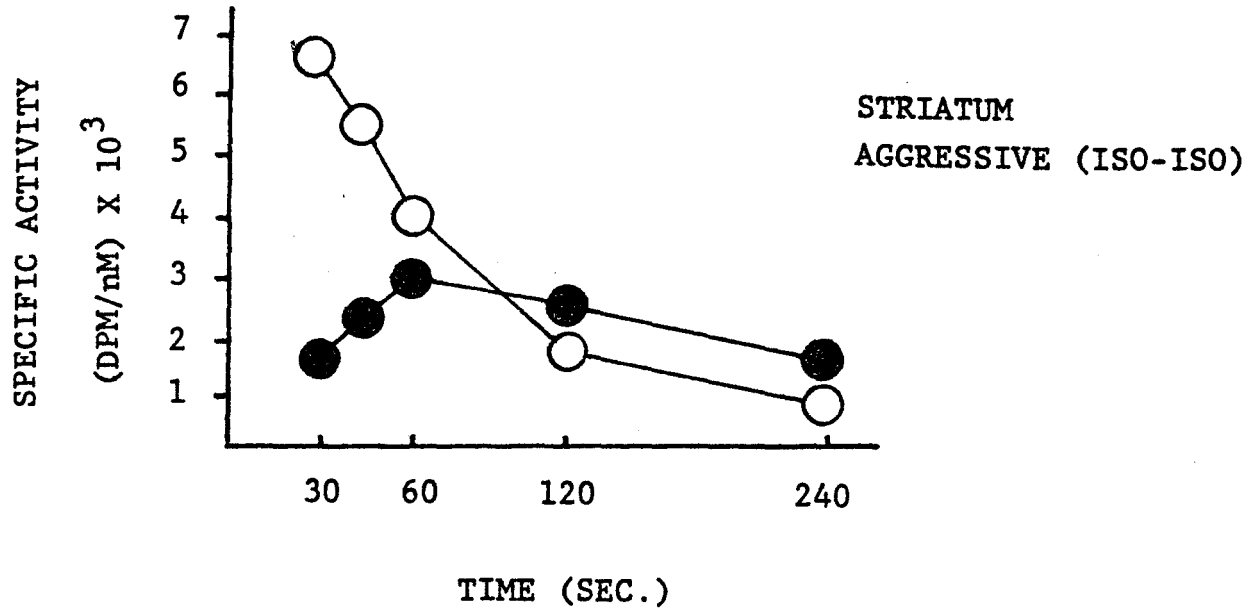
STRIATAL CH AND ACH SPECIFIC ACTIVITY LEVELS (DPM/nM) X 10^3

Striatum

Behavioral Group	CH;ACH	30 sec	45 sec	60 sec	120 sec	240 sec
Aggressive	CH	6595 ± 235 (5-52)	5399 ± 109 (6-63)	4004 ± 98 (5-59)	1808 ± 74 (5-63)	871 ± 48 (4-40)
Aggressive	ACH	1707 ± 58 (5-52)	2362 ± 60 (6-63)	2986 ± 178 (5-59)	2526 ± 45 (5-63)	1669 ± 32 (4-40)
Non-aggressive	CH	7553 ± 244 (5-60)	6317 ± 107 (6-72)	4802 ± 77 (5-58)	2940 ± 155 (5-60)	1541 ± 62 (4-48)
Non-aggressive	ACH	1642 ± 88 (5-60)	2800 ± 58 (6-72)	4002 ± 77 (5-58)	4007 ± 85 (5-60)	2268 ± 102 (4-48)

(x-y): x = Number of data points; y = Number of animals

FIGURE 5B


SPECIFIC ACTIVITY
(DPM/nM) X 10^3
7
6
5
4
3
2
1
30
60
120
240
TIME (SEC.)
STRIATUM
AGGRESSIVE (ISO-ISO)

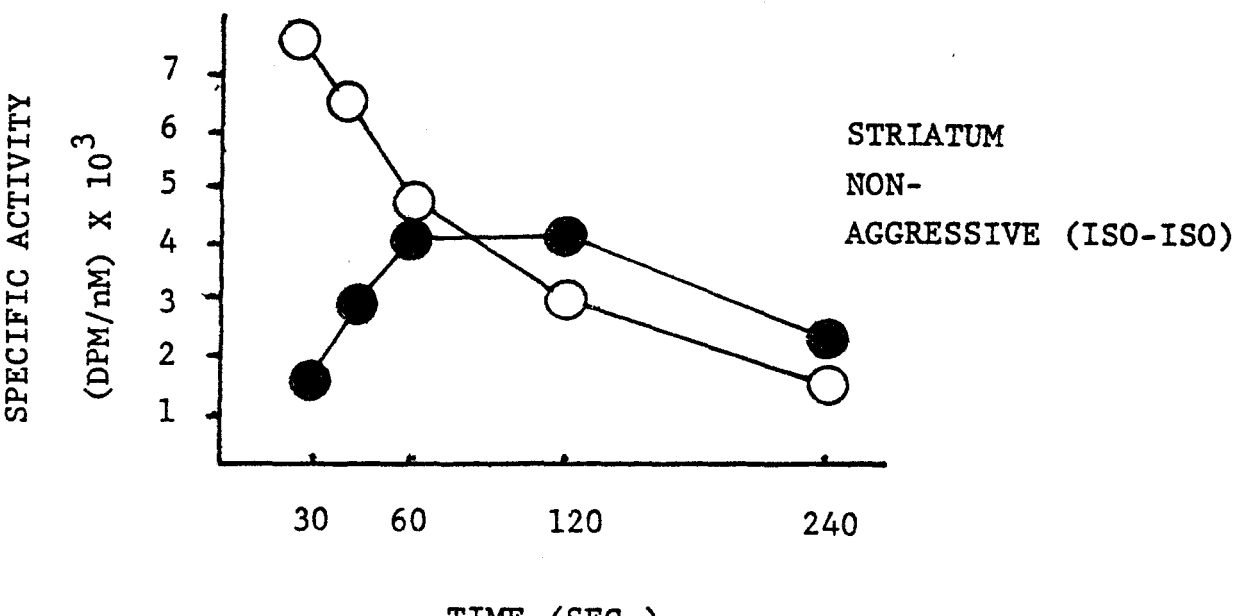

SPECIFIC ACTIVITY
(DPM/nM) X 10^3
7
6
5
4
3
2
1
30
60
120
240
TIME (SEC.)
STRIATUM
NON-
AGGRESSIVE (ISO-ISO)

TABLE 5C

TEST FOR STEADY-STATE CONDITIONS; CHOLINE AND ACETYLCHOLINE SPECIFIC ACTIVITY LEVELS IN THALAMUS OF AGGRESSIVE (ISO-ISO) AND NON-AGGRESSIVE (ISO-ISO) MICE 30 SEC., 45 SEC., 60 SEC., 120 SEC., AND 240 SEC., AFTER INJECTION OF TRITIATED CHOLINE

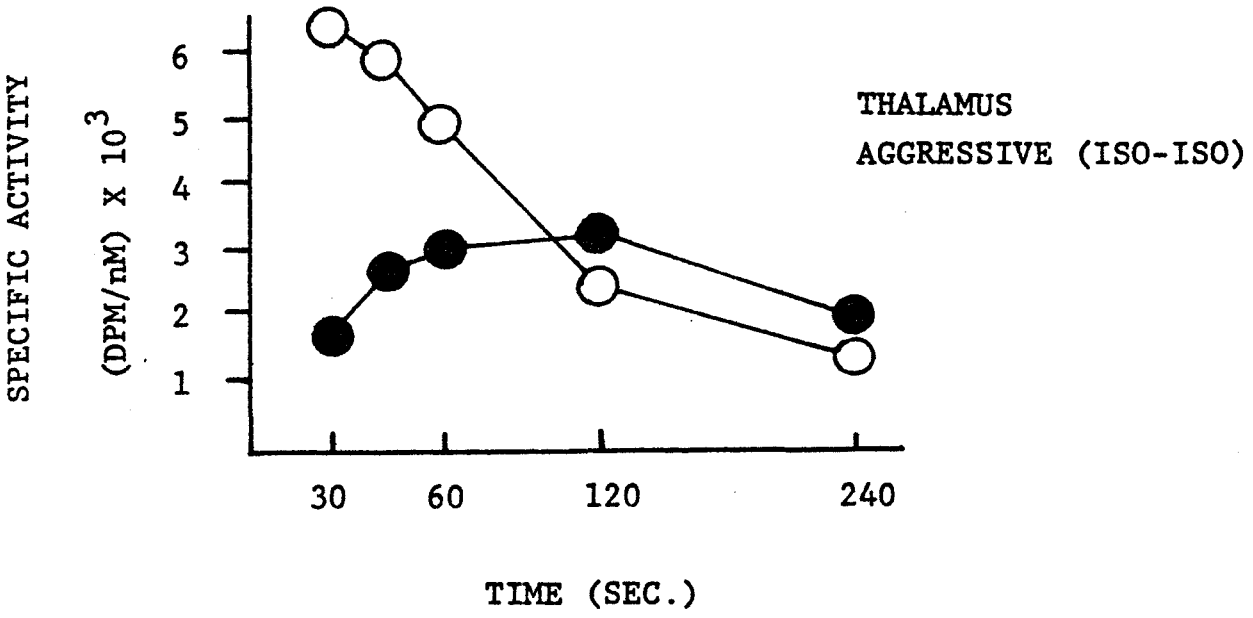
THALAMIC CH AND ACH SPECIFIC ACTIVITY LEVELS (DPM/nM) X 10^3

Thalamus

Behavioral Group	CH;ACH	30 sec	45 sec	60 sec	120 sec	240 sec
Aggressive	CH	6262 ± 291 (5-50)	5855 ± 199 (6-60)	4808 ± 110 (5-60)	2200 ± 97 (5-60)	1261 ± 71 (4-40)
Aggressive	ACH	1662 ± 52 (5-50)	2797 ± 135 (6-60)	2993 ± 39 (5-60)	3040 ± 93 (5-60)	1813 ± 74 (4-40)
Non-aggressive	CH	6125 ± 253 (5-52)	5601 ± 78 (6-61)	5002 ± 237 (5-62)	3128 ± 241 (5-60)	2025 ± 101 (4-40)
Non-aggressive	ACH	1079 ± 46 (5-52)	1802 ± 75 (6-61)	2260 ± 112 (5-62)	3463 ± 162 (5-60)	2970 ± 270 (4-40)

(x-y): x = Number of data points; y = Number of animals

FIGURE 5C


SPECIFIC ACTIVITY
(DPM/nM) X 10^3
6
5
4
3
2
1
30
60
120
240
TIME (SEC.)
THALAMUS
AGGRESSIVE (ISO-ISO)

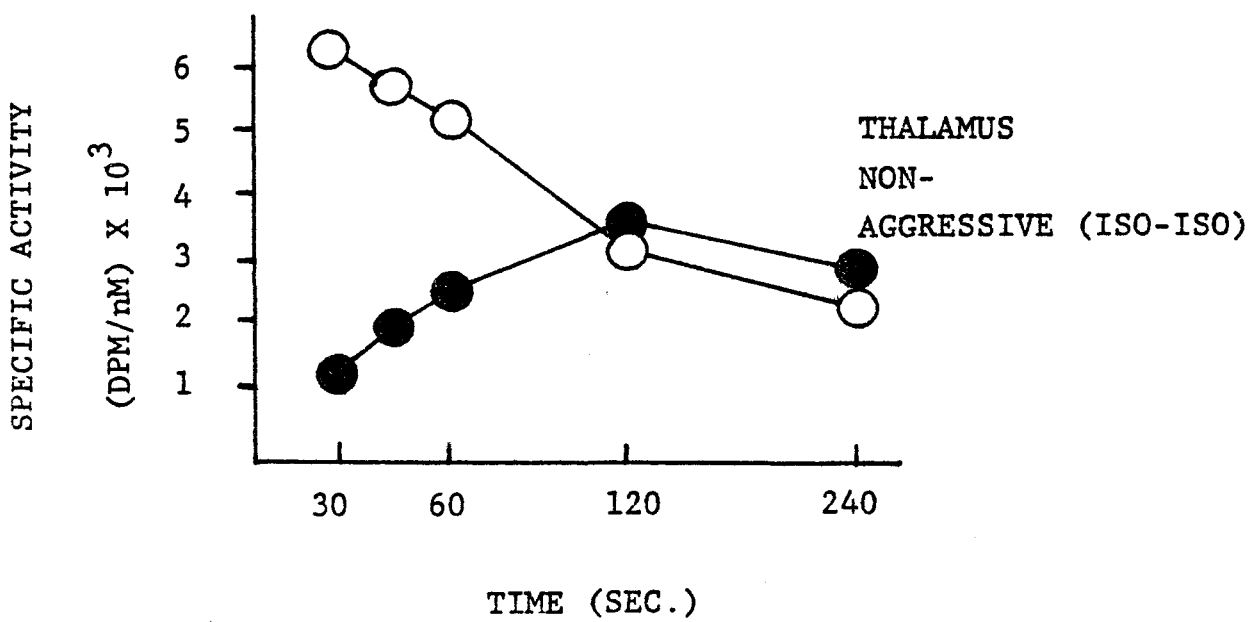

SPECIFIC ACTIVITY
(DPM/nM) X 10^3
6
5
4
3
2
1
30
60
120
240
TIME (SEC.)
THALAMUS
NON-
AGGRESSIVE (ISO-ISO)

