

The Contribution of Age and Sex to Emotionality in Two Strains of Rats Bred
for Differences in Amygdala Kindling Susceptibility

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ABSTRACT

Previous studies on rodent emotionality indicated that age and sex contribute to this phenomenon and the amygdala is a neural structure important for fear. The present study was interested in determining if these variables were involved with two strains of rats that were selectively bred for their differences in amygdala excitability as seen in their differential rates of amygdala kindling. Both sexes of the kindling-prone (Fast) and kindling-resistant (Slow) rats were tested in the elevated plus maze and open field as juveniles at 30 and 32 days of age, respectively, and subsequently as adults at 90 and 92 days of age, respectively. Following these two tests in the elevated plus maze and open field, the same rats were trained and tested in the conditioned emotional response unit as adults over the course of 13 days. Following fear conditioning, rats underwent extinction for an additional 13 days. Results indicated that there were little sex differences in the elevated plus maze, open field and conditioned emotional response unit. There were age differences recorded in the elevated plus maze and open field, suggesting that younger rats are less fearful than older rats in both strains. Older animals of both strains increased total arm entries in the elevated plus maze compared to younger animals. In addition, Fast rats tended to be less fearful at both juvenile and adult ages, and fearfulness and activity tended to be independent measures. The Fast rats spent more time in the center of the elevated plus maze at both ages. Fast rats tended to spend more time in the center zone and entered the center zone of the open field more often than Slow animals. In the conditioned emotional response test, Slow rats had lower suppression scores than Fast animals during both fear conditioning and extinction. This was particularly prevalent in

the females, whereas the males of each strain had similar suppression scores. It was concluded that Fast rats were less fearful than Slow rats and it was suggested that selection for focal excitability or seizure susceptibility to amygdala kindling contributed to these differences.

ACKNOWLEDGEMENTS

Although this thesis used rodents in order to understand fear, the general theme in behavioural neuroscience is to acquire knowledge from an evolutionarily related species and apply that knowledge to our own kind. Indeed, kindling is an animal model that seeks to understand and, perhaps solve the problem of human temporal lobe epilepsy. The deep need to understand humanity and the fascination with epilepsy have co-existed in antiquity and many of man's neurological diseases has shed some light on his place in the universe. Like many scientists of human nature, it is this deep rooted urge in solving the problem of "what man is" that drives my research and as that great novelist and epileptic Dostoevsky once noted "[m]an is a mystery: if you spend your entire life trying to puzzle it out, then do not say you have wasted your time." Enough said!

Michael S. Gazzaniga once stated "[p]sychology itself is dead" and the "odd thing is that everyone but its practitioners knows about the death of psychology." Indeed, mind (or soul) does not exist. It is a fictitious human creation misdirecting our behaviour. So what is man? There may not be any real answer to this question but there may be some truths and perhaps the following quotes are closer to our veridical selves. The first is by Dostoevsky and a second by Nietzsche who indicated that Dostoevsky had shed more light on human nature than any psychologist and from what I can gather this statement is just as true today. The third and fourth are by the novelists Steinbeck and Lawrence, respectively.

[M]an is pre-eminently a creative animal, predestined to strive consciously for an object and to engage in engineering...[m]an likes to make roads and to create, that is a fact beyond dispute (Fyodor Dostoevsky).

To the despisers of the body will I speak a word. That they despise is caused by their esteem. What is it that created esteeming and despising and worth and will? The creating Self created for itself esteeming and despising, it created for itself joy and woe. The creating body created for itself spirit, and as a hand to its will. Even in your folly and despising ye each serve your Self, ye despisers of the body. I tell you, your very Self wanteth to die, and turneth away from life. No longer can your Self do that which it desireth most: - create beyond itself. That is what it desireth most; that is all its fervour. (Friedrich Nietzsche).

The last clear function of man...minds aching to create beyond the single need - this is man...[t]o build a wall, to build a house, a dam... (John Steinbeck).

It is the desire of the human male to build up out of his own self and his own belief and his own effort something wonderful. Not merely something useful. Something wonderful. Even the Panama Canal would never have been built simply to let ships through. It is a pure disinterested craving of the human male to

make something wonderful, out of his own head and his own self and his own soul's faith and delight, which starts everything going" (David H. Lawrence).

Indeed, this thesis is just that, a human (brain, not mind or soul, and behaviour) creation and there are a number of people who contributed to it for whom I wish to thank.

I had the great opportunity to be supervised by Dr. Dan C. McIntyre who has guided my wandering brain and at the same time has given me freedom in scientific investigation. Thanks for correcting my, how should I say this, numerous "brainfarts" and I did come into your office to hear your stories.

I would like to thank my family members (and I mean all my family members and to remain scientifically neutral, displayed by age from oldest to youngest) for their ongoing encouragement - Dad, Mom, Dale, Dave, Darrell, Brenda, Sheila, Cyndi, Tara (for introducing me to Freud and the joy of writing), Pam, Wes, Brett, and all my nieces and nephews. I would also like to thank Berg for spending several nights during the week playing checkers with me on MSN. I don't think you will reach my calibre buddy! I would also like to thank Kaede for prying me away from my studies and teaching me to enjoy activities outside the field of neuroscience. I am fascinated by your work and unwavering determination in pursuing the metastatic mechanisms of cancer cells. I have all the belief in the world that you will make a substantial contribution to the field.

Once again, thanks everyone.

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Introduction

Much of what we know about the neural components that have been given the descriptive name emotional, are derived from injuries, disorders or diseases of the nervous system. Indeed, the relation between emotion and abnormal brain structure and function can be found in the root of the word pathology, *pathos*, which is the Greek term for emotions or passions especially pity or melancholy (*pathologia* is the study of emotions). It was thought by the Stoics that emotions were the disease of the soul and thus the soul should be free from pathos (*apatheia*). One particular neural disorder that has shed considerable light on our understanding of the brain's involvement with emotions has been epilepsy, particularly the temporal lobe type. It was the keen eye of Hippocrates who observed the close relation between emotion and epilepsy.

