THE EFFECTS OF ENVIRONMENTAL CONDITIONS AND BRAIN MICRO STRUCTURE ON AGONISTIC BEHAVIOUR DISPLAYED IN RATS SUBSEQUENT TO SEIZURE INDUCTION BY LITHIUM AND PILOCARPINE

by:
Dawn C. Desjardins

Submitted in partial fulfillment of the requirements
for the Degree of Master of Science in Biology

School of Graduate Studies
Laurentian University
Sudbury, Ontario
May, 1998

Acknowledgments

Throughout the duration of my Master's program, and my entire education up to this point, I have received a tremendous amount of support, encouragement and guidance. I would first like to acknowledge Dr. M.A. Persinger, my supervisor of this project and of my undergraduate thesis. Dr. M.A. Persinger introduced me to research and taught me the value of creativity, clarity of thought, and persistence. He also introduced me to a new realm of thinking, while still respecting me as an individual. In my opinion there are too few individuals like Dr. M.A. Persinger, who can truly instill the wonder and joy of research to those that are willing to learn.

I would also like to thank my committee members, Dr. G. Parker, Dr. R. Michel and Dr. S. Fairgrieve. Each of them has contributed new enlightening ideas and techniques which have molded my thesis into what it is today. I am truly grateful for the guidance and continued support from each of these skilled professors.

I would like to acknowledge my external examiner, Dr. K. Makarec, who has had a key role in the derivation of many of the techniques I have used in this thesis. Dr. K. Makarec provided many enlightening comments and challenging questions which broadened my outlook on this project and research in general.

There are also the Neuroscience research team and associates that I would like to thank. Many of these individuals have supported and challenged me throughout my undergraduate and master's degrees. This team has taught me the value of diversity in problem solving. A special thanks goes out to Linda St. Pierre who also assisted me in data collection.

Lastly I would like to acknowledge my family and friends who truly gave me the courage to attain my goals. Although all of these individuals played a role in my success, I will mention only a few. Diane Desjardins was my mother and continued mentor. She has always been and continues to be the source of my inspiration. My parents, Gordon Desjardins and Rosanna Desjardins are a never-ending source of love and support for everything I choose to do. Together they have made my goals attainable. Lastly I would like to thank Chantell McAloney who has been my guide and companion in research and in life. Thank you all!

Sincerely,

Dawn C. Desjardins

Table of Contents

List of Figures

Title **Page**

List of Tables

Title **Page**

CHAPTER 1

Introduction

Aggressive behaviour has been considered to be a fundamental biological property that allows the survival of the most adaptable and competitive members within a species (Lorenz, 1966). The intensity of various forms of aggression between males of a species frequently determines which individual will dominate the group, control the food, and impregnate the females. Consequently, aggressive or agonistic behavior strongly influences the survival of the individual and the genetic composition of the population.

Considering the importance of aggressive behaviour in the survival of the individual and specific groups within a species, one would expect these behaviours to be mediated by several structures within the brain. In fact, one would expect a neuromatrix or functional system of structures within the limbic system, diencephalon, subcortical telencephalon, and even the cerebral cortex that would serve as the anatomical substrate for this type of behaviour. Single lesions within one of these structures would not necessarily affect the entire system.

Excitotoxic death of neurons due to self-stimulation of intrinsic or endogenous pathways results in necrosis within groups of neurons that are functionally related. By comparing the quantitative changes in the different types of agonistic behaviours with the quantitative changes in brain microstructure, a more naturalistic relationship between structure and function might be achieved. This thesis was designed to examine the social

factors, neuroanatomical pathways, and potential reversible treatments of aggressive behaviour that emerged after an autogenic brain injury induced by seizures following a single systemic injection of lithium and pilocarpine. The purpose of this thesis was not to focus on epilepsy, but to focus on the histological result of brain damage from lithium/pilocarpine-induced seizures as a means of understanding the biological significance of the consequential aggression in male rodents.

Literature Cited

Brain Injury from Induction of Seizures by Systemic Lithium and Pilocarpine

Epilepsy is the product of excessive electrical activity within the brain which may be the result of such conditions as, a head injury, a brain tumor, a vascular problem and/or genetic predisposition. Epilepsy is expressed as episodic reoccurrences of paroxysmal neurological and behaviourial manifestations that are accompanied by neuropathological changes which consequently alter the life of the epileptic. In order to study both the behavioural and neuropathological consequences that occur from this condition, a model that produces chronic recurrent epilepsy is advantageous.

Honchar *et al.* (1983) developed a procedure that evoked seizure activity resulting in damage to brain tissue within functionally related systems of the diencephalon and telencephalon. A combination of two drugs were used for this purpose: lithium (an anti-manic agent) and pilocarpine (a muscarinic agonist). If 3 mEq/kg of lithium chloride was injected subcutaneously 24 hours (hr) before the systemic injection of 30 mg/kg of

pilocarpine, the rat would display a sequence of head nods, then myoclonic jerks and finally rearing, rapid forelimb movement (paddling), and falling. Without the antecedent injection of lithium approximately 300 mg/kg of pilocarpine was required to produce these same effects (Turski *et al.*, 1989).

Pilocarpine affects the receptors that sequester acetylcholine (ACh). These ACh receptors constitute the cholinergic system of the central nervous system. Cholinergic fibers in rats have been found primarily in the cerebral cortex, thalamus, interpeduncular nucleus of the midbrain, the basal ganglia, and the limbic system (Mendelsohn & Paxinos, 1991). The regions in the limbic system receiving cholinergic input include the primary olfactory cortex and bulb, septum, hippocampus, amygdala, entorhinal-pyriform cortex, insula, orbitofrontal and temporal lobes (Cooper *et al.*, 1991; Mendelsohn & Paxinos, 1991).

Pilocarpine acts directly on postsynaptic muscarinic receptors and evokes responses similar to those of low doses of ACh or anti-AChE (acetylcholinesterase) agents (Honchar *et al.*, 1983). Olney *et al.* (1983) found that injection of pilocarpine directly into the basolateral amygdala of the rat induced seizure activity, which in turn produced edema and acute necrosis in several brain regions.

Lithium is widely used in the treatment of bipolar depressive disorders. Initially it inhibits adenyl cyclase activity by interfering with the enzyme's magnesium binding sites (Seigel *et al.*, 1994). Goodman and Gilman (1980) have reported that lithium increases 1) permeability of the blood brain barrier, 2) choline uptake (causing a greater production of acetylcholine), and 3) the uptake of noradrenaline. Lithium has been reported to reduce

seizure thresholds in those individuals susceptible to temporal lobe seizure (Honchar *et al.*, 1983).

Both Honchar *et al.* (1983), and Persinger *et al.*, (1993) have found high mortality rates when seizures were evoked by the systemic injection of lithium and pilocarpine. These rates ranged from 50-70% within 72 hours of pilocarpine injection. Persinger *et al.* (1993) found that the administration of 30 mg/kg of acepromazine, neuroleptic, could diminish mortality rate to less than 20%. Such reduction of mortality reduced the confounding problems such as skewed results or improper sample size that result when studying the relatively few surviving rats. Because more animals survived, the sample size was also sufficient to systematically evaluate the behavioral consequences of the correlative, seizure-induced brain injury (Persinger *et al.*, 1993).

According to results from Persinger *et al.* (1993), the rats did not recover immediately from the impact of the induction of seizures. General recovery, defined as a return to normal eating and grooming, took approximately 7 to 10 days. During the first 24 hours, the animals were mainly comatose and hypothermic. There was also substantial weight loss (15 -20%) for up to 48 hours following the induction of seizures even though overt manifestations, such as myoclonic jerks, were not evident. By the second day, the subjects were able to eat food dissolved in water, and by days 5 to 7 they began to eat solid food and drink from a water bottle.

Depending upon the time since the induction of the seizures, different cytopathologies emerged. Persinger *et al.* (1993) reported a progressive necrosis reflecting a specific pattern of damage that occurred and progressed with time since

seizure induction. This progression was also evident within the lateral ventricles. A positive correlation was reported between the enlargement of the lateral ventricles (ventricle area divided by the area of coronal section) and the postseizure time (Persinger, 1993). Other cytopathologies observed by Persinger *et al.* (1993) included massive swelling of neuronal structures, degeneration of neuronal cell bodies, neuronal tissue loss or cystic lesions (in animals that survived longer), reactive gliosis, and the appearance of crystalline-like, densely stained material (containing calcium) within thalamic structures. The most frequently and severely damaged structures were the entorhinal and pyriform cortices, the substantia nigra (reticulata), the dorsomedial thalamic nuclear group, and the posterior thalamic group.

There was also damage within the amygdala. The areas of the amygdala that displayed the most damage included the anterior group, the medial group, and the basolateral group, respectively. The central amygdala displayed no qualitative damage, at least within the limits of light microscopy (400 X) (Persinger *et al*, 1993).

Persinger (1991) suggested that the delay in the increased intermale aggression obeserved by these rats (commencing at 7 to 10 days subsequent to seizure induction and reaching an asymptote at approximately 20 days) was compatible with the spread of damage within the amygdala and could not be exclusively attributed to the weakness or incapacitation associated with the initial induction of the seizure.

Brain Damage by Lithium/Pilocarpine Seizures

Characteristic brain damage from seizure in rats is induced by the combination of lithium followed by pilocarpine as previously described. The syndromes produced by both lithium and pilocarpine together and by pilocarpine alone are metabolically, and neuropathologically indistinguishable (Clifford, 1987). However, the syndrome is produced more reliably and with less mortality by the combination of lithium and pilocarpine (Clifford, 1987). It has been suggested that lithium may act by interfering with the metabolism of the second messengers called phosphoinositides which play an important role in synaptic function. Berridge *et al.* (1982) stated that lithium alters the metabolism of the phosphoinositides by inhibiting the conversion of *myo*-inositol 1-phos-phatase into *myo*-inositol by the enzyme *myo*-inositol 1-phosphatase. Hydrolyses of the phosphoinositides have been found to be dependant on calcium (Rhee *et al.*, 1989), and has been found to play an integral role in synaptic function of muscarinic receptors. Berridge *et al.*, 1982 suggested that lithium alters the activity of muscarinic receptors which allow for the subsequent lowering of seizure thresholds to pilocarpine administration.

Pilocarpine is a muscarinic cholinergic agonist which exacerbates cholinergic effects on hippocampal neurons (Liu *et al.*, 1994). Studies performed by Liu *et al.* (1994) have reported that acetylcholine induces a slow membrane depolarization and a simultaneous increase in membrane resistance of hippocampal pyramidal neurons which are associated with an increase in the frequency of burst discharge. These researchers hypothesized that pilocarpine induces a muscarinic excitatory effect on hippocampal pyramidal neurons resulting in the prolonged sustained seizure activity that characterizes

the acute phase of the pilocarpine effect. Researchers have found the subsequent brain damage could not be attributed to pilocarpine but was more likely due to an excitotoxic effect mediated by glutamate (Clifford *et al.*, 1987; Jope *et al.*,1992; Liu *et al.*, 1994).

Glutamate is a major excitatory amino acid neurotransmitter in the mammalian central nervous system (Siegel *et al.*, 1994). It mainly reacts with NMDA receptors (Siegel *et al.*, 1994). These researchers stated that excessive amounts of glutamate release contributes to neuronal injury, through over activation of NMDA receptors, which is termed excitotoxicity. Siegel *et al.* (1994) listed two distinct events in glutamate-induced neuronal injury. First, exposure of neurons to glutamate may have caused an acute neuronal swelling resulting from the depolarization-mediated influx of sodium, chloride and water. This process was reversible if glutamate is removed from the system. The degree to which this event contributed to neuronal injury was unclear, but it has been suggested that water entry causes osmotic lysis, which may have disrupted neuronal function. The second event was characterized by excessive calcium influx primarily via excitatory amino acid receptor channel activation. The elevation of intracellular calcium has been known to activate lipases, phospholipases, proteases and protein kinases, each of which, if not properly regulated, could have produced considerable cellular damage as observed in the lithium and pilocarpine seizure model. According to Siegel *et al.*, (1994) glutamate neurotoxicity has three stages: first, overstimulation of glutamate receptors, leading to a set of immediate cellular derangements; second, amplification where the intensity of these derangements is increased and the involvement of other neurons; and third, expression, the destruction cascade directly responsible for neuronal cell death.

Agonistic Behaviour

Agonistic behaviour has been a topic of interest throughout the twentieth century. Research has explored many facets of this subject: conspecific aggression characteristics, isolation, dominant and submissive behaviour, intruder aggression, pain induced aggression, anxiety and aggression, and techniques in neuroscience on aggression. The following paragraphs include a brief outline of previous research on agonistic behaviour.

Conspecific Aggression Characteristics

Rodents exhibited three stages of agonistic behaviour according to Scott and Fredericson (1951): preliminary behaviour, agonistic and defensive behaviour(displayed by opponents), and escape behaviour. Preliminary behaviour included piloerection, which gave the animal a false expanded look. Agonistic behaviour consisted of biting and boxing. Biting was defined by Desjardins and Persinger (1995) as dental contact which produced squeaking and tissue damage. Boxing involved two animals standing on their haunches facing each other (usually with their mouths open and their front paws out in front). Another agonistic behaviour was chasing, in which one animal ran away while another chased and then struck and bit with the teeth (Scott & Fredericson, 1951). Another agonistic behaviour was termed offensive sideways movements; these movements were described as the attacker displaying lateral movements that allowed the agonist access to the defensive rat's back (Blanchard & Blanchard, 1981).

Defensive and escape behaviours are often similar to agonistic responses. These behaviours were described by Scott and Fredericson in 1951 as subjects squeaking when

attacked, and, if given the opportunity, running and hiding. If cornered, the rat would rear up in a boxing stance and remain motionless until attacked. This boxing could be classed as both aggressive and defensive in rats, because both parties boxed during agonistic behaviour. In some instances a rat would lay on his back exposing vital areas in an extremely submissive posture. Blanchard and Blanchard (1981) suggested that this vulnerable position blocked preferred attack sites from an aggressor. These preferred attack sites were all located on the dorsal surface but were localized to different regions depending on the experimental conditions. A dominant colony male would attack an intruder anywhere on the dorsal surface while the intruder would respond by localizing his attack to the head of his opponent (Blanchard & Blanchard, 1981). If the agonistic behaviour was a result of pain from shock, both dominant and intruder subjects would restrict their attack to the head region (Blanchard & Blanchard, 1981).

Vocalizations also accompany agonistic behaviour in rodents. Rats emit pure tone ultrasonic vocalizations between 20 to 200 kHz during agonistic encounters (Vivian & Meczek, 1993). Vivian and Meczek (1993) conducted studies in which they devocalized some subjects and deafened others. These researchers concluded that the ultrasonic vocalizations do not alter aggressive behaviour suggesting that other components of social interaction may be involved in the intitiation and perseverance of agonistic episodes.

Pellis *et al.* (1996) have studied the uses of vision by rats in social interactions such as play fighting. These researchers discovered that sightless rats would still take part in play fighting. However, the playful activity was atypical. The enucleated rats initiated more play than the rats that could see (Pellis *et al.*, 1996). These researchers concluded

that vision was not used in the organization of play fighting. During this experiment the vibrissae were cut to decrease the effects of tactile information on the observed behaviour. Tactile sense could provide valuable information to the rat during attack, but offensive and defensive behaviour was still occurring in rats that had local anesthetic injected into skin of preferred attack sites (Pellis & Pellis, 1987) and rats that had their vibrissae cut (Pellis *et al.*, 1996).

The only sensory system that, upon removal, eliminates agonistic behaviour in rodents is olfaction (Ropartz, 1968; Rowe & Edwards, 1971; Edwards *et al.*, 1992). These researchers have demonstrated that bilateral removal of the olfactory bulbs eliminated intermale fighting in rodents. These results suggested that olfaction was imperative for the organization of agonistic behaviour in rodents.

In all instances of agonistic encounters in rats, both biting and boxing are consistent and could be used as a valid measure of the frequency of agonistic encounters. The involvement of the sensory systems in the organization and propagation of agonistic encounters should be considered upon analyzing neural correlates of aggression.

Isolation

A number of studies found that prolonged rearing in social isolation resulted in increased aggressiveness in various species, eg., in fish (Kuo, 1967; Archer, 1988); quail (Kuo, 1960; Archer, 1988); Asian song thrushes, kogs (Kuo, 1967; Archer, 1988); and rhesus monkeys (Mason, 1960, 1963; Harlow *et al.*, 1965; Archer, 1988). Aggression in adult male rodents increased following an absence of peer-group contact between birth

and weaning (Denenberg, 1973; Archer, 1988). These results suggested that social interactions between male litter mates exerted an inhibitory effect on later aggressive behaviour (Archer, 1988). Namikas and Wehmer (1978) compared males which had been reared with male or female litter mates and found that those reared with females were more aggressive in young adulthood, again suggesting an inhibiting effect of social interactions between male litter mates (Archer, 1988). Several researcher have suggested that rats learned to restrain their attacks on other males as a result of punishment and the development of fighting strategies (Taylor, 1980; Bekoff, 1981; Archer, 1988).

Scott and Fredericson (1951) also studied the concept of learned inhibition between rodents that were raised together. This inhibiting effect was relative to the social context of rearing conditions. For example, these researchers cited several studies that investigated the effects of cross-fostering in rodents from non-aggressive to aggressive strains of rodents. The results indicated a strain-dependent influence on adult aggression (Denenberg, 1973; Archer, 1988; Scott & Fredericson, 1951). Non-aggressive strains of rodents reared with an aggressive strain exhibited increased adult aggression, and the reverse was also found (Denenberg, 1973; Archer, 1988; Scott and Fredericson, 1951).

Scott and Fredericson (1951) hypothesized that young rodents which were raised together, and which did not fight up to 30 days of age, formed a strong habit of not fighting after having been placed together. This characteristic was sufficient to overcome stimulation which under other conditions, would normally produce aggression. Individuals singly-housed did not have the opportunity to experience learned inhibition and were therefore more aggressive towards conspecific males (Scott and Fredericson, 1951).

Brain (1975) examined both the physiology and the neurochemistry of isolated rodents, and found that isolated rodents have heavier testicles and prostate glands than grouped rodents (suggesting a higher androgen production), reduced serotonin and epinephrine turnover, and reduced free fatty acids (indicating higher adrenal activity). Welch and Welch (1971) concluded that the tendency to fight was diminished by impairing the biosynthesis of neural catecholamines and serotonin, which may have related the changes in both serotonin and norepinephrine to the reported isolation effects on aggressiveness. Brain (1975) stated that neural changes in serotonin may exert profound influences on the functioning of the rodent pituitary-adrenocortical axis, indicating a relationship between these changes in putative neurotransmitter concentrations and the production of glucocorticoid. This literature suggested that the increased frequency of agonistic behaviour found in isolated individuals was due to both behavioral and physiological changes, consequential to early rearing conditions.

Dominant and Submissive Behaviour

Examination of dominant and submissive behaviour indicated that early aggressive experience played an important role in predicting future behavioral responses to conflict (Archer, 1988). Dominance was considered as a description of behaviours that occurred within the individual that predicted the likelihood of winning a conspecific aggressive encounter (Archer, 1988). Dominance has been deemed as the result of learning and previous experience (Archer, 1988; Scott & Fredericson, 1951). According to Blanchard and Blanchard (1981) dominant male rats showed characteristic features during

aggression. If an intruder was introduced, initially the dominant rat approached and sniffed the stranger; once the rat had perceived the odor, piloerection occurred, and this was shortly followed by biting and chasing (Blanchard & Blanchard, 1981).

Lorenz (1966) stated that dominant rats benefitted by facing limited opposition when acquiring such resources as food and mates. The cost to the dominant male, because he is typically the first to respond to intruders, included risk from intruder and predator attack (Lorenz, 1966). The energy spent defending status, particularly from closely matched subordinates, was also considered to be a high cost (Blanchard & Blanchard, 1990).

A submissive individual has been typically classed as any subject in a close social environment that is not the dominant. Submissive behaviour in rats was described by Blanchard and Blanchard (1990) as retreat, rearing on hind legs, and rolling over and exposing the ventral surface (which was not normally attacked), thus reducing access to preferred biting sites. Typically, the subordinate engaged in escape or defensive behaviour during aggressive encounters (Scott & Fredericson, 1951).

Focus has traditionally been placed on the advantages of dominance in social settings. However, Blanchard and Blanchard (1990) have described the advantages of subordinates in social settings as well. Because a dominant male would spend copious amounts of time defending territory, subordinates could access available resources (Blanchard & Blanchard, 1990). From a theoretical point of view it was assumed that the dominant/submissive relationship was caused by simple conditioning (Ginsburg & Allee, 1942). The dominant animal formed the habit of attack and the subordinate animal formed

the habit of escape or defense (Ginsburg & Allee, 1942).

Uhrich (1940) and Ginsburg and Allee (1942) indicated that previous success reinforced the dominant condition, whereas previous defeat produced a subordinate animal (Scott & Fredericson, 1951). They also found that these roles could be reversed. A succession of bad defeats caused a dominant animal to become subordinate, and a subordinate animal could be trained to be dominant through the introduction of successive easy victories (Scott & Fredericson, 1951). Hence a researcher could train subjects in order to examine their reinforcement of fighting strategies.

Using this training technique, Scott and Fredericson (1951) determined that once a fight was started, the two animals reinforced each other's fighting by mutual injury, until one became badly hurt and responded with escape behaviour. The victor kept attacking, thereby reinforcing escape behaviour with further injury. When this situation was repeated, reinforcement of fighting occurred in one animal, who became dominant, and reinforcement of escape or defensive posture occurred in the other, who became subordinate (Scott & Fredericson, 1951). The consequences of the defeat were very effective, such that generalization to other opponents occurred. This generalization was tested by Ginsburg and Allee (1942). They subjected albino mice to several defeats twice a day for 8 successive days by an aggressive black mouse strain. The albino mice assumed the submissive position following defeat, however, once matched with non-aggressive black mice of a different strain, the albino mice continued to display submissive behaviour despite the lack of potential harm.

Social status has been demonstrated not only in the behaviours but in the

physiology of rodents as well. Rodents which have been defeated displayed chronic elevations of adrenocorticotrophic hormone, which predisposed the animal towards showing lower levels of aggressiveness and demonstrating a potentiality for subordinate behaviour (Welch and Welch, 1971; Brain, 1972, 1975). Typically, once exposed to a subsequent opponent the defeated subjects assumed a submissive posture (Welch & Welch, 1971). Brain (1975) also reported that circulating androgens were elevated in dominant or isolated rodents indicating that the aggressiveness of these animals may be higher than that of defeated mice or the majority of group-housed animals, due to the stimulatory effects of these hormones on agonistic behaviour.