TABLE 5D

TEST FOR STEADY-STATE CONDITIONS; CHOLINE AND ACETYLCHOLINE SPECIFIC ACTIVITY LEVELS IN HIPPOCAMPUS OF AGGRESSIVE (ISO-ISO) AND NON-AGGRESSIVE (ISO-ISO) MICE 30 SEC., 45 SEC., 60 SEC., 120 SEC., 240 SEC., AFTER INJECTION OF TRITIATED CHOLINE

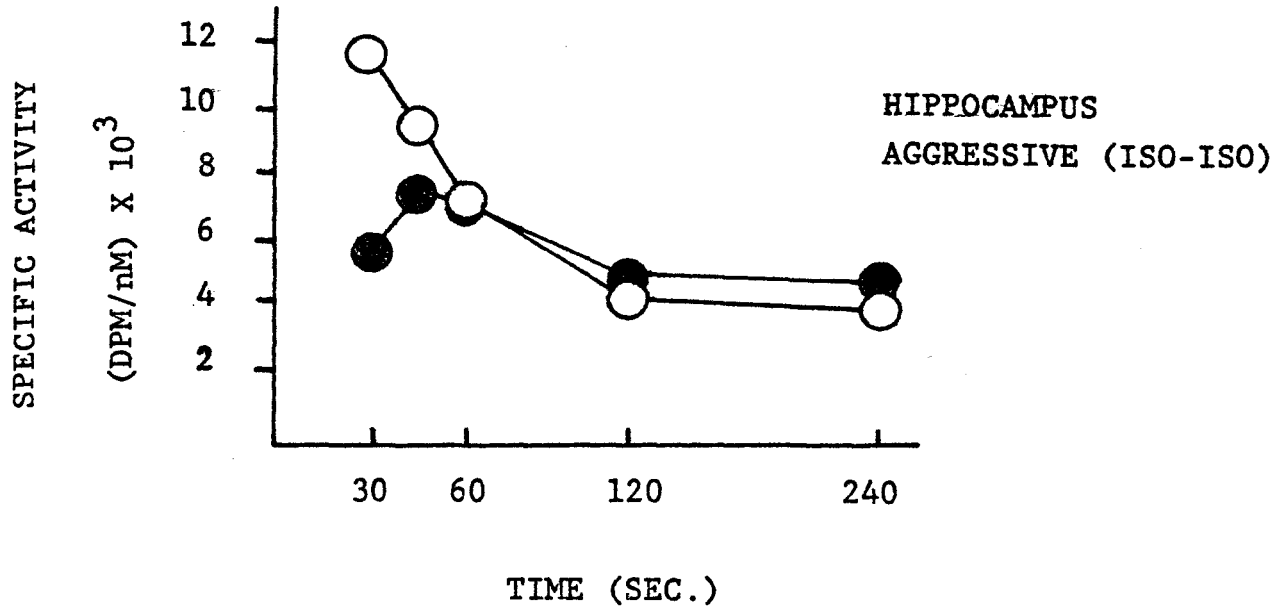
HIPPOCAMPAL CH AND ACH SPECIFIC ACTIVITY LEVELS (DPM/nM) X 10^3

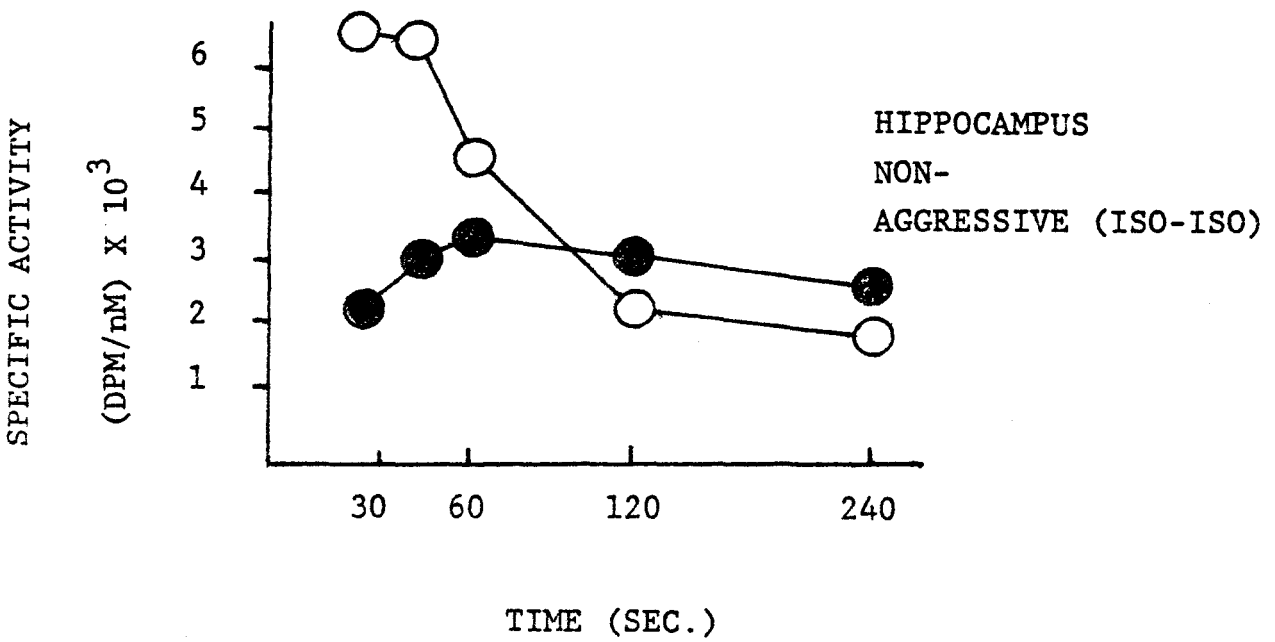
Hippocampus

Behavioral Group	CH;ACH	30 sec	45 sec	60 sec	120 sec	240 sec
Aggressive	CH	11589 ± 200 (5-56)	9465 ± 341 (6-64)	7200 ± 84 (5-60)	4002 ± 489 (5-62)	3743 ± 670 (4-40)
Aggressive	ACH	5604 ± 212 (5-56)	7254 ± 187 (6-64)	6808 ± 159 (5-60)	4506 ± 96 (5-62)	4407 ± 448 (4-40)
Non-aggressive	CH	6554 ± 418 (5-50)	6428 ± 124 (6-60)	4408 ± 161 (5-60)	2178 ± 163 (5-58)	1826 ± 354 (4-40)
Non-aggressive	ACH	2048 ± 141 (5-50)	2947 ± 178 (6-60)	3220 ± 105 (5-60)	3022 ± 133 (5-58)	2621 ± 274 (4-40)

(x-y): x = Number of data points; y = Number of animals

FIGURE 5D


SPECIFIC ACTIVITY
(DPM/nM) X 10^3
12
10
8
6
4
2
30
60
120
240
TIME (SEC.)
HIPPOCAMPUS
AGGRESSIVE (ISO-ISO)


SPECIFIC ACTIVITY
(DPM/nM) X 10^3
6
5
4
3
2
1
30
60
120
240
TIME (SEC.)
HIPPOCAMPUS
NON-
AGGRESSIVE (ISO-ISO)

stated, therefore, that an appropriate precursor-product relationship exists for the choline-acetylcholine determinations carried out in the experiments carried out for this dissertation (see Results Section II-V).

II. RESULTS FOR "NO FIGHT" MICE: UNPAIRED, AGGREGATED AND UNPAIRED ISOLATED

1. ENDOGENOUS CH AND ACH LEVELS OF "NO FIGHT" MICE

A. ENDOGENOUS CHOLINE LEVELS FOR "NO FIGHT" UNPAIRED, AGGREGATED MICE - COMPARISON OF BRAIN PARTS

Table 6A shows endogenous choline levels of the septum, striatum, thalamus, and hippocampus for "NO FIGHT," unpaired, aggregated mice. Septal choline levels (40.8 nM/g) were significantly ($p < 0.01$) higher than those in other areas; thalamic (30.1 nM/g) and striatal (28.1 nM/g levels were intermediate; and hippocampal choline levels were significantly lower ($p < 0.01$) than those of other brain areas. This sequence of choline levels (septal-highest, thalamic and striatal-intermediate, and hippocampal-lowest) was found also in the case of "NO FIGHT", unpaired, isolated mice (cf. next section, II.2.).

B. ENDOGENOUS CHOLINE LEVELS FOR "NO FIGHT" UNPAIRED, ISOLATED MICE: COMPARISON OF BRAIN PARTS

Table 6A also shows endogenous choline values of the septum, striatum, thalamus, and hippocampus for "NO FIGHT", unpaired, isolated-mice. Septal choline levels

TABLE 6A

ENDOGENOUS CHOLINE LEVELS OF "NO FIGHT" MICE: UNPAIRED, AGGREGATED AND UNPAIRED, ISOLATED BRAIN REGION CH LEVELS (nM/g + S.E.M.); n = 5

	CH	
Brain Area	Unpaired, Aggregated	Unpaired, Isolated
Septum	40.8 ± 1.7	41.3 ± 0.7
Striatum	28.1 ± 0.6	27.9 ± 0.5
Thalamus	30.1 ± 1.1	30.5 ± 1.4
Hippocampus	22.5 ± 0.7	22.7 ± 0.8

* = $p < 0.05$; ** = $p < 0.01$

41.3 nM/g) were significantly higher ($p < 0.01$) than those of other areas. Thalamic (30.5 nM/g) and striatal (27.9 nM/g) levels were significantly lower than the septal levels but were not significantly different from one another. Hippocampal choline levels (22.7 nM/g) were found to be the lowest of the values for the four brain areas investigated.

C. ENDOGENOUS CHOLINE LEVELS - COMPARISON OF "NO FIGHT" MICE; UNPAIRED, AGGREGATED AND UNPAIRED, ISOLATED

Table 6A sets up the comparison between choline levels of the septum, striatum, thalamus, and hippocampus of "NO FIGHT", unpaired, aggregated mice, on the one hand, and "NO FIGHT', unpaired, isolated mice, on the other. In all four brain areas no significant difference between choline levels was found for these two groups of "NO FIGHT" mice. It can be stated, therefore, that isolation has no effect on endogenous choline levels of the septum, striatum, thalamus, and hippocampus of CF-1, male mice. The sequence of values for choline levels in the 4 brain parts was identical in the case of "NO FIGHT", unpaired aggregated mice and "NO FIGHT", unpaired isolated mice.

D. ENDOGENOUS ACETYLCHOLINE LEVELS FOR "NO FIGHT", UNPAIRED, AGGREGATED MICE - COMPARISON OF BRAIN PARTS

Table 6B shows that for "NO FIGHT", unpaired, aggregated mice, acetylcholine levels were highest in the striatum

TABLE 6B

ENDOGENOUS ACETYLCHOLINE LEVELS OF "NO FIGHT" MICE: UNPAIRED, AGGREGATED AND UNPAIRED, ISOLATED

BRAIN REGION ACH LEVELS (nM/g + S.E.M.); n = 5

	ACH	
Brain Area	Unpaired, Aggregated	Unpaired, Isolated
Septum	12.8 ± 0.5	12.7 ± 0.7
Striatum	16.8 ± 0.6	16.9 ± 0.7
Thalamus	11.8 ± 0.5	11.8 ± 0.5
Hippocampus	7.3 ± 0.3	7.3 ± 0.4

* = $p < 0.05$; ** = $p < 0.01$

(16.8 nM/g), intermediate in the septum (12.8 nM/g) and thalamus (11.8 nM/g), and lowest in the hippocampus (7.3 nM/g).