Melancholics ordinarily become epileptics, and epileptics melancholics: of these two states what determines direction is the direction the malady takes; if it bears upon the body, epilepsy, if upon the intelligence, melancholy (Lewis, 1934).

The "intellectual aura" or the psychical phenomena accompanied with abnormal neuronal activity at the seizure focus that often precede the complete development of the seizure was not commonly thought of as a sign of epilepsy within the medical profession prior to Hughlings-Jackson. Jackson describes a young lady who had "intellectual auras" prior to full seizure development and when she had inquired about this experience with a medical doctor he "said that her case was one of hysteria" (Hughlings-Jackson, 1888). Hughlings-Jackson did not approve of the term "intellectual aura" because the nomenclature could be confused with the "crude sensations" which were the warnings (auras) of epilepsy. Some common "crude sensations" that preceded a seizure included

vertigo or giddiness, epigastrium, smells, voices, and tastes or more accurately, movements that made it seem as if an epileptic individual tasted something. Jackson indicated that he did not observe a seizure produced in an individual when it was preceded by the sensation of taste. For Jackson the "epileptic discharge" was associated with the "crude sensations" and not the "intellectual auras".

It is scarcely likely that one thing, an epileptic discharge, should be the physical condition for a sudden stench in the nose - a crude sensation - and also the physical condition for an infinitely more elaborate psychical state. I submit that the former occurs during the discharge, and that the latter is owing to but slightly raised activity of healthy nervous arrangements consequently on "loss of control" (Hughlings-Jackson, 1880)

In order to lessen the confusion, Jackson adopted the phrase "dreamy state", a term that his patients used to describe these phenomena. "Dreamy states" are in some sense a part of a normal individual's life and one of Jackson's patients uses a Dickens quote to describe this state.

We have all some experience of a feeling which comes over us occasionally, of what we are saying and doing having been said or done before, in a remote time - of our having become surrounded, dim age ago, by the same faces, objects, and circumstances - of our knowing perfectly what will be said next, as if we suddenly remembered it (Hughlings-Jackson, 1888).

In addition, to the "dreamy states", Jackson observed that other psychical states are accompanied by seizures. One of these states was fear as indicated by a patient of his.

The things around me seem to be moving; and if I am reading, the book will appear to be going from me, when at once I feel as if all must be a dream, though well knowing at the same time it must be reality...through it all the fear of some impending catastrophe seems to be hanging over me (Hughlings-Jackson, 1880).

Interestingly, Jackson mentioned that fear was often, but not always, preceded by the

epigastric sensation and he further notes that "[a]fter some slight paroxysms beginning either by the epigastric sensation or by fear without it, the patient's bowels are moved: a fact, I submit, of some significance" (Hughlings-Jackson, 1880). Jackson also indicated that, although he did not observe the antithesis of fear, that is anger, some of his patients told him "that at the onset of their seizures they feel they must attack someone, or have a hatred against some person present. As anger is the emotion of combat, this is equivalent to confessions of feeling of anger." (Hughlings-Jackson, 1880).

After Hughlings-Jackson, Penfield, through his thorough studies on electrical stimulation of the temporal lobes, described the reminiscent "dreamy states" as experiential phenomena and those that are illusions and emotional as interpretive phenomena (Penfield, 1954; Penfield, 1958). Still later, Gloor, Olivier, Quesney, Andermann, and Horowitz (1982) had placed the illusions and emotions under the heading of experiential phenomena. Furthermore, Gloor pointed out that "[a]mong the manifestations of temporal lobe epilepsy psychic phenomena are the most intriguing. They are the only manifestations evoked by the cerebral seizures which relate to the patient's personal identity".

Gloor and his colleagues were able to show that besides stimulating the temporal lobes as Penfield showed, experiential phenomena could be elicited by stimulating the limbic system, especially the amygdala, with an electrode. It was found, by electrical stimulation, that the most observed experiential phenomenon was the emotion fear. In a 22 year old male patient, they stimulated the right amygdala three consecutive times with increased intensity. The initial stimulation consisted of a 1 milliamp (mA) current and

the patient "experienced something that he found difficult to describe but finally likened to a feeling of falling into water." After another stimulation with no warning, the patient "immediately opened his mouth with an astonished look on his face, sat up, and said that now he knew what it was: it was the feeling of being at a picnic in Brewer Park in Ottawa" (Gloor et al., 1982). It was in this park where a "big fellow" had submersed the patient's head under water and that the patient was reliving when his brain was electrically stimulated. A while later the patient was stimulated again in the right amygdala, this time with a 2 mA current and "[h]is face turned pale and he looked frightened or sad." The young man felt as if he were about to fall off a cliff.

It was one of those feelings, a feeling of being someplace very far away, definitely noon. It is an atmosphere I often experience during my 'petit mal' attacks. It recalls to mind the day in the country with Tracy [a girl who lived next door to him] and brother Jamie. It was very spooky, but it was far away. It was out by the sea and high up on a cliff, a feeling as if I were going to fall. It was a scary feeling (Gloor et al., 1982).

The third stimulation was a 3 mA current and "[h]e again became nauseated, felt that he was somewhere in the country with Tracy at a place where he had been before, and felt that it was dark and raining. He was extremely frightened and pale, and pleaded not to repeat the stimulation" (Gloor et al., 1982). Each stimulation produced fear in the presence of water. With the first stimulation there was the memory of the boy's head held under water at the park, the second stimulation he felt as if he would fall off a cliff into the water below and finally the third stimulation a feeling of fear when it was "dark and raining". Fear is the most commonly described aura of temporal lobe epilepsy and stimulation of the medial temporal lobes.

Patients often show behaviours that would be described as being fearful, yet they

do not indicate that they were aware of any feeling of fear. At times a sufferer of temporal lobe epilepsy may show a face resembling a person in fear or produce a sound, such as a cry that can be described as fearful. Furthermore, temporal lobe epileptics may produce defensive behaviours or may flee indicating that they may be afraid. Also fearful expressions and vocalizations can be provoked by amygdala stimulation (Gloor, 1990). These patients, although may appear afraid, do not describe themselves as being frightened.