Intruder Aggression

The introduction of an unfamiliar male into an established social group was one method by which researchers could observe aggression in both social and isolated housing conditions. Lorenz (1963) stated that rats are social animals which recognize members of their social family by smell alone. If a rat intruded on an unfamiliar social family, the family would not react to the intruder until the intruder's smell was recognized as foreign. At this point the family members would attack the intruder until it retreated or until it was killed. During the attack, family members would mistakenly attack one another, until they recognized odor-similarities (Lorenz, 1963).

Blanchard and Blanchard (1990) reported that intruders were attacked by group males only, and usually by the dominant male of the group first. If the intruder's odor was masked by an artificial scent, the intensity of fighting was drastically reduced (Denenberg,

1973; Connor, 1972, Guillet and Chapouthier, 1996). Hurst *et al.* (1994) mentioned that urinary odors have a strong inherited component that reduced differences in family member odors. This component allowed for a larger discrepancy and for easier recognition between related and non-related individuals.

Fanselow (1985) stated that rats released an innately recognized odor in response to a variety of natural and unnatural aversive events. Valenta and Rigby (1968) discovered that in response to aversive electric shocks, rats released odors that conspecifics could discriminate from the odors of unstressed rats. Odor from dominant male urine provoked a higher frequency of attacks than that from non-aggressive subordinates (Hurst *et al.*, 1993). This increased frequency of attack may have been due to the fact that dominant males marked their territories by urine more frequently than did subordinates (Hurst *et* al., 1993), and there was a difference in the quality of the urine based on status that was detected by other males (Jones & Nowell, 1973; Hurst *et al.*, 1993).

Lorenz (1963) found that a group member who had been removed and masked with non-group member odor was attacked upon being returned to the original group. The masked member did not react as an intruder does with flight and defense on first encountering the group, because it was assumed that the subject recognized the group smell as his own. However, the group members attacked and sometimes killed the masked individual as they would have done to an intruder (Lorenz, 1963).

Pain-Induced Aggression

Pain-induced aggression has typically included observations of aggressive encounters following foot or tail shock. Brain (1981) argued that shock-induced aggression showed a more offensive form of agonistic behaviour. Blanchard and Blanchard (1981) found opposite effects. The latter researchers claimed that shock-induced aggression was not offensive but defensive in form. These observational differences may have been due to differences in intensities of the shock, or in the rat's physiological state , such as anxiety levels which have been found to reduce pain thresholds, at the time of the shock treatment (Archer, 1988). Scott and Fredericson (1951) viewed pain-induced aggression in the natural habitat, and suggested that slight pain may have been a primary stimulus that evoked fighting, whereas intense pain evoked defense and escape. They noted that once a fight was started, the two animals reinforced each other's fighting through injury, until one experienced intense pain and tried to escape while the other, who had experienced slight pain, continued to attack. Pain-induced aggression could be tested and observed under conditions of group or isolated housings, as well as in plentiful or deprived resource (i.e. food, territory, mate) conditions.

Anxiety and Aggression

Areas of the brain reported to comprise the main neural substrate of fear or anxiety have included the amygdala, the hypothalamus, and the midbrain pariaqueductal grey (Beckett & Marsden, 1995). These researchers also found that electrical stimulation of

these nuclei elicited a defense response. This defense response was defined as an escape component which was supported by cardiovascular and analgesic changes. Cardiovascular changes such as fluctuations in heart rate or blood pressure can provide indirect information on activity of the autonomic nervous system, which plays a major role in the response to stress (Sgoifo *et al.*, 1994). Sgoifo *et al.* (1994) found that rats displayed sinus tachycardia during aversive social conditions such as conspecific attack. Studies performed by Griebel *et al.* (1996) suggested that the frequency of biting was a result of both increased anxiety and defense response. These researchers also discovered that compounds effective against generalized anxiety and panic attacks significantly attenuated the frequency of biting. The benzodiazepine receptor has been implicated in this effect and has been shown to play an important role in anxiety, stress, seizures, and sleep (Haefely, 1990; Weerts *et al.*, 1993). Benzodiazepines reduced both defensive and escape oriented behaviours that were the result of pain (foot shock) and/or isolation (Cole & Wolf, 1966; Krsiak & Sulcova, 1990; Weerts *et al.*, 1993). These attenuation effects of benzodiazepines on aggression were dose and drug dependent suggesting a threshold and specific receptor subtype response within associated brain nuclei.

Techniques in Neuroscience Research on Aggression

Commonly, neuroscientists electrically stimulate an area of the brain in order to observe the behavioral changes that ensue. In examining aggression, researchers have stimulated various regions of the limbic system, particularly the amygdala and the hypothalamus, in order to observe an increase or reduction in aggression in prepared

environmental settings. The details of various findings using this method are described in the next segment on neural correlates of aggression.

Some researchers have also studied the metabolism of radioactively labeled glucose in order to determine which neural centers were most active during behavioral displays. Radiolabelled 18-F-2-deoxyglucose (FDG) can be used in order to perform positron emission tomography. The FDG is taken in by the cells more metabolically active during agonistic episodes, which are displayed in an image available for analysis by the researcher.

Pharmacologically, researchers have administered local infusions of medications in order to examine their effects on agonistic behaviour. Local drug infusions have been administered through stereotaxic procedures, which allow for accurate placement. The benefits of this procedure include the penetration of the blood brain barrier to allow passage of drugs which would ordinarily be excluded and determination of the functional role of the specified medication in specific nuclei within various regions of the brain (Myers, 1966). Myers (1966) conducted several studies to determine the volume of medication to be used in micro injection protocols, so as not to produce lesions or diffusion of the drug along the entire cannula track. The recommended volume was in the order of 0.5 micro liters (Myers, 1966). One micro liter occupied a spherical area having a diameter of 1.1 - 1.9 mm which, if placed accurately, would not affect neighboring nuclei (Myers, 1966).

Medication which would reduce aggression has often been used clinically in the human population to avoid social conflict. Typically these medications have included

serotonin uptake inhibitors, benzodiazepines, anti-epileptic and antipsychotic drugs. The type of drug administered has been dependent upon other symptoms displayed by the patient that coincided with the aggressive episodes. Further research is needed in order to assist these individuals with uncontrolled rage, in order to maintain proper social functioning.

Upon studying conspecific aggression, careful consideration of the technique used to induce agonistic encounters must be taken. Agonistic behaviours, as described earlier, have been induced by seizure induction of lithium and pilocarpine, pain, anxiety, isolation, and the introduction of an intruder. This thesis utilized the lithium and pilocarpine seizure induction method to not only study the resultant agonistic behaviour, but to further define neural correlates of the resultant behaviour.

Neural Correlates of Aggression

The diversity of aggression research has revealed that there is no real "aggression center" within the brain, but instead a network of neuronal connections beginning in the limbic system and travelling to other areas which integrates and organizes aggressive behaviour (Restak, 1984). Areas typically examined for aggression include portions of the amygdala and hypothalamus. Other possibilities include portions of the thalamus, limbic system, and telencephalon. Earlier studies have utilized electrical stimulation techniques within these regions to observe induced behavioral changes. More recently, pharmacological agents have been administered through microinjections and/or cannula placement to observe behaviour at the level of receptor subtypes. Both the amygdala and

the hypothalamus are composed of various nuclei, some of which are implicated in aggression. The following paragraphs discuss the results of various studies aimed at elucidating the neural correlates of aggression.

According to Krettek and Price (1978), the amygdala holds a central position in a variety of limbic circuits that have been implicated in aggression and defense. These researchers noted that the amygdala has mono-synaptic projections to the ventral medial hypothalamus, to the bed nucleus of the stria terminalis, and to the ventral hippocampus. These projection sites have also been implicated in aggression. Accordingly Krettek and Price (1978) stated that individual differences in the defensive responses to natural threats arose from the combination of degree of amygdala response to complex threat, and the distribution of that response to the various efferent targets of the amygdala.

The amygdala is made up of various nuclei which have been implicated in various functions other than aggression, such as heart regulation, emotionality, olfaction, hormone regulation, defense reaction, learning, territoriality, rage, sleep, awakening, neurotransmitter phenomena, sexual behaviour, and ovulation (Restak, 1984). The multitude of behavioral involvement invested in the amygdala created difficulty in pinpointing source and/or mechanism in any one of the aforementioned functions.

Vochteloo and Koolhaas (1987) used discrete electrolytic lesions within the specific nuclei of the amygdala and concluded that the medial amygdaloid nucleus was involved in social behaviour including intraspecific aggression, avoidance of a dominant, and sexual behaviour. Koolhaas *et al.* (1990) built on these findings by investigating efferents of this area. These researchers concluded that the medial amygdala received

major afferents from the accessory olfactory bulb, which is involved in processing species-specific olfactory signals from the vomeronasal organ. These researchers also stated that the medial amygdala was a source of efferent projections to the ventrolateral aspects of the ventromedial hypothalamic nucleus, an area which other researchers (Kruk *et al.*, 1984) reported to be involved in aggression. Other areas of the amygdala that were reported to be involved in aggression included both the basolateral complex and the central amygdala. Lesion studies performed by Oakes and Coover (1997) led them to conclude that both the basolateral and central amygdala were involved in fear, defensive, and offensive behaviour. Davis *et al.* (1994) stated that the projections to the central amygdala were the major intra-amygdaloid target of the basolateral complex, which was critical for autonomic and somatic responses produced by stimuli that were previously paired with aversive events. Fox and Sorenson (1994) also agreed that the amygdala was involved with fear responses and have cited literature describing the behavioral effects of bilateral central amygdaloid lesions. The resultant behaviour included freezing in open areas (Grijalva *et al.*, 1990) or in response to natural predators (Blanchard & Blanchard, 1972), heart rate changes (Kapp *et al.*, 1982), and potentiated startle (Hitchcock & Davis, 1986). Also utilizing lesion techniques, Fox and Sorenson (1994) attributed a function of the central and basolateral amygdala to be the expression of analgesia in response to diverse environmental challenges. The main areas of the amygdala involved in aggression included the medial, basolateral, and central portions.

In lesion studies often other nuclei, besides the nucleus of interest, are also damaged in the process resulting in behavioural changes non-related to the nucleus in

question. Careful consideration of the neurochemistry can confirm and further strengthen lesion study findings.

Davis *et al.* (1992) stated that the central nucleus contained GABA as the main neurotransmitter, along with a variety of neuropeptides, and that the basolateral nucleus also contained copious amounts of GABA and neuropeptides, along with choline acetyltransferase. These researchers have also observed dopaminergic innervation of the central nucleus, and direct cortical input to the central GABA neurons, which in turn projected to the medial central nucleus and the brainstem. These projections to the brainstem were implicated in the initiation of the autonomic and somatic components of the fear reaction. In order to study the role of GABA in the central and basolateral nuclei, Davis *et al.* (1992) locally infused benzodiazepine, which facilitated GABA transmission. This treatment resulted in evidence that the anticonflict effects of benzodiazepines, in general, occurred after local infusion into the basolateral nuclei, and not the central nuclei. These researchers concluded that the output neurons of the central nucleus to brainstem nuclei, known to be involved in the autonomic and somatic aspects of conditioned fear, were tightly regulated by GABA and other inhibitory transmitters, the disruption of which greatly amplified fear and stress.

Desjardins and Persinger (1995) found the number of neurons in the central medial nucleus of the amygdala was correlated ($r=.92$) with the number of aggressive bite attacks for male Wistar rats. This study utilized the lithium pilocarpine epilepsy model, which induced damage to all amygdaloid nuclei except the central (Persinger *et al.*, 1993). Bedard and Persinger (1995) suggested that the inhibitory intra-amygdaloid afferents to

the central amygdala were damaged by the epileptic seizures, thereby disinhibiting the central amygdala. The consequence to this pharmacologically based procedure was an increase in conspecific biting frequency, which corresponded to the neuron number within the central amygdala (Desjardins and Persinger, 1995).

The hypothalamus has been found to have a fundamental role in agonistic behaviour. According to Parent (1996), the hypothalamus is involved in the coordination of the autonomic nervous system, the regulation of body temperature, the maintenance of water balance, the control of the anterior pituitary, the control of reproductive functions, the control of growth, the control of food intake, the control of emotional behaviour, and sleep. Parent attributed the hypothalamic involvement in the control of emotional behaviours to its involvement in the coordination of the autonomic nervous system. All emotions had an autonomic basis. Aggression was found during sympathetic stimulation, which was controlled by the lateral and posterior hypothalamic regions (Parent, 1996). Stimulation of the lateral and posterior hypothalamus induced sympathetic nervous system responses such as pupil dilation, piloerection, increased heart rate, increased blood pressure, increased respiration, somatic struggling movements, and inhibition of the gut and bladder (Parent, 1996). Different types of emotional responses were elicited from different parts of the hypothalamus. Roeling *et al.* (1994) stated that electrical stimulation of the intermediate hypothalamic area and the ventrolateral pole of the ventromedial hypothalamic nucleus elicited attack behaviour. Parent (1996) stated that flight responses were most readily evoked from lateral regions of the anterior hypothalamus, while aggressive responses are reportedly evoked by stimulating the

ventromedial nucleus (Adamec, 1990). Koolhaas *et al.* (1990) stated that electrical stimulation of both the ventrolateral and the ventromedial hypothalamus most readily elicited aggressive behaviour. Parent (1996) speculated that since these responses were evoked by stimulation, the cerebral cortex and the thalamus must have played important roles in these behaviours.

In humans the frontal cortices have been implicated in aggression. The degree of social contact within human societies demands that not only specific aggressive behaviours, but also context dependent aggressive behaviours, be inhibited or expressed depending on given circumstances. This behaviour requires modulation and intervention by the frontal cortex, as the hypothalamus is not equipped for the planning and memory that is involved (Restak, 1984). Complex associations with sensory components, such as human aggression inhibition, have been found to be routed through the thalamus to and from the cortex (Parent, 1996). Roeling *et al.* (1994) have implicated the dorsomedial thalamic nucleus in aggressive behaviour in rats. They noted that the medial dorsal thalamus appeared to receive a larger afferent projection from the intermediate hypothalamic area than from any other hypothalamic nucleus. One of the main projection areas of the medial dorsal thalamus was the prefrontal cortex, which was reported to exert inhibitory control over hypothalamically elicited attack behaviour. The medial and central segments of the mediodorsal nucleus of the thalamus were found to be reciprocally interconnected with the prefrontal cortex, the agranular insular cortex, the pyriform cortex, the ventral pallidum and the amygdaloid complex (Ray & Price, 1992). On this basis, Ray and Price (1992) hypothesized that the mediodorsal thalamus would relay

information between the ventral forebrain nuclei and the cortical regions. With the use of retrograde tracers, Ray and Price (1992) discovered that the pyriform cortex and the amygdala also have direct projections to the prefrontal and agranular insular cortices. This triangular organization provides a mechanism whereby the dorsomedial thalamus enables the highest functional levels of the brain to control the more primitive elements of mental activity such as are represented in emotional reactions (Leonard, 1972).

The interconnections between the areas of the hypothalamus and the amygdala involved in aggressive behaviours have also been examined. Roeling *et al.* (1994) used a microinjection of phaseolus vulgaris-leucoagglutinin (an anterograde tracer) in order to determine inter-connections of interest. These researchers found that fibers from the intermediate hypothalamus project mostly to the medial amygdala, but also project sparsely to the central amygdala. It was also reported that the ventromedial hypothalamus innervates portions of the medial, basolateral and central amygdala (Roeling *et al.*, 1994).

Pharmacologically, Kruk (1991) discovered that the most pronounced local infusion drug effects on aggressive behaviour in the hypothalamus are drugs with an affinity for serotonin receptors, possibly the serotonin 1B receptor. Nikulina and Popova (1986) have noted that serotonin was also involved in the predatory response of mice and mink. Kruk (1991) stated that drugs that had high affinities for serotonin 1 receptors selectively inhibited hypothalamic-mediated attack in both sexes and in different strains of rats. Kruk (1991) explained that these drugs also reduced naturally aggressive interactions provoked by the introduction of an intruder endangering offspring or territory. He did not claim that these serotonin antagonists inhibited all aggressive behaviours,

however he did claim that they inhibited the most violent parts of the agonistic pattern. Bandler (1969) discovered that direct injections of carbachol, Ach-plus-eserine, or neostigmine into sites of the lateral hypothalamus facilitated frog and mouse-killing responses in rats (DeFeudis, 1974), thereby suggesting a cholinergic role in attack. Brody *et al.* (1969) reported similar findings using local injections of glutamate, a potent CNS stimulant. DeFeudis (1974) cited that intra-hypothalamic injections of chlorpromazine (an adrenergic blocking agent) also caused aggressiveness and attack in rats. From this research DeFeudis (1974) suggested that adrenergic mechanisms may have played a significant role in the central mediation of aggressive behaviour.

In summary, studies on agonistic behaviour have indicated that the main regions involved in observed aggressive responses are the central, basolateral, and medial amygdala, and the intermediate, ventromedial and ventrolateral hypothalamus. The neurotransmitters GABA and dopamine are implicated in the amygdala-mediated aggressive responses. Serotonin is implicated in the inhibition of hypothalamic aggressive responses and cholinergic stimulation was implicated in the elicitation of hypothalamic aggressive responses. These results have been obtained through local infusion, stimulation, or lesion techniques. The literature suggestes that complex sensory information, specifically information involved with the initiation of agonistic behaviour, is relayed to the frontal cortex via the thalamus, which is then processed and relayed to the amygdala and hypothalamus from the frontal cortex via the thalamus. If aggression is required, then either the amygdala, the hypothalamus or both together stimulate the sympathetic nervous system via the brainstem nuclei, and the animal then launched into

action. This literature provides only a general picture of the complexities of aggression. Studies have yet to reveal many of the details and releasing stimuli of the intricate associations inducing or inhibiting the numerous aggressive behaviours available to one individual. The behaviours can range from body positioning and subtle physiological reactions to overt attack or submission. In order to examine these intricate associations, researchers will be required to look at systems within the brain rather than employing single local techniques.

Chapter 2

Changes in Social Agonistic Behaviour Dependent on Environmental Conditions, Subsequent to Brain Damage Induced by Means of the Lithium Pilocarpine Epilepsy Rat Model: Implications for Early Social Integration After Brain Damage

Abstract

Research has indicated a higher frequency of agonistic behaviour displayed by lithium and pilocarpine induced epileptic rats (experimental) than in non-epileptic rats (controls) within group housing environments. This research indicated that lithium and pilocarpine induced epileptic rats group housed with control subjects, 21 days after seizure induction, in a ratio of greater than 33% control to epileptic displayed a significantly lower frequency of agonistic behaviour. This decrease in agonistic behaviour frequency, observed in experimental subjects, was the result of constant exposure of epileptic subjects to two or more control subjects in a group housing environment with 6 subjects per cage. If the experimental subjects were group housed with controls 32 days following seizure induction, the agonistic behaviour was no longer reduced in frequency. This study suggested that, in terms of social behaviour, early integration subsequent to brain injury is crucial for acceptable social functioning.

Previous research has indicated that a single injection of 3mEq/kg of lithium followed 4 hours later by a single subcutaneous injection of 30mg/kg pilocarpine induced tonic/clonic seizures which were accompanied by brain damage in structurally and functionally related nuclei. The brain damage induced by the lithium and pilocarpine epilepsy rat model invoked behavioural changes that occurred during the weeks that followed the seizure induction (Persinger *et al.*,1993). Persinger *et al.*,(1993) discovered that 7 to 10 days subsequent to seizure induction, intragroup aggression increased. About 20% of the rats housed in pairs displayed synchronous stereotyped movements with their partners. The durations of the agonistic episodes ranged between 10 and 15 minutes (Persinger *et al.*, 1993). In about 20% of the animals, the aggression was extreme and they were separated to remain in compliance with the endpoints of the protocol as defined by the guidelines of the Canadian Council on Animal Care (Persinger *et al.*, 1993). The interictal aggression resulted in body lesions localized mainly to the ears and face, and pinpoint lesions to the tail (Persinger *et al.*, 1993). The facial lesions were found in regions comparable to two situations: 1) those resulting from intruders who were aggressing towards dominant colony males, and 2) those of dominant colony males who had undergone shock-induced fighting, which was considered offensive in nature (Blanchard & Blanchard, 1981; Archer, 1988).

Social aspects of aggression in controls has been defined by Miczek *et al.* (1994) as antisocial behaviour resulting primarily from exposure to aversive environmental events.

Blanchard and Blanchard (1981) have indicated that male laboratory rats tend to form dominance relationships involving consistent, long-term asymmetries in offensive and defensive behaviours between the members of a specific dyad. The consistency of these relationships enables a highly successful prediction of future behaviour, both in regards to the agonistic relationships for a particular male dyad and for other behaviours as well (Blanchard and Blanchard, 1981). Establishment and maintenance of a territory, along with group formation and the enforcement of social hierarchies are some of the critical functions of aggressive acts, postures, and displays (Miczek *et al.*, 1994). Various social interactions affecting conspecific aggression have been studied within these functions.

Elements of social interaction are dependent upon sensory stimulation. Sensory stimulation within group housing environments for rats include vision, touch, vocalizations, and smells. Studies of each sensory system alone to determine its involvement in the organization of agonistic behaviour have revealed that vision (Pellis *et al.*, 1996), touch (Pellis *et al.*, 1996) and vocalizations (Vivian & Meczec, 1993) do not singularly affect agonistic encounters. The only sensory system that, upon its removal, affects the frequency of agonistic encounters is olfaction. Although olfaction has been studied singularly, without the possibility of measuring interactions between this and other senses in agonistic encounters, studies focusing on olfaction have implied that this sensory system is strongly involved with the organization of agonistic behaviours in rodents.

This study has focused on aggression within the lithium/pilocarpine epilepsy model. Many cases of brain injury involve damage within multiple brain regions which may evoke subsequent necrosis in structurally and functionally related nuclei. This

approach to brain function is considered ecologically relevant in light of the typical clinical manifestations of traumatic or idiopathic brain injury (Persinger *et al.*, 1993). Early intervention may serve to decrease these secondary clinical effects and they allow for an increased probability for social reintegration. This study was designed to examine the effects of environmental changes, viewed as early social integration, on aggression after brain damage.

Methodology

Animals

Adult male Wistar rats (350 g, approximately 70-110 days old; Charles River, St. Constance, Quebec) were held in groups of one to three under a 12 hour light: 12 hour dark cycle (light 0800-2000 hr) with food (Purina Rat Chow) and water available *ad libitum*. One group of rats were untreated (controls n=42 for study 1 and n=6 for study 2) while the other group (experimental n=42 for study 1 and n=12 for study 2) received brain damage by lithium/pilocarpine induced seizures. Individual rats were subcutaneously (s.c.) injected with lithium chloride (3 mg/kg/ 10 ml saline; Sigma) followed 4 hours later by a s.c. injection of pilocarpine (30 mg/kg/ 10 ml saline; Sigma). The presence of seizures was visually confirmed. The subjects were observed to rear and display tonic-clonic contractions at approximately 30 min. after the pilocarpine injection. After the injections and seizure induction, the rats were allowed to recover for 10 days. To enhance their recovery, the rats received a s.c. injection of acepromazine (30mg/kg/ 10 ml saline; Sigma) 1 hour after the pilocarpine.