The difference between striatal acetylcholine levels and septal, thalamic, and hippocampal acetylcholine levels was significant ($p < 0.01$); the difference between hippocampal acetylcholine levels and septal, striatal, and thalamic acetylcholine levels was also significant ($p < 0.01$). Septal (12.8 nM/g) and thalamic acetylcholine levels (11.8 nM/g) did not differ significantly. However, septal acetylcholine levels were significantly different ($p < 0.01$) from the striatal and hippocampal acetylcholine levels; similarly, thalamic acetylcholine levels were significantly different from striatal and hippocampal acetylcholine levels. As in the case of endogenous choline levels (cf. Table 6A), endogenous acetylcholine levels for "NO FIGHT", unpaired, aggregated mice display a three-tiered relationship; however, in the case of acetylcholine the sequence was, in order of decreasing importance: striatum, septum, thalamus, and hippocampus, which differs from the sequence observed in the case of choline (see Section II.1. and Table 6A). This hierarchical arrangement was also observed in the case of "NO FIGHT", unpaired, isoalted mice (see the following Section).

E. ENDOGENOUS ACETYLCHOLINE LEVELS FOR "NO FIGHT" MICE; UNPAIRED, ISOLATED - COMPARISON OF BRAIN PARTS

Endogenous acetylcholine levels in the septum, stri-

atum, thalamus, and hippocampus of "NO FIGHT", unpaired, isolated mice are shown in Table 6B. Striatal levels were highest (16.9 nM/g), septal and thalamic levels were intermediate (12.7 nM/g, and 11.8 nM/g, respectively); and hippocampal levels were lowest (7.3 nM/g). Striatal and hippocampal acetylcholine levels differed significantly ($p < 0.01$) from those in all other brain parts of this group of "NO FIGHT" mice; septal and thalamic levels did not differ significantly from each other.

F. ENDOGENOUS ACETYLCHOLINE LEVELS - COMPARISON OF "NO FIGHT" UNPAIRED, AGGREGATED AND UNPAIRED, ISOLATED MICE

In Table 6B, acetylcholine levels in the septum, striatum, thalamus, and hippocampus of "NO FIGHT" unpaired, aggregated mice are compared with those of "NO FIGHT", unpared, isolated mice. No significant difference was found between these two groups of mice with regard to acetylcholine brain levels of the four brain areas. It can be stated that isolation has no effect on endogenous acetylcholine levels in the septum, striatum, thalamus, and hippocampus of CF-1, male mice. Furthermore, one order of values for ACh levels in the 4 brain parts was identical in the two groups of mice.

2. ACH TURNOVER OF "NO FIGHT" MICE

A. ACETYLCHOLINE TURNOVER VALUES FOR "NO FIGHT", UNPAIRED, AGGREGATED MICE - COMPARISON OF BRAIN PARTS

Table 6C shows acetylcholine turnover values for the septum, striatum, thalamus, and hippocampus of "NO FIGHT", unpaired, aggregated mice. Highest acetylcholine turnover values characterized the septum (31.3 nM/g/hr); intermediate values were found for hippocampus (22.5 nM/g/hr); and lowest acetylcholine turnover was found in the thalamus and striatum (16.5 nM/g/hr, and 15.7 nM/g/hr, respectively). Septal acetylcholine turnover value (31.3 nM/g/hr) was significantly ($p < 0.01$) different from all other turnover values for this group of mice. Hippocampal acetylcholine turnover values (22.5 nM/g/hr) were significantly different ($p < 0.05$) from thalamic (16.5 nM/g/hr) and striatal (15.7 nM/g/hr) as well as septal acetylcholine turnover values (31.3 nM/g/hr), ($p < 0.01$). Striatal (15.7 nM/g/hr) and thalamic (16.5 nM/g/hr) acetylcholine turnover values did not differ significantly from one another; however, there were significant differences with regard to acetylcholine turnover values between these two (striatal, thalamic) brain parts and either septum (31.3 nM/g/hr; $p < 0.01$) or hippocampus (22.5 nM/g/hr; $p < 0.05$).

B. ACETYLCHOLINE TURNOVER VALUES FOR "NO FIGHT", UNPAIRED, ISOLATED MICE - COMPARISON OF BRAIN PARTS

Table 6C concerns "NO FIGHT", unpaired, isolated mice. The highest acetylcholine turnover value was found

TABLE 6C

ACETYLCHOLINE TURNOVER VALUES FOR "NO FIGHT" MICE: UNPAIRED, AGGREGATED AND UNPAIRED, ISOLATED

BRAIN REGION ACH TURNOVER VALUES (nM/g/min + S.E.M.) n = 5

	ACH TURNOVER	
Brain Area	Unpaired, Aggregated	Unpaired, Isolated
Septum	31.3 ± 1.0	31.8 ± 1.7
Striatum	15.7 ± 0.9	15.4 ± 0.7
Thalamus	16.5 ± 0.8	18.6 ± 1.5
Hippocampus	22.5 ± 1.4	22.2 ± 1.4

* = $p < 0.05$; ** = $p < 0.01$

for the septum (31.8 nM/g/hr). The descending order of values of turnover for the remaining brain parts was as follows: Hippocampus (22.2 nM/g/hr), thalamus (18.6 nM/g/hr), and striatum (15.4 nM/g/hr).

Septal turnover values (31.8 nM/g/hr) were found to be significantly different from the remaining turnover values ($p < 0.01$). Hippocampal acetylcholine turnover values (22.2 nM/g/hr) were found to be significantly different ($p < 0.05$) from striatal (15.4 nM/g/hr) and thalamic (18.6 nM/g/hr) acetylcholine turnover values. Striatal values (15.4 nM/g/hr) were not significantly different from thalamic (18.6 nM/g/hr) acetylcholine turnover values.

C. ACETYLCHOLINE TURNOVER VALUES - COMPARISON OF "NO FIGHT", UNPAIRED, AGGREGATED AND UNPAIRED, ISOLATED MICE

Table 6C shows acetylcholine turnover values obtained for the septum, striatum, thalamus, and hippocampus of "NO FIGHT", unpaired, aggregated mice (shown in the left-hand column) and "NO FIGHT", unpaired, isolated mice (shown in the right-hand column). No significant difference was observed between septal values for "NO FIGHT", unpaired, aggregated mice (3.13 nM/g/hr) and for "NO FIGHT", unpaired, isolated mice (31.8 nM/g/hr). Likewise, no significant differences were found when striatal, thalamic, and hippocampal values for "NO FIGHT", unpaired, aggregated mice were compared with values obtained for "NO FIGHT", unpaired, isolated mice. For either group of "NO FIGHT" mice the

descending order of brain part turnover values was: septum (31.3 and 31.8 nM/g/hr), hippocampus (22.5 and 22.2 nM/g/hr), thalamus (16.5 and 18.6 nM/g/hr), and striatum (15.7 and 15.4 nM/g/hr).

Isolation, therefore, has been shown to have no effect on acetylcholine turnover values in the four brain areas tested.

D. SUMMARY - RESULTS FOR "NO FIGHT", UNPAIRED, AGGREGATED AND "NO FIGHT", UNPAIRED, ISOLATED MICE

The purpose of obtaining endogenous choline and acetylcholine levels and acetylcholine turnover values from the septum, striatum, thalamus, and hippocampus of "NO FIGHT", unpaired, aggregated and "NO FIGHT", unpaired, isolated mice was to determine what effect, if any, isolation has on choline and acetylcholine levels and acetylcholine turnover values. It must be emphasized that the 3-day, paired fighting session was not implemented for either group (cf. Methods); thus, the mice in question did not experience a fighting episode.

The results described in this section can be summarized as follows:

1) Endogenous choline levels were highest in the septum, intermediate in the thalamus and striatum, and lowest in the hippocampus; this was found in the case of both "NO FIGHT", unpaired, aggregated and "NO FIGHT", unpaired, isolated mice.

2) Endogenous acetylcholine levels were highest in the striatum, intermediate in the septum and thalamus, and lowest in the hippocampus; again, this was found to be true for both "NO FIGHT", unpaired, aggregated and "NO FIGHT", unpaired, isolated mice.

3) The brain part ranking (highest to lowest) of acetylcholine turnover values for both "NO FIGHT", unpaired, aggregated and "NO FIGHT", unpaired, isolated mice was: septum, hippocampus, thalamus, and striatum.

4) Endogenous choline levels, endogenous acetylcholine levels, and acetylcholine turnover values in all four brain areas showed no significant differences when "NO FIGHT", unpaired, aggregated mice were compared with "NO FIGHT", unpaired, isolated mice. Thus, isolation had no effect on these parameters.

III. RESULTS FOR PAIRED, AGGREGATED MICE PAIRED IN AGGRESSION TEST; NON-AGGRESSIVE

1. ENDOGENOUS CH AND ACH LEVELS IN PAIRED, NON-AGGRESSIVE ("NO FIGHT") MICE

A. ENDOGENOUS CHOLINE LEVELS FOR PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE - COMPARISON OF BRAIN PARTS

Table 7A shows endogenous choline levels to be highest in the septum (38.8 nM/g) and lowest in the hippocampus (23.3 nM/g) of paired, aggregated (non-aggressive) mice; these mice were paired, following aggregation, but did not exhibit aggression in the pairing test. Striatal and thalamic choline levels were similar 30.9 and 30.4 nM/g, respectively) and intermediate as compared to striatal and hippocampal choline levels. Thus, the choline levels shown in Table 7A, which are similar to those displayed by "NO FIGHT", unpaired, aggregated and "NO FIGHT", unpaired, isolated mice (see Results, II), display a three-tiered arrangement. All three tiers of choline levels were significantly different from one another ($p < 0.01$)

B. ENDOGENOUS CHOLINE LEVELS FOR PAIRED, AGGREGATED NON-AGGRESSIVE) MICE COMPARED TO "NO FIGHT", UNPAIRED, AGGREGATED AND "NO FIGHT", UNPAIRED, ISOLATED MICE

Table 7A also compares endogenous choline levels for paired, aggregated (non-aggressive) mice with endogenous

TABLE 7A

ENDOGENOUS CHOLINE LEVELS FOR UNPAIRED, AGGREGATED; UNPAIRED, ISOLATED; AND PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE BRAIN REGION CH LEVELS (nM/g + S.E.M.); n = 4

	CH		
Brain Area	Unpaired, Aggregated	Unpaired, Isolated	Paired, Aggregated
Septum	38.8 ± 1.2	43.2 ± 3.8	38.8 ± 0.5
Striatum	29.9 ± 1.1	27.6 ± 2.2	30.9 ± 1.3
Thalamus	30.6 ± 0.9	30.8 ± 1.7	30.4 ± 0.5
Hippocampus	22.4 ± 1.6	22.3 ± 0.2	23.3 ± 0.4

* = $p < 0.05$; ** = $p < 0.01$

choline levels for "NO FIGHT", unpaired, aggregated and "NO FIGHT", unpaired, isolated mice. There were no significant differences in any of the four brain areas tested between these 3 groups of mice.

C. ENDOGENOUS ACETYLCHOLINE LEVELS FOR PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE - COMPARISON OF BRAIN PARTS

Table 7B shows endogenous acetylcholine levels in the septum, striatum, thalamus, and hippocampus for paired, aggregated (non-aggressive) mice, i.e. mice that did not exhibit aggression following aggregation period and pairing. A different three-tiered arrangement can be seen here as compared to that for choline levels of the paired, aggregated mice. Striatal acetylcholine levels (17.3 nM/g), which were the highest among the four brain areas, were significantly higher ($p < 0.01$) than the other three brain areas. The values for these brain areas were: septum, 13.0 nM/g (different at the 5% significance level from the value for the striatum); thalamus, 11.6 nM/g (different from the striatal level at the 1% significance level); and hippocampus, 7.2 nM/g (different from the striatal level at the 1% significance level). Septal and thalamic acetylcholine levels were not significantly different from each other but were significantly different from striatal and hippocampal levels. The hippocampus had the lowest acetylcholine level, 7.2 nM/g; this level was significantly lower ($p < 0.01$) than that for thalamic, septal, and striatal acetylcholine levels in this group of mice.

TABLE 7B

ENDOGENOUS ACETYLCHOLINE LEVELS FOR UNPAIRED, AGGREGATED; UNPAIRED, ISOLATED; AND PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE

BRAIN REGION ACH LEVELS (nM/g + S.E.M.); n = 4

	ACH		
Brain Area	Unpaired, Aggregated	Unpaired, Isolated	Paired, Aggregated
Septum	11.9 ± 1.2	14.6 ± 1.1	13.0 ± 1.2
Striatum	17.9* ± 0.9	23.0 ± 4.4	17.3 ± 1.1
Thalamus	12.0 ± 0.8	11.9 ± 0.8	11.6 ± 0.4
Hippocampus	7.2 ± 0.5	7.8 ± 0.9	7.2 ± 0.6

* = $p < 0.05$; ** = $p < 0.01$

D. ENDOGENOUS ACETYLCHOLINE LEVELS FOR PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE COMPARED TO "NO FIGHT", UNPAIRED, AGGREGATED AND "NO FIGHT", UNPAIRED, ISOLATED MICE

Table 7B also compares acetylcholine levels for paired, aggregated (non-aggressive) mice with those for "NO FIGHT", unpaired, aggregated and "NO FIGHT", unpaired, isolated mice. For all four brain areas tested there were no significant differences between these 3 groups; thus, acetylcholine levels for the paired, aggregated (non-aggressive) mice did not differ from levels exhibited by "NO FIGHT", unpaired mice whether aggregated or isolated.