Temporal lobe epilepsy and electrically stimulating the temporal lobe area has provided invaluable information about the neural mechanisms that are described as emotional especially by that word fear. It has become apparent that the amygdala is an important contributor to fear in humans and refined techniques and methodology are being used to uncover the amygdala's specific role in fright. As Penfield noted, "epilepsia still has secrets to reveal. She has much to teach us if we will only listen" (Penfield, 1975).

A common procedure to alleviate intractable temporal lobe epilepsy is surgical removal, *en bloc*, of the anteromedial temporal lobe, which consists of the hippocampus, amygdala, and overlying temporal lobe cortex. Carefully conducted studies on human patients that have undergone anteromedial temporal lobectomy have revealed deficits in emotional processing.

LaBar, LeDoux, Spencer, and Phelps (1995) assessed emotional learning for simple and complex conditional discrimination tasks in epileptics that had unilateral temporal lobectomy. Patients had roughly 70-80% of their amygdala removed, all of the

hippocampus, including the parahippocampal gyri on either the right or left side. In the simple discrimination procedure, a tone (conditioned stimulus/CS) either co-terminated with a white burst of noise (CS+) or did not co-terminate with a white burst of noise (CS-). At the end of conditioning, the subjects were then asked if they could explain or interpret the procedure in order to test their declarative knowledge of the paradigm. Skin conductance responses (SCR) were measured. It was found that controls showed normal conditioning, while the temporal lobectomy subjects had impaired conditioning to the simple discrimination task. When asked if they could explain the procedure, they showed declarative knowledge of the rules involved. That is, they understood that one tone was followed by the burst of white noise and another tone was not, suggesting to the authors that declarative knowledge alone is not sufficient to produce fear conditioning.

The complex conditional discrimination task involved a green light that preceded the CS tone, which was immediately followed with a white burst of noise and red light that was followed by a tone and was not followed with a burst of white noise. At the end, the subjects were again asked to indicate what the task was about in order to test their declarative knowledge. In this task the unilateral temporal lobectomy subjects were observed to have deficits in SCR scores to the green light compared to controls, similar to those scores in the previous experiment. In addition, the temporal lobectomy patients understood the rules of the task, that the green light preceded the tone and predicted the white burst of noise. This again suggested that declarative knowledge of the rules is not enough to engage fear conditioning. In contrast to fear conditioning to a burst of white noise, it was found that eyelid conditioning was preserved in unilateral temporal

lobectomy subjects (Daum, Channon, & Gray, 1992). However, eyelid conditioning tends to be a function of the cerebellum and brain stem in animals (Lavond, Kim, & Thompson, 1993).

Another study further revealed that bilateral temporal lobectomy obstructs emotional recognition, especially for fear (Scott, Young, Calder, Hellowell, Aggleton, et al., 1997). Patient D.R., a woman who had suffered from epilepsy from the age of 28, had stereotaxic operations conducted to target the amygdala on both the right and left sides of her brain. Magnetic resonance imaging indicated that her lateral nucleus of the amygdala was spared lesions and the right amygdala had a small lesion at the posterior region. It was found in an earlier study that she was unable to recognize fearful faces that were presented to her and to a lesser extent she was unable to recognize angry and disgusted facial features (Calder, Young, Rowland, Perret, Hodges, et al., 1996). In a follow up study she was tested on her intonation pattern recognition for vocal affect. She was able to discriminate between same non-word and word pairs such as 'zog-zog' and 'house-house' and different non-word and word pairs such as 'zog-zeg' and 'house-mouse'. She had impairment in the detection of emotional tone of voice, for instance, if a sentence was read in a happy, angry or sad tone. She had further difficulty in detecting differences in tone when sentences were read as a question, exclamation or a statement and she had deficits in recognizing familiar voices of famous people. D.R. also showed impaired recognition of neutral words, such as carpet, that were spoken in an angry or fearful tone and it was observed that she had deficits in detecting non-word sound patterns that implied emotionality (i.e., laughing for happiness, growling for anger,

etc.), while she was not hindered in other non-word sound patterns.

D.R. was further assessed on her memory for an emotionally laden anecdote (Papps, Calder, Young & O'Carroll, 2003). A story consisted of a neutral beginning, an emotional middle (i.e., an accident occurred to one of the characters in the story) and an ending dealing with the consequences of the disturbing middle portion. A week later the control group and D.R. were questioned, without warning, on the context of the stories. The controls and D.R. were also read 15 words five times. These words were from a list of infections, negative emotions, dangerous weapons, etc. and after each trial the subjects tried to recollect as many of the words as possible. After the fifth trial the words were followed by a "distracter list" and subjects were required to recall these distracter words. After this they were again instructed to immediately recall the 15 words prior to the distracter words and then again 20 minutes after the distracter words.

D.R. was able to recognize the emotional middle portion of the story when given multiple choice questions on it a week later and there was no difference in her scores compared to healthy controls. She was able to recall affective verbal words as well as controls. She had the ability to recall long-term emotional memories of anecdotes and words yet previous studies indicated that she had deficits in recognition for fearful faces. The authors suggested that affective recognition and memory may have separate neural mechanisms although these mechanisms may still rely on the amygdala since D.R. had some of her amygdala intact. In contrast to the above study, it has been stated that affective perception or recognition and memory may have the same neural mechanisms (Anderson & Phelps, 2001).

Memories for emotional words depend on the ability to arouse the individual. Phelps, LaBar and Spencer (1997) investigated memory for affective words and for neutral words that are part of emotional sentences. Subjects were patients who had unilateral anteromedial temporal lobectomy in order to treat refractory complex partial seizures. Words in the first experiment were of positive (i.e., lucky, funny, joke), neutral (stamp, spare, chair) and negative (error, fool, false) valence. The control and experimental subjects were to rate the valence of the word on a scale from 1 to 5 with 1 being negative and 5 being positive.