Experimental Design

Study 1:

Siezured ____ Recovery _____Change cage _____ Habituation _____ Observations
10 days selected ratio 11 days 11 days

Study 2:

Siezured ____ Recovery _____Change cage _____ Habituation _____ Observations
10 days all experimental 11 days 11 days
___ Change cage _____ Habituation _____ Observations
3 exp. : 3 contr. 11 days 11 days

Housing Conditions

Following the 10 day recovery period, animals were placed in groups of six in Plexiglas cages (101.5 x 101.5 x 50.5cm) under a 12 hour light: 12 hour dark cycle (light 0800-2000 hr) with food (Purina Rat Chow) and water available *ad libitum*. In Experiment 1 rats were held for 21 days in one of 7 different ratios of control to experimental (seizured) animals: (1) 6:0, (2) 5:1, (3) 4:2, (4) 3:3, (5) 2:4, (6) 1:5 and (7) 0:6 (n=2, for each ratio condition). Agonistic behaviours (described below) of the rats were observed over the last 11 consecutive holding days for 1 hour each night between 2200-2400 hr under red fluorescent lighting. In order to determine if the reduction in agonistic behaviour exhibited by the experimental subjects, exposed to control subjects was dependent on early integration after brain injury, a second experiment was performed. Experiment 2 animals were held in groups of 6 experimental (seizured) subjects for 21 days following recovery and their agonistic behaviours observed over the last 11 consecutive holding days for 1 hour each night between 2200-2400 hr under red

flourescent lighting. They were then divided and held in two groups of 3 controls and 3 experimental animals per cage for an additional 23 days. One hour behavioural observations were carried out on the last 11 consecutive nights. In both experiments rats were individually tail marked with a black marker to distinguish them under the red lighting conditions.

Behavioural Observations

Agonistic behaviour is generally considered to include the behaviours of threat, attack, submission, and withdrawal (Scott & Fredericson, 1951). The specific agonistic behaviours scored here included biting, boxing and mounting. Biting was defined as dental contact with another rat, that resulted in squeaking or tissue damage in the recipient (Desjardins & Persinger, 1995). A 3 second inter-bite interval was allotted before the next bite was scored. Boxing was defined as two animals rearing while facing one another with both forefeet off the bedding. Both subjects required at least one forefoot back onto the bedding before another boxing bout was scored. Mounting was defined as one subject grasping another with both forefeet and delivering a sequence of at least three pelvic thrusts against the other.

Data Analysis

In Experiment 1, the means were taken for all subjects which included the experimental subjects and the control subjects in each cage condition for the entire 11 day observation period. A one-way analysis of variance with a polynomial subcommand was

performed in order to determine the statistical differences and trend components between cage conditions for all subjects. A second series of one-way analyses were performed in order to determine statistical differences between cage conditions for all subjects, the experimental subjects alone and the control subjects alone.

In Experiment 2, the mean frequencies for the various agonistic behaviours in the first 11 and last 11 experimental days were determined. A multivariate analysis of variance, one level repeated, with a one-way analysis of variance *post hoc* was performed on the means of each of the two observation periods to determine if there were significant differences in observed behaviours between the two groups.

Results

Experiment 1

Subjects maintained in each of the cage conditions did not exhibit any noticeable alterations in feeding, drinking or self-grooming behaviours in the various groups across the 11 days. During the light period the rats in the various groups were noted to be sleeping in a single group at one end of the cage, with no single subject isolated from this social behaviour for more than 2 consecutive days.

Table 1.1 lists the means and standard deviations for the three observed behaviours for each cage condition during the 11-day observation period. A one-way analysis of variance was calculated for the mean observed behaviours between the seven groups for each of the behaviour types observed.

Table 1.1: Agonistic Behaviours Exhibited by Different Combinations of Control and Experimental Subjects per Hour, Over 11 Days.

Group	Cage conditions	bites/hour (mean ± sd)	boxing/hour (mean ± sd)	mounts/hour (mean ± sd)
1	6 control: 0 experimental	2.24 ± 0.23	6.16 ± 4.14	0.27 ± 0.27
2	5 control: 1 experimental	0.78 ± 0.01	5.42 ± 0.14	0.52 ± 0.31
3	4 control: 2 experimental	1.02 ± 0.24	7.74 ± 3.30	1.50 ± 1.00
4	3 control: 3 experimental	2.39 ± 0.52	9.16 ± 2.11	3.66 ± 0.95
5	2 control: 4 experimental	2.01 ± 0.23	11.06 ± 4.49	1.05 ± 0.93
6	1 control: 5 experimental	8.68 ± 3.04*	26.74 ± 8.66*	0.83 ± 0.92
7	0 control: 6 experimental	11.00± 0.83*	27.47 ± 1.13*	4.18 ± 4.76

* significantly different from other housing conditions at $p<.01$.

Analysis by Cage condition

In order to determine statistical differences between overall cage conditions, an analysis of variance was performed. Group 6 and 7 indicated a significantly higher frequency of observed biting than all other groups [$F(6,7)=22.45$, $p<.001$]. Group 6 and 7 also indicated a significantly higher frequency of observed boxing than groups 1, 2, 3 and 4 [$F(6,7)=9.90$, $p<.01$]. There were no significant differences in the frequency of mounting.

Analysis of Control and Experimental Subjects Seperately by Cage Condition

Biting, boxing, and mounting means for the control subjects and experimental subjects within each cage condition were calculated and analyzed separately using one-

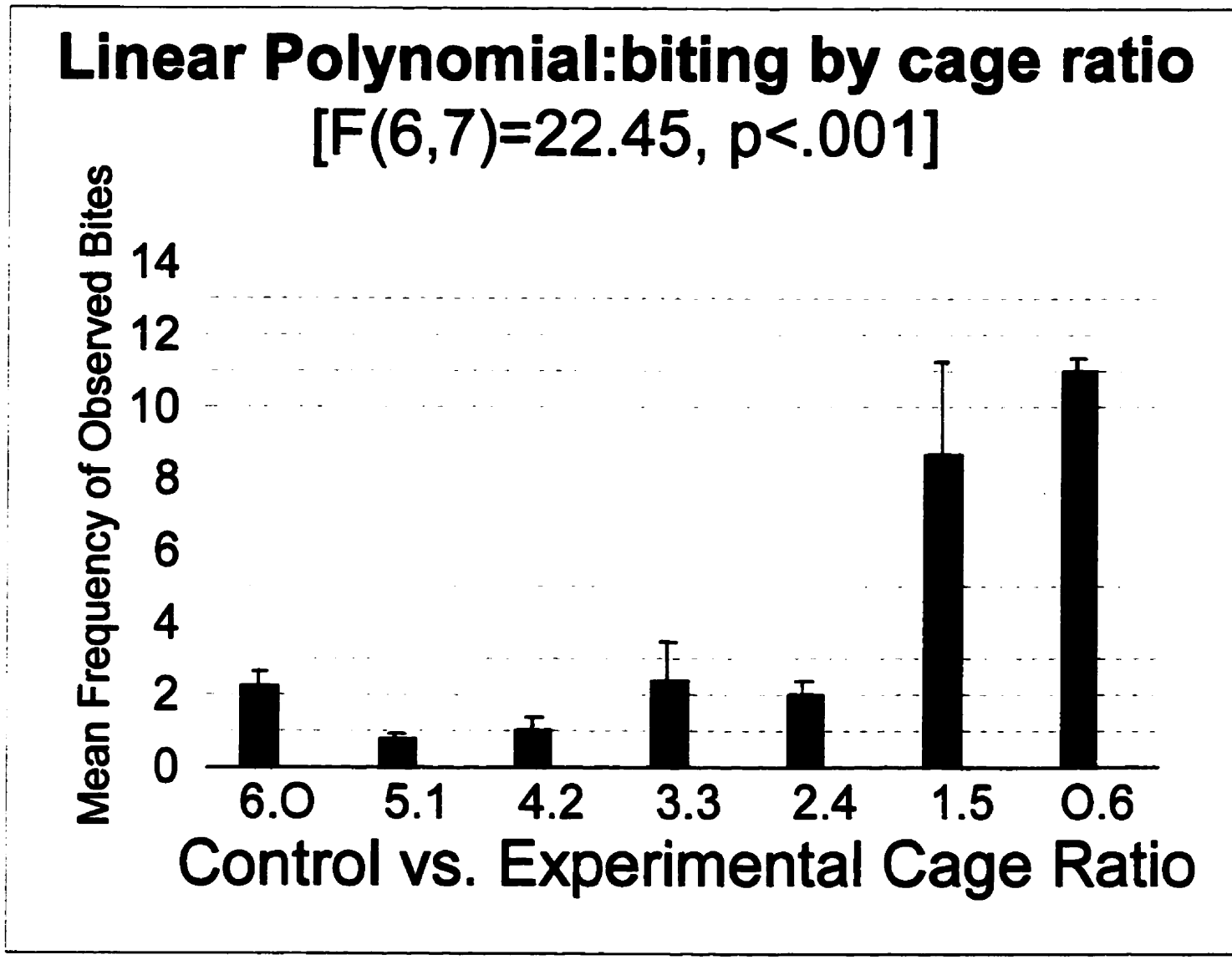
way analysis of varience. The results for experimental subjects indicated a significantly higher frequency of observed biting [$F(6,7)=15.04$, $p<.01$] for group 6 and 7 than for all other groups. For boxing, groups 6 and 7 indicated a significantly higher frequency of observed boxing than groups 1, 2 and 4 [$F(6,7)=9.00$, $p<.01$]. Mounting indicated no significant differences [$F(6,7)=.97$, $p=.50$] The results for control subjects indicated that group 6 displayed a higher frequency of biting [$F(6,7)=15.44$, $p<.01$], and a higher frequency of boxing [$F(6,7)=17.88$, $p<.001$] than in all other groups. Group 3 indicated a significantly higher frequency of mounts than all groups except group 5 [$F(6,7)=7.03$, $p<.05$].

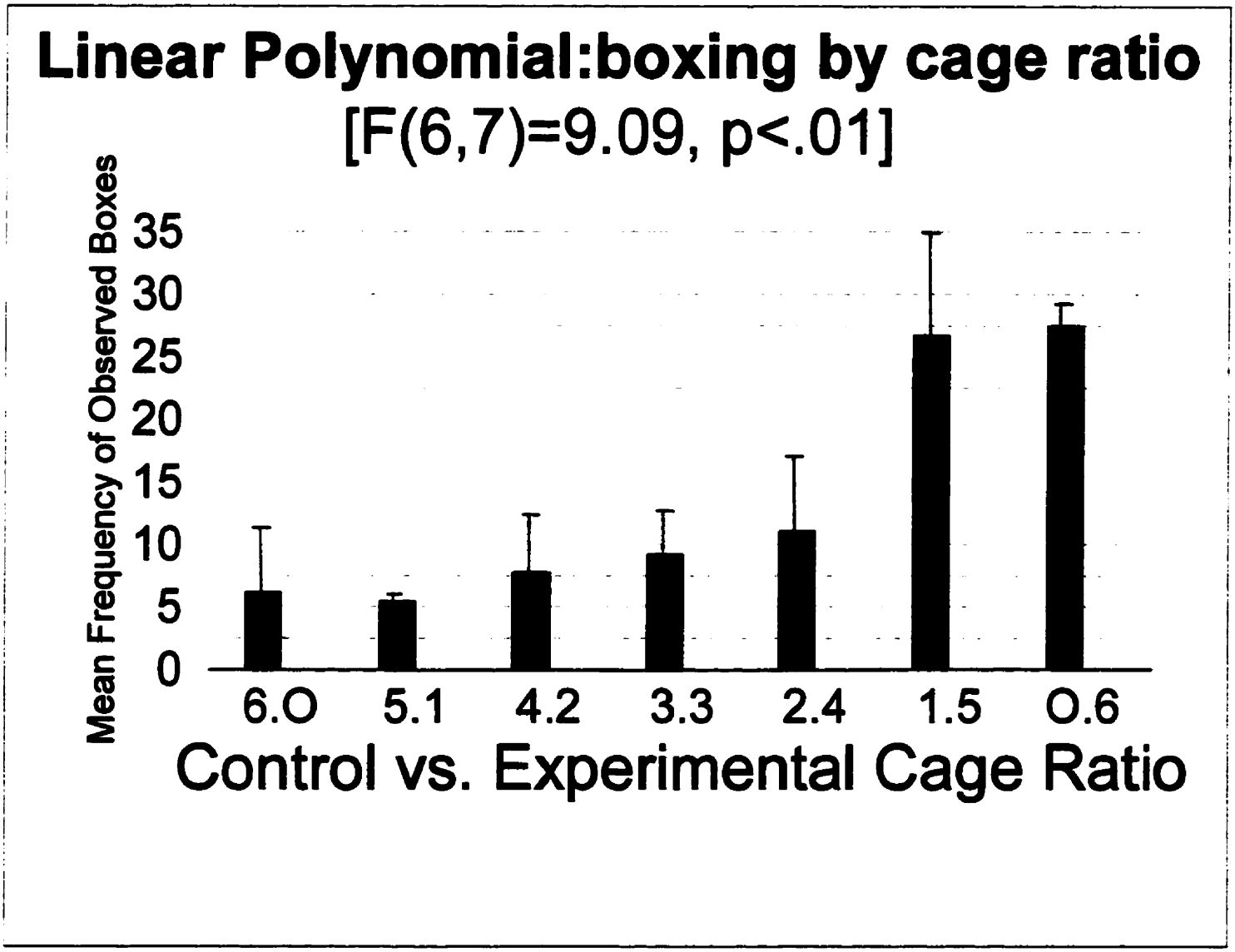
A polynomial one-way analysis was performed to examine the trend components of the behavioural observation data. The results indicated a significant linear equation (Fig. 1.1) for bites [$F(6,7)=22.45$, $p<.001$] and a significant linear equation (Fig. 1.2) for boxing [$F(6,7)=9.90$, $p<.01$].

Experiment 2

The frequency of the various agonistic behaviours exhibited by the 6 experimental subjects was not significantly different than the frequency of observed behaviour exhibited by the 0:6 control to experimental ratio cage condition in Experiment 1 of this study. A multivariate analysis of variance with one level repeated was performed on observed behaviours of these experimental subjects. The repeated measure included the two observation periods. The first 11 day observations consisted of the 0:6 control to experimental condition and the second 11 day observations consisted of the 3:3 control to

experimental condition. This analysis revealed no significant differences [$F(6,5)=1.34$, $p>.05$] in behaviour within the two groups.


Linear Polynomial:biting by cage ratio
[F(6,7)=22.45, p<.001]
Mean Frequency of Observed Bites
14
12
10
8
6
4
2
0
6.O
5.1
4.2
3.3
2.4
1.5
O.6
Control vs. Experimental Cage Ratio


Linear Polynomial:boxing by cage ratio
[F(6,7)=9.09, p<.01]
Mean Frequency of Observed Boxes
35
30
25
20
15
10
5
0
6.O
5.1
4.2
3.3
2.4
1.5
O.6
Control vs. Experimental Cage Ratio

Discussion

The present study examined agonistic behaviours in group-housed rats which had received intrinsic brain damage through lithium and pilocarpine induced seizures. This study revealed that (i) experimental (seizured) rats displayed significantly greater frequencies of agonistic behaviours than control rats and (ii) the frequency of agonistic behaviours was dependent on social housing conditions. Maximum frequencies of agonistic behaviours were evident in groups that consisted entirely of experimental (seizured) animals, with the frequency of agonistic behaviours decreasing as the ratio of experimental to control animals decreased. It is assumed that the presence of control subjects is responsible for the reduction in agonistic behaviour and not simply the reduction in number of experimental subjects exposed to one another causing this effect. This assumption is based upon two former studies. Both studies observed high frequencies of agonistic encounters between experimental subjects within the same or similar study cage including a sample size of 3 experimental subjects only (unpublished data) and 4 experimental subjects only (Persinger, 1997). Considering that fewer subjects were observed per cage and high frequencies of agonistic encounters occured, than the reduction of agonistic behaviour can only then be attributed to the presence of control subjects. This reduction of agonistic behaviour frequency suggests that some component of social interaction between the control rats is inhibiting the frequency of agonistic behaviours in the brain damaged rats, or conversely that increased interactions between experimental (seizured) rats either augments or induces agonistic behaviours.

The present results are consistent with and extend prior investigations indicating that a single subcutaneous injection of lithium followed 4 hours later by a single subcutaneous injection of pilocarpine resulted in a significantly higher frequency of agonistic behaviour commencing 7 to 10 days following this seizure induction procedure (Persinger *et al.*, 1993). Persinger (1991) suggested that the delay in the introduction of increased aggression in the rats (commencing at 7 to 10 days subsequent to seizure induction and reaching an asymptote at approximately 20 days) was compatible with the spread of damage within the amygdala due to increased brain damage, subsequent to seizure episodes. Other researchers who have damaged portions of the amygdala using lesion techniques have also found a functional role of this nucleus in agonistic encounters (Blanchard & Blanchard, 1972; Fox & Sorenson, 1994; Oakes & Coover, 1997).

The present findings are also consistent with the results of studies, using a variety of species, that have revealed that social factors can exert a modulating affect on agonistic behaviour. Conspecific aggression in rodents has been shown to depend upon the behavioural and sensory cues of the opponents (Ginsburg & Allee, 1942; Guillot & Chapouthier, 1996).

It has been reported that rats release specific odors in response to aversive stimulation, which can be discriminated by conspecifics (Brown, 1979). It was the components of these odors which included steroid metabolites, prostaglandins, fatty acid chains, peptides and proteins, that affected the behaviour of conspecifics (Fanselow, 1985; Herrada & Dulac, 1997). Halpern (1987) has indicated two different classes of pheromones within the odors that affect behaviour: "releasing" pheromones that induced

immediate behavioural changes in mating or agonistic behaviours, and "priming" pheromones that elicited long-term and mostly endocrine modifications such as induction or inhibition of female estrous (Herrada & Dulac, 1997). Although the components of the odors and their effects on the experimental subjects were not measured here, the literature suggests that further examination of these variables may lead to insight into the involvement of olfaction in the resultant inhibitory response on agonistic behaviour observed in lithium and pilocarpine brain damaged rats exposed to 2 or more controls in a population of 6 subjects.

Both biting and boxing are common elements in behavioural research of agonistic encounters in rats. Biting is often classed as offensive in nature. This behaviour is often observed in dominant subjects that would chase their opponent and then would strike and bite with the teeth (Scott & Fredericson, 1951). Blanchard and Blanchard (1981) have demonstrated preferred biting sites for dominant subjects. These preferred sites were all located on the dorsal surface but were localized to different regions depending on the experimental conditions. A dominant colony male would attack an intruder anywhere on its dorsal surface, but if the agonistic behaviour was a result of pain from shock, dominant subjects would restrict their attack to the head region (Blanchard and Blanchard, 1981). Defensive bites were also observed in rats, but the focus of this behaviour was restricted to the opponent's facial region only (Blanchard and Blanchard, 1990). Boxing has been commonly classed as defensive in nature. Scott and Fredericson (1951) stated that, if cornered, a rat would rear up in a boxing stance and remain motionless until attacked. Blanchard and Blanchard (1990) also described upright boxing posture as defensive

behaviour. However, these researchers stated that boxing can be classed as both aggressive and defensive in rats, because both parties boxed during agonistic behaviour. This study analyzed the frequency of observed biting and boxing in the subjects without distinguishing between offense and defense. Offensive and defensive behaviours were not distinguished here due to the furiosity of agonistic encounters. This would have created great difficulty in distinguishing the subtleties of distinction between offensive and defensive behaviours within such a large (n=6) sample size per cage. Due to this lack of distinction, the dominant male in each housing condition could not be distinguished, and therefore this study could not determine if this status position was held primarily by control or experimental subjects.

The frequency of conspecific mounting was also recorded in this study. Scott and Fredericson (1951) stated that sexual mounting and chasing appeared very similar to an attack, and in some cases this would start fights between males. Although these researchers mentioned that mounting may have had an involvement in the initiation of agonistic behaviour, they did not include it in their description of patterns of agonistic behaviour. The potential (initial) involvement of mounting in agonistic behaviour coincides with the results of this study. Although mounting was observed prior to agonistic encounters, this behaviour did not follow the same trend as biting and boxing. Mounting was not found to be significantly higher in frequency for experimental subjects than that of controls. However, mounting was also observed prior to agonistic encounters for the control subjects as well. This suggests an alternate source and pathway, not affected by seizure induction from lithium and pilocarpine, within the brains of these

subjects which is not nesessarily a component of agonistic behaviour.

This study also suggests that the inhibitory response on agonistic behaviour in experimental subjects due to cage conditions occurs during the recovery period. No significant decrease in agonistic behaviour was displayed by experimental subjects that were group housed with 50% controls 32 days after seizure induction. This maintenance of agonistic behaviour frequency was contrary to the significant reduction in agonistic behaviour displayed by experimental subjects housed with controls earlier (11 days vs. 32 days) subsequent to seizure induction. These results suggest that the inhibitory effect on observed agonistic behaviour affects related neural networks for social functioning undergoing secondary necrosis subsequent to brain damage. This suggests that early integration may be critical for normal social functioning within this seizure model.

Chapter 3

Discrimination of Experimental Cage Conditions and Micro structural Involvement in Aggressive Behaviour Based on Neurohistological Analysis of Lithium/Pilocarpine Induced Epileptic Rats.