2. ACH TURNOVER IN PAIRED, NON-AGGRESSIVE ("NO FIGHT") MICE

A. ACETYLCHOLINE TURNOVER VALUES FOR PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE - COMPARISON OF BRAIN PARTS

Table 7C reveals that, in the case of paired, aggregated (non-aggressive) mice, septal acetylcholine turnover values (32.8 nM/g/min) were significantly higher ($p < 0.01$) than those for the other three brain areas. Hippocampal acetylcholine turnover values (24.3 nM/g/min) were second highest; they were significantly higher than the thalamic (20.3 nM/g/min; $p < 0.05$) and striatal (18.5 nM/g/min; $p < 0.01$) values. Thalamic acetylcholine turnover values were third highest albeit they did not differ significantly from the lowest, striatal acetylcholine turnover values.

TABLE 7C

ACETYLCHOLINE TURNOVER VALUES FOR UNPAIRED, AGGREGATED; UNPAIRED, ISOLATED; AND PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE

BRAIN REGION ACH TURNOVER VALUES (nM/g/min + S.E.M.) n = 4

	ACH TURNOVER		
Brain Area	Unpaired, Aggregated	Unpaired, Isolated	Paired, Aggregated
Septum	40.0 ± 2.6**[1]	40.9 ± 2.5**[1]	32.8 ± 1.8 **[1]
Striatum	16.2 ± 1.1	13.9 ± 2.3	18.5 ± 1.4
Thalamus	17.0 ± 2.0	17.9 ± 1.1	20.3 ± 1.5
Hippocampus	23.3 ± 2.0	21.3 ± 1.3	24.3 ± 1.9

* = $p < 0.05$; ** = $p < 0.01$

Intergroup comparison: 1

B. ACETYLCHOLINE TURNOVER VALUES FOR PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE COMPARED TO "NO FIGHT", UNPAIRED, AGGREGATED AND "NO FIGHT", UNPAIRED, ISOLATED MICE

As can be seen from Table 7C, acetylcholine turnover values of the septum of paired, aggregated (non-aggressive) mice were significantly ($p < 0.05$) lower than those for either "NO FIGHT", unpaired, aggregated and "NO FIGHT", unpaired, isolated mice: 32.8 nM/g/min, compared to 40.0 and 40.9 nM/g/min, respectively.

Hippocampal acetylcholine turnover values for paired, aggregated (non-aggressive) mice did not differ significantly from the values for "NO FIGHT", unpaired, aggregated or "NO FIGHT", unpaired, isolated mice.

Thalamic acetylcholine turnover values for paired, aggregated (non-aggressive) mice did not differ significantly from those for "NO FIGHT", unpaired, aggregated or "NO FIGHT", unpaired, isolated mice.

There was no significant difference between turnover values for the striatum of paired, aggregated (non-aggressive) mice and the values for the striatum of "NO FIGHT", unpaired, aggregated and unpaired, isolated mice. Thus, only in the case of the septum did the turnover values differ between paired, aggregated mice and unpaired mice, whether isolated or aggregated.

3. SUMMARY - RESULTS FOR ENDOGENOUS CHOLINE AND ACETYLCHOLINE LEVELS AND ACETYLCHOLINE TURNOVER VALUES FOR PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE COMPARED TO "NO FIGHT", UNPAIRED, AGGREGATED AND "NO FIGHT", UNPAIRED ISOLATED MICE

Results for this section can be summarized as follows:

1) There was no significant difference in endogenous choline levels when paired, aggregated (non-aggressive) mice were compared to "NO FIGHT", unpaired, aggregated and "NO FIGHT", unpaired, isolated mice in all four brain areas tested. Septal levels were highest, striatal and thalamic levels were intermediary, and hippocampal levels were lowest in the case of paired, aggregated mice.

2) There was no significant difference in endogenous acetylcholine levels in any of the four brain areas tested when paired aggregated (non-aggressive) mice were compared to "NO FIGHT", unpaired aggregated and "NO FIGHT", unpaired, isolated mice. Striatal levels were highest, septal and thalamic levels were intermediary, and hippocampal levels were lowest in the cases of paired, aggregated mice.

3) Septal acetylcholine turnover values for paired, aggregated (non-aggressive) mice were significantly lower ($p < 0.05$) than turnover values for "NO FIGHT", unpaired, aggregated and "NO FIGHT", unpaired, isolated mice. No significant differences in acetylcholine turnover values were found in the hippocampus, thalamus and striatum when these groups were compared. Septal values were highest, hip-

pocampal values were intermediary, and thalamic and striatal values were lowest in the case of the paired aggregated mice.

IV. RESULTS FOR ISOLATED MICE PAIRED WITH AGGREGATED MICE - AGGRESSIVE WINNERS (ISO-AGG) AND NON-AGGRESSIVE LOSERS (ISO-AGG)

1. ENDOGENOUS CH AND ACH LEVELS IN PAIRED WINNERS AND LOSERS

A. ENDOGENOUS CHOLINE LEVELS FOR WINNING (ISO-AGG) AND LOSING (ISO-AGG) MICE - COMPARISON OF BRAIN PARTS

Table 8A shows endogenous choline levels for WINNING (ISO-AGG) mice paired with LOSING (ISO-AGG) mice (which were aggressive and non-aggressive, respectively). Mice determined to be aggressive (WINNERS) were found invariable among the isolated, and mice determined to be non-aggressive (LOSERS) were invariably found among the aggregated mice. For the WINNING (ISO-AGG) mice, septal choline levels (48.9 nM/g) were significantly higher than thalamic (30.5 nM/g), stiatal (25.5 nM/g), and hippocampal (16.6 nM/g) choline levels; also, these three endogenous choline levels were significantly different ($p < 0.05$) when compared to one another.

Table 8A also shows endogenous choline levels for LOSING (ISO-AGG) mice. No significant difference was found between 3 of the 4 brain areas tested for this group: striatum (32.6 nM/g), septum (32.3 nM/g), and hippocampus (30.3 nM/g). Only the thalamus (24.3 nM/g) was significantly different ($p < 0.05$) than the other 3 aforementioned brain parts.

TABLE 8A

ENDOGENOUS CHOLINE LEVELS FOR UNPAIRED, AGGREGATED; UNPAIRED, ISOLATED; PAIRED, AGGREGATED (NON-AGGRESSIVE); AGGRESSIVE (ISO-AGG); AND NON-AGGRESSIVE (ISO-AGG).

BRAIN REGION CH LEVELS (nM/g ± S.E.M.); n = 4

	CH				
Brain Area	Unpaired, Aggregated	Unpaired, Isolated	Paired, Aggregated	Winners (ISO-AGG) Aggressive	Losers (ISO-AGG) Non-Aggressive
Septum	38.8±1.1	43.2±1.2	38.8±0.5	48.9±0.4 **1	32.3±0.6 **1
Striatum	29.9±1.1 **2	27.6±0.3 *3	30.9±0.3 **2	25.5±0.2 **2	32.6±0.6 *3
Thalamus	30.6±0.9 **4	30.8±0.7 **4	30.4±0.5 **4	30.5±0.4 **4	24.3±0.5 **4
Hippocampus	22.4±0.6 **5	22.3±1.2 **5	23.3±0.9 **5	16.6±0.4 **5	30.3±1.0 **5

(ISO-AGG): Isolated mice paired with aggregated mice; isolated mice winners

(ISO-AGG): Isolated mice paired with aggregated mice; aggregated mice losers

* = $p < 0.05$; ** = $p < 0.01$

Intergroup comparison: 1,2,3,4,5

B. ENDOGENOUS CHOLINE LEVELS FOR WINNING (ISO-AGG) AND LOSING (ISO-AGG) MICE COMPARED WITH "NO FIGHT"-UNPAIRED, AGGREGATED: "NO FIGHT"-UNPAIRED, ISOLATED; AND PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE

Septal choline levels for WINNING (ISO-AGG), (48.9 nM/g) and LOSING (ISO-AGG), (32.3 nM/g) mice were significantly different ($p < 0.01$) from septal choline levels for "NO FIGHT"-unpaired, aggregated (38.7 nM/g), "NO FIGHT"-unpaired, isolated (43.2 nM/g), and paired, aggregated (non-aggressive)(38.8 nM/g) mice. Septal choline levels for WINNING (ISO-AGG) mice were significantly higher than those of the other groups: LOSING (ISO-AGG); "NO FIGHT"-unpaired, aggregated; "NO FIGHT"-unpaired, isolated; and paired, aggregated (non-aggressive) mice. Conversely septal choline levels for LOSING (ISO-AGG) mice were significantly lower than those of the other groups: WINNING (ISO-AGG); "NO FIGHT"-unpaired, aggregated; "NO FIGHT"-unpaired, isolated; and paired, aggregated (non-aggressive) mice.

Striatal choline levels for WINNING (ISO-AGG) mice were significantly lower than all other groups; whereas, striatal choline levels for LOSING (ISO-AGG) mice were significantly ($p < 0.01$) higher than those for unpaired, aggregated ($p < 0.05$); unpaired, isolated; paired, aggregated; and LOSING (ISO-AGG) mice.

Table 8A shows no significant difference for thalamic endogenous choline levels when WINNING (ISO-AGG) mice (30.5

nM/g) were compared with "NO FIGHT"-unpaired, aggregated mice (30.6 nM/g); "NO FIGHT"-unpaired, isolated mice (30.8 nM/g); and paired, aggregated (non-aggressive) mice (30.4 nM/g). Thalamic endogenous choline levels for LOSING (ISO-AGG) mice (24.3 nM/g) were found to be significantly lower ($p < 0.01$) than those for "NO FIGHT"-unpaired, aggregated; "NO FIGHT"-unpaired, isolated; and paired, aggregated (non-aggressive) mice.

It can be seen from the table that septal endogenous choline levels reflect a dipole relationship with hippocampal endogenous choline levels with respect to the outcome (WINNING or LOSING) of the fighting session. Septal choline levels (48.9 nM/g) for WINNING (ISO-AGG) mice were significantly ($p < 0.01$) higher than septal choline levels (32.3 nM/g) for LOSING (ISO-AGG) mice while the opposite is true for the hippocampus. Hippocampal choline levels (16.6 nM/g) for WINNING (ISO-AGG) mice were significantly ($p < 0.01$) lower than hippocampal choline levels (30.3 nM/g) for LOSING (ISO-AGG) mice.

C. ENDOGENOUS ACETYLCHOLINE LEVELS FOR WINNING (ISO-AGG) AND LOSING (ISO-AGG) MICE-COMPARISON OF BRAIN PARTS

Table 8B shows no significant differences between endogenous acetylcholine levels for the septum, striatum, and thalamus when WINNING (ISO-AGG) and LOSING (ISO-AGG) mice were compared. Only hippocampal acetylcholine levels differed sig-

TABLE 8B

ENDOGENOUS ACETYLCHOLINE LEVELS FOR UNPAIRED, AGGREGATED; UNPAIRED, ISOLATED; PAIRED, AGGREGATED (NON-AGGRESSIVE); AGGRESSIVE (ISO-AGG); AND NON-AGGRESSIVE (ISO-AGG).

BRAIN REGION ACH LEVELS (nM/g ± S.E.M.); n = 4

	ACH				
Brain Area	Unpaired, Aggregated	Unpaired, Isolated	Paired, Aggregated	Winners (ISO-AGG) Aggressive	Losers (ISO-AGG) Non-Aggressive
Septum	11.9±1.2	14.6±1.5	13.0±1.2	14.9±1.3	11.4±1.1
Striatum	17.9±0.9	23.0±4.2**1	17.3±0.2	16.9±0.3**1	16.8±3.2**1
Thalamus	12.0±0.8*2	11.9±0.8*2	11.6±0.4*2	11.2±1.0*2	14.9±0.7*2
Hippocampus	7.2±0.5	7.8±1.0	7.2±0.6	5.4±1.0*3	10.0±0.9*3

(ISO-AGG): Isolated mice paired with aggregated mice; isolated mice winners

(ISO-AGG): Isolated mice paired with aggregated mice; aggregated mice losers

* = $p < 0.05$; ** = $p < 0.01$

Intergroup comparison: 1,2,3

nificantly ($p < 0.05$): 5.4 nM/g for WINNERS (ISO-AGG) compared to 10.0 nM/g for the LOSERS (ISO-AGG).