The left temporal lobectomy (LTL) patients tended to show reduced recall for words compared to control and right temporal lobectomy (RTL) patients but this was not significant indicating normal recall for temporal lobectomy patients (LTL and RTL) overall. RTL, LTL and control groups had higher recall for positive and negative words compared to neutral words. There was no difference in SCR scores for words between groups and no difference between groups for rating the valence of the word from 1 (negative) to 5 (positive).

In the second experiment, subjects were instructed to surround neutral words in positive, negative or neutral sentences. For instance, a subject may be asked to place a neutral word (i.e., chair) in a negative context ("When I sat in the chair, it broke and I hurt my back"). After completing the sentences and conversing with the experimenter the subjects were asked to recall, without warning, the neutral words. Furthermore, control and experimental groups were to rate the valence of the sentences from 1 to 5. The rating of sentences on a scale of 1 (negative) to 5 (positive) showed that all groups

rated negative sentences more negatively and positive sentences more positively. All groups tended to show greater recall for words that were embedded in affective sentences compared to neutral sentences although LTL patients had attenuated recall performance compared to RTL patients and control groups.

The lack of differences between groups in the first experiment may be that the words are not affectively strong but are only semantically emotional, so that they are not shocking to the subjects. They are not provoking and therefore not relying on the amygdala. Perhaps more disturbing words would produce a recall deficit in individuals who have undergone temporal lobectomy since it has been shown that arousal enhances memory consolidation over time (Eysenck, 1976; Heurer & Reisberg, 1992).

LaBar and Phelps (1998) tested memory recall for arousal words in patients that had unilateral temporal lobectomy. In this experiment subjects were shown 20 arousing and 20 neutral words. Arousing words consisted of profanities, sexually explicit words, socially taboo words, etc. SCRs were measured and the words rated by subjects from 1 (not at all arousing) to 4 (very arousing). Recall was determined by rating the familiarity of the word from 0 (not at all familiar) to 6 (very familiar). All subjects rated the affective words as more arousing than the neutral words. However, it was noticed that after 1 hour, the control group showed enhanced memory recall for the emotionally arousing words where the LTL and RTL patients did not. The RTL patients actually tended to move in the opposite direction, that is, they had reduced memory recall after one hour.

Studies on epileptics have shown that the amygdala plays an important role in

emotionality and one of the most common experiential phenomena described by epileptic patients is the feeling of fear. At times these patients may express fearful behaviours without being aware of feeling afraid. Electrically stimulating the brain with an electrode produces many of the experiential phenomena that are produced during an epileptic seizure. In particular, stimulation near the amygdaloid complex is often associated with feelings of fear although, similar to convulsive episodes, patients may act as if they are afraid after electrical stimulation yet they are not aware of feeling afraid. Epileptics that have been treated by surgical removal of the anterior temporal cortex and the medial structures have deficits in memory for arousing words, fearful facial recognition, and fearful auditory recognition. That the amygdala is part of the emotional circuitry, especially for fear, is strongly supported by animal research.

Animal Studies

There is a keen interest in localizing emotionality within the nervous system and genetic makeup of an animal. Since the seminal work of Calvin Hall (1938) in selectively breeding rats for differences in emotionality based on the open field test, there have been a number of rodent strains developed in order to further understand emotion. For instance, the Maudsley Reactive (MR) strain has been found to defecate (defecation is indicative of fear in rodents) more in the open field, show greater bar pressing suppression in the conditioned emotional response test and in a task involving an unsignaled footshock compared to the Maudsley Non-Reactive (MNR) strain. However, scores in the elevated plus maze (EPM) have been inconsistent between strains. It has

been observed that the MR rats showed greater anxiety in the EPM, while other studies have shown no strain differences. Also, the MR strains have been observed to be more fearful in approaching a novel stimulus compared to the MNR strain (see Blizard and Adams for review, 2002).

Another line of rats selectively bred for differences in high- (HAB) and low- (LAB) anxiety behaviours performed differently in a number of emotionally based tests. For instance, HAB rats have shown higher anxiety scores in the EPM, open field, black/white box, social interaction test, and modified hole board (see Landgraf and Wigger for review, 2002). However, the LAB rats were as fearful as the HAB rats in the acoustic startle test (Yilmazer-Hanke, Wigger, Linke, Landgraf & Schwegler, 2004). Other lines of rats selectively bred for emotionality include the Roman (Chauloff, Castanon & Mormède, 1994) and Saradinian alcohol preferring (Colombo, Agabio, Lobina, Reali, Zocchi, et al., 1995) lines. Since selective breeding is not restricted to discerning a single gene, but almost certainly a number of different genes contribute to the desired differences between strains, it is not uncommon that animals selected for one trait also exhibit alterations in another. For instance, rats selectively bred for amygdala kindling also show differences in a number of behaviours such as aggression, attention and fear (McIntyre, Poulter & Gilby, 2002).

Kindling and Fear

Kindling is an animal model of temporal lobe epilepsy (Goddard, McIntyre & Leech, 1969; Kalynchuk, 2000). Initial stimulation to a brain structure, usually the

amygdala, with high frequency, and low intensity pulses of electrical current produces sub-convulsive after-discharges. Repeated exposure over days to the current, at the focus of stimulation, produces longer after-discharges that reach beyond the original focus of stimulation and induces convulsions. In their paper, Goddard, McIntyre and Leech (1969) described '[t]he progressive changes that result from repeated electrical stimulation...as the "kindling effect."' Kindling has been shown to occur in a number of species including the rat, mouse, frog, cat, dog and monkey (McIntyre, Poulter & Gilby, 2002; McNamara, Byrne, Dasheiff & Fitz, 1980; Racine, 1978). The progressive nature of the kindling effect, as observed in the electroencephalogram (EEG) and its associated convulsions, has been classified into 5-stages (Racine, 1972). Stage 1 is marked with facial clonus, Stage 2 by head nodding, Stage 3 involves contralateral forelimb clonus, Stage 4 with forelimb clonus and rearing and Stage 5 is noted with Stage 4 descriptors plus falling. Later, Pinel and Rovner (1978) added three additional stages to Racine's five stages. Pinel and Rovner's Stage 5 includes forelimb clonus, rearing and falling once, Stage 6 is marked with forelimb clonus, rearing and multiple falling, Stage 7 has Stage 6 behavioural descriptors plus running fits and Stage 8 involves any of Stages 1-7 with bouts of tonus.