Abstract

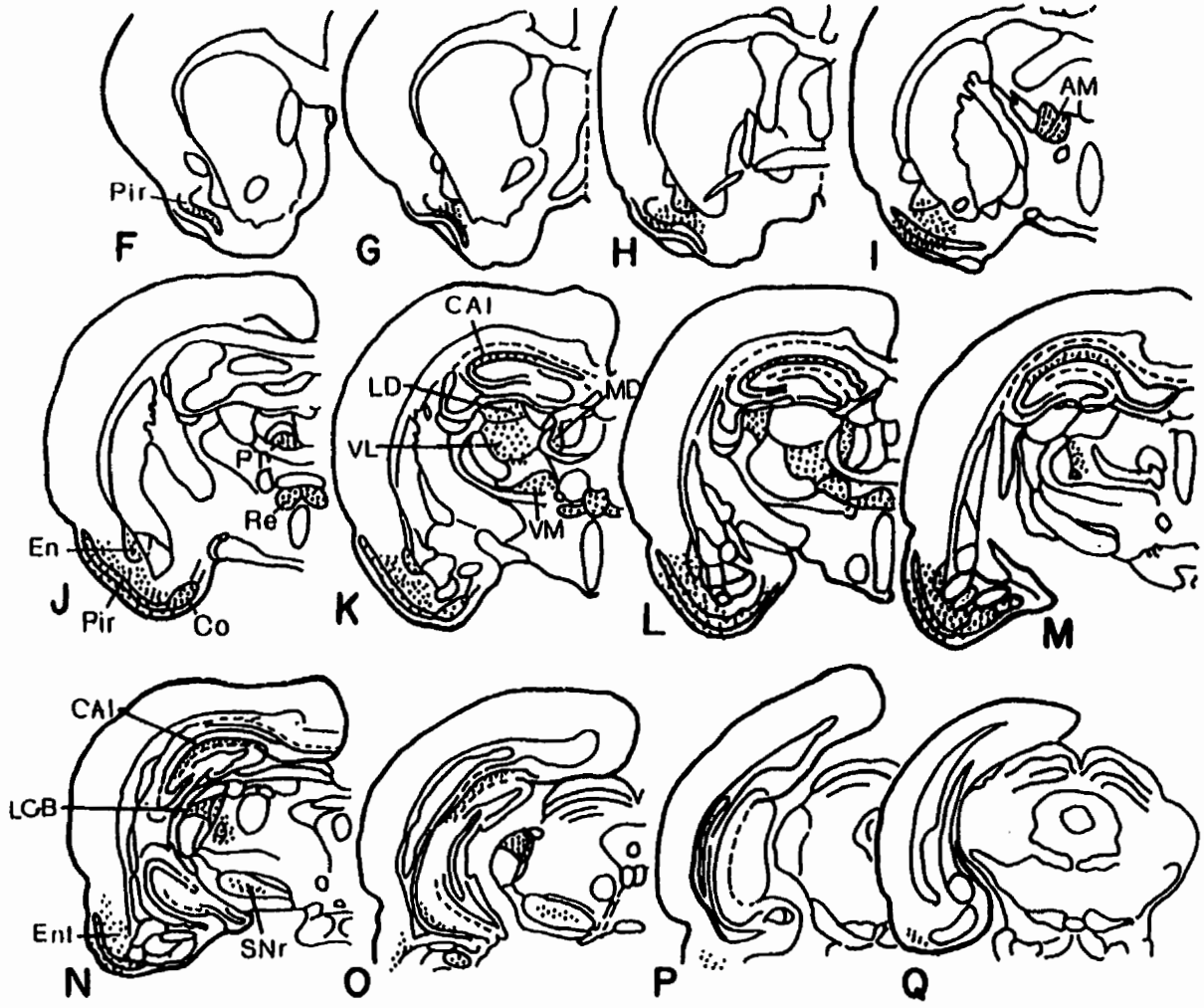
This study was designed to neurohistologically determine microstructure involvement in aggressive behaviour as well as to discriminate between brain damaged rats exposed to separate cage conditions. Male Wistar rats received a subcutaneous injection of 3mEq/kg of lithium, followed 4 hours later by a subcutaneous injection of 30 mg/kg of pilocarpine. This sequence of injections initiated a seizure and consequent brain damage. Subjects were divided into three groups: 1) those housed with a 0:6 ratio of experimental subjects to control subjects (control group), 2) those housed with a 3:3 ratio of experimental subjects to control subjects (mixed group), and 3) those housed with a 6:0 ratio of experimental subjects to control subjects (experimental group). Based upon a quantitative histological analysis of healthy neurons, chromatolytic neurons, oligodendrocytes, astroglia, and microglia within selected areas of the hypothalamus, thalamus, and amygdala, a discriminant analysis predicted cage conditions of the experimental subjects with 90 % accuracy. The discriminant function was based on the number of healthy neurons within the medial dorsal medial thalamus, microglia within the perifornical nucleus, and oligodendrocytes within the basal lateral amygdala.

Behaviourally these rats exhibited a significant difference in the frequency of aggressive behaviour that was measured by biting another animal and by boxing. The experimental group exhibited a higher frequency of biting and boxing than both the mixed group and the control group. Interactions between the cage conditions and the quantitative cell counts within the hypothalamus and thalamus suggest that environmental changes lead to subtle micro-structural changes within related brain regions. This study suggests that environmental conditions affect the quantitative microstructure of the brain, and can be crucial in allowing for normal social recovery subsequent to brain injury.

Introduction

Previous research has demonstrated extreme inter-male aggression commencing 7 to 10 days subsequent to a single subcutaneous injection of lithium (3mEq/kg), followed 4 hours later by a single subcutaneous injection of pilocarpine (30mg/kg) (Persinger *et al., 1993).* This increase in frequency of aggressive episodes asymptoted between postseizure days 15 and 20 (Persinger *et al.*, 1993). This lithium/pilocarpine epilepsy model invoked insidious brain damage which continued to evolve for weeks after the seizure induction (Harrigan *et al.*, 1990). The damage included neuronal necrosis, neuronal dropout, and reactive gliosis (Persinger *et al.*, 1993), which extended to portions of the diencephalon and telencephalon (Bureau *et al.*, 1994) (Figure 2.1) that are structurally and functionally related (Persinger *et al.*, 1993). Histological examination of these related brain regions

>80% DAMAGE



Structures in which damage (stippled areas) was observed in more than 80% of all brain in which seizures had been induced. Coronal sections F through Q are arranged in a rostral to caudal direction. Cortical nuclei: Pir, piriform; En, endopiriform, Ent, entorhinal. Thalamic nuclei: PT, paratenial, Re, reuniens, LD, lateral dorsal, VL, VM, ventral, LGB, lateral geniculate body. SNr, substantia nigra, reticulata. CA1, hippocampus.

has lead to further insight on the extent to which individual nuclei within a related system can affect behaviour as a whole. For example radial arm maze task errors correlated (rho=.80) with the extent of damage to the mediodorsal thalamus and not with other nuclei such as the amygdala, basal ganglia, insular cortices and other thalamic nuclei (Persinger *et al.*, 1993).

Histological studies on aggression have implicated portions of the amygdala (Koolhaas *et al.*, 1990; Adamec, 1990; Nikulina, 1991; Adamec, 1991; Desjardins & Persinger, 1995), the hypothalamus (Adamec, 1990; Kruk, 1991; Nikulina, 1991; Adamec, 1991; Roeling *et al.*, 1994) as well as the thalamus (Roeling *et* al., 1994) in agonistic behaviour. In a recent lithium/pilocarpine induced seizure study, the number of neurons within the central medial amygdala correlated (r=.92) with the level of observed aggression, defined by the number of bites per hour (Desjardins, & Persinger, 1995). Bedard and Persinger (1995) have hypothesized that the disinhibition of the central nucleus of the amygdala, due to subsequent damage to areas that inhibit the central amygdaloid nucleus from the lithium/pilocarpine seizure induction, is a major contributor to the enhanced observed aggression. Other areas of the amygdala have been implicated in aggression, such as the medial amygdala (Koolhaas, 1990) and the basolateral complex (Adamec, 1990). The medial amygdaloid nucleus is noted to be involved with social behaviour, including intraspecific aggression (Koolhaas, 1990) and the basolateral complex, specifically the more medial portion, is implicated in the more defensive aspects of aggression (Adamec, 1990).

The hypothalamus is referred to as the integrator of behaviour and associated

autonomic and endocrine responses (Roeling *et al.*, 1994). The various projections between areas of the hypothalamus and the amygdala are implicated with the associated aggression (Adamec, 1990; Nikulina, 1991; Adamec, 1991; Kruk, 1991; Roeling *et al.*, 1994). The hypothalamic nuclei associated with aggression are the ventromedial (Adamec, 1990, 1991; Kruk, 1991; Roeling *et al.*, 1994), the lateral (Nikulina, 1991; Kruk, 1991), and the intermediate hypothalamic area (Roeling *et al.*, 1994). Electrical stimulation of the ventromedial hypothalamus has been shown to be involved in the production of defensive responses to a threat (Adamec, 1990).

Pharmacological studies have implicated both the lateral hypothalamus (Nikulina, 1991) and the intermediate hypothalamic area (Roeling *et al.*, 1994) in attack behaviour. Due to the extensive connections between the hypothalamus and the amygdala and their involvement in the various behaviours exhibited during aggression, both nuclei have been implicated as the source of aggression.

The thalamus has also been implicated in agonistic behaviour. Bandler (1971) suggested that lesions of the dorsomedial thalamus increased aggressive behaviour. Anterograde and retrograde tracing of the dorsomedial thalamus has revealed extensive reciprocal connections between the dorsomedial thalamus, forebrain, amygdala, hypothalamus and pyriform cortices (Ray & Price, 1992). Leonard (1972) has suggested that through the extensive connections of the dorsomedial thalamus with the frontal cortex and the hypothalamus, the dorsomedial thalamus provides a mechanism whereby the highest functional levels of the brain are enabled to control the more primitive elements of mental activity, such as are represented in emotional reactions. More recent studies also

included the reciprocal connections of the amygdala and pyriform cortex in this theory of emotional control (Ray & Price, 1992).

Recent research has indicated a significant reduction in aggression exhibited by lithium and pilocarpine induced epileptic rats (experimental subjects) upon constant exposure and interaction with non-epileptic rats (control subjects) (Desjardins *et al.*,unpublished data). Due to the dramatic differences in levels of observed aggression exhibited in these rats as compared to those experimental subjects not exposed to control subjects, histological differences would be expected within the hypothalamus, thalamus and amygdala. By quantitatively examining healthy neurons, chromatolytic (non-healthy) neurons, oligodendrocytes, astroglia and microglia, this study was designed not only to further define the involvement of the aforementioned neuronal groups in aggression but also to delineate any histological differences within these areas that would reflect the significantly different levels of observed agonistic behaviour between study groups.

Methodology

Animals

Adult male Wistar rats (350 g, approximately 70-110 days old; Charles River, St. Constance, Quebec) were held in groups of one to three under a 12 hour light: 12 hour dark cycle (light 0800-2000 hr) with food (Purina Rat Chow) and water available *ad libitum.* One group of rats were untreated (controls n=10) while the other group (experimental n=20) received brain damage by lithium-pilocarpine induced seizures.

Individual rats were subcutaneously (s.c.) injected with lithium chloride (3 mg/kg/ 10 ml saline; Sigma) followed 4 hours later by an s.c. injection of pilocarpine (30 mg/kg/ 10 ml saline; Sigma). To enhance their recovery the rats received an s.c. injection of acepromazine (30mg/kg/ 10 ml saline;) 1 hour after the pilocarpine. The rats were allowed to recover for 10 days after the injections and seizure induction. Not all subjects were seizured on the same day due to the availability of observation cages. Therefore the subjects were seizured in groups of six once an observation cage became available. This resulted in three seizure periods, one in the summer, one in the fall and one in the winter.

The lithium and pilocarpine injection combination used here has been previously shown to induce seizures in rats and affect their brain integrity (Honchar *et al.*, 1983). The presence of seizures was visually confirmed. The subjects were observed to rear and display tonic-clonic contractions at approximately 30 min. after the pilocarpine injection.

Housing Conditions

Following the 10 day recovery period, animals were placed in groups of six in Plexiglas cages (101.5 x 101.5 x 50.5cm) under a 12 hour light: 12 hour dark cycle (light 0800-2000 hr) with food (Purina Rat Chow) and water available *ad libitum*. Rats were held for 21 days in one of three different ratios of control to experimental (seizured) animals: (1)6:0 (control group), (2) 3:3 (mixed group) and (3) 0:6 (experimental group) (n=2, for condition 1 and 3, and n=4 for condition 2). Not all subjects were used in the histological portion of this study, which will be defined later in the histology section. Agonistic behaviours (described below) of the rats were observed over the last 11

consecutive holding days for 1 hour each night between 2200-2400 hr under red fluorescent lighting.

Behavioural Observations

Agonistic behaviour was considered here to include behaviours entailing threat, attack, submission and withdrawal (Scott & Fredericson, 1951). The specific agonistic behaviour observed here were biting, boxing and mounting. Biting was defined as dental contact with another rat that resulted in squeaking or tissue damage in the recipient (Desjardins & Persinger, 1995). There was a 3 second inter-bite interval required (as previously defined) before the next bite was scored as separate. Boxing was defined as two animals rearing while facing one another with both forefeet off the bedding. Both subjects required at least one forefoot back onto the bedding before another box was scored. Mounting was defined as one subject grasping another with both forefeet and delivering a sequence of at least three pelvic thrusts against the other.

Histology

At the end of the total observation period, the subjects were decapitated, and their brains were removed. Ten brains, picked randomly from Group 1, fifteen brains picked randomly from each condition from Group 2 (10 experimental and 5 control) and 10 brains picked randomly from Group 3 were fixed in ethanol-formalin-acetic acid and embedded in paraffin wax. The thirty brains were cut at 10 micrometers coronally, and representative sections were selected at approximately every 50 micrometers, and stained

with toluidine blue O. The number of healthy neurons, chromatolytic neurons, oligodendrocytes, astrocytes, and microglia were counted per field at 1000 x magnifications (oil immersion) for one to five fields, depending on the size of the nucleus. The hypothalamic, thalamic, and amygdaloid nuclei that were included in this study included: lateral hypothalamus (LH); ventral medial hypothalamus ventral lateral portion (VMHVL); medial preoptic nucleus (MPO); the lateral preoptic nucleus (LPO); periventricular hypothalamus (Pe); posterior hypothalamus (PH); perifornical nucleus (PeF); ventromedial thalamus (VM); central dorsomedial thalamus (MDC); medial dorsomedial thalamus (MDM); central lateral amygdala (CeL); medial anterior amygdala (MeA); central medial amygdala (CeM); basal lateral anterior amygdala (BLA); basal lateral ventral amygdala (BLV); basal medial amygdala (BM); medial posterior ventral amygdala (MePV); and the lateral ventral lateral amygdala (LaVL). The mapping of these nuclei were defined by the atlas of Paxinos and Watson (1986).

Data Analysis

Means were calculated for the frequency of biting, boxing, and mounting for each subject over the 11 day observation period within each of the three cage conditions. A oneway analysis of variance (ANOVA) was calculated for each behaviour by cage condition. Means were also calculated for each of the five cell types (healthy neurons, chromatolytic neurons, oligodendrocytes, astroglia and microglia) per mm squared for the length (rostral to caudal) of each brain nucleus. For the purposes of histological examination, group two was divided into control-mixed and experimental-mixed variables.

An analysis of variance with a one-way *post hoc* was performed on the five cell types per nucleus by cage condition (control, control-mixed, experimental and experimental-mixed) in order to determine if there were any significant interactions and/or significant differences in cell type frequency across cage conditions. Two stepwise discriminant analyses were performed: first between all experimental subjects, and all control subjects and then between experimental-mixed subjects and experimental subjects in order to determine the accuracy of discriminating the two groups based upon cell type frequencies. A factor analysis of the five cell types for each nucleus was performed for all subjects to determine if the five cell types share common sources of variance that may reflect previous literature findings on degenerative changes commonly observed subsequent to brain damage. A factor analysis using the mean frequency of the five cell types for each nucleus as predictors for the mean frequency of biting, boxing and mounting was performed. Pearson correlation coefficients examining the relationships between the five cell types for each nucleus and the three agonistic behaviours were computed.

Results

Subjects maintained in each of the three cage conditions did not exhibit any noticeable alterations in feeding, drinking or self-grooming behaviours across the 11 days. During the light period the rats within each cage condition were noted to be sleeping in a single group at one end of the cage, with no single subject isolated from this social behaviour for more than two consecutive days.

Table 2.1 lists the means and standard deviations for the three observed behaviours for each cage condition during the 11-day observation period. A one-way analysis of variance was calculated for the mean observed behaviours between the three groups. Group 3 indicated a significantly higher frequency of observed biting [$F(5,3)=43.12$, $p<.001$] and boxing [$F(5,3)=40.11$, $p<.01$] than the other two cage conditions. There were no significant differences in the frequency of mounting.

Table 2.1: Agonistic Behaviours Exhibited by Three Different Combinations of Control and Experimental Subjects Over 11 Days.

Group	Cage conditions	bites per hour (mean ± sd)	boxing per hour (mean ± sd)	mounts per hour (mean ± sd)
1	6 control: 0 experimental	2.24 ± 0.23	6.16 ± 4.14	2.27 ± 0.27
2	3 control: 3 experimental	3.85 ± 0.35	6.78 ± 4.30	3.67 ± 2.63
3	0 control: 6 experimental	11.00 ± 0.83**	27.47 ± 1.13**	4.18 ± 4.76

** significantly different from other housing conditions at $p<.01$.

The mean numbers of healthy neurons, chromatolytic neurons, oligodendrocytes, astroglia, and microglia per field for each structure were calculated and expressed as numbers of cells per mm-sq. In order to determine the effects of environmental and experimental condition on the microstructure of the brain, a seies of one-way analyses of variance were performed on the five cell types per nucleus by cage condition. The means,

standard deviations and F values are listed in Table 2.2.

The experimental subjects had significantly higher numbers of observed microglia than the control subjects in the following regions: the basal lateral anterior amygdala (BLA) [$F(3,21)=8.38$, $p<.01$], the posterior hypothalamus (PH)[$F(3,21)=7.57$, $p<.01$], the perifornical nucleus (PeF) [$F(3,21)=6.59$, $p<.001$], the medial dorsomedial thalamus (MDM) [$F(3,21)=4.57$, $p<.05$], the central medial amygdala (CeM) [$F(3,21)=7.32$, $p<.05$], the central lateral amygdala (CeL) [$F(3,21)=5.48$, $p<.001$], the basal lateral ventral amygdala (BLV) [$F(3,21)=7.15$, $p<.001$], the lateral anterior ventral lateral amygdala (LaVL) [$F(3,21)=6.54$, $p<.001$], the ventral medial hypothalamus (VMHVL) [$F(3,21)= 9.22$, $p<.001$], the medial posterior ventral hypothalamus, (MePV) [$F(3,21)=5.13$, $p<.05$] and the basal medial amygdala (BM) [$F(3,21)=4.48$, $p<.05$]. Two other cell types showed significance: 1) healthy neurons in the medial posterior ventral amygdala (MePV) [$F(3,21)= 6.69$,$p<.01$] where control subjects had significantly more healthy neurons than the experimental subjects from cage condition 2 (3 control: 3 experimental), and the medial dorsal medial thalamus (MDM) [$F(3,21)= 6.05$,$p<.01$] where all control subjects displayed significantly more healthy neurons than all experimental subjects and 2) oligodendrocytes in the lateral ventral lateral amygdala (LaVL)[$F(3,21)= 4.68$, $p<.01$] where experimental subjects from cage condition 1 had significantly more oligodendrocytes in this area than control subjects from cage condition 3.

Table 2.2: Incidence of Cell Types Within Brain Nuclei Associated With Agonistic Behaviours for Different Cage Conditions.

Area	Cell Type	Cont-unif.		Cont-mix		Seiz-mix		Seiz-unif.		F
		X	SD	X	SD	X	SD	X	SD	value
LH	healthy neur.	324	108	336	51	340	88	376	88	.49
	chrom. neur.	124	37	144	45	140	39	137	44	.23
	oligo.	148	18	192	124	150	45	142	44	.78
	astrocyte	606	91	510	102	582	78	600	95	1.32
	microglia	74	11	72	36	113	55	129	52	2.51
Pe	healthy neur.	792	212	872	180	765	194	832	227	.36
	chrom. neur.	323	68	317	94	297	94	305	92	.11
	oligo.	173	38	163	39	181	43	168	40	.30
	astrocyte	408	72	315	63	361	54	367	76	1.60
	microglia	77	26	85	22	103	58	109	58	.60
PH	healthy neur.	728	273	808	234	680	206	744	281	.31
	chrom. neur.	216	73	288	66	296	105	284	81	.40
	oligo.	200	49	208	52	204	72	176	60	1.02
	astrocyte	392	107	520	28	332	92	372	84	5.60*
	microglia	48	18	56	46	152	82	176	57	7.57*
PeF	healthy neur.	600	106	744	189	664	219	664	194	.47
	chrom. neur.	264	67	320	94	272	65	224	68	2.10
	oligo.	152	34	192	66	164	40	176	85	.41
	astrocyte	544	159	424	67	464	127	496	91	1.06
	microglia	88	34	64	61	136	47	176	57	6.59*

VM	healthy neur.	512	150	459	95	392	87	383	154	1.52
	chrom. neur.	147	56	157	29	132	42	125	39	.78
	oligo.	163	48	144	17	179	53	163	40	.72
	astrocyte	499	36	504	110	547	139	567	89	.65
	microglia	80	25	80	54	251	192	323	253	2.92
MDC	healthy neur.	472	203	504	166	324	148	280	229	2.23
	chrom. neur.	152	66	192	66	160	38	132	63	1.30
	oligo.	176	22	184	61	184	54	192	41	.13
	astrocyte	472	96	488	173	408	53	424	125	.79
	microglia	120	49	88	66	512	420	752	814	2.46
MDM	healthy neur.	432	137	512	100	216	85	280	198	6.05*
	chrom. neur.	136	67	224	78	172	96	100	28	3.80
	oligo.	200	63	168	34	236	93	188	71	1.16
	astrocyte	488	18	480	177	360	108	404	189	1.24
	microglia	96	92	88	87	672	445	584	454	4.57*
CeM	healthy neur.	960	495	1216	143	984	199	948	129	1.49
	chrom. neur.	320	126	360	172	252	76	240	80	1.89
	oligo.	200	56	192	52	228	54	248	68	1.33
	astrocyte	360	120	352	52	380	93	388	105	.20
	microglia	72	34	80	117	288	130	188	78	7.32*
CeL	healthy neur.	1155	135	1021	132	928	177	931	165	2.75
	chrom. neur.	307	62	315	80	216	58	233	90	3.08
	oligo.	173	30	165	38	237	62	231	58	3.32
	astrocyte	325	35	339	97	316	65	320	87	.11
	microglia	53	27	85	47	195	82	204	111	5.48*