For the WINNING (ISO-AGG) mice, striatal acetylcholine levels were highest (16.9 nM/g), septal acetylcholine levels were next (14.9 nM/g), thalamic acetylcholine levels were third (11.2 nM/g), and hippocampal acetylcholine levels were lowest (5.4 nM/g) in sequence. All of these groups were significantly different ($p < 0.05$) from one another.

With regard to endogenous acetylcholine levels for LOSING (ISO-AGG) mice, striatal (16.8 nM/g) and thalamic (14.9 nM/g) levels were highest and did not differ significantly from each other. Septal (11.4 nM/g) levels were significantly ($p < 0.05$) lower than thalamic (14.9 nM/g) and striatal (16.8 nM/g) acetylcholine levels; and hippocampal (10.0 nM/g) levels were significantly ($p < 0.05$) lower than thalamic and striatal levels but were not significantly different from septal acetylcholine levels.

D. ENDOGENOUS ACETYLCHOLINE LEVELS FOR WINNING (ISO-AGG) AND LOSING (ISO-AGG) MICE COMPARED WITH "NO FIGHT"-UNPAIRED, AGGREGATED: "NO FIGHT"-UNPAIRED, ISOLATED: AND PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE

Table 8B shows that there is no significant difference when septal endogenous acetylcholine levels for WINNING (ISO-AGG) and LOSING (ISO-AGG) mice were compared with sep-

tal acetylcholine levels for "NO FIGHT"-unpaired, aggregated; "NO FIGHT"-unpaired, isolated; and paired, aggregated (non-aggressive mice.

Striatal acetylcholine levels for WINNING (ISO-AGG), (16.9 nM/g) and LOSING (ISO-AGG), (16.8 nM/g) mice were significantly ($p < 0.01$) lower than striatal levels for unpaired, isolated mice.

Thalamic acetylcholine levels for LOSING (ISO-AGG), (14.9 nM/g) mice were significantly ($p < 0.05$) higher than the acetylcholine levels in the other four behavioral groups (see Table 8B).

Hippocampal acetylcholine levels of WINNING (ISO-AGG) mice (5.4 nM/g) were significantly lower than those from unpaired, aggregated (7.2 nM/g), unpaired, isolated (7.8 nM/g) and paired, aggregated (7.2 nM/g) mice. However, hippocampal acetylcholine levels for LOSING (ISO-AGG) mice were significantly ($p < 0.05$) higher than the acetylcholine levels in the other four behavioral groups.

2. ACH TURNOVER IN WINNERS AND LOSERS

A. ACETYLCHOLINE TURNOVER VALUES FOR WINNING (ISO-AGG) AND LOSING (ISO-AGG) MICE - COMPARISON OF BRAIN PARTS

Table 8C shows that septal acetylcholine turnover values for WINNING (ISO-AGG) mice (39.4 nM/g/min) were highest, thalamic values (16.8 nM/g/min) were second, hippocampal values (15.9 nM/g/min) were third, and striatal values (12.4

TABLE 8C

ACETYLCHOLINE TURNOVER VALUES FOR UNPAIRED, AGGREGATED; UNPAIRED, ISOLATED; PAIRED, AGGREGATED; (NON-AGGRESSIVE); AGGRESSIVE (ISO-AGG); AND NON-AGGRESSIVE (ISO-AGG).

BRAIN REGION ACH TURNOVER VALUES (nM/g/MIN ± S.E.M.); n = 4

	ACH TURNOVER				
Brain Area	Unpaired, Aggregated	Unpaired, Isolated	Paired, Aggregated	Winners (ISO-AGG) Aggressive	Losers (ISO-AGG) Non-Aggressive
Septum	40.0±2.6	40.9±2.5	32.8±1.8 **1	39.4±1.5 **1	16.7±1.4 **1
Striatum	16.2±1.1	13.9±1.6	18.5±0.4	12.4±0.3 *	15.7±1.2
Thalamus	17.0±2.0	17.9±1.2	20.3±2.1	16.8±1.5	9.6±1.4 *2
Hippocampus	23.3±1.0	21.3±1.3	24.3±0.5	15.9±0.3 **3	31.8±1.8 **3

(ISO-AGG): Isolated mice paired with aggregated mice; isolated mice winners

(ISO-AGG): Isolated mice paired with aggregated mice; aggregated mice losers

* = $p < 0.05$; ** = $p < 0.01$

Intergroup comparison: 1,2,3

nM/g/min) were fourth in sequence. Septal acetylcholine turnover values were significantly ($p < 0.01$) different than the values from the other three brain areas; also, striatal acetylcholine turnover values were significantly different ($p < 0.01$) than values from the other three brain areas. Thalamic and hippocampal acetylcholine turnover values did not differ significantly from one another, however.

Table 8C also shows that hippocampal acetylcholine turnover values for LOSING (ISO-AGG) mice (31.8 nM/g/min) were highest, septal values (16.7 nM/g/min) were second, striatal values (15.7 nM/g/min) were third, and thalamic values (9.6 nM/g/min) were fourth in sequence. Hippocampal acetylcholine turnover values were significantly different ($p < 0.01$) than values from the remaining 3 brain areas with regard to LOSING (ISO-AGG) mice. Thalamic acetylcholine turnover values were also significantly different from the other brain areas. Septal and striatal acetylcholine turnover values did not differ significantly, however.

B. ACETYLCHOLINE TURNOVER VALUES FOR WINNING (ISO-AGG) AND LOSING (ISO-AGG) MICE COMPARED WITH "NO FIGHT"-UNPAIRED, AGGREGATED; "NO FIGHT"-UNPAIRED, ISOLATED; AND PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE

As seen from Table 8C, septal acetylcholine turnover values for WINNING (ISO-AGG) mice (39.4 nM/g/min) were sig-

nificantly higher ($p < 0.01$) than acetylcholine turnover values for LOSING (ISO-AGG) mice (16.7 nM/g/min) and grouped, aggregated (non-aggressive) mice (32.8 nM/g/min; $p < 0.05$); however, septal acetylcholine turnover values for WINNING (ISO-AGG) mice (39.4 nM/g/min) were not significantly different than "NO FIGHT"-unpaired, aggregated (40.0 nM/g/min) or "NO FIGHT"-unpaired, isolated (40.9 nM/g/min) values. Septal acetylcholine turnover values for LOSING (ISO-AGG) mice (16.7 nM/g/min) were significantly lower ($p < 0.01$) than septal acetylcholine turnover values for WINNING (ISO-AGG)(39.4 nM/g/min); "NO FIGHT"-unpaired, aggregated (40.0 nM/g/min); "NO FIGHT"-unpaired, isolated (40.9 nM/g/min); and grouped, aggregated (non-aggressive) (32.8 nM/g/min) mice.

Striatal acetylcholine turnover values for WINNING (ISO-AGG) mice (12.4 nM/g/min) were significantly lower (p 0.05) than striatal acetylcholine turnover values for "NO FIGHT"-unpaired, aggregated (16.2 nM/g/min); grouped, aggregated (non-aggressive)(18.5 nM/g/min); and LOSING (ISO-AGG) (15.7 nM/g/min) mice. Striatal acetylcholine turnover values for LOSING (ISO-AGG) mice did not differ significantly from acetylcholine turnover values from "NO FIGHT"-unpaired, aggregated (16.2 nM/g/min); "NO FIGHT"-unpaired, isolated (13.9 nM/g/min); or grouped, aggregated (non-aggressive)(18.5 nM/g/min) mice.

Thalamic acetylcholine turnover values for WINNING

(ISO-AGG) mice (16.8 nM/g/min) were significantly higher ($p < 0.01$) than thalamic acetylcholine turnover values for LOSING (ISO-AGG) mice (9.6 nM/g/min); however, thalamic acetylcholine turnover values did not differ significantly from those turnover values for "NO FIGHT"-unpaired, aggregated (17.0 nM/g/min); "NO FIGHT"-unpaired, isolated (17.9 nM/g/min); or paired, aggregated (non-aggressive) mice (20.3 nM/g/min). Thalamic acetylcholine turnover values for LOSING (ISO-AGG) mice (9.6 nM/g/min) however, were significantly ($p < 0.01$) lower than thalamic acetylcholine turnover values for "NO FIGHT"-unpaired, aggregated (17.0 nM/g/min); "NO FIGHT"-unpaired, isolated (17.9 nM/g/min); or grouped, aggregated (non-aggressive)(20.3 nM/g/min) mice.

Hippocampal acetylcholine turnover values for WINNING (ISO-AGG) mice (15.9 nM/g/min) were also shown in Table 8C and were found to be significantly lower ($p < 0.01$) than hippocampal acetylcholine turnover values for LOSING (ISO-AGG) mice (31.8 nM/g/min); "NO FIGHT"-unpaired, aggregated (23.3 nM/g/min) mice; "NO FIGHT"-unpaired, isolated (21.3 nM/g/min) mice; and paired, aggregated (24.3 nM/g/min) mice. Conversely, hippocampal acetylcholine turnover values for LOSING (ISO-AGG) mice (31.8 nM/g/min) were significantly higher ($p < 0.01$) than acetylcholine turnover values for "NO FIGHT"-unpaired, aggregated (23.3 nM/g/min); "NO FIGHT"-unpaired isolated (21.3 nM/g/min); and paired, aggregated (24.3 nM/g/min) mice.

3. SUMMARY - RESULTS FOR ENDOGENOUS CHOLINE AND ACETYLCHOLINE LEVELS AND ACETYLCHOLINE TURNOVER VALUES FOR WINNING (ISO-AGG) AND LOSING (ISO-AGG) MICE COMPARED WITH "NO FIGHT"-UNPAIRED, AGGREGATED; "NO FIGHT"-UNPAIRED, ISOLATED; AND PAIRED-AGGREGATED (NON-AGGRESSIVE) MICE

Results for this section can be summarized as follows:

1) Septal choline levels for WINNING (ISO-AGG) mice were significantly higher than septal choline levels for LOSING (ISO-AGG); NO FIGHT-unpaired, aggregated; "NO FIGHT"-unpaired, isolated; and paired, aggregated (non-aggressive) mice. Septal choline levels for LOSING (ISO-AGG) mice were significantly lower than septal choline levels for "NO FIGHT"-unpaired, aggregated; "NO FIGHT"-unpaired, isolated; and paired, aggregated (non-aggressive) mice. Striatal choline values for WINNING (ISO-AGG) mice were significantly lower than striatal levels for other groups; whereas, choline levels from LOSING (ISO-AGG) mice were higher than those from unpaired, aggregated; unpaired, isolated; paired, aggregated; and WINNING (ISO-AGG) mice. Thalamic endogenous choline levels for LOSING (ISO-AGG) mice were found to be significantly lower than those levels for "NO FIGHT"-unpaired, aggregated; "NO FIGHT" -unpaired, isolated; and paired, aggregated (non-aggressive) mice. Hippocampal choline levels for WINNING (ISO-AGG) mice were significantly lower than hippocampal choline levels for LOSING (ISO-AGG) mice.

2) Hippocampal acetylcholine levels of LOSING (ISO-AGG) mice were significantly higher than hippocampal acetylcholine levels of "NO FIGHT"-unpaired, aggregated; "NO FIGHT"-unpaired, isolated; paired, aggregated (non-aggressive); and WINNING (ISO-AGG) mice.

3) Septal acetylcholine turnover values for WINNING (ISO-AGG) mice were significantly higher than acetylcholine turnover values for LOSING (ISO-AGG) mice. Striatal acetylcholine turnover values for WINNING (ISO-AGG) mice were significantly lower than striatal acetylcholine turnover values for LOSING (ISO-AGG) mice. Thalamic acetylcholine turnover values for WINNING (ISO-AGG) mice were significantly higher than thalamic acetylcholine turnover values for LOSING (ISO-AGG) mice. Hippocampal acetylcholine turnover values for WINNING (ISO-AGG) mice were found to be significantly lower than hippocampal acetylcholine turnover values for LOSING (ISO-AGG) mice.

V. RESULTS FOR ISOLATED MICE PAIRED WITH ISOLATE MICE - AGGRESSIVE WINNERS (ISO-ISO) AND NON-AGGRESSIVE LOSERS (ISO-ISO) - IMMEDIATE AND 7-DAY SACRIFICE FOLLOWING ^{3}H-CHOLINE INJECTION

1. CH AND ACH LEVELS

A. ENDOGENOUS CHOLINE LEVELS FOR WINNING (ISO-ISO) AND LOSING (ISO-ISO) MICE-IMMEDIATE AND 7-DAY SACRIFICE - COMPARISON OF BRAIN PARTS; INTER- AND INTRAGROUP COMPARISON

Endogenous choline levels in the septum of WINNING (ISO-ISO) mice for both immediate and 7-day sacrifice groups were significantly ($p < 0.01$) higher than choline levels in the septum of LOSING (ISO-ISO) mice: 52.7 and 51.4 nM/g compared with 31.9 and 31.7 nM/g, respectively. No significant differences were found for striatal choline levels between WINNING (ISO-ISO) and LOSING (ISO-ISO) groups of mice.