It has been observed that kindling of certain neural structures contribute to changes in emotional behaviours. For instance, McIntyre and Molino (1972) tested six different groups of rats in a conditioned emotional response (CER) task. Three control groups included 1) a group of normal animals, 2) rats that had bilateral implantation of electrodes within the amygdala but stimulation was absent and 3) rats that had unilateral

lesions of the amygdala with electrode placement in the amygdala contralateral to the lesion and this group was also not stimulated. Three additional treatment groups included 4) animals with unilateral amygdala lesions with electrode placement and 3Hz stimulation in the amygdala contralateral to the lesion, 5) a group where rats were treated the same as the last but received a 60Hz stimulation instead of 3Hz and 6) a final group received bilateral amygdala lesions. After surgery all rats were trained to bar press for food and this was interrupted with a 3 minute tone that co-terminated with a shock. It was observed that the three control groups and the unilateral lesion with 3Hz stimulation group acquired the conditioned emotional response or conditioned suppression. That is, after a few trials their lever pressing behaviour was significantly reduced during the presentation of the tone. However, the unilateral lesion and 60Hz stimulation treatment group and the bilateral lesion group did not show conditioned suppression of bar pressing with the repeated presentation of the tone-shock suggesting impairment in fear learning. In addition, it has been observed that kindling increases susceptibility to stomach ulcer development when an animal is exposed to stressful events (Henke & Sullivan, 1985).

More recently, it has been shown that kindling alters emotional scores in ecologically based tests of fear and anxiety. Kalynchuk and Meaney (2003) kindled three groups of male Long-Evans rats with a different number of electrical stimulations to the left amygdala. One group received 20 stimulations and these animals reached at least Stage 4, 8 to 16 times. The second group received 60 stimulations and reached Stage 4 or greater roughly 27 to 52 times and a third group received 100 stimulations reaching Stage 4 or greater roughly 90 times. The 100 stimulations were determined by observing that

this is the maximum number of stimulations before spontaneous convulsions begin to appear (Kalynchuk, 2000). A control group was added and these animals were sham operated. It was observed that rats receiving 60 or 100 stimulations crossed fewer lines than did the 20 stimulation group. Also, the animals stimulated 60 and 100 times resisted being captured by the experimenter more than did the sham stimulated group and the 100 stimulation group was more likely to resist being captured compared to the rats which received 20 stimulations. The 60 and 100 stimulation groups engaged in more fleeing behaviour than did the sham stimulated group or the 20 stimulation group. The investigators indicated that the level of anxiety was directionally proportional to the number of stimulations. Increased emotionality is observed with increased stimulation. In another study by Kalynchuk, Pinel and Treit (1998), long term kindling (i.e., 99 stimulations) of the left basolateral amygdala significantly reduced the number of lines crossed in the open field test and increased the resistance to be captured, indicating that left basolateral amygdala kindling increases fear. However, when tested in the elevated plus maze, the kindled rats showed a significant increase in open arm entries and a significant increase in time spent in open arms compared to the sham amygdala stimulated rats. In order to solve the paradoxical findings between the open field and elevated plus maze results, the authors suggested that open arm entries in the elevated plus maze represent increased fear. That is, the rats were attempting to escape the elevated plus maze via the open arms.

Adamec and Morgan (1994) came to a different conclusion when kindling the left amygdala. These authors short-term kindled male Wistar rats in either the left or right

medial amygdala or either the left or right basolateral amygdala. Testing in the elevated plus maze took place once rats reached four Stage 5 convulsions and one week passed since the last stimulation. It was observed that kindling the right medial nucleus did not produce any differences in anxiety compared to kindling the right basolateral nucleus. Likewise, there was no difference in emotionality between left medial amygdala kindling and left basolateral amygdala kindling. There was a difference in the left amygdala kindled rats compared to the control group that had left amygdala electrode implantation but stimulation was absent. The left amygdala kindled group spent more time in the open arms of the elevated plus maze suggesting a decrease in fear. In contrast, the right amygdala kindled rats tended to spend less time in the open arms compared to the right implanted control group. In addition to the location of amygdala kindling (i.e. right or left hemisphere), baseline anxiety prior to kindling also influences post-kindling anxiety-like behaviours.

Adamec and Young (2000) tested male Wistar rats in the elevated plus maze to determine if pre-kindling anxiety levels influence post-kindling anxiety-like behaviours. After surgery but prior to kindling, rats were tested in the elevated plus maze in order to determine baseline anxiety. After kindling, the rats were retested in the plus maze and it was noticed that the animals who had spent higher than normal time in the open arms in the test prior to kindling (i.e., less fearful rats) had significantly reduced their open arm time in the test after kindling. In contrast, the rats that spent less time in the open arms prior to kindling (i.e., fearful rats) showed no change in their post-kindling plus maze behaviours. Although the amygdala is an important contributor to disturbances in

emotionality via short-term and long-term kindling, another prominent brain structure that also has been found to alter emotionality when kindled is the perirhinal cortex, which lies laterally adjacent to the lateral nucleus of the amygdala and with which it communicates.

Hannesson, Howland, Pollock, Mohapel, Wallace et al. (2005) kindled male Long-Evans hooded rats in the right or left anterior perirhinal cortex. After three Stage 5 seizures were reached the animals were tested in the open field and elevated plus maze seven or eight days after the last stimulation. The authors found that when tested in the open field, the kindled rats entered the central area less often than did the control group and the kindled group tended to spend less time in the center compared to the control group. These findings were supported by the behaviours scored in the elevated plus maze. Kindled rats spent less time in the open arms and entered the open arms less often than did control rats. Kindling of the perirhinal cortex did not alter the total activity in the open field and elevated plus maze. The ability of perirhinal and amygdala kindling to alter emotionality in rodents is explained in part by the observation that the amygdala and perirhinal cortex have many neuronal connections with each other (McDonald, 1998; Burwell, Witter & Amaral, 1995). In addition, the amygdala and perirhinal cortex have been shown to kindle faster than other brain structures (Kelly & McIntyre, 1996; McIntyre, Kelly & Armstrong, 1993; McIntyre, Kelly & Dufresne, 1999).