BLA	healthy neur.	629	60	651	66	656	154	640	125	.06
	chrom. neur.	200	58	232	93	196	48	197	54	.46
	oligo.	163	22	181	35	173	50	196	45	.83
	astrocyte	301	67	296	65	247	45	219	60	3.33
	microglia	56	50	64	57	192	80	195	68	8.38*
BLV	healthy neur.	472	44	516	114	546	139	506	121	.47
	chrom. neur.	156	68	172	39	190	60	172	59	.41
	oligo.	148	34	148	46	206	49	242	94	3.44
	astrocyte	240	40	256	56	264	42	248	58	.33
	microglia	64	38	96	62	252	95	194	98	7.15*
MePV	healthy neur.	1104	278	1440	254	868	273	932	196	6.69*
	chrom. neur.	360	85	464	207	352	132	352	141	.82
	oligo.	160	49	192	18	184	28	208	80	.94
	astrocyte	272	59	320	85	272	72	240	50	1.66
	microglia	40	28	24	22	300	161	400	331	5.13*
LAVL	healthy neur.	624	178	648	77	444	234	376	191	3.25
	chrom. neur.	256	146	216	92	252	132	192	62	.65
	oligo.	160	28	168	34	240	68	252	63	4.68*
	astrocyte	344	61	312	104	220	63	232	101	3.39
	microglia	64	36	48	44	424	288	444	237	6.54*
VMHVL	healthy neur.+	1016	201	1053	272	1136	328	1160	375	.25
	chrom. neur.	376	159	453	189	368	178	290	106	.99
	oligo.	184	36	267	228	176	61	155	50	1.11
	astrocyte	304	73	307	116	288	72	330	88	.27
	microglia	40	00	40	69	120	49	150	42	9.22*

MPO	healthy neur.+	1160	56	1176	224	1272	606	1240	392	.07
	chrom. neur.	420	142	344	83	348	147	296	107	.70
	oligo.	200	00	176	46	180	51	156	52	.67
	astrocyte	400	113	192	72	296	71	328	75	5.10*
	microglia	80	46	88	66	108	66	88	56	.25
LPO	healthy neur.+	440	00	440	63	436	117	512	132	.91
	chrom. neur.	120	56	136	67	144	96	156	97	.12
	oligo.	180	28	152	34	168	70	156	35	.24
	astrocyte	460	28	560	85	648	142	568	116	1.77
	microglia	100	28	96	36	136	68	108	68	.61
MeA	healthy neur.+	627	101	840	224	656	170	635	166	1.72
	chrom. neur.	280	00	256	96	244	72	230	92	.31
	oligo.	173	23	176	36	204	40	190	36	.94
	astrocyte	360	120	312	66	420	71	425	132	1.77
	microglia	53	23	64	22	176	87	275	238	2.92
BM	healthy neur.+	790	360	800	162	495	200	595	184	2.78
	chrom. neur.	370	213	248	91	295	167	260	158	.54
	oligo.	200	33	160	40	230	46	255	111	1.90
	astrocyte	300	52	360	69	330	73	245	114	2.20
	microglia	60	40	48	34	365	234	335	241	4.48*

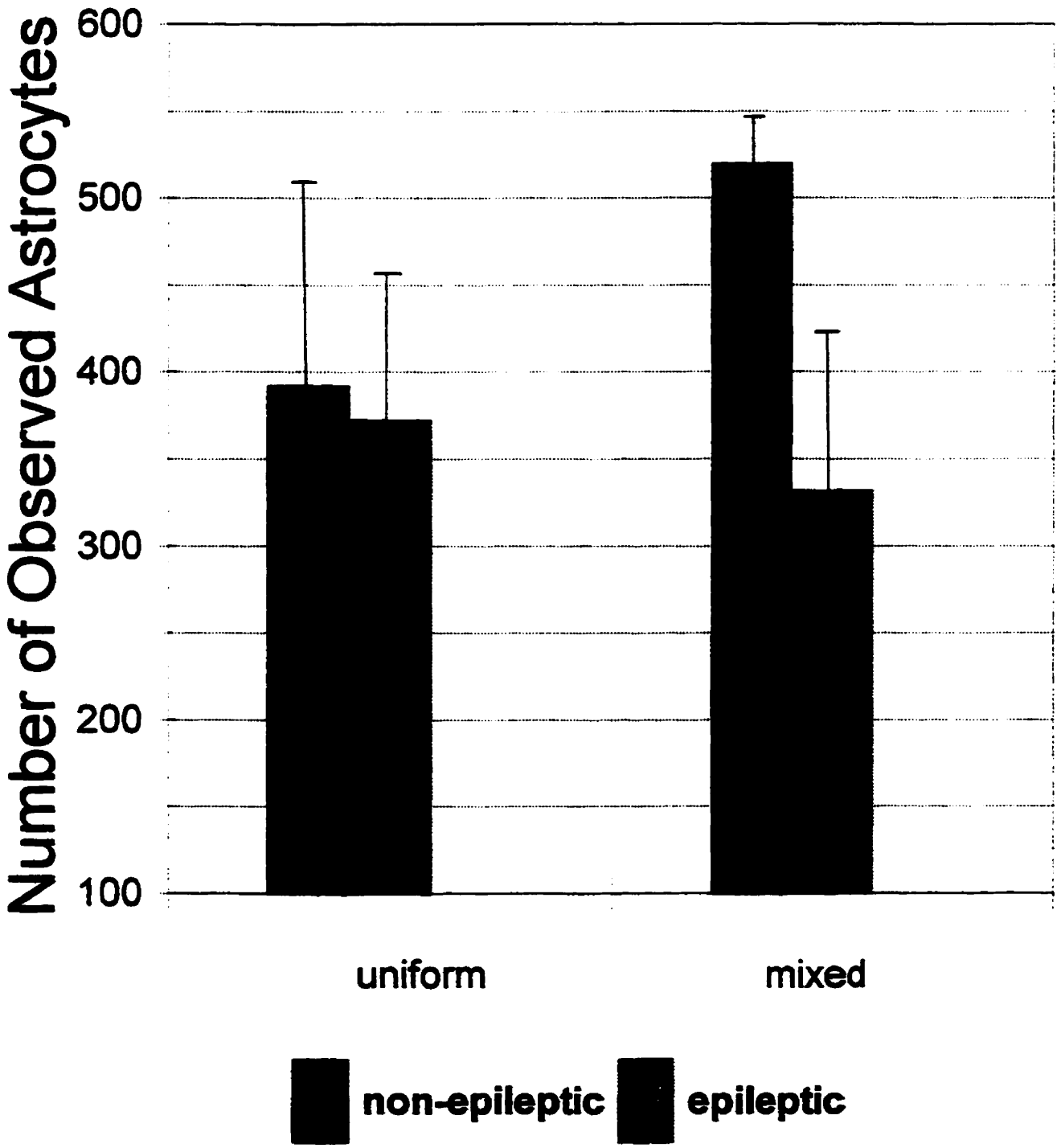
*p<.05

+denotes missing subjects due to nucleus being short in the rostral-caudal plane. Missing subjects are as follows: vmHvl -2 heterogeneous non-epileptics and -2 homogeneous epileptics, MPO -3 homogeneous non-epileptics, LPO -3 homogeneous non-epileptics, MeA -2 homogeneous non-epileptics and -2 homogeneous epileptics, BM -1 homogeneous epileptic and -2 from each epileptic group.

In order to pursue the housing effects on brain microstructure, an analysis of variance was performed on the numbers of different cell types per nucleus by group which displayed three significant interactions (Table 2.2). In the posterior hypothalamus, the number of astrocytes showed an increasing trend in controls from Group 2 (mixed) as compared to the control group. In the experimental population, astrocytes did not change in Group 2 (mixed) condition as compared to the Group 3 (experimental) condition [$F(3,26)=6.5$, $p<.05$, eta sq=.08] (Figure 2.2). In the medial dorso- medial thalamus, chromatolytic neurons showed an increasing trend in the mixed control group as compared to the control group. In the experimental population, chromatolytic neurons showed an increasing trend in the mixed condition as compared to Group 3 (experimental) [$F(3,26)=8.4$, $p<.01$, eta sq.12] (Figure 2.3). In the medial preoptic nucleus of the hypothalamus, observed astrocytes showed a decreasing trend in the mixed control group as compared to the control group. In the experimental population; astrocytes showed a decreasing trend in the mixed condition as compared to Group 3 (experimental) [$F(3,26)=6.1$, $p<.05$, eta sq=.40] (Figure 2.4).

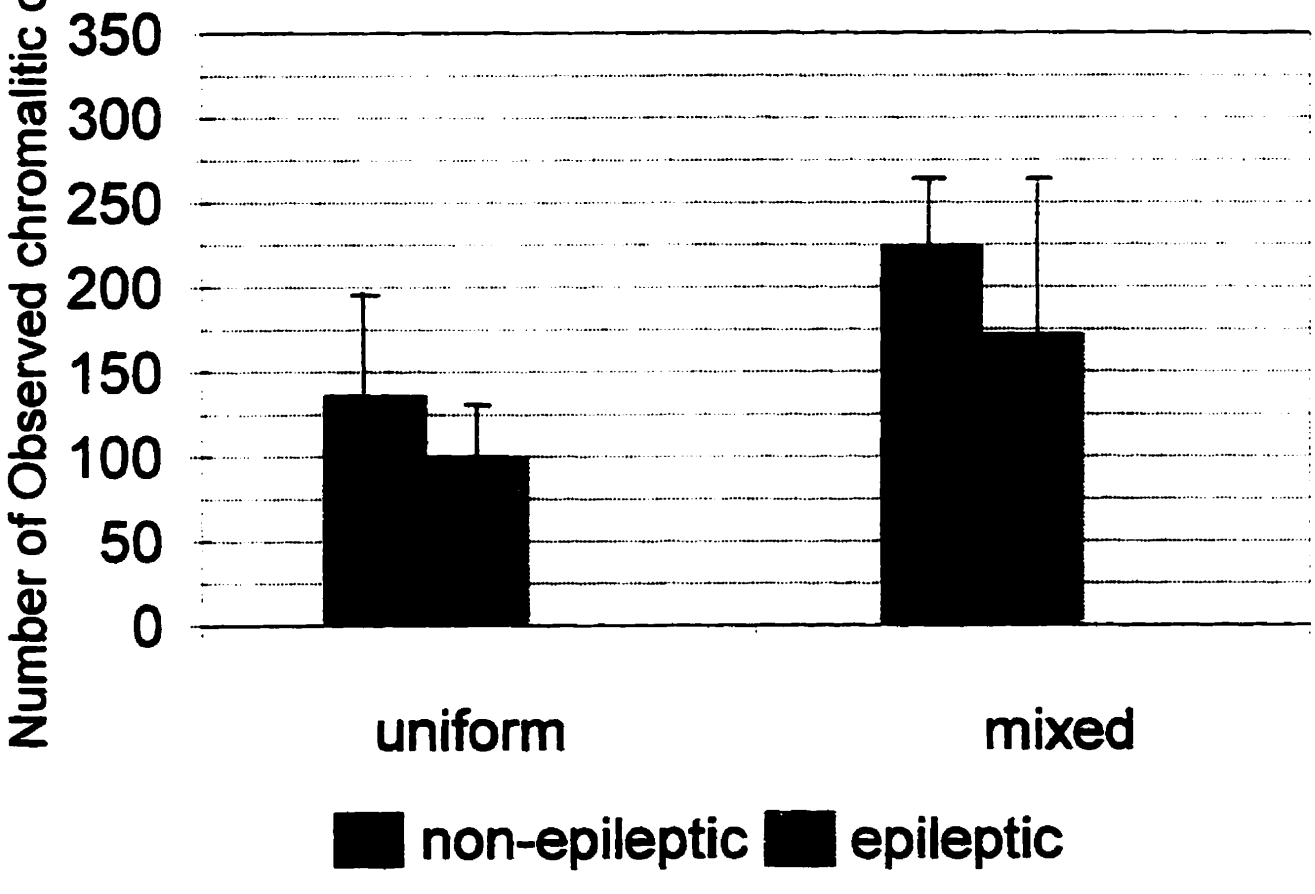
PH Astrocytes by Group Interaction

[F(3,26)=6.5, p<.05, eta=.08]



MDM chromalitic neurons by group

[F(3,26)=8.4, p<.01, eta=.12]



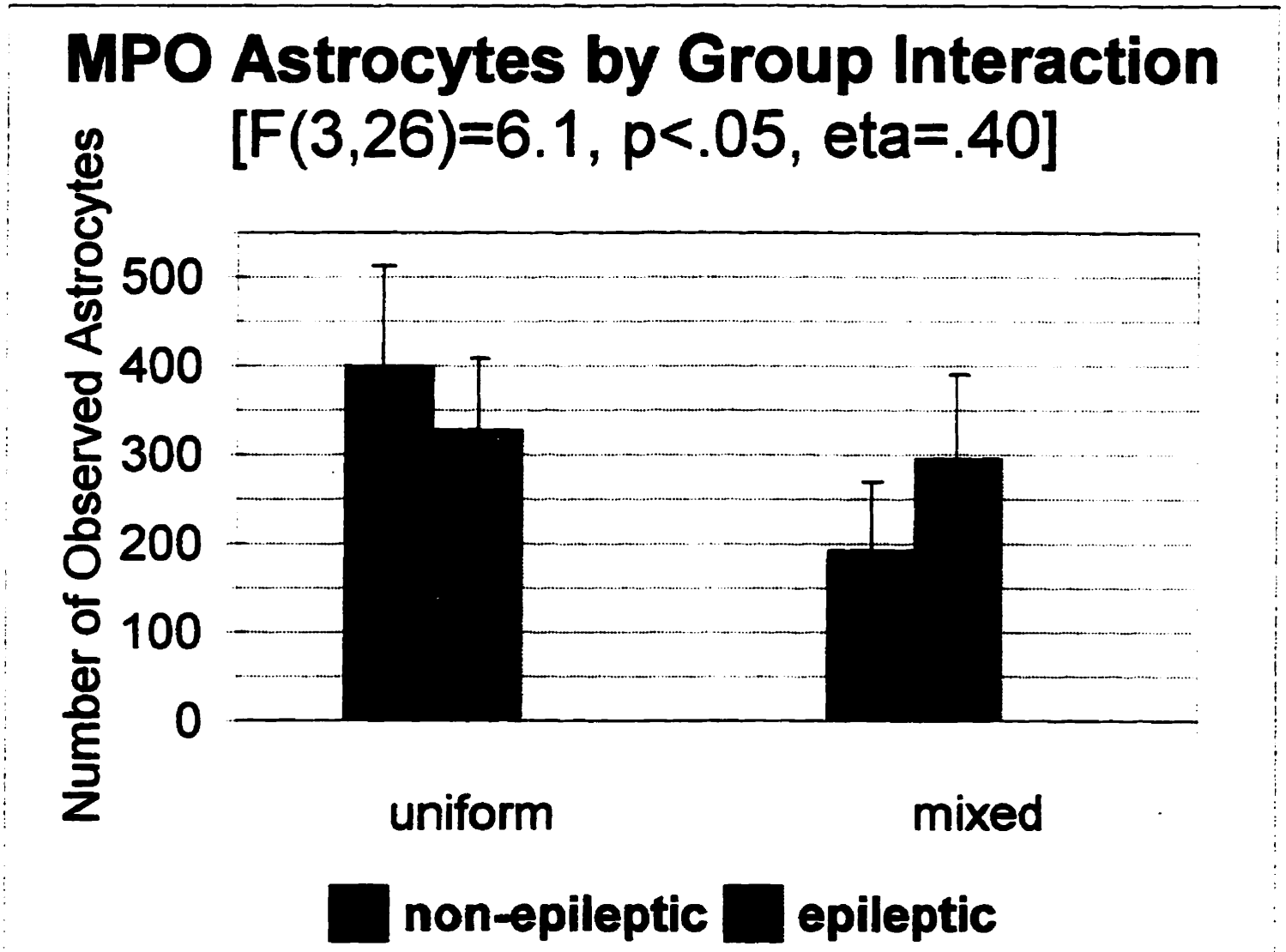

MPO Astrocytes by Group Interaction
[F(3,26)=6.1, p<.05, eta=.40]
Number of Observed Astrocytes
500
400
300
200
100
0
uniform
mixed
non-epileptic
epileptic

Table: 2.3 Interaction of Cell Types Within Selected Brain Nuclei With Varying Cage Conditions

Nucleus	Cell Type	F.	p.	Eta	Group	X	Sd.
PH	astrocytes	6.5	<.05	.08			
					control	392	108
					control-mixed.	520	28
					experimental	372	84
					experimental-mixed	332	92
MDM	chrom. neurons	8.4	<.01	.12			
					control	136	67
					control-mixed	224	78
					experimental	100	28
					experimental-mixed	172	96
MPO	astrocytes	6.1	<.05	.40			
					control	400	113
					control-mixed	192	72
					experimental	328	75
					experimental-mixed	296	71

To define the microstructures that differentiates the control subjects (n=10) from the experimental subjects (n=20), a discriminant function analysis was performed to predict subject condition. The prediction was made on the basis of the five cell types per nucleus (healthy neurons, chromatolytic neurons, oligodendrocytes, astroglia, and microglia for the various portions of the hypothalamus, thalamus, and amygdala listed

above).

The stepwise discriminant function included three variables. The variables were microglia in the basal lateral amygdala (BLAm), see (Figure 2.5) for examples of how these areas appeared, oligodendrocytes in the lateral ventral lateral amygdala (LAVLo), and the healthy neurons in the medial dorsal medial thalamus (MDMn). The standardized canonical discriminant functions, Wilk's lambda, and change in Rao's V are listed in Table 2.4a. The experimental subjects were predicted by a high discriminant score (D) using the equation D=(0.50*BLAM) - (0.91*MDMn) + (1.02*LAVLo)-2.64, with 100% correct prediction.

Another discriminant function analysis was performed to predict cage condition of experimental subjects with one group being the experimental group (cage condition 3) and the other group being the experimental mixed group (cage condition 2). The prediction was made on the basis of the five cell types per nucleus (healthy neurons, chromatolytic neurons, oligodendrocytes, astrocytes, and microglia for the various portions of the hypothalamus, thalamus, and amygdala listed above).

The stepwise discriminant function included three variables. The variables were microglia in the perifornical nucleus of the hypothalamus (PeFm), oligodendrocytes in the basal lateral amygdala (BLAo), and the chromatolytic neurons in the medial dorsal medial thalamus (MDMn). The standardized canonical discriminant functions, Wilk's lambda, and change in Rao's V are listed in Table 2.4b. The experimental group (cage condition 3) was predicted by a high discriminant score (D) using the equation D=(0.99*PeFm) -

(1.11*MDMN) + (1.21*BLAo)-5.55, with 90% correct prediction. For examples of how these areas appeared see (Figure 2.6).

Table 2.4a: Discriminant Functions That Distinguish Experimental Subjects From Control Subjects.

Variable	Wilk's lambda	Change in Rao's V	Discrim. Canon. fn.
BLAm	.51	26.99	.50
LAVLo	.36	50.28	1.02
MDMn	.21	104.23	-.91

canonical corr.=.89, chi square= 41.14, p<.001

Table 2.4b: Discriminant Functions That Distinguish Cage Conditions (2 or 3) for Experimental Subjects.

Variable	Wilk's lambda	Change in Rao's V	Discrim. Canon. fn.
MDMc	.78	5.15	.78
BLAo	.61	11.56	.61
PEFm	.37	30.21	.37

canonical corr.=.79, chi square=16.25, p<.001

Figure 2.5: Coronal sections of the basolateral amygdala at 100x and 400x of control subjects (left) and experimental subjects (right). This figure depicts the basolateral amygdala of a control subject at 100x (top left) and at 400x (bottom left) and a experimental subject at 100x (top right) and at 400x (bottom right). The increase in microglia in the experimental subject is evident qualitatively from the photographs.

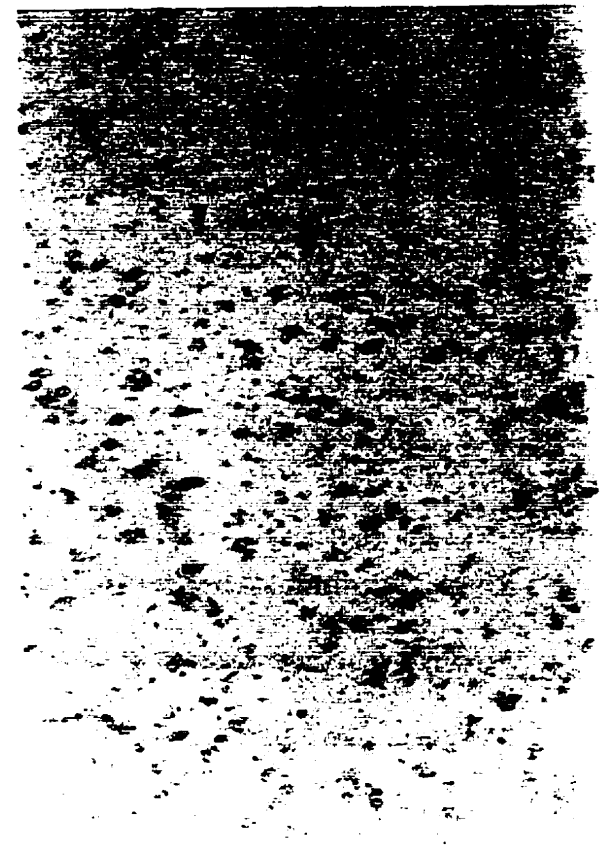
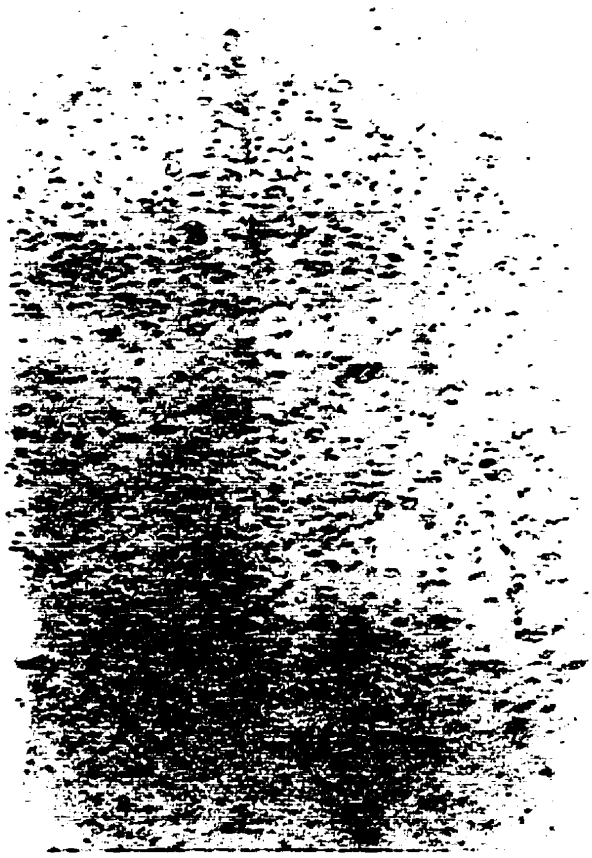
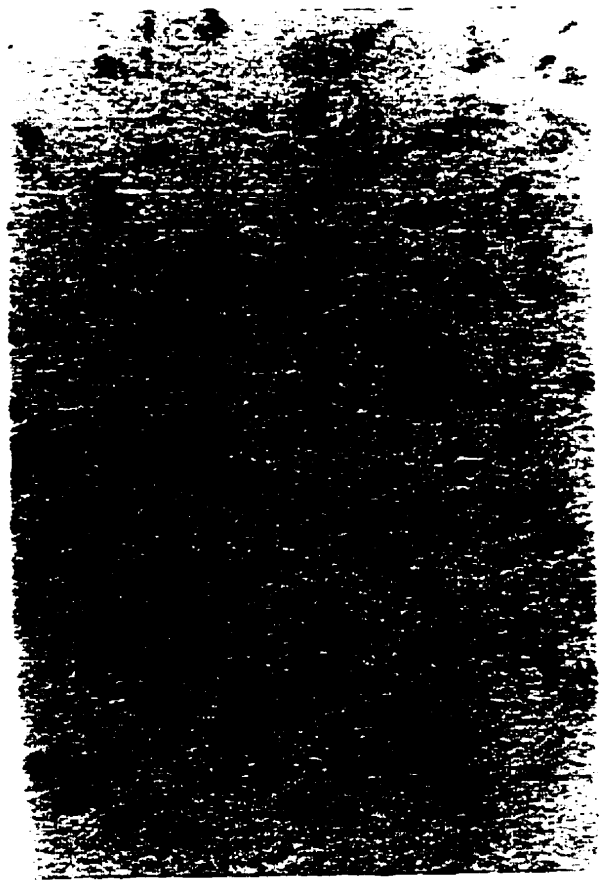


Figure 2.6: Coronal sections of the medial dorsomedial thalamus at 100x and 400x of experimental subjects housed in condition 2 (left) and seizured subjects housed in condition 3 (right). This figure depicts the medial dorsomedial thalamus of an experimental subject housed in condition 2 at 100x (top left) and at 400x (bottom left) and an experimental subject housed in condition 3 at 100x (top right) and at 400x (bottom right). The decrease in chromatolytic neurons in the experimental subject housed in condition 3 is evident qualitatively from the photographs.