Thalamic choline levels for WINNING (ISO-ISO) mice sacrificed immediately after test (31.2 nM/g) and those of WINNING (ISO-ISO) mice sacrificed 7 days after test (31.5 nM/g) were significantly ($p < 0.01$) higher than thalamic choline values obtained from LOSING (ISO-ISO) Mice, whether sacrificed immediately after test (24.7 nM/g) or 7 days after test (23.9 nM/g).

Thalamic choline levels for WINNING (ISO-AGG) (30.5

TABLE 9A

ENDOGENOUS CHOLINE LEVELS FOR WINNING (ISO-ISO) AND LOSING (ISO-ISO) MICE - IMMEDIATE AND 7-DAY SACRIFICE. BRAIN REGION CHOLINE LEVELS (nM/g + S.E.M.); n = 5

CH

	Immediate Sacrifice		7-Day Sacrifice	
Brain Area	Winners (ISO-ISO) Aggressive	Losers (ISO-ISO) Non-Aggressive	Winners (ISO-ISO) Aggressive	Losers (ISO-ISO) Non-Aggressive
Septum	52.7 ± 1.2**[1]	31.9 ± 0.8**[1]	51.4 ± 1.3**[2]	31.7 ± 0.7**[2]
Striatum	27.2 ± 0.8**[3]	31.8 ± 0.4**[3]	27.2 ± 0.7**[4]	31.8 ± 0.4**[4]
Thalamus	31.2 ± 1.0**[5]	24.7 ± 0.5**[5]	31.5 ± 1.2**[6]	23.9 ± 0.5**[6]
Hippocampus	19.8 ± 0.5**[7]	29.0 ± 1.6**[7]	19.7 ± 0.4**[8]	28.9 ± 1.7**[8]

Non-paired student t test: *p < 0.05; **p < 0.01
Intergroup comparison: 1,2,3,4,5,6,7,8

nM/g) mice and WINNING (ISO-ISO) (30.7 nM/g) mice did not differ significantly. Hippocampal choline levels present a different picture; WINNING (ISO-ISO) animals, whether sacrificed immediately or 7 days after test, display choline levels of 19.8 nM/g, and 19.7 nM/g, respectively. These hippocampal choline levels do not differ significantly from levels of "NO FIGHT"-unpaired, aggregated (22.4); "NO FIGHT"-unpaired, isolated (22.3 nM/g); and paired, aggregated (non-aggressive) (22.3 nM/g) mice. However, hippocampal choline levels from WINNING (ISO-ISO) mice whether sacrificed immediately or 7 days after test, do differ significantly (p 0.01) from the higher hippocampal choline levels of LOSING (ISO-ISO) mice whether sacrificed immediately (29.0 nM/g) or 7 days after test (28.9 nM/g).

B. ENDOGENOUS ACETYLCHOLINE LEVELS FOR WINNING (ISO-ISO) AND LOSING (ISO-ISO) MICE - IMMEDIATE AND 7-DAY SACRIFICE - COMPARISON OF BRAIN PARTS; INTER- AND INTRAGROUP COMPARISON

As can be seen from Table 9B septal, thalamic and hippocampal acetylcholine levels for WINNING (ISO-ISO) mice differ significantly (p 0.01) from levels of LOSING (ISO-ISO) mice; this was true for both the immediate and 7-day sacrifice groups. Septal values from WINNING (ISO-ISO) (immediate sacrifice; 14.6 nM/g) and 7-day sacrifice (14.7 nM/g) animals were higher (p 0.01) than those septal acetylchol-

TABLE 9B

ENDOGENOUS ACETYLCHOLINE LEVELS FOR WINNING (ISO-ISO) AND LOSING (ISO-ISO) MICE - IMMEDIATE AND 7-DAY SACRIFICE.

BRAIN REGION ACH LEVELS (nM/g + S.E.M.); n = 5

ACH

	Immediate Sacrifice		7-Day Sacrifice	
Brain Area	Winners (ISO-ISO) Aggressive	Losers (ISO-ISO) Non-Aggressive	Winners (ISO-ISO) Aggressive	Losers (ISO-ISO) Non-Aggressive
Septum	14.6 ± 0.9**[1]	10.2 ± 0.5**[1]	14.7 ± 0.9**[2]	10.1 ± 0.4**[2]
Striatum	17.5 ± 1.0	17.9 ± 1.3	17.5 ± 1.1	17.9 ± 1.3
Thalamus	10.9 ± 0.9**[3]	14.1 ± 0.7**[3]	11.0 ± 0.6**[4]	14.2 ± 0.7**[4]
Hippocampus	5.9 ± 0.5**[5]	8.9 ± 0.4**[5]	5.9 ± 0.6**[6]	8.8 ± 0.5**[6]

Non-paired student t test: *p < 0.05; **p < 0.01
Intergroup comparison: 1,2,3,4,5,6

ine values from LOSING (ISO-ISO) (immediate sacrifice; 10.2 nM/g) and 7-day sacrifice (10.1 nM/g) animals. Thalamic acetylcholine levels were lower for WINNING (ISO-ISO) (immediate sacrifice; 10.9 nM/g) and 7-day sacrifice (11.0 nM/g) mice compared to thalamic acetylcholine levels for LOSING (ISO-ISO) (immediate sacrifice; 14.1 nM/g) and 7-day sacrifice (14.2 nM/g) mice. The same pattern holds for hippocampal acetylcholine levels when WINNING (ISO-ISO) (immediate sacrifice; 5.9 nM/g) (7-day sacrifice; also 5.9 nM/g) mice were compared to LOSING (ISO-ISO) (immediate sacrifice; 8.9 nM/g) (7-day sacrifice; 8.8 nM/g) mice. No significant differences were found when striatal acetylcholine levels were compared between WINNING (ISO-ISO) and LOSING (ISO-ISO) mice with regard to immedate and 7-day sacrifice groups.

2. ACH TURNOVER

A. ACETYLCHOLINE TURNOVER VALUES FOR WINNING (ISO-ISO) AND LOSING (ISO-ISO) MICE - IMMEDIATE AND 7-DAY SACRIFICE - COMPARISON OF BRAIN PARTS; INTER- AND INTRAGROUP COMPARISON

Table 9C shows no significant differences for striatal acetylcholine turnover values when WINNING (ISO-ISO) and LOSING (ISO-ISO) mice of both the immediate and 7-day sacrifice groups were compared with each other and with "NO FIGHT" -unpared, isolated; and paired, aggregated (non-aggressive) mice (see Table 7A).

Septal, thalamic, and hippocampal acetylcholine turn-

TABLE 9C

ACETYLCHOLINE TURNOVER VALUES FOR WINNING (ISO-ISO) AND LOSING (ISO-ISO) MICE - IMMEDIATE AND 7-DAY SACRIFICE

BRAIN REGION ACH TURNOVER VALUES (nM/g/MIN + S.E.M.); n = 5

	ACH TURNOVER			
	Immediate Sacrifice		7-Day Sacrifice	
Brain Area	Winners (ISO-ISO) Aggressive	Losers (ISO-ISO) Non-Aggressive	Winners (ISO-ISO) Aggressive	Losers (ISO-ISO) Non-Aggressive
Septum	39.8 ± 1.3**[1]	17.1 ± 0.6**[1]	42.6 ± 0.7**[2]	16.7 ± 0.8**[2]
Striatum	14.2 ± 0.6	13.9 ± 0.7	14.1 ± 1.1	14.3 ± 0.9
Thalamus	16.6 ± 0.3**[3]	8.8 ± 0.6**[3]	18.2 ± 0.7**[4]	9.2 ± 1.0**[4]
Hippocampus	19.1 ± 0.5	18.2 ± 1.0	19.2 ± 0.8**[5]	27.2 ± 2.7**[5]

Non-paired student t test: *p < 0.05; **p < 0.01
Intergroup comparison: 1,2,3,4,5

over values for WINNING (ISO-ISO) mice did not differ significantly from "NO-FIGHT"-unpaired, aggregated; "NO FIGHT"-unpaired, isolated; and paired, aggregated (non-aggressive) mice; this was true whether for mice sacrificed immediately or 7 days after test. However, septal and thalamic turnover values of LOSING (ISO-ISO) animals were significantly ($p < 0.01$) lower than values of "NO FIGHT"-unpaired, aggregated; "NO FIGHT"-unpaired, isolated; and paired, aggregated (non-aggressive) mice; this was also true whether for mice sacrificed immediately or 7 days after test.

When comparing acetylcholine turnover values of immediate and 7-day sacrifice animals, only one comparison was significantly different ($p < 0.01$): hippocampal acetylcholine turnover values of LOSING (ISO-ISO) (immediate sacrifice; 18.2 nM/g/min) mice were significantly lower than hippocampal acetylcholine turnover values of LOSING (ISO-ISO) (7-day sacrifice; 27.2 nM/g/min) mice.

3. SUMMARY - RESULTS OF CHOLINE AND ACETYLCHOLINE LEVELS AND ACETYLCHOLINE TURNOVER VALUES OF WINNING (ISO-ISO; IMMEDIATE AND 7-DAY SACRIFICE) AND LOSING (ISO-ISO; IMMEDIATE AND 7-DAY SACRIFICE) MICE COMPARED WITH WINNING (ISO-AGG) MICE; LOSING (ISO-AGG) MICE; "NO FIGHT"-UNPAIRED, AGGREGATED MICE; "NO FIGHT"-UNPAIRED, ISOLATED MICE; AND PAIRED, AGGREGATED (NON-AGGRESSIVE) MICE

Results for this section can be summarized as follows:

1) Endogenous choline levels in the septum of WINNING

(ISO-ISO) mice for both immediate and 7-day sacrifice groups were significantly higher than choline levels in the septum of LOSING (ISO-ISO) mice. Thalamic choline levels for WINNING (ISO-ISO) mice sacrificed immediately after test and those of WINNING (ISO-ISO) mice sacrificed 7 days after test were significantly higher than thalamic choline levels obtained from LOSING (ISO-ISO) mice, whether sacrificed immediately after test or 7 days after test. Hippocampal choline levels from WINNING (ISO-ISO) mice whether sacrificed immediately or 7 days after test were significantly lower than hippocampal choline levels of LOSING (ISO-ISO) mice whether sacrificed immediately or 7 days after test.

2) Thalamic acetylcholine levels were significantly lower for WINNING (ISO-ISO; immediate sacrifice) and 7-day sacrifice mice compared to thalamic acetylcholine levels for LOSING (ISO-ISO) (immediate sacrifice) and 7-day sacrifice mice. The same patterns hold for hippocampal acetylcholine levels when WINNING (ISO-ISO) (immediate sacrifice) mice were compared to LOSING (ISO-ISO) (immediate sacrifice and 7-day sacrifice) mice.

3) Hippocampal acetylcholine turnover values of LOSING (ISO-ISO) (immediate sacrifice) mice were significantly lower than hippocampal acetylcholine turnover values of LOSING (ISO-ISO) (7-day sacrifice mice).

DISCUSSION

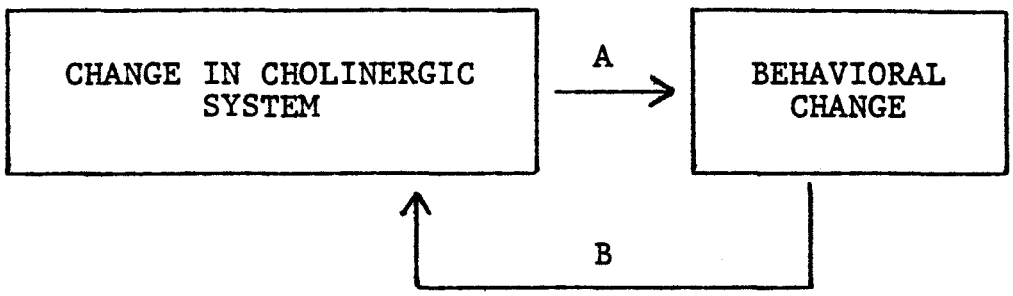
I. BEHAVIORAL RESULTS

A. EXPERIMENTAL DESIGN

Numerous investigators (Bandler, 1972; Igic et al., 1970; DeSouza and Neto, 1978), have manipulated the cholinergic system biochemically to produce behavioral changes. Bandler (1970), facilitated frog-killing behavior in rats via intrahypothalamic carbachol injections. This carbachol induced facilitation of killing was blocked by systemically administered atropine sulfate. Yoshimura and Ueki (1977), were able to suppress isolation-induced mouse-killing behavior in rats with intraperitoneal injections of atropine and scopolamine. The research strategy employed by these investigators is illustrated in Step A, Figure 2 changes in the cholinergic system produce behavioral changes.