Sex , Age and Fear

Age (Macri, Adriani, Chiarotti & Laviola, 2002; Vataeva, 2003) and sex (Johnston & File, 1991; Palanza, 2001) differences have been observed in rodent fear.

Generally, rodent fear tends to increase with age and males display more fear-like behaviour than do females. However, inconsistencies arise depending on the type of apparatus used. In addition, sex differences in fear-like behaviours appear developmentally between weanling and adulthood.

Beatty and Fessler (1975) tested male and female albino rats at 22, 46 and 71 days old in the open field. It was found that previous to 50 days of age there were no sex differences in the number of rearings (increased rearing indicates decreased fear) or the number of lines crossed (increased line crosses represents less fear). These differences emerged roughly at 50 days of age. Masur, Schutz & Boerngen, (1980) examined male and female Wistar rats at 30, 45, 60, 90 and 100 days of age in the open field. They found no differences in rearing or the number of lines crossed at days 30 or 45 but differences emerged on day 60. It was further noticed that males decreased their number of lines crossed and rearing where females remained at their youthful levels. At 60 days of age males had defecated more than females and females reduced their defecation scores compared to their youthful scores where male defecation scores remained constant among the different ages.

In another study, Imhof, Coelho, Schmitt, Morato and Carobrez (1993) examined the emotionality scores of male and female Wistar rats at 45, 60, 90, 120 and 150 days of age in the elevated plus maze. It was observed that independent of sex, rats 60 days of age or younger were less fearful than rats 60 days or older. This interpretation was based on the observation that younger rats had more open arm entries and spent more time in the open arms. Sex differences in the number of open arms visited, time spent in the

open arms and total arm entries occurred at 90 days of age. The authors noted that the male scores for all three measures decreased between the ages of 60 and 90 days of age where the female scores for the number of open arms dropped between 90 and 120 days of age and there were no sex differences between these two scores at 120 days of age. Although it appears that males are more fearful than females in the open field and elevated plus maze, other tests of fear do not entirely confirm these results. Johnston and File (1991) found that in the Vogel test, male Wistar rats were less anxious than females and in the elevated plus maze females displayed less anxiety-like scores. In the social interaction test, there was no sex difference found in fear. Furthermore, in the hole board, a test of motor activity and exploration (i.e., head-dipping), there was no sex difference in Wistar rats (Fernandes, Gonzalez, Wilson & File, 1999). In addition, it has been shown that females are more anxious than male rats in response to potential dangers (i.e., cat odours) in an anxiety/defensive battery test (Blanchard, Shepherd, Carobrez & Blanchard, 1991).

Proposal

The purpose of this study was to examine more closely the emotional responses of two strains of rats that were selectively bred for their susceptibility to amygdala kindling. Previous studies clearly indicate that the kindling-prone (Fast) strain kindle faster than the kindling-resistant (Slow) strain due to repeated electrical amygdala stimulation. The kindling-prone strain requires 20% - 40% fewer stimulations to produce a Stage 5 seizure and the kindling-resistant strain require 200% - 300% more stimulations compared to

original parental controls (Racine, Steingart & McIntyre, 1999). This difference in kindling rates may be explained in part by differences in GABA_A expression between the two strains. It has been observed that the Fast animals have an over expression of the immature receptor subunits α_2 , α_3 , and α_5 compared to controls and the Slow strain exhibit an over expression of the adult subunit α_1 (Poulter, Brown, Tynan, Willick, William & McIntyre, 1999). In addition to the differences in kindling and GABA_A subunit expression between the strains, behavioural differences in fear and stress related tests were also observed. In the elevated plus maze, adult Slow rats made significantly fewer open arm entries and total arm entries than did the Fast rats (Mohapel & McIntyre, 1998). Slow rats were also less likely to explore a novel environment measured by the amount of time it took the animals to elevate their head over the wall of an open box. In the same study, Slow rats left the centre of the open field more quickly than the Fast rats and showed a decrease in crossing lines after repeated exposure to the open field over days. There was no change in the number of lines crossed for the Fast rats over the course of four days. Furthermore, in the inhibitory avoidance task, Slow rats exhibited less movement than the Fast rats and in the one-way avoidance task Slow animals initially took longer to escape shock than did the Fast rats presumably because of their behavioural proclivity to freeze (Mohapel & McIntyre, 1998). Together these results indicated that the Slow rats were more fearful than the Fast strain. In addition, when exposed to two different types of stressors, the two strains exhibited different behavioural profiles. The Fast rats actively struggled when exposed to a physical stressor (i.e., restraint) and exhibited startle and freezing behaviour when exposed to a psychological

stressor (i.e., ferret), where Slow rats were passive and immobile during the physical stressor and more active during the psychological stressor (Anisman, Lu, Song, Kent, McIntyre et al., 1997; McIntyre, Kent, Hayley, Merali & Anisman, 1999).

The medial amygdala (MeA) is a sexually dimorphic nucleus. It has been observed that the nuclear volume of this structure is larger in males than in females (Mizukami, Nishizuka & Arai, 1983) and that its synaptic organization is also differentiated between sexes (Nishizuka & Arai, 1983). The neuroendocrine system may be mediated by posterodorsal MeA connections with the hypothalamus and reproductive and defensive behaviours may be regulated by anterodorsal and anteroventral MeA connections with the hypothalamus (Canteras, Simerly & Swanson, 1995).

Since it has been postulated that the selective breeding of the Fast and Slow rats based on amygdala kindling may have altered certain sexual differentiated behaviour produced by the amygdala (Reinhart, Pellis & McIntyre, 2004), this proposed study was particularly interested in investigating sex differences in anxiety within the two strains. Because sex differences in anxiety are largely a function of ontogeny, this proposed study was also interested in following the development of anxiety at two different ages (pre-puberty and post-puberty) in the Fast and Slow strains. In experiment 1, animals were tested at 30 and 90 days of age in the elevated plus maze (EPM) and at 32 and 92 days of age in the open field test. In experiment 2, the same animals were tested in the CER unit at 103 - 106 days of age.