Neuronal damage is followed by fundamental degenerative changes in the brain. In order to determine if the brain microstructure shares common sources of variance that may reflect these changes, a factor analysis was employed. The factor analysis included observed numbers of healthy neurons, chromatolytic neurons, oligodendrocytes, astrocytes, and microglia for each nucleus. Two factors emerged: chromatolytic neurons and microglia sharing the same source of variance; and neurons and microglia sharing an inverse source of variance. Table 2.5 reports factor loadings and the percent of explained variance for each nucleus.

Table: 2.5 Factor Loadings and Percent of Explained Variance for Each Factor for Cell Type Within Each Nucleus.

Nucleus	Factor 1		Factor 2		Factor 3	
	cell type	loading	cell type	loading	cell type	loading
LH	healthy neur.	.84	chrom. neur.	.46		
	oligo.	.82	microglia	.94		
% expl.	41.9%		65.3%			
Pe	healthy neur.	.84	chrom. neur.	.56		
	oligo.	.85	microglia	.88		
% expl.	40.7%		70.3%			
PH	healthy neur.	.86	chrom. neur.	.76	astrocytes	.92
	oligo.	.89	microglia	.88		
% expl.	31.1%		58.1%		79.6%	

PeF	healthy neur. .62	chrom. neur. .76	
	oligo. .81	microglia .88	
	astrocytes .76		
% expl.	**36.1%**	**60.9%**	
VM	healthy neur. -.65	oligo. .85	
	chrom. neur. .79	astrocytes .87	
	microglia .84		
% expl.	**41.7%**	**68.0%**	
MDC	healthy neur. -.87	chrom. neur. .74	
	astrocytes .79	oligo. .74	
	microglia .86		
% expl	**43.1%**	**67.1%**	
MDM	healthy neur. -.91	chrom. neur. .86	astrocytes .95
	microglia .89	oligo. .80	
% expl.	**38.5%**	**63.2%**	**83.8%**
CeM	healthy neur. .72	chrom. neur. .69	
	astrocytes -.80	microglia .82	
% expl.	**30.3%**	**54.6%**	
CeL	chrom. neur. .91	healthy neur. .85	microglia .98
	astrocytes .83	oligo. .85	
% expl.	**34.9%**	**64.8%**	**86.2%**
BLA	chrom. neur. .64	healthy neur. -.64	
	oligo. -.81	microglia .74	
	astrocytes .79		
% expl.	**36.2%**	**63.6%**	

BLV	microglia .88	oligo. -.85	
	chrom. neur. .75	astrocytes .78	
	healthy neur. .45		
% expl.	**32.3%**	**59.6%**	
MePV	chrom. neur. -.81	healthy neur. -.47	astrocytes .94
	oligo. .77	microglia .91	
% expl.	**35.7%**	**59.0%**	**80.9%**
LaVL	healthy neur. -.90	chrom. neur. -.85	
	astrocytes .75	oligo. .89	
	44.9%	**77.5%**	
VMHVL	healthy neur. .91	chrom. neur. .95	
	oligo. .82		
	astrocytes .66		
	microglia .71		
% expl.	**49.6%**	**71.8%**	
MPO	healthy neur. .94	chrom. neur. .85	
	astrocytes -.62	microglia .79	
	oligo. .73		
% expl.	**41.5%**	**71.8%**	
LPO	oligo. .80	chrom. neur. .96	healthy neur. .91
	astrocytes .73		
	microglia .73		
% expl.	**36.5%**	**59.6%**	**82.7%**
MeA	healthy neur. .58	chrom. neur. -.76	
	astrocytes .81	oligo. .60	

	microglia -.78		
% expl.	**34.0%**	**59.4%**	
BM	healthy neur. -.68	oligo. .89	
	chrom. neur. .89	astrocytes .83	
	microglia .95		
% expl.	**47.1%**	**81.1%**	

A multiple regression was performed to predict agonistic behaviour observed as bites, boxing, and mounting using all cell types from each nucleus as predictors. No variables entered the equation in this analysis.

A subsequent multiple regression using the factor scores, listed above, in order to predict agonistic behaviour was employed. The results indicated that the factor variable including chromatolytic neurons and microglia of the lateral hypothalamus predicted mounts [r squ=.21, B=-2.18, const.=3.51, F=4.63, $p<.05$], both bites and boxing was not predicted. In order to predict bites and boxing, the data for each variable was standardized per cage and transformed using z scores. The regression indicated that the microglia in the medial dorsal medial thalamus predicted bites [r squ.=.22, B=.05, const.=-.57, F=4.82, $p<.05$], and oligodendrocytes in the medial dorsal medial thalamus predicted boxing [r squ.=.30, B=.26, const. =-1.38, F=7.23, $p<.05$]. No other variables entered the equation.

In order to determine a relationship between cell quantity and agonistic behaviour a Pearson r correlation was performed. All chosen nuclei and cell types counted were

analyzed with the three observed behaviours: biting, boxing, and mounting. The results for all experimental subjects and control subjects summarized in Tables 2.6 and 2.7, respectively.

Table 2.6: Correlation of Cell Number With Agonistic Behaviour Frequency for Experimental Subjects.

Nucleus	Cell Type	Biting	Boxing	Mounting
VMHVL	microglia	-.68*		
MPO	oligodendrocytes		.48*	
PEF	chromato. neurons	.44*		
VM	astrocytes			-.45*
VM	microglia			-.45*
MDC	microglia			-.49*
MDM	oligodendrocytes		.53*	

*** indicates significance less than .05.**

Table 2.7: Correlation of Cell Number With Agonistic Behaviours for Control Subjects.

Nucleus	Cell Type	Biting	Boxing	Mounting
LH	astrocytes			-.76*
MPO	astrocytes			-.80*
PE	astrocytes		-.66*	
PH	chromato. neurons			.65*
PEF	healthy neurons	.79**		
CEL	healthy neurons		-.70*	
CEL	microglia		.67*	
MEA	healthy neurons	.78*		
BM	oligodendrocytes		-.74*	
MEPV	healthy neurons			.08**

The values depicted in Tables 2.6 and 2.7 indicate the same trend when employing a Spearman rho correlation.

Discussion

This study has replicated previous behavioural findings observed with lithium and pilocarpine seizure induced (experimental) subjects. Those experimental subjects housed in groups indicated an increase in agonistic behaviour (Persinger *et al.*, 1993), and those experimental subjects housed with 50% control subjects during the recovery period

indicated an absence of extreme levels of agonistic behaviour.

The significant differences in cell types by cage condition indicated a trend: the epileptic rats showed increased amounts of microglia in brain areas that involve autonomic control and decreased amounts of healthy neurons in the medial amygdala which is concerned with social behaviour (Adamec, 1990). However, only one nucleus was relevant in the prediction of agonistic behaviour, observed as bites, which was the frequency of microglia in the medial dorsomedial thalamus. The medial dorsomedial nucleus holds an interesting position in emotional states. This nucleus has extensive connections with the amygdala, the hypothalamus, and the cortex. Leonard (1972) has described that this nucleus is a modulator nucleus, an area that allows the higher functions such as planning and inhibition to control lower functions such as those observed in emotion. Findings from this study strengthen this description. This study demonstrated that increased damage within the medial dorsomedial thalamus could predict the frequency of biting displayed by seizured subjects using multiple regression.

Previous research (Desjardins & Persinger, 1995) studying agonistic behaviour subsequent to seizure induction by lithium and pilocarpine resulted in a correlation between the frequency of agonitic encounters with the number of central amygdala neurons. This correlation was not replicated in this study, nor was the cell counts within the central amygdala able to predict the observed frequency of agonistic encounters in the subjects. These differences may have been due to subtle changes in experimental procedure. In the present study, the total numbers of neurons per nucleus were divided

into healthy neurons and chromatolytic neurons (those that could not be classed confidently into either group were not included). This exclusion of non- classed neurons resulted in decreased numbers of neurons per brain, which may have been vital to the prediction. Differences in neuronal counts were also found between study groups, which may have been due to environmental conditions during seizure induction. Three different researchers induced the seizures within different annual seasons, which may have had an effect on the initial seizure damage. In addition, the previous study the subjects were restrained immediately subsequent to seizure induction and this did not occur during this study. These discrepancies may have had an impact on the number of neurons within the amygdala.

Another difference was that in this study a lower number of sections were counted per brain due to the increased sample size and the variable length of the central nucleus among rats. The difference in sample frequency should not have affected this study because all central amygdaloid sections had previously predicted agonistic behaviour frequency. This decrease in sample sections may have been relevant to prediction of aggression frequency if key subdivisions of the central amygdala involved with the observed agonistic behaviour were excluded from the sample. In order to verify counting consistency within each nucleus, which could have explained the inability to predict agonistic behaviour, a factor analysis was performed. A general pattern of damage was discovered in the factor analysis results. In most cases, microglia and chromatolytic cell frequencies loaded on the same factor and the healthy neurons loaded on another factor.

This is consistent with the literature on degeneration and was represented here as a measure of internal consistency between cell counts.

The effect of environmental changes on brain micro-structure was demonstrated by the ability to discriminate between the experimental subjects in the mixed (cage condition 2) and non-mixed (cage condition 3) cage conditions with 90% accuracy. The areas used to discriminate the groups included the basal lateral amygdala which is concerned with defensive behaviour (Adamec, 1990), the perifornical hypothalamic nucleus which is concerned with autonomic control, and the medial dorsal medial thalamus concerned with autonomic control (Paxinos, 1995) and the modulation of emotional behaviour (Leonard, 1972). The focus here is on the medial dorsal medial thalamus because the discriminant is based on the chromatolytic neurons and not glial cells as in the other two nuclei. It is the neurons that are mainly responsible for the observed behaviour. Anterograde and retrograde tracing of the medial portion of the dorsomedial thalamus has indicated that this region has reciprocal connections with the frontal cortices (Roeling *et al.*, 1994) the amygdala (anterior, basolateral and central regions) (Groenewegen, 1988) and the hypothalamus, including the perifornical nucleus (Allen & Cechetto, 1992). Leonard (1972) has stated that it is these connections of the dorsal medial dorsal thalamus which allow the fronal cortices to exert inhibitory control over the amygdala and the hypothalamus. Leonard (1972) also described the dorsal medial dorsal thalamus, in this context as a modulator for such behaviours as aggression. It is therefore logical to assume that as more damage has been done to this nucleus, then higher frequencies of agonistic

encounters may emerge.

The one-way analysis of variance results from Table 2.2 indicated a significant difference in the number of healthy neurons within the medial dorsomedial thalamus between the control and experimental conditions, but no significant differences in neuron numbers were found between the mixed and non-mixed experimental conditions. However, the discriminant analysis used the number of chromatolytic neurons in the medial dorsomedial thalamus to help define the two experimental conditions. In the non-mixed group significantly high frequencies of agonistic behaviour were observed, whereas in the mixed group low frequencies of agonistic behaviour were observed. The discriminant analysis defined the non-mixed experimental condition by a lower number of chromatolytic neurons. This was also evident by the interaction between the number of chromatolytic neurons within the medial dorsomedial nucleus between housing conditions (Fig.2.2). This suggests that after a significant loss of neurons to the medial dorsomedial thalamus (in the order of 50%) the remaining number of neurons could have had profound effects on the behaviour of the subjects. The environmental conditions may have affected the neurons which are in a "weak" condition, such as the chromatolytic neurons, and were vulnerable to subtle changes.

The observed numbers of astroglia decreased in the control and experimental mixed conditions as opposed to the non-mixed conditions. The medial preoptic area in the rat is involved in gonadosteroid function (Paxinos, 1995). The production of gonadosteroids can be affected by environmental conditions such as changes in

dominant/subordinate status. Hurst *et al.* (1993) demonstrated that the quality of urinary odors (reflecting hormonal changes) can change in response to social subordination, and Retana-Marquez *et al.* (1996) demonstrated that stress alone can induce suppression of testosterone secretion. These environmental conditions would seem likely to affect the neurochemistry of the brain as opposed to the microstructure. Previous research on astrocytes has concluded that these cells control the extracellular concentrations of glutamate, GABA, and biogenic amines (Parent, 1996), and display a high degree of phenotypic plasticity. Parent (1996) stated that astrocytes are able to respond to various stimuli by changing their form, functional state, and even their rate of multiplication. The current study suggests that changes in housing conditions can affect proliferation of astrocytes within the medial preoptic nucleus.

Upon examination of these results it is clear that the environment has an impact on the microstructure of the brain. Subtle changes in the environment can produce changes in the frequency of glial cells, astrocytes in particular, in different areas of the brain. The incidence of microglia within different nuclei is accompanied by cell damage and loss, and may have little to do with the environment. The profound changes in behaviour observed in brain-damaged subjects stems from environmental factors affecting the number of neurons within brain nuclei. The impact is not apparent until substantial loss of neuron number to key nuclei has occurred. At this point slight differences in proportion of vulnerable cells can have a strong impact on the behaviours exhibited by the individual. This was observed within the medial dorsomedial nucleus of the thalamus. The healthy

neurons in the experimental condition, although significantly lower in number than in the control condition, did not discriminate between mixed and non-mixed cage condition. It was the number of chromatolytic neurons that discriminated between mixed and non-mixed experimental conditions. This coincides with research in areas of debilitating diseases such as Parkinson's, where the symptoms of such are not observed until 80% of the dopaminergic cells within the substantia nigra compacta have been lost.

Chapter 4

The Effects of Local Administration of Tetrodotoxin to the Central Amygdala on Behaviour in Lithium Pilocarpine Induced Epileptic Rats

Abstract

Previous research has indicated that the central amygdala is involved in the frequency of aggressive behaviour in lithium and pilocarpine induced epileptic rats. The lithium/pilocarpine seizure model damages portions of the amygdala which inhibit the activity of the central amygdala. The central amygdala which modulates agonistic behaviour would then be disinhibited. In order to further define this involvement, the sodium channel blocker tetrodotoxin (TTX) was utilized to inhibit the central amygdala, with the use of a cannula and mini-osmotic pump. Presurgery aggressive behaviours were maintained following the cannula placement and a saline pump baseline period. Once TTX was pumped into the central amygdala, biting, boxing and activity level measured by the approximated distance traveled in meters per hour significantly decreased. Subsequent to the termination of the TTX delivery, these behaviours indicated no significant difference from the saline pump baseline measures. Once the effects of activity were covaried, the effects for biting and boxing were no longer significant. These results indicated that TTX

reduced overall activity of the subjects which subsequently decreased the frequency of observed aggression.

Introduction

Previous research has implicated the amygdala, particularly the basolateral and central nuclei, in mammalian aggression (Oakes & Coover, 1996). The central amygdala has been shown to be involved with the expression of fear, which could lead to defensive aggressive episodes (Fox & Sorenson, 1994). Various projections to the amygdala have been involved with the transmission of sensory stimuli associated with aversive events. Davis *et al.* (1994) stated that the projection to the central amygdala was the major intra-amygdaloid target of the basolateral complex, which is critical for autonomic and somatic responses produced by stimuli that were previously paired with aversive events. The interconnections of the amygdala allow for appropriate behavioural responses to afferent sensory information (Savander *et al.*, 1997). Savander *et al.* (1997) have reported that the amygdala of rodents contains interconnections to the contralateral side which allows for bi-hemispheric responses to sensory stimuli. Consequently these interconnections may also allow for the spread of seizure activity from one amygdala to the other (Savander *et al.*, 1997).

Seizure-induced brain damage using subcutaneous injections of lithium and

pilocarpine has been shown to increase the frequencies of agonistic behaviours (Persinger *et al.*, 1993). Desjardins and Persinger (1995), utilizing this model of brain damage, have shown that the incident of bites is highly positively correlated (r=.92) with the number of neurons within the central medial amygdala. Bedard and Persinger (1995) have indicated that the accompanied increase in aggression observed in subjects exposed to this treatment may have been due to the disinhibiton of the central medial amygdala. Various nuclei that inhibit the central amygdala are damaged due to seizure, which would allow the central nucleus to be disinhibited and the subjects would therefore display increased agonistic behaviour frequencies. These findings from lithium/pilocarpine treated subjects imply that the central amygdala may be the source of the increased aggression observed for these rats.

Circumscribed damage by lesioning is the most common method of determining the involvement of specific brain nuclei in the mediation of behavioural response. Another potential method of determining involvement is the use of tetrodotoxin microinjections into the nucleus in question. Tetrodotoxin is a reversible sodium - channel - dependent activity blocker which has been shown to produce reversible inactivation of a brain nucleus in a nanomolar range (Bermudez-Rattioni *et al.*, 1991). Upon inhibition of the sodium channels, a neuron can not produce an action potential, however the cell can still survive, and once the TTX has dissipated can resume normal functioning. This study examines the reversible effects of neural application of the sodium channel blocker tetrodotoxin on the agonistic behaviours of lithium and pilocarpine induced epileptic rats

by inhibition of the central amygdaloid nucleus..

Methodology

Animals

Adult male Wistar rats (350 g, approximately 70-110 days old; Charles River, St. Constance, Quebec) were held in groups of three under a 12 hour light: 12 hour dark cycle (light 0800-2000 hr) with food (Purina Rat Chow) and water available *ad libitum.*

Seizure Induction

Individual rats were subcutaneously (s.c.) injected with lithium chloride (3 mg/kg/ 10 ml saline; Sigma) followed 4 hours later by a s.c. injection of pilocarpine (30 mg/kg/ 10 ml saline; Sigma). To enhance their recovery the rats received a s.c. injection acepromazine (30mg/kg/ 10 ml saline; Sigma) 1 hour after pilocarpine administration. The rats were allowed to recover for 10 days after the injections and seizure induction.

The lithium and pilocarpine injection combination used here has been previously shown to induce seizures in rats and affect brain integrity (Honchar *et al.*, 1983). Seizure onset was confirmed by the subject rearing, followed by tonic-clonic contractions.

Recovery and Habituation

The subjects were returned to their original cage (three rats per cage) for 10 days of recovery. During this time they were given soft, liquified food (Purina Rat Chow mixed with water) for seven days and then returned to regular hard food (Purina Rat Chow). On day ten following seizure induction the subjects were transferred in groups of six to a clear plexiglass cage (101.5 x 101.5 x 50.5cm) and allowed 11 days to become habituated.

Behavioural Observations

Twenty-one days subsequent to seizure induction, nightly 1 hour observation periods began and lasted for 6 evenings under red light conditions. Behaviours recorded included: the frequency of biting defined as dental contact which produced tissue damage and/or squeaking from the recipient (Desjardins & Persinger, 1995), the frequency of boxing defined as two or more subjects rearing with both front feet off of the bedding (a subsequent boxing episode was not counted until all parties had at least one front foot back onto the bedding), the frequency of mounting defined as one subject grasping another with both front feet and delivering a series of three or more pelvic thrusts, the frequency of one subject grooming another, the duration of self-grooming, the frequency of eating and drinking and the distance the subject travelled. The subject acquired one distance score once it travelled the equivalent of one quarter the cage length during the observation period.

Surgery

The subjects underwent standard stereotaxic surgery 27 days after seizure induction. Unilateral cannulas (7.2 mm in length) were implanted with the tips located dorsal to the central amygdala using the following coordinates: -3.16 mm caudal, 4.4 mm lateral and 7.2 mm ventral from bregma (modified for 500 g rats from Paxinos and Watson, (1986)). The cannulas were anchored to the skull with jewelers screws and dental cement. A mini-osmotic pump implanted subcutaneously delivered 0.5 micro liters per hour of sterile saline (0.9% NaCl) for 14 days following this surgical procedure. The drug (TTX) and saline vehicle were delivered at 0.5 micro liters per hour in order to localize the effects of the TTX to the central amygdala. Studies performed by Salinas *et al.* (1996) demonstrated, using ibotenic acid, that no more than 0.5 micro liters should be delivered to brain nuclei (Myers, 1966). This low level was required in order to prevent lesions in neuronal tissue from too high of drug delivery volume, and to ensure the drug does not diffuse along the entire cannula track missing the target nucleus completely (Myers, 1966). The pump was attached to the cannulas by silastic tubing 2.8 cm in length with an inner diameter of 0.032 cm in order to ensure that pump contents would reach the target nucleus within 24 hours.

Time Chart of Treatment

Siezured_____Change cage ___ habituation__**observations**_ surgery___ **saline treatment**

10 days 11 days 6 days 6 days 6 days

recovery observation

change pump____**TTX treatment**_____ allow pump to drain___**no treatment**

1 day 6 days 1 day 6 days

recovery observation observation

Treatment

As indicated by the following time chart, the subjects were seizured using lithium and pilocarpine as described above. They were allowed 10 days to recover from the seizure before they were moved to the long plexiglass cage. The subjects were allowed to habituate to the new environment for 11 days. The subjects were observed for 6 days prior to surgery for an initial baseline reference. Following the cannula placement surgery the subjects received saline at a rate of 0.5 ul per hour for a total of 12 days. The post surgery observations occurred during the last 6 days of the saline treatment. The subcutaneous mini-osmotic pump was then replaced with a second subcutaneous mini-osmotic pump which contained tetrodotoxin (6.0 ng/ul; Sigma). The tetrodotoxin was delivered at a rate of 0.5 ul per hour for 7 days. The behavioural observations occurred during the last 6 days of the tetrodotoxin treatment. Post-tetrodotoxin behavioural observations began 9 days subsequent to the implantation of the tetrodotoxin mini-osmotic pump and occurred for 6 consecutive evenings.