The basic reasearch strategy employed in this dissertation, however, involves behavioral manipulations producing changes in the cholinergic system (see Step B, Figure 2 Changes in septal, striatal, thalamic and hippocampal choline and acetylcholine levels and acetylcholine turnover values were observed following 3-day fighting sessions. One

FIGURE 2



GENERAL RESEARCH STRATEGIES:

A: Change in cholinergic system produces behavioral change.

B: Behavioral change produces change in cholinergic system.

example of a similarly-designed experiment was reported by Consolo and Valzelli (1970). Here, choline acetylase (ChA) and monoamine oxidase (MAO) activities were recorded from 5 brain areas of both non-aggressive, aggregated and aggressive, isolated mice. Isolation-induced aggressive behavior had no effect on enzyme activity. In another experiment, which was not concerned with aggression, Burgel et al. (1978) observed choline uptake in two groups of rats; one group of rats was habituated to an experimental milk-drinking situation and the other consisted of naive animals. High-affinity hippocampal choline uptake of the habituated rats exceeded that of the naive animals whereas striatal values were not affected. The results support the notion that the high affinity uptake of choline may vary depending on the behavioral situation.

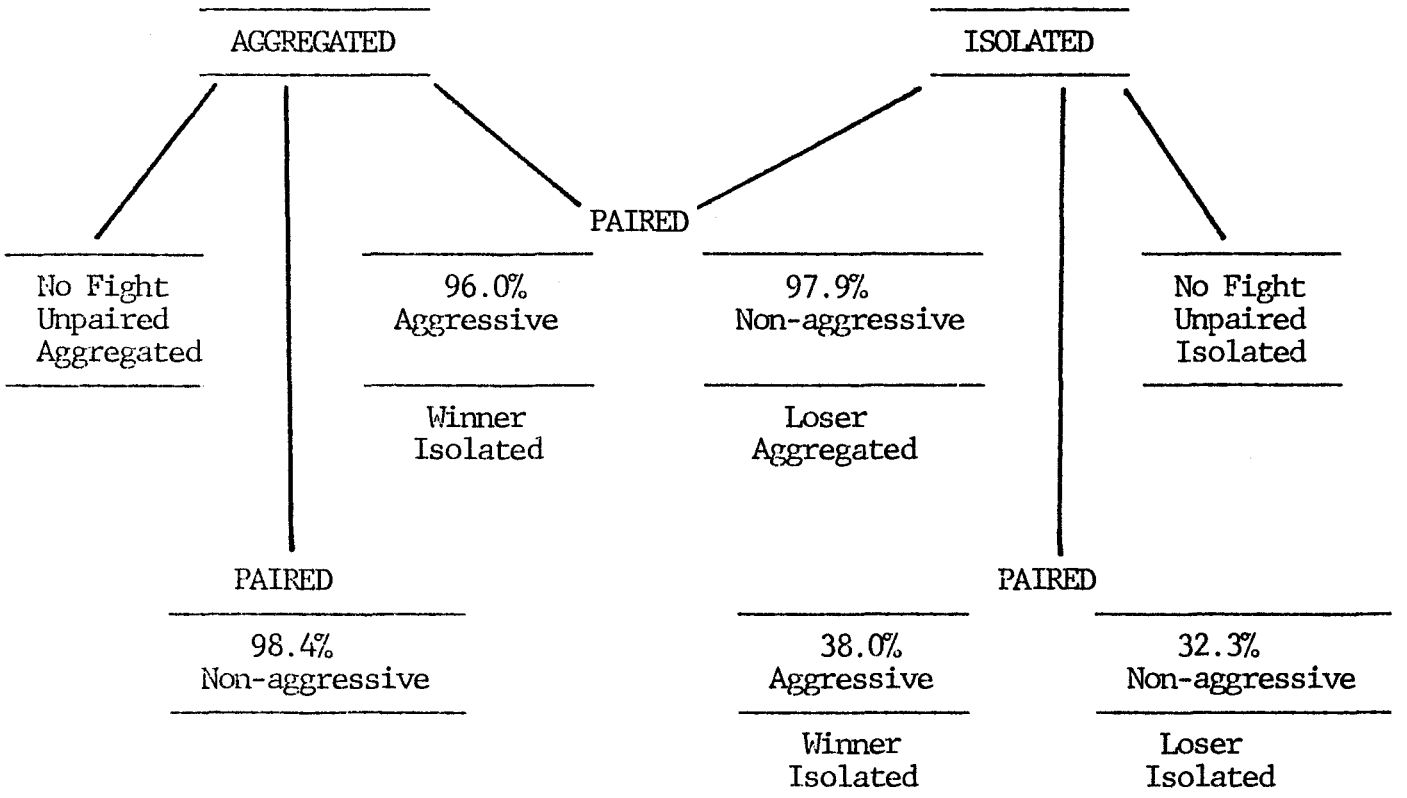
B. BEHAVIORAL DATA

A recap of the behavioral results have been illustrated in Figure 3. The behavioral data can be summarized as follows:

1) When aggregated mice were paired 98.4% were non-aggressive. These animals did not fight. 1.6% of the animals tested were not evaluated biochemically since they exhibited split behavior (cf. Methods).

2) When isolated mice were paired with aggregated mice, 96.0% of the isolated mice were winners, or aggressive. 97.9% of the aggregated mice were losers, or non-aggressive.

FIGURE 3


AGGREGATED
ISOLATED
PAIRED
No Fight
Unpaired
Aggregated
96.0%
Aggressive
Winner
Isolated
97.9%
Non-aggressive
Loser
Aggregated
No Fight
Unpaired
Isolated
PAIRED
98.4%
Non-aggressive
PAIRED
38.0%
Aggressive
Winner
Isolated
32.3%
Non-aggressive
Loser
Isolated

3) When isolated mice were paired, 38.0% were winners, or aggressive; 32.3% were losers, or non-aggressive; and 29.7% were neither winners or losers and therefore were not tested biochemically.

4) One group of aggregated mice were not paired: NO FIGHT; and one group of isolated mice were not paired: NO FIGHT. (Biochemical data were obtained from these two groups to determine what effect, if any, isolation had on choline and acetylcholine levels and acetylcholine turnover values.)

It is apparent from these data that aggregated mice did not fight when paired, whereas isolated mice fought. However, isolated mice were less aggressive when paired with aggressive animals. Highly aggressive mice have been known to signal a willingness to fight by displaying behaviors such as rearing onto hind legs and tail rattling (Micyek and O'Donnel, 1978). It is possible that these behaviors signal a readiness to fight to the opponent which may reduce the opponent's aggressive tendencies (Krysiak, 1976). On the other hand, the failure of an opponent to display these fighting cues while showing signs of timidity such as squealing and escape behavior (Krysiak, 1975), apparently encourage aggression and fighting attacks. These signs of timidity have been observed in aggregated mice when paired with isolated mice. The high degree of aggressivity observed from this pairing in isolated mice may result from timidity cues displayed by the aggregated opponent.

The high degree of aggressivity (96.0%) reported in this dissertation, with regard to isolated mice paired with aggregated mice, is in agreement with data reported by Cairns and Scholy (1973). However, Krysiak (1979), reported that only 40.0% of isolated mice exhibited aggressive behavior when paired with aggregated mice. This low percentage of aggressive animals may be due to the lack of inbreeding in the experimental animals. Randomely-bred male albino Swiss mice were used in Krysiak's experiments. The Mus musculus CF-1 strain used in my experiments were inbred for aggressive behavior (cf. Karczmar et al., 1973).

In conclusion, isolation has been shown to induce aggressive behavior in CF-1 male mice, but the degree of aggressivity is dependent upon the inherent aggressivity of the testing partner. Nearly all (96.0%) of the isolated mice were aggressive when paired with non-aggressive, aggregated partners. However, approximately one-third (38.0%) of the isolated mice were aggressive when paired with aggressive, isolated partners.

II. NEUROCHEMICAL RESULTS

A. ACETYLCHOLINE LEVELS AND ACETYLCHOLINE TURNOVER

1. General Turnover Concepts

The observation that acetylcholine in the central nervous system can be labeled with a radioactive precursor injected into an animal in the periphery contributed to the development of the isotopic approach to brain acetylcholine turnover. Such an approach was first described by Dross and Kewitz (1966), and was subsequently expanded by Schuberth et al. (1969, 1970).

According to Schuberth et al. (1969), tritium labeled Me-^{3}H-choline was injected into mice (100 µCi/mouse) and incorporation of radioactivity into brain acetylcholine was measured at various times following the injection. The data were expressed in terms of ^{3}H-acetylcholine as percent of ^{3}H-choline in the brain.

An important precondition in all turnover calculations is that steady-state concentrations of brain choline and acetylcholine are not perturbed during the experimental procedure. Early studies in whole brain of mice (Jenden et al., 1974), and guinea pigs (Haubrich et al., 1974), indicated that

steady-state levels of choline and acetylcholine in the brain do not change when animals are injected with a pulse injection of radiolabeled choline. More recent data (Cohen et al., 1969; Racagni et al., 1975), provide evidence that brain acetylcholine content increases when choline concentration ($\geq$500μM) in plasma are elevated. Thus intravenous administration of high levels of radiolabeled choline could induce artificial elevation of brain acetylcholine and consequently prevent steady-state choline and acetylcholine levels from being applicable in acetylcholine turnover calculations. Therefore, it is essential that steady-state conditions are maintained at very early times following the administration of the radiolabeled precursor.

Accordingly, it was necessary to demonstrate the existence of steady-state conditions after injection of 73.5 μCi ^{3}H-choline used for acetylcholine turnover (cf. Section IV, B., Review of Related Literature). Tables 2A-B (cf. Results) show choline and acetylcholine levels for aggressive and non-aggressive mice (isolated mice, paired) in the four brain areas studied following injection of 0.25 ml of 0.9% saline solution. Tables 3A-D (cf. Results) show choline and acetylcholine levels for aggressive and non-aggressive mice in the four brain areas studied which were determined 30 sec., 45 sec., 60 sec., 120 sec., and 240 sec., after injection of 0.25 ml of μM ^{3}H-choline. If steady-state conditions were perturbed (Zilnersmit, 1960; Hanin and Costa, 1976), choline

and acetylcholine levels for the mice injected with ^{3}H-choline (cf. Tables 3A-D), would differ from the choline and acetylcholine levels obtained from the mice injected with 0.9% saline solution (cf. Tables 2A-B). In comparing these two sets of data it is apparent that no significant differences in choline or acetylcholine levels exist. The steady-state has been preserved and there is reasonable evidence that the biochemical techniques used to obtain turnover data are sound.

It was also important to show that plots (specific activity versus time) of specific activities obtained from the five time points (described in the preceding paragraphs) resemble those from other investigators in this area (Jenden et al., 1974; Hanin and Costa, 1976).

Tables 4A-D show choline and acetylcholine specific activity values for aggressive and non-aggressive mice in the four brain areas studied for the 5-point analysis. Graphs 4A-D depict these specific activity values plotted versus time. These plots of specific activities resemble those of Jenden et al. (1974); Hanin and Costa, (1976); and Racagni et al. (1975). Choline specific activity is shown to increase exponentially before decreasing. Also, acetylcholine specific activity increases exponentially (albeit at a lower rate than choline specific activity), intersects the choline specific activity curve near its zenith, then diminishes slowly. It should be noted that the initial exponential increase in choline specific activity can be observed in the first 30 secs., following injection of tritiated choline. This initial expo-

nential increase, therefore, cannot be represented in most of the graphs since it was not possible to sacrifice the animals sooner than 30 sec after ^{3}H-choline injection. (Graph 4-A1 is the only exception where the 30 sec time point occurs during the exponential increase in choline specific activity.)

2. Acetylcholine Turnover in Aggression

To date, no literature has been reported involving brain part acetylcholine turnover evaluations in isolation-induced aggression mice. In fact, very few data in the existing literature have a bearing on this dissertation. However, some data are indirectly related to the work presented in this dissertation. For example, pharmacological manipulation of the striatum and cortex has been shown to produce regional differences in brain acetylcholine turnover values (Racagni et al., 1974). Also, it has been well documented that isolation-induced aggression in mice can be inhibited by anticholinergic drugs (Groblewski et al., 1973), and by septal lesions (Slotnick and McMullen, 1972). Placement of acute septal lesions in rats (Atweh and Kuhar, 1976), lowers hippocampal acetylcholine turnover. Septal-lesioned rats also exhibit increased shock-induced fighting (Eichelman and Thao, 1971). These contrasting behavioral effects suggest that different neural systems may be critical in the facilitation or inhibition of different aggressive paradigms in the rat and the mouse.