Experiment 1

Methods and Procedures

Animals

Rats were bred from two parental strains, Fast and Slow, at Carleton University (Life Sciences Research Building). These strains were first developed from a parental cross of Long-Evans Hooded and Wistar rats and selectively bred over 11 generations for their susceptibility to amygdala kindling (Racine, Steingart, & McIntyre, 1999). There were 10 Fast and 10 Slow females isostrain paired and housed in an environmentally controlled vivarium (temperature ~20 °C; humidity ~50%) 23 days post-natal in opaque plastic tubs (44 cm long X 24 cm wide X 20 cm deep) bedded with wood chips (Pro-Chips by P.W.I. Industries Inc.). Similarly, 10 Fast and 10 Slow males were isostrain paired and housed in opaque plastic tubs 23 days post-natal. All animals had free access to food (rat chow) and water (tap water) and were placed on a 12:12 light/dark cycle (lights came on at 8:00 am) throughout the experiment. At 27 days of age, odd-numbered rodents were marked with a black permanent marker at the base of the tail for identification purposes. Even-numbered rodents were treated in the same fashion although they were pseudo-marked with a dry marker. This process was repeated as needed throughout the experiments. All experimental manipulations were in accordance with the Canadian Council on Animal Care and a protocol approved by the Carleton University Animal Care Committee.

Apparatus

Elevated Plus maze

A grey plexiglas elevated plus maze (EPM) consisted of four (two open and two closed) arms that were at right angles from each other and extended from a central location and as a result formed a plus-shape. The two open arms (45 cm long X 14 cm wide) were situated across from each other and likewise the two closed arms (45 cm long X 14 cm wide) were situated across from each other. In addition, the closed arms consisted of 40 cm high walls running along the sides and back of the arms leaving the ceiling open. The EPM was raised 50 cm from the floor. Rodents naturally avoid open arms and prefer closed arms and as a result open arm entries imply reduced fear and anxiety where closed arm entries imply increased fear and anxiety (Handley & McBlane, 1993; Handley & Mithani, 1984). Animals were placed singly in the center of the EPM facing an open arm and behaviours were taped from a video camera directly above the apparatus. The duration of testing was five minutes. The opaque tubs in which the animals were housed were placed on a cart and subsequently wheeled from their housing room to a neutral area just outside the testing room. Each animal was carried separately by hand into the room. Testing took place in a closed room that was well lit. The experimenter left the room once the animal had been placed on the maze and testing began. Later the experimenter re-entered the room directly after testing was completed. The maze was cleaned after every run with a damp cloth and warm water. Testing commenced at 8:15 am each day when running animals for the elevated plus maze. Rats were weighed (see Table 1) and tested at 30 days of age and additionally at 90 days of

age. Behaviours scored included the number of total arm entries (reflecting total activity), percent time spent in open arms, percent of open arm entries, number of closed arm entries, and percent time spent in the center of the maze. Percent time spent in the open arms was calculated by dividing the time, in seconds, spent in the open arms over the total time of testing (i.e., 300 secs). Similarly, percent time spent in the center of the maze was computed by dividing the time spent in the center of the maze over total testing time. The percent number of open arm entries was determined by dividing the total number of open arm entries over the total number of open and closed arm entries in a run. Arm and center entries and exits were scored once all four paws of the animal were respectively in or out of the arm. The experimenter scored all behaviours by watching videotapes of the recorded tests.

Open Field

The open field was composed of a white open-top box with 50 cm high walls made of wood and a 100 x 100 cm floor made of Plexiglas. The floor was marked off with black lines into 25 equal squares that had 20 x 20 cm dimensions. A video camera was mounted directly above the open field so behaviours could be scored at a later date. Testing took place in a closed room that was well lit. Opaque tubs containing two rats each were placed on a cart and wheeled to a neutral area and individually carried into the room by hand. Once animals were placed in the center square testing took place and the experimenter left the room and only returned after a session terminated (i.e., 5 min). Testing commenced at 8:15 am. The open field was cleaned by a damp cloth and warm

water. Rats were weighed (see Table 1) and tested at 32 and 92 days of age.

Behaviours scored included percent time spent in the center zone of the box (described as the inner 9 squares), number of center entries (described as leaving the peripheral zone), and number of lines crossed. Percent time, in seconds, spent in the center was calculated by dividing the time spent in the center over the total time of testing (i.e., 300 secs). An animal was said to have crossed a line when all four paws were clearly over a marked line. In addition, if an animal crossed diagonally into a square, that is, over a corner of the square, two lines were counted.

Data Analysis

Behaviours scored (i.e, dependent variables) in the EPM and open field were analyzed using a repeated measures design. The within factor for the EPM and open field was Age and between factors were Strain and Sex. In addition, if interactions were observed than pair-wise comparisons were conducted with paired sample t-tests. Alpha levels were maintained at .05.

Results

Repeated measures indicated that there was no Sex difference or Sex X Strain interaction in the total number of arms entered in the EPM. However, a significant Strain difference [$F(1, 36) = 89.71$; $p < .0001$] was detected indicating that Fast rats made more total arm entries than did Slow rats (Figure 1). In addition, there was a significant difference in Age [$F(1, 36) = 15.89$; $p < .001$] with older animals making more arm

Table 1:

Mean Weights of Males and Females for Fast and Slow Strains
at Juvenile and Adult Ages

Group	Age	Weight (g)		
		<u>M</u>	<u>S.E.M.</u>	<u>n</u>
Fast/Male	30	63.70	2.68	10
	32	73.60	3.19	10
	90	356.50	7.70	10
	92	358.50	8.02	10
Fast/Female	30	69.40	2.56	10
	32	78.50	2.70	10
	90	233.40	2.85	10
	92	233.10	5.57	10
Slow/Male	30	76.00	2.13	10
	32	85.50	2.23	10
	90	399.80	2.54	10
	92	403.70	2.49	10
Slow/Female	30	65.40	2.17	10
	32	73.40	2.32	10
	90	209.20	2.87	10
	92	210.30	3.74	10

entries than younger animals (Figure 1). There were no Age X Strain, Age X Sex, and Age X Strain X Sex interactions.