Statistical Analysis

The mean and the standard deviation of each observed behaviour was calculated for each treatment condition. A multivariate analysis of variance was executed for the mean behaviours across the treatment conditions.

Results

A repeated multi variate analysis of variance was performed to determine if there was a differential relationship across treatments with respect to observed behaviours including: frequency of biting, boxing, mounting, grooming others, eating, drinking, duration of self-grooming, and total distance moved. The four treatments include: before surgery, after surgery with saline injection, after saline with tetrodotoxin injection, and after tetrodotoxin (TTX). The mean of the behaviours for each treatment observation block is summarized in Table 3.1.

Table 3.1 Effects of Tetrodotoxin (TTX) or Saline Vehicle on Agonistic Behaviour.

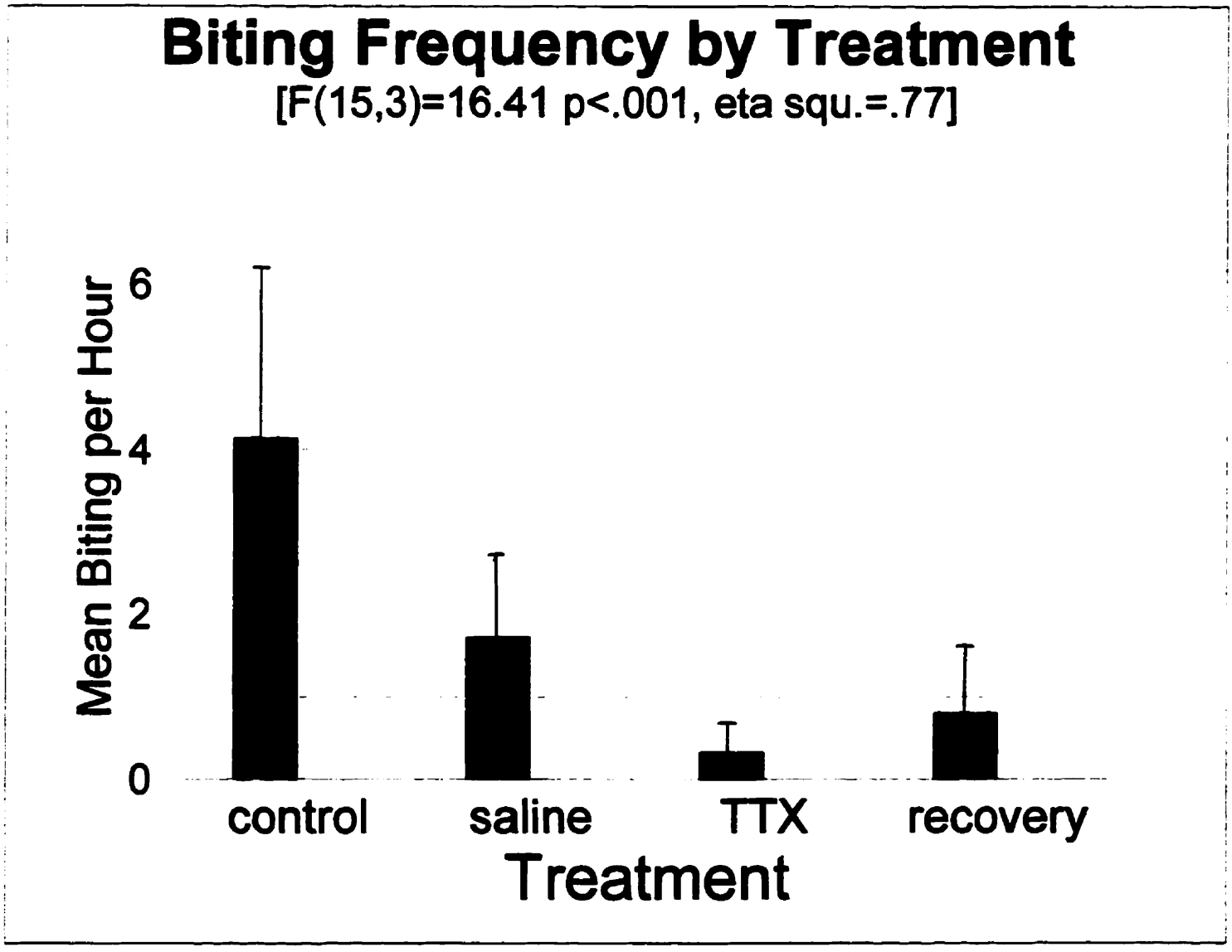
	Before Surgery	After Surgery with Saline	After Saline with TTX	After TTX
	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)
Bites	4.14 ± 2.09	1.73 ± 0.56	0.33 ± 0.30	0.81 ± 0.80
Boxing	11.19 ± 7.56	6.61 ± 1.29	1.83 ± 2.05	4.25 ± 3.31
Mounts	0.77 ± 0.51	0.50 ± 0.41	1.11 ± 1.30	0.44 ± 0.28
Grooming Others	4.14 ± 1.14	6.14 ± 0.93	8.08 ± 1.97	5.92 ± 2.26
Self Grooming*	2.62 ± 0.05	2.41 ± 0.11	2.54 ± 0.01	2.55 ± 0.09
Total Movement+	58.64 ± 10.12	32.11 ± 2.44	23.55 ± 8.30	29.78 ±4.70
Eating	2.44 ± 0.79	3.08 ± 1.23	3.05 ± 0.74	2.67 ± 0.57
Drinking	2.97 ± 0.26	2.30 ± 0.61	2.64 ± 0.81	1.50 ± 0.20

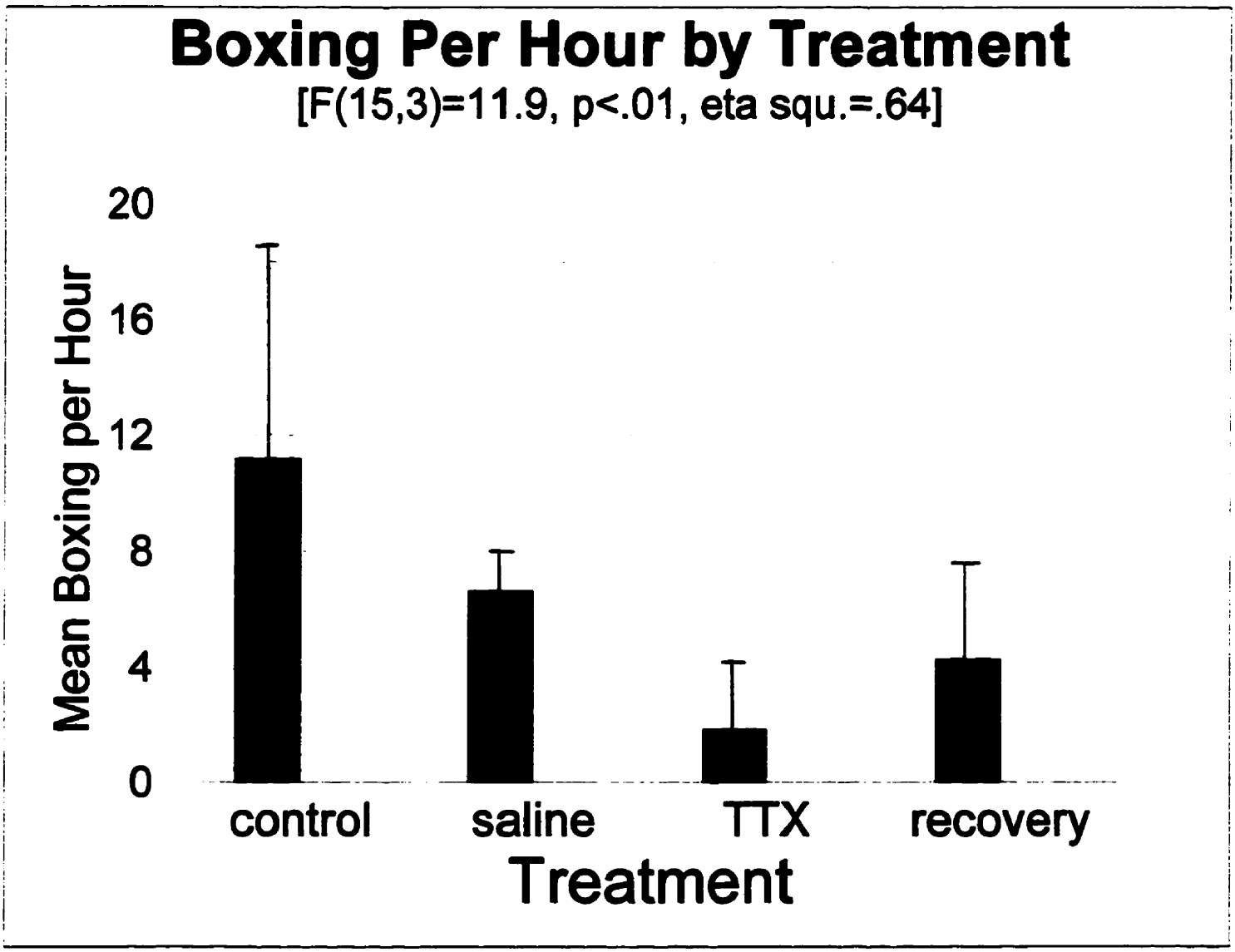
* indicates duration in log of mean seconds per hour

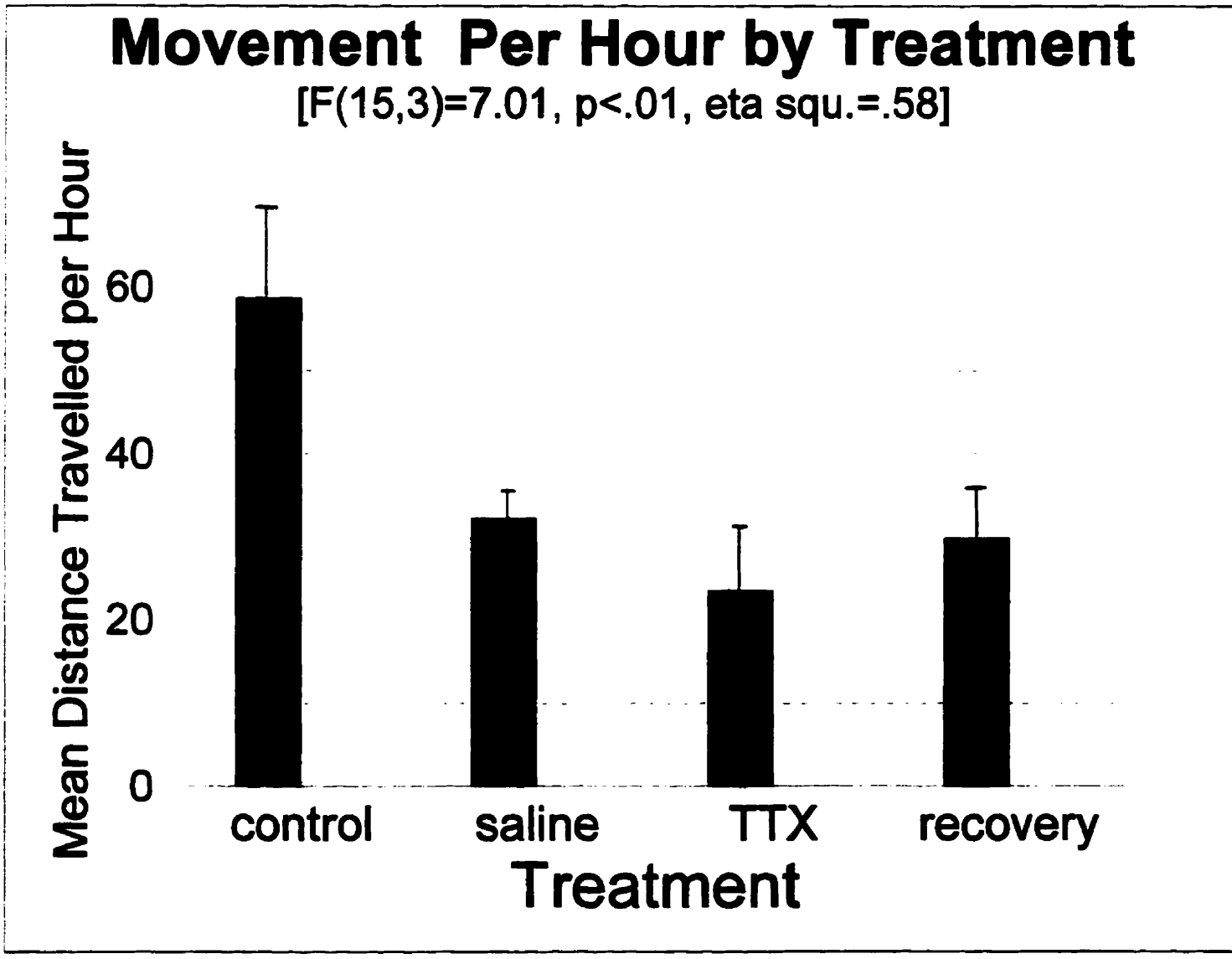
+ indicates mean of total distance in metres covered per hour, the other behaviours with no indicators are mean frequency per hour.

A significant main effect was found between the control observations and the TTX observations for bites [$F(15,3)=16.41$, $p<.001$, eta squ.=.77], with the control observations displaying significantly more bites per hour than the TTX observations (Figure 3.1). A significant main effect was also found between the control observations and the TTX observations for boxing [$F(15,3)=8.75$, $p<.01$, eta squ.=.64] with the control

group displaying significantly more boxing behaviour per hour than the subjects receiving TTX(Figure 3.2). A significant main effect was found between the control observations and both the TTX observations and the recovery observations for the total observed movement [F(15,3)=7.01. P<.01. Eta squ.=.58] with the control group displaying significantly more locomotion per hour than the subjects receiving TTX (Figure 3.3). Once biting and boxing were covaried with the total observed movement, these significant main effects were no longer observed.


Biting Frequency by Treatment
[F(15,3)=16.41 p<.001, eta squ.=.77]
Mean Biting per Hour
6
4
2
0
control
saline
TTX
recovery
Treatment


Boxing Per Hour by Treatment
[F(15,3)=11.9, p<.01, eta squ.=.64]
Mean Boxing per Hour
20
16
12
8
4
0
control
saline
TTX
recovery
Treatment


Movement Per Hour by Treatment
[F(15,3)=7.01, p<.01, eta squ.=.58]
Mean Distance Travelled per Hour
60
40
20
0
control
saline
TTX
recovery
Treatment

Discussion

This study indicates that administration of TTX decreased the frequency of biting and boxing per hour from the post-surgery control condition. The other behavioural components to this study included the duration of self-grooming, the frequency of grooming conspecifics, the frequency of eating and drinking and mounting. No significant differences were found for these other behaviours across treatments. Van Erp *et al.* (1994) reported that grooming was one method for rats to reduce arousal after a stressful situation. They reported a significant increase in self-grooming behaviour subsequent to stress induced by experimenter handling. These researchers described this increase in grooming behaviour to be significantly higher for subordinate subjects than for dominant subjects. Blanchard *et al.* (1993) showed that anxious rats displayed decreases in the frequency of eating and drinking. Changes in the duration of self-grooming, and the frequency of eating and drinking did not occur in this study. Changes in anxiety-based behaviour were anticipated due to results of lesional studies of the central medial amygdala that indicated that this region was involved in fear and anxiety (Fox & Sorenson, 1994; Oakes & Coover, 1996). The frequency of eating and drinking and the duration of self-grooming did not change significantly across treatments. The only recorded behavioural changes that occurred upon administration of TTX included the total movement per hour and the frequency of biting and boxing. These results suggest that the agonistic behaviours observed in lithium and pilocarpine induced epileptic subjects is not a defensive reaction to fear or constant high levels of anxiety. If this were the case, and

increase in grooming, and a decrease in eating and drinking would have been observed during the TTX treatment condition coinciding with the changes in agonistic behaviour.

The significant decrease in biting and boxing during the TTX administration was not significant once the effects of movement were co-varied. Haller (1995) has reported that energetic costs can constrain levels of aggression. He has listed three points demonstrating the relationship between aggression and energy metabolism: 1) animals prepare themselves metabolically for fighting; 2) there is a substantial energy mobilization during the fight; and 3) the metabolic consequences of the fight and the optimal use of energy resources may be of crucial importance regarding the survival of the animals. Haller (1995) stated that the control of aggression and the control of energetic processes related to aggression may have common factors suggesting the possibility of a behavioural-metabolic coregulation process. This study involved surgery and the use of the sodium channel blocking drug TTX. A decrease in locomotor activity was observed in this study suggesting that the administration of TTX decreased overall arousal, which subsequently decreased the frequency of biting and boxing for the subjects.

Another possible cause for the decreased general arousal may have been due to the amount of TTX administered. Previous research has injected TTX into specific brain nuclei with a cannula and single injection system which reported no significant decreases in arousal. This study delivered a constant flow of 0.5 micro liters per hour for 7 days. Although the delivery was maintained at 0.5 micro liters, the TTX was given at a constant rate. This increase in overall amount of TTX may have caused the TTX to saturate the

central amygdala and it may have travelled to other regions, such as along the cannula to affect cortical regions. This increase may have decreased general arousal of the subjects and therefore may be too high a concentration to observe a bonifide effect of treatment. There was also a decreasing trend of arousal following the initial surgery. The frequency of biting and boxing was not significantly reduced, however the total distance covered per hour was significantly reduced in the post surgery (saline) treatment. This suggests that a longer recovery period may have been required.

The results of this experiment indicated no significant increase in agonistic behaviour or total distance covered per hour following the TTX treatment. Examination of the increasing trend in these behaviours during this recovery period suggests that perhaps the TTX was not fully metabolized from the central amygdala. The pumps were allowed to drain, however they were not removed or blocked in any way making it entirely possible that some subjects were still receiving TTX during this observation period. Future studies would benefit from a lower delivery rate, a longer recovery time from surgery and complete cessation of TTX delivery following the TTX treatment period.

Chapter 5

Summary and Conclusion

This thesis combined behavioural observations and histology in order to further define the mechanism of conspecific agonistic behaviour observed in rats with lithium and pilocarpine induced epilepsy. The significant agonistic behaviours included biting, which defined as dental contact, which produced a lesion and squeaking in the recipient (Desjardins & Persinger, 1995), and boxing which was defined as two subjects rearing while facing each other with both forefeet off the bedding. The frequencies of these two agonistic behaviours were significantly higher in housing conditions that contained only seizured subjects when compared to housing conditions that contained only non-epileptic subjects (controls). This suggested that the epileptic subjects were prone to displaying agonistic behaviour which was evident in the volatile environment of six epileptic subjects housed together.

In order to observe changes in the frequency of aggression, the cage conditions were altered. Subjects were housed in one of seven types of cage conditions: six seizured rats (7), five seizured rats and one control rat (6), four seizured rats and two control rats (5), three seizured rats and three control rats (4), two seizured rats and four control rats (3), one seizured rat and five control rats (2), and six control rats (1). The subjects in cage conditions 7 and 6 displayed a significantly higher frequency of agonistic behaviour than all other cage conditions. This significant increase in cage conditions 6 and 7

suggested a modulatory response on the frequency of biting and boxing which was dependent on the ratio of exposure to control subjects.

After 1 month, subsequent to seizure induction had passed, the inhibitory effect of housing seizured subjects with control subjects on agonistic behaviour was no longer observed. This suggested that environmental conditions may have detrimental and lasting effects on recovery from brain damage. During recovery, the neurons which have been damaged can either die or recuperate to a healthy condition. At this time, it is possible that subtle environmental changes can affect the microstructure of the brain.

Histological analysis of the brains of epileptic rats housed uniformly (conditon 7), epileptic rats housed with control rats (condition 4), and control rats (condition 1) indicated that the thalamus, hypothalamus, and amygdala were involved in the observed agonistic behaviour. The frequency of healthy neurons, chromatolytic neurons, oligodendrocytes, astrocytes and microglia, were counted within selected regions of the hypothalamus, thalamus, and amygdala. Statistical analysis of the data resulted in a significant 100% discriminant function between subjects housed in conditions 1 and 7. This discriminant function indicated a high score for subjects housed in condition 7, which resulted in more microglia within the basolateral amygdala, more oligodendrocytes within the lateral ventral lateral amygdala, and fewer healthy neurons within the medial dorsomedial thalamus. There was extensive damage to the brains of the seizured rats. However, it was these three nuclei that defined the epileptic condition in the discriminant analysis. According to Ray and Price (1992) and Groenewegen *et al.*(1990) there are

reciprocal connections between the basolateral and lateral amygdala with the medial dorsomedial thalamus. These regions are also reciprocally connected to the prefrontal cortex. Leonard (1972) has implied that these connections are involved with the ability of higher functional centers to control the more primitive elements of mental activity such as emotional expression.

Once the brains of epileptic rats in condition 7 and epileptic rats housed in conditon 4 (3 controls: 3 epileptic) were analyzed, a discriminant function could correctly discriminate between the two groups with 90% accuracy. This discriminant function indicated a high score for subjects housed in conditon 7 which resulted in fewer chromatolytic neurons within the medial dorsomedial thalamus, more microglia within the perifornical hypothalamic nucleus, and more oligodendrocytes within the basolateral amygdala. The differences between these two groups were strictly environmental. Due to the change in environmental conditions, a significant difference in agonistic behaviour occurred between the two groups. In this analysis a lower number of chromatolytic neurons in the medial dorsomedial thalamus comprised a portion of the discriminant equation. This was contradictory to what was expected until the number of healthy neurons between the two groups was examined. Significant differences in number of healthy neurons were not found between these groups. The only discrepancy between the number of neurons between conditions 7 and 4 was between the number of chromatolytic neurons. The subjects housed in condition 4 had a higher incidence of neurons (defined by the discriminant analysis) in the medial dorsomedial thalamus. Although chromatolytic

neurons are damaged, they are still functioning. These results inferred that once a large portion of the neuron frequency in the medial dorsomedial thalamus has been destroyed then further reductions in the number of neurons within this area can be detrimental to the control of emotional states, such as agonistic behaviour. In condition 7 there was a lower overall number of neurons in the medial dorsomedial thalamus than in condition 4.