3. Acetylcholine Levels and Turnover Values and Interpretation of the Present Data

This section entails a discussion of choline and acetylchoine levels and acetylcholine turnover values obtained from the septum, striatum, thalamus, and hippocampus of unpaired, isolated mice; unpaired, aggregated mice; paired, aggregated mice; winners and losers resulting from an isolated versus aggregated pairing; and winners and losers resulting from an isolated versus isolated pairing.

It was necessary to determine what effects, if any, isolation had on endogenous choline and acetylcholine levels and acetylcholine turnover values prior to evaluating the effect of fighting and of its outcome (which yields winners and losers). When unpaired, aggregated (NO FIGHT) and unpaired, isolated (NO FIGHT) mice were compared with regard to the biochemical parameters, no significant differences were found. These findings are in agreement with the results of earlier investigations (Ruchesbusch and Brunet-Tallon, 1966; Garattini et al., 1969; Consolo and Valzelli, 1970; cf. Karczmar et al., 1973). (cf. Results, Section II.).

When paired, aggregated mice were compared to unpaired, aggregated and unpaired, isolated mice with respect to choline and acetylcholine levels and acetylcholine turnover values, the only significant difference was in the septal acetylcholine turnover value for paired, aggregated mice (32.8 nM/g/min; Table 7C). Even though the paired, aggre-

gated mice were paired for aggression testing after being housed in aggregate, they did not fight (98.4% were non-aggressive; Figure 3). Pairing these mice in the aggression test, therefore, could be said to lower septal acetylcholine turnover.

Major differences in biochemical data were observed when data from isolated mice paired with aggregated mice were compared to data from unpaired, isolated and unpaired, aggregated mice (see Tables 8A, 8B, 8C). WINNING (ISO-AGG) and LOSING (ISO-AGG) mice exhibited opposite effects with regard to septal, striatal, and hippocampal choline levels. Septal choline levels for WINNING (ISO-AGG) mice (48.9 nM/g) were significantly higher and septal choline levels for LOSING (ISO-AGG) mice (32.3 nM/g) were significantly lower than septal choline levels of unpaired, aggregated (38.8 nM/g); unpaired, isolated (43.2 nM/g); and paired, aggregated (38.8 nM/g) mice. However, hippocampal choline levels for WINNING (ISO-AGG) mice (16.6 nM/g) were significantly lower and hippocampal choline levels for LOSING (ISO-AGG) mice (30.3 nM/g) were significantly higher than hippocampal choline levels of unpaired, aggregated (22.4 nM/g); unpaired, isolated (22.3 nM/g); and paired, aggregated (23.3) mice.

Similar to hippocampal choline levels, striatal choline levels for WINNING (ISO-AGG) mice (25.5 nM/g) were significantly lower and striatal choline levels for LOSING (ISO-AGG) mice were significantly higher than striatal choline levels for unpaired, aggregated (29.9 nM/g) and paired, aggregated (30.9 nM/g) mice.

Thalamic choline levels for LOSING (ISO-AGG) mice (24.3 nM/g) were significantly lower than thalamic choline levels for unpaired, aggregated (30.6 nM/g); unpaired, isolated (30.8 nM/g) and paired, aggregated (30.4 nM/g) mice.

In summary, the major changes in choline levels for the four brain areas investigated were observed in LOSING (ISO-AGG) and, to a lesser extent, WINNING (ISO-AGG) mice.

With regard to acetylcholine levels, hippocampal acetylcholine levels for WINNING (ISO-AGG) mice (5.4 nM/g) were significantly lower and hippocampal acetylcholine levels for LOSING (ISO-AGG) mice (10.0 nM/g) were significantly higher than hippocampal acetylcholine levels for unpaired, aggregated (7.2 nM/g); unpaired, isolated (7.8 nM/g); and paired, aggregated (7.2 nM/g) mice. In this context, the major differences found in hippocampal acetylcholine levels parallels hippocampal choline level differences for WINNING (ISO-AGG) and LOSING (ISO-AGG) mice.

The same pattern also applies when acetylcholine turnover values for WINNING and LOSING mice are compared to acetylcholine turnover values for mice that did not have a fighting experience (see Table 8C). Hippocampal acetylcholine turnover values for WINNING (ISO-AGG) mice (15.9 nM/g/min) were significantly lower and hippocampal acetylcholine turnover values for LOSING (ISO-AGG) mice (31.8 nM/g/min) were significantly higher than hippocampal acetylcholine turnover values for unpaired, aggregated (23.3 nM/g/min);

unpaired, isolated (21.3 nM/g/min); and paired, aggregated (24.3 nM/g/min) mice. In this context, the data resembles comparative choline levels for WINNING and LOSING mice (see Table 8A); where WINNING mice exhibit higher septal and lower hippocampal levels, and LOSING mice exhibit lower septal and higher hippocampal choline levels when compared to unpaired, aggregated; unpaired, isolated; and paired, aggregated mice.

However, some of these results do not appear to be consistent. For example, striatal choline levels (see Table 8A) for LOSING (ISO-AGG) mice were significantly higher than striatal choline levels for unpaired, aggregated and unpaired, isolated mice; whereas, striatal acetylcholine turnover values (see Table 8C) for LOSING (ISO-AGG) mice did not differ significantly from striatal acetylcholine turnover values for unpaired, aggregated and unpaired, isolated mice. In this example, comparative changes in striatal choline levels were not parallel by similar comparative changes in striatal acetylcholine turnover values when LOSING (ISO-AGG) mice were compared to unpaired, aggregated and unpaired, isolated mice.

Similar patterns can be seen in WINNING and LOSING mice sacrificed immediately and 7-days subsequent to ^{3}H-choline injection (see Tables 9A, 9B, and 9C for intragroup comparisons). WINNING (ISO-ISO) mice show elevated septal and thalamic choline and acetylcholine turnover levels when compared to LOSING (ISO-ISO) mice. However, decreased striatal choline levels for WINNERS (immediate sacrifice; ISO-ISO)

is not paralleled by decreased striatal acetylcholine turnover values for WINNERS (immediate sacrifice; ISO-ISO). Also, increased striatal choline levels for LOSERS (immediate and 7-day sacrifice; ISO-ISO) is not paralleled by increased striatal acetylcholine turnover values for LOSERS (immediate and 7-day sacrifice; ISO-ISO).

Concerning the hippocampus, decreased hippocampal choline levels (immediate and 7-day sacrifice; ISO-ISO) for WINNERS is not paralleled by decreased acetylcholine turnover values for WINNING (immediate sacrifice; ISO-ISO) mice when compared to LOSERS (immediate sacrifice; ISO-ISO); however increased hippocampal choline levels for LOSERS (7-day sacrifice; ISO-ISO) are paralleled by increased hippocampal acetylcholine value for LOSERS (7-day sacrifice; ISO-ISO) when compared to WINNERS.

B. RECONCILIATION OF TURNOVER DATA WITH REGARD TO CHOLINERGIC AGONISTS AND ANTAGONISTS AND THEIR EFFECT ON BEHAVIOR

1. Effect of Cholinergic Agonists on Aggression

Indirect evidence may indicate that increased neuronal cholinergic activity can be associated with aggressive behavior in most species (Beleslin and Samardzic, 1979; Berntson et al., 1976). It may be proposed that cholinergic agonists that increase aggression (biting attacks) in cats do so by increasing neuronal activity (Berntson et al., 1976).

Similarly, direct stimulation of the thalamus by carbachol induces aggressive behavior in the rat (Bandler, 1970). Also, the intraventricular administration of carbachol (Beleslin and Samardzic, 1979) induces aggressive displays in the cat. Extrapolation of these data to the data presented in this dissertation can be formulated as follows. Aggressive mice (WINNERS) in present experiments exhibit acetylcholine turnover values in the septum and thalamus which are similar (not significantly different) to those of unpaired mice, while the septum and thalamus of non-aggressive (LOSERS) mice reveal significantly ($p < 0.05$) lower ACh turnover values when compared to aggressive (WINNERS) and unpaired mice (cf. Table 8C). The focus here is on the decreased cholinergic activity of the non-aggressive LOSERS which may be a reflection of the decreased ACh turnover.

The 'relative' differences in cholinergic activity is the important factor. In Bandler's experiments, it can be postulated that the aggressive behavior displayed by the animals receiving the cholinergic agonist, carbachol, is produced by the resultant increase in thalamic cholinergic activity. It may be speculated that these aggressive animals have a relatively higher level of cholinergic activity than the controls, which did not receive the carbachol pretreatment. Data presented in this dissertation reveal the same relative differences: septal and thalamic (cf. Table 8C) ACH turnover values which may reflect cholinergic activity levels, are relatively higher for aggressive (WINNING) animals when compared to non-aggressive (LOSING) animals. In

addition, septal acetylcholine turnover values for WINNING mice were significantly higher than septal acetylcholine turnover values for "control" mice: unpaired, aggregated and unpaired isolated. Elevated septal acetylcholine values for WINNING mice therefore, may reflect increased cholinergic activity in animals that have won a fighting session.

2. Effect of Cholinergic Antagonists on Aggression

It is well known that cholinergic antagonists suppress aggressive behavior. The drugs in question are muscarinic rather than nicotinic antagonists.

The structure activity relationship of anticholinergic drugs with regard to the ability to inhibit isolation-induced aggression in the mouse was investigated by Groblewski et al. (1973). Anticholinergic compounds have been listed in decreasing order of potency with regard to the ability to inhibit isolation-induced aggression; the sequence was as follows: scopolamine, atroscine, l-hyoscyamine, atropine, apoatropine, homatropine and eucatropine. Tertiary forms of these drugs were more efficacious than quaternary forms, presumably because the former but not the latter readily cross the blood-brain barrier (Malick and Barnett, 1975). Atropine and scopolamine have been shown to suppress isolation-induced aggression in rats (Yoshimura and Ueki, 1976). Furthermore, Barnett et al. (1970) antagonized isolation-induced aggression in mice by administering antihistamines possessing potent anticholinergic activities. It is evident

from this literature that anticholinergic drugs are potent inhibitors of aggressive behavior. However, there exists a paucity of biochemical data with regard to this paradigm.

The data presented for non-aggressive (LOSERS) mice may reflect inhibition of cholinergic activity, thus resembling the situation that occurs with anticholinergic treatment. Septal and thalamic acetylcholine turnover values were less than those of aggressive (WINNERS) mice. This decreased turnover could be interpreted as decreased cholinergic activity.

In conclusion, the data presented in this dissertation show that biochemical changes were produced by behavioral changes. Animals which were not paired and did not fight did not exhibit changes in ACh, Ch, or acetylcholine turnover levels as compared to paired animals, while profound neurochemical changes could be attributed to a fighting experience; these neurochemical changes were most evident in animals that were losers in the fighting experience. Clearly, decreased septal and thalamic ACh turnover values resulted from engaging in the fighting experience and losing.

SUMMARY

In general, only fighting animals exhibited major changes in the biochemical parameters. Of the fighting animals (ISO-AGG and ISO-ISO), the LOSING mice (ISO-AGG and ISO-ISO; see Tables 8A-C, 9A-C) exhibited most of the biochemical changes. It is apparent that the biochemical changes in regional choline, acetylcholine, and acetylcholine turnover values depicted here occur as a result of the experimental animals engaging in a fighting session and losing. Mice that did not fight (unpaired, aggregated; unpaired isolated; and paired, aggregated) did not exhibit significant biochemical changes in the four brain areas studied.

It has been postulated that various behavioral paradigms are linked to changes in choline transport and uptake mechanisms (Brugel and Rommelspacher, 1978). In fact, it has been suggested (Feighly and Hamilton, 1971; Haggbloom et al., 1974), that septo-hippocampal neurons exhibit increased high affinity choline uptake in the hippocampus during behavioral inhibition. Herein lies the most concrete evidence for a meaningful inter-

pretation of the data presented in this dissertation.

Mice that have been determined to be non-aggressive (LOSERS) when paired in the course of a fighting test consistently showed increased hippocampal choline levels and increased hippocampal acetylcholine turnover values (see Tables 8A, 8C, 9A, and 9C). If non-aggression can be interpreted as a form of behavioral inhibition, then the increased cholinergic activity seen in the hippocampus of non-aggressive LOSING mice (see Tables 8A, 8C, 9A, and 9C) may be a product of increased choline uptake in the hippocampus.

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APPROVAL SHEET

The dissertation submitted by Gary C. Magistrelli has been read and approved by the following committee:

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The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

11/24/1981 A. G. Karczmar
Date Director's Signature