The resultant microstructural changes between the two seizured conditions stem from environmental conditions. The changes in environmental conditions included exposure to control subjects during the recovery period. Previous literature inferred that the component of social interaction which most likely induced these results was, perhaps, olfactory related. Analysis of olfactory projections indicated major projections to the mediodorsal thalamus and amygdala (Leonard, 1972; Ray & Price, 1992). There are also reciprocal connections between the entorhinal cortex, the dorsomedial thalamus and the prefrontal cortex which have been implicated in the control of emotional expression (Leonard, 1972; Ray & Price, 1992). Perhaps some component of the olfactory cues of control subjects stimulated the olfactory bulbs and entorhinal cortices. These two areas activate reciprocal connections to the dorsomedial thalamus, amygdala, and prefrontal cortex which, in the lithium/pilocarpine model, stimulation of these areas by exposure to control subjects may have prevented further damage to the chromatolytic neurons within the dorso medial thalamic nucleus. This hypothesis would have to be determined in further studies in this area.

The brains of the epileptic and control subjects in condition 4 (3:3 ratio) were

analyzed with the control subjects in condition 1 and seizured subjects in condition 7. The results indicated a significant interaction for the frequency of astrocytes in the preoptic hypothalamic nucleus. For both the seizured and control groups, a decrease in astrocytes was observed in condition 4. The biggest discrepancy was found between conditions (1 and 4) for the control subjects. The medial preoptic area in the rat is involved in gonadosteroid function (Paxinos, 1995). The production of gonadosteroids can be affected by environmental conditions such as stress (Hurst *et al.*, 1993; Retana-Marquez *et al.*, 1996). Stress-inducing changes in environmental conditions would likely affect neurochemistry before it would affect microstructure. Previous research on astrocytes demonstrated that these cells participate in the regulation of the extracellular environment (Parent, 1996). Parent (1996) has indicated that astrocytes respond to various stimuli by changing their form, their functional state, and their rate of multiplication. This thesis has demonstrated that environmental conditions has induced changes in the number of astrocytes in the region of the medial preoptic nucleus.

Chapter Four in this thesis was intended to further define the involvement of the central medial amygdala in agonistic behaviour by treatment with TTX. This study involved observations of the frequency of biting, boxing, mounting, eating, drinking, grooming others, the duration of rest, and movement. This study included observations of the same seizured subjects that were group housed under four different conditions: 1) before cannula placement surgery, 2) after cannula placement surgery with saline administration, 3) after cannula placement surgery with TTX administration, 4) after TTX

administration. The results indicated a significant decrease in the frequency of biting, boxing, and the duration of movement during TTX administration. The increase in biting, boxing, and movement could indicate a possible anxious state in the epileptic subjects. This hypothesis was not supported once biting and boxing were covaried with the mean distance the subjects moved per hour. Unfortunately, this study has indicated that administration of 0.5 micro liters per hour of TTX (6 ng/ul) for 7 days has an overall sedative effect on the subjects. The accompanying decrease in agonistic behaviour was most likely an effect of global sedation. The involvement of the central medial amygdala could not be determined by this study.

Overall this thesis has demonstrated that factors affecting social interaction can influence brain microstructure. In control subjects, environmental conditions may affect proliferation of astrocytes which is consistent with the literature. In epileptic individuals, environmental conditions can induce small changes in medial dorsomedial thalamic neuron frequency which can have a dramatic effect on agonistic behaviour. The environmental conditions inducing frequency changes in the medial dorsomedial thalamus may involve olfactory, or some interaction of sensory stimuli, based social interactions between epileptic and control subjects.

References

Adamec, R.E. Role (1990). of the amygdala and medial hypothalamus in spontaneous feline aggression and defense. *Aggressive Behavior, 16*, 207-222.

Adamec, R.E. (1991). Partial kindling of the ventral hippocampus: identification of changes in limbic physiology which accompany changes in feline aggression and defense. *Physiology and Behavior, 49*,443-453.

Archer, J. (1988). Mechanisms of protective aggression and their relation to function. In *The behavioural biology of aggression.* (Eds. Barlow, G.W., Bateson, P.P.G., & Oppenheim, R.W.) Pp.44-68. Cambridge University Press.

Bandler, R.J. (1969). Facilitation of aggressive behaviour in the rat by direct cholinergic stimulation of the hypothalamus. *Nature, 224*, 1035-1036.

Bandler, R.J. (1971). Direct chemical stimulation of the thalamus: effects on aggressive behavior in the rat. *Brain Research, 26*, 81-93.

Bedard, A.W., & Persinger, M.A. (1995). Prednisolone blocks extreme intermale social aggression in amygdaloid-damaged rats: implications for the central nucleus, corticotrophin releasing factor, and electrical seizures. *Psychological Reports 77*: 3-9.

Beckett, S., & Marsden, C.A. (1995). Computer analysis and quantification of periaqueductal grey-induced defense behavior. *Journal of Neuroscience Methods, 58*, 157-161.

Bekoff, M. (1981). Development of agonistic behaviour: ethological and ecological aspects. In Multidisciplinary approaches to aggression research. Amsterdam: Elsevier North Holland.

Bermudez-Rattioni, F. & McGaugh, J.L. (1991). Insular cortex and amygdala lesions differentially affect acquisition on inhibitory avoidance and conditioned taste aversion. *Brain Research, 549*, 165-170.

Bernstein, I.S. (1981). dominance: the baby and the bathwater. *The Behavioral and Brain Sciences, 4*, 419-429.

Blanchard, D.C., Blanchard, R.J.; Lee, M.C.& Williams, G. (1981). Taming in the wild Norway rat following lesions in the basal ganglia. *Physiology and Behavior, 27*, 995-1000.

Blanchard, D.C. & Blanchard, R.J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. *Journal of Comparative Physiological Psychology, 81*, 281-290.

Blanchard, D.C., Hori, K., Rodgers, R.J., Hendrie, C.A., & Blanchard, R.J. (1989). Attenuation of defensive threat and attack in wild rats (*Rattus rattus*) by benzodiazepines. *Psychopharmacology, 97*, 397-401.

Blanchard, D. C. & Blanchard, R.J. (1990). Behavioral correlates of chronic dominance-subordination relationships of male rats in a seminatural situation. *Neuroscience and Biobehavioral Reviews, 14*, 455-462.

Brain, P. (1975). What does individual housing mean to a mouse? *Life Sciences, 16*, 187-200.

Brain, P, F. (1981). Attempts to determine the status of electroshock-induced attack in male laboratory mice. *Behavioural Processes, 6*, 171-189.

Bureau, Y.R. J., Peredery, O.& Persinger, M.A. (1994). Concordance of quantitative damage within the diencephalon and telencephalon following systemic pilocarpine (380 mg/kg) or lithium (3 mEq/kg) pilocarpine (30 mg/kg) induced seizures. *Brain Research, 648*, 265-269.

Clifford, D.B., Olney, W.J., Maniotis, A., Collins, R.C. & Zorumski, C.F. (1987). The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures. *Neuroscience, 23*, (3), 953-968.

Cole, H.G. & Wolf, H.H. (1966). The effects of some psychotropic drugs on conditioned avoidance and aggressive behaviors. *Psychopharmacologia, 8*, 389-396.

Connor, J. (1972). Olfactroy control of aggressive and sexual behavior in the mouse (*Mus musculus L.*). *Psycoanalytical Science, 27*, 1-3.

Cooper, J.R., Bloom, F. E.& Roth, R.H. (1991). Acetylcholine. In The *biochemical basis of neuropharmacology*. Oxford University Press.

Davis, M. (1992). The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. *The International Journal of Progressive Sciences, 13*, 35-41.

DeFeudis, F.V. (1974). Cholinergic roles in motivated behaviours. In Central cholinergic systems and behaviour. London Academic Press.

Denenberg, V.H., Hudgens, G.A.& Zarrow, M.X. (1963). Mice reared with rats: modification of behavior by early experience with another species. *Science, 143*, 380.

Denenberg, V.H. (1973). Developmental fectors in aggression. In The control of aggression. Chicago.

Desjardins, D. & Persinger, M.A. (1995). Association between intermale social aggression and cellular density within the central amygdaloid nucleus in rats with lithium/pilocarpine-induced seizures. *Perceptual and Motor Skills, 81*, 635-641.

Edwards, D.A., Nahai, F.R., Wright, P. (1992). Pathways linking the olfactory bulbs with the medial preoptic anterior hypothalamus are important for intermale aggression in mice. Brief Communication, *Behaviour and Biology, 48*, 611, 615.

Fanselow, M.S. (1985). Odors released by stressed rats produce opiod analgesia in unstressed rats. *Behavioral Neuroscience 99*,(3) 589-592.

Fox, R.J., & Sorenson, C.A. (1994). Bilateral lesions of the amygdala attenuate analgesia induced by diverse environmental challenges. *Brain Research, 648*, 215-221.

Ginsburg, B, & Allee, W.C. (1942). Some effects of conditioning on sicial dominance and subordination in inbred strains of mice. *Physiological Zoology, 15*, 485-506.

Gosling, L.M., & McKay, H.V. (1990). Competitor assessment by scent matching: an experimental test. *Behavioral Ecology and Sociobiology, 26*, 415-420.

Griebel, G., Blanchard, C.D. & Blanchard, R. J. (1996). Evidence that the behaviors in the mouse defense test battery relate to different emotional states: a factor analytic study. *Physiology and Behavior, 60*, (5), 1255-1260.

Grijalva, C.V., Levin, E.D., Morgan, M., Roland, B. & Martin, F.C. (1990). Contrasting effects of centromedial and basolateral amygdaloid lesions on stress-related responses in the rat. *Physiology and Behavior, 48*, 495-500.

Groenewegen, H.J., Berendse, H.W., Wolters, J.G., & Lohman, A. H. M. (1990). The anatomical relationship of the prefrontal cortex with the striatopallidal system, the thalamus and the amygdala: evidence for a parallel organization. *Progress in Brain Research, 85*, 95-116.

Guillot, P.V., & Chapouthier, G. (1996). Olfaction, GABAergic neurotransmission in the olfactroy bulb, and intermale aggression in mice: modulation by steroids. *Behavior*

Genetics, 26,(5), 497-504.

Haefely, W. (1990). The GABA-benzodiazepine interaction fifteen years later. Neurochemical Research, 15, (2), 169-174.

Halpern, M. (1987). The organization and function of the vomeronasal system. *Annual Reviews of Neuroscience, 10,* 325-362.

Harlow, H.F., Dodsworth, R.O., & Harlow, M.K. (1965). Total isolation in monkeys. *Proceedings of the National Academy of Sciences, 54,* 90-97.

Harrigan, T., Peredery, O., & Persinger, M.A. Failure to acquire an inhibitory task following seizure-induced brain damage. *Perceptual Motor Skills, 70,* 268-270; 1990.

Herrada, G., & Dulac, C. (1997). A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. *Cell, 90,* 763-773.

Hichcock, J.M., & Davis, M. (1986). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. *Behavioral Neuroscience, 100,* 11-22.

Honchar, M.P., Olney, J.W., & Sherman, W.R. (1983). Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. *Science, 220,* 323-325.

Hurst, J.L., Fang, J., & Barnard, C.J. (1993). The role of substrate odours in maintaining social tolerance between male house mice, *Mus musculus domesticus. Animal Behavior, 45,* 997-1006.

Hurst, J. L., Fang, J., & Barnard, C.J. (1994). The role of substrate odours in maintaining social tolerance between male house mice, *Mus musculus*: relatedness, incidental kinship effects and the establishment of social status. *Animal Behavior, 48,* 157-167.

Jones, R.B., & Nowell, N.W. (1973). Aversive and aggression-promoting properties of urine from dominant and subordinate male mice. *Animal Learning and Behavior, 1,* 207-210.

Kapp, B.S., Gallagher, M., Underwood, M.D., McNall, C.L.& Whitehorn, D. (1982). Cardiovascular responses elicited by electrical stimulation of the amygdala central nucleus in the rabbit. *Brain Research, 234,* 251-262.

Koolhaas, J.M., van den Brink, T.H.C., Roozendaal, B.& Boorsma, F. (1990). Medial amygdala and aggressive behavior: Interaction between testosterone and vasopressin. *Aggressive Behavior, 16,* 223-229.

Krettek, J.E., & Price, J.L. (1978). A description of the amygdaoid complex in the rat and cat with observations on intra-amygdaloid axonal connections. *Journal of Comparative Neurology, 178,* 255-280.

Krsiak, M., & Sulkova, A. (1990). Differential effects of six structurally related benzodiazepines on some ethological measures of timidity, aggression and locomotion in mics. *Psychopharmacology, 101,* 396-402.

Kruk, M.R., Van der Laan, C. E., Meelis, W., Phillips, R.E., Mos, J., & Van der Laan, C. E. (1984). Brain stimulation induced agonistic behaviour: a novel paradigm in ethopharmacological aggression research. In Miczed, K.A. Kruk, M.R., Olivier, B., eds. *Ethopharmacological aggression research. Progress in clinical and biological research, 167.* New York Alan Liss.

Kruk, M.R. (1991). Ethology and pharmacology of hypothalamic aggression in the rat. *Neuroscience and biobehavioral Reviews, 15,* 527-538.

Kuo, Z.Y. (1960). Studies on the basic factors in animal fighting. IV. Developmental and environmental factors affecting fighting in quails. *Journal of Genetic Psychology, 96,* 225-239.

Kuo, Z.Y. (1967). *The dynamics of development: An epigenetic view.* New York Random House.

Lagerspetz, K. & Mettala-Portin, R. 1968 Studies on the aggressive behaviour of mice. *Annales Academiae Scientiarum Fennicae, series B, 131,(3),* 7-131;

Leibowitz, S. F., Miller, N. E. (1969). Unexpected adrenergic effect of cholopromazine: eating elicited by injection into rat hypothalmus. *Science, 165,* 609-611.

Leonard, C.M. (1972). The connections of the dorsomedial nuclei. *Brain, Behaviour and Evolution, 6,* 524-541.

Liu, Z., Nagao, T., Desjardins, G.C., Gloor, P., & Avoli, M. (1994). Quantitative evaluation of neuronal loss in the dorsal hippocampus in rats with long-term pilocarpine seizures., *Epilepsy Research, 17,* 237-247.

Mason, W.A. (1960). The effect of social restriction on the behavior of rhesus monkeys. 1. Free social behavior. *Journal of Comparative and Physiological Psychology. 53*, 582-589.

Mason, W.A. (1963). The effects of environmental restriction on the social development of rhesus monkeys. In *Primate Social Behavior*, ed. Southwick, C. H. pp. 161-173. Princeton: Van Nostrand.

Mendelsohn, F.A. & Paxinos, G. (1991). *Trends in Neuroscience, 42*,(1), 245-260.

Miczek, K.A., Weerts, E., Haney, M., & Tidey, J. (1994). Neurobiological mechanisms controlling aggression: preclinical developments for pharmacotherapeutic interventions. *Neuroscience and Biobehavioral Reviews, 18*, (1), 97-110.

Myers, R.D. (1966). Injection of solutions into cerebral tissue: relation between volume and diffusion. *Physiology and Behavior, 1*, 171-174.

Namikas, J., & Wehmer, F. (1978). Gender composition of the litter affects behavior of male mice. *Behavioral Biology, 23*, 219-224.

Nikulina, E.M. (1991). Neural control of predatory aggression in wild and domesticated animals. *Neuroscience and Biobehavioral Reviews, 15*, 545-547.

Oakes, M.E., & Coover, G.D. (1997). Effects of small amygdala lesions on fear, but not aggression, in the rat. *Physiology and Behavior, 61*,(1), 45-55.

Olney, J.W., Gubareff, T. & Labruyere, J. (1983). Seizure-related brain damage induced by cholinergic agents. *Nature, 301*, 520-522.

Parent, M.B., & McGaugh, J.L. (1994). Posttraining infusion of lidocaine into the amygdala basolateral complex impairs retention of inhibitory avoidance training. *Brain Research, 661*, 97-103.

Parent, A. (1996). Hypothalamus. In *Carptenter's human neuroanatomy*. 1011pp. London. Wiliams and Wilkins.

Paxinos, G. (1995). Amygdala and extended amygdala. pp 495-572 ed. Paxinos, G. In *The Rat Nervous System*, Academic Press.

Pellis, S.M., McKenna, M.M., Pellis, V.C., Prusky, G.T. & Whishaw, I.Q. (1996). Uses of vision by rats in play fighting and other close-quarter social interactions. *Physiology and*

Behavior, 59, (4), 905-913.

Persinger, M.A., Makarec, K. & Bradley, J.-C. (1988). Characteristics of limbic seizures evoked by peripheral injections of lithium and pilocarpine. *Physiology and Behavior, 44*, 27-37.

Persinger, M.A., Bureau, Y.R.J., Kostakos, M., Peredery, O., & Falter, H. (1993). Behaviors of rats with insidious, multifocal brain damage induced by seizures following single peripheral injections of lithium and pilocarpine. *Physiology and Behavior, 53*, 849-866.

Persinger, M.A. (1997). Geomagnetic variables and behaviour: LXXXIII, increased geomagnetic activity and group aggression in chronic limbic epileptic male rats. Perceptual and Motor Skills, 85, 1376-1378.

Ray, J.P., & Price, J.L. (1992). The organization of the thalamocortical connections of the mediodorsal thalamic nucleus in the rat, related to the ventral forebrain-prefrontal cortex topography. *The Journal of Comparative Neurology, 323*, 167-197.

Restak, R. (1984). Stress and emotion. In *The brain*. Books Toronto 371pp. Bantam.

Retana-Marquez, S., Salazar, E. D. & Velazquez-Moctezuma, J. (1996). Effect of acute and chronic stress on masculine sexual behavior in the rat. *Psychoneuroendocrinology, 21*, 39-50.

Rhee, S.G., Suh, P. G., Ryu, S.H., & Lee, S.Y. (1980). Studies of inositol phospholipid-specific phospholipase C. *Science, 244*, 546-550.

Roeling, T.J., Veening, J.G., Kruk, M.R., Peters, J.P.W., Vermelis, M.E.J., & Nieuwenhuys, R. (1994). Efferent connections of the hypothalamic "aggression area" in the rat. *Neuroscience, 59*,(4), 1001-1024.

Ropartz, P. (1968). The relation between olfactory stimulation and aggressive behavior in mice. *Animal Behaviour, 16*, 97-100.

Rowe, F.A. & Edwards, D.A. (1971). Olfactory bulb removal: influences on the aggressive behaviours of male mice. *Physiology and Behavior, 7*, 889-892.

Salinas, J.A., Parent, M.B., & McGaugh, J.L. (1996). Ibotenic acid lesions of the amygdala basolateral complex or central nucleus differentially effect the response to reductions in reward. *Brain Research, 742*, 283-293.

Sandnabba, N.K. (1995). Predatory aggression in male mice selectively bred for isolation-induced intermale aggression. *Behavioural Genetics, 25*,(4), 361-366.

Savander, V., Ledoux, J.E. & Pitcanen, A. (1997). Interamygdaloid projections of the basal and accessory basal nuclei of the rat amygdaloid complex. *Neuroscience, 76*,(3), 725-735.

Scott, J.P., & Fredericson, E. (1951). The causes of fighting in mice and rats. *Physiological Zoology*, 24,(4), 273-309.

Siegel, G.J., Agranoff, B.W., Albers, R.W., & Molinoff, P.B. (1994). Epileptic seizures. p 885-898 In *Basic Neurochemistry*. Raven Press.

Sgoifo, A., Aimi, B., Parmigiani, S., Manghi, M. & Musso, E. (1994). Behavioral and electrocardiographic responses to social stress in male rats. *Physiology and Behavior, 55*, (2), 209-216.

Taylor, G.T. (1980). Reinforcement and intraspecific aggressive behavior. *Behavioral and Neural Biology*, 27, 1-24.

Valenta, J.G. & Rigby, M.K. (1968). Discrimination of the odour of stressed rats. *Science, 161*, 599-601.

Vivian, J.A., & Miczek, K.A. (1993). Morphine attenuates ultrasonic vocalization during agonistic encounters in adult male rats. *Psychopharmacology, 111*, 367-375.

Vochteloo, J.D. & Koolhaas, J.M. (1987). Medial amygdala lesions in male rats reduce aggressive behavior: interference with experience. *Physiology and Behavior, 41*,99-102.

Weerts, E.M., Tornatzky, W., & Miczek, K.A. (1993). "Anxiolytic" and "anxiogenic" benzodiazepines and beta-carbolines: effects on aggressive and social behavior in rats and squirrel monkeys. *Psychopharmacology, 110*, 451-459.

Welch, B.L. & Welch, A.S. (1968). Differential activation by restraint stress of a mechanism to conserve brain catecholamines and serotonin in mice differing in excitability. *Nature, 218*, 575-577.

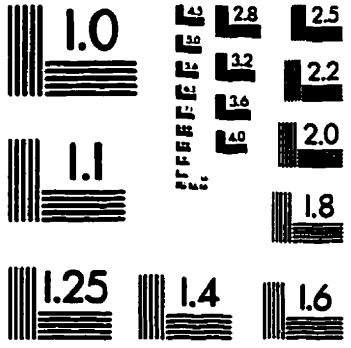
Appendix

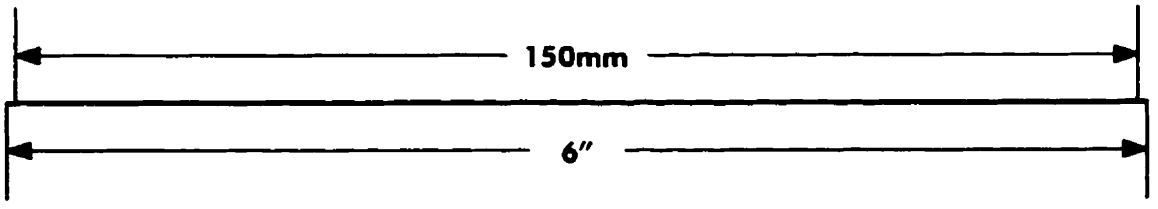
Appendix 1

The following is a list of names and abbreviations used in chapter 3 of this thesis.

Name	Abbreviation
lateral hypothalamus	LH
ventral medial hypothalamus ventral lateral	VMHVL
medial preoptic nucleus	MPO
the lateral preoptic nucleus	LPO
periventricular hypothalamus	Pe
posterior hypothalamus	PH
perifornical nucleus	PeF
ventromedial thalamus	VM
central dorsomedial thalamus	MDC
medial dorsomedial thalamus	MDM
central lateral amygdala	CeL
medial anterior amygdala	MeA
central medial amygdala	CeM
basal lateral anterior amygdala	BLA
basal lateral ventral amygdala	BLV
basal medial amygdala	BM
medial posterior ventral amygdala	MePV
lateral ventral lateral amygdala	LaVL

TEST TARGET (QA-3)





APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989