For closed arm entries in the EPM there was a significant difference in Strain [$F(1, 36) = 78.22; p < .0001$] with Fast animals making more closed arm entries than Slow animals (Figure 2), but there was no significant Sex difference or Strain X Sex interaction. A significant difference was detected for the within factor Age [$F(1, 36) = 31.12; p < .0001$], indicating that older rats made more closed arm entries than did the younger animals (Figure 2). There were no Age X Strain, Age X Sex and Age X Strain X Sex interactions.

In the elevated plus maze the increased number of total arm entries observed in the Fast rats indicate that this strain is more active than the Slow animals and both strains increase their activity with age. This is further supported by the finding that Fast rats had more closed arm entries than Slow rats and older animals made more closed arm entries than did the younger animals.

A significant difference was detected for the percent time spent in the center of the EPM for the between factor Strain [$F(1, 36) = 11.89; p < .01$]. Fast rats spent more time in the center area of the EPM than did Slow rats (Figure 3). There was no Sex difference or Strain X Sex interaction. There was an Age difference [$F(1, 36) = 22.77; p < .0001$] with younger animals spending more time in the center than older animals (Figure 3). In addition, there were no Age X Strain, Age X Sex, and Age X Strain X Sex interactions for time spent in the center of the EPM.

For the percent time spent in the open arms, a significant difference was detected

Figure 1: Total Number of Arm Entries in the Elevated Plus Maze made by Males and Females in the Fast and Slow Strains

Mean \pm Std. Error

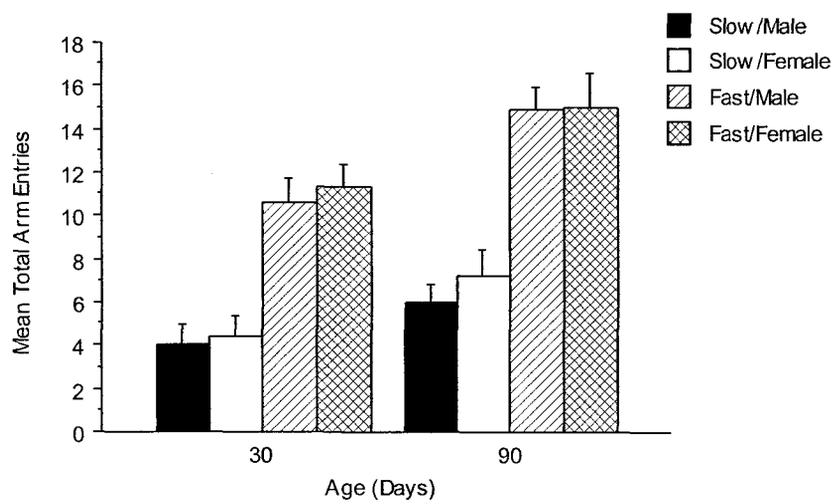
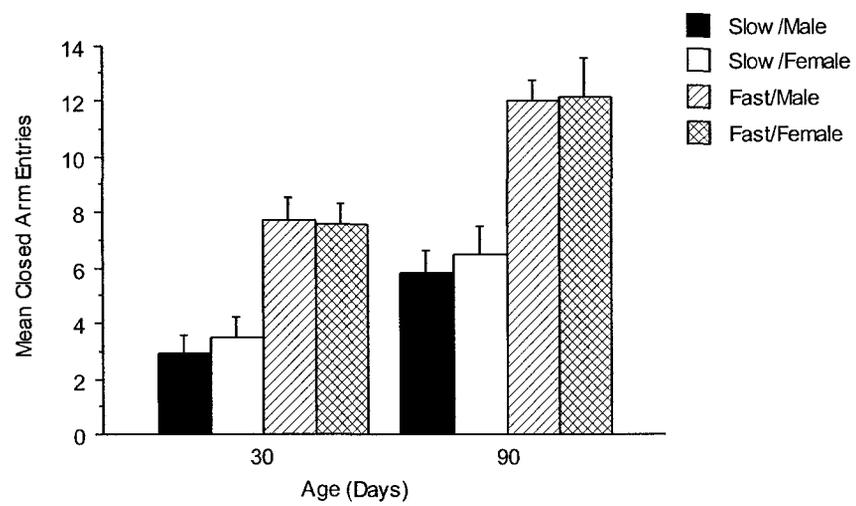


Figure 2: Mean (\pm Std. Error) Number of Closed Arm Entries made in the Elevated Plus Maze by Males and Females in the Fast and Slow Strains



for the between factor Strain [$F(1, 36) = 23.26; p < .0001$], but not for Sex. Fast animals spent more time in the open arms than did Slow animals (Figure 4). Furthermore, there was no Strain X Sex interaction for the time spent in the open arms. A significant difference was observed for Age [$F(1, 36) = 5.38; p < .05$], but no Age X Strain, Age X Sex or Age X Strain X Sex interactions were detected. Younger animals spent more time in the open arms than did the older animals (Figure 4).

There was a significant difference observed for the between factor Strain [$F(1, 36) = 15.73; p < .001$], but not for Sex and no Strain X Sex interaction was found for the percent number of open arm entries. Fast animals entered the open arms more often than did the Slow animals (Figure 5). In addition, there was a significant difference in Age [$F(1,36) = 18.59; p = .0001$], but there were no Age X Strain, Age by Sex or Age X Strain X Sex interactions. Here young rats made more open arm entries than did older rats (Figure 5).

The Fast strain appear to be less fearful than the Slow strain as indicated by their tendency to stay in the open arms longer and make more open arm entries than the Slow animals. Similarly, younger animals spent more time in the open arms and made more open arm entries and as a result were less fearful than the older animals. Since the younger animals and Fast rats spent more time in the center of the EPM, increased scores in this zone may also reflect attenuated fear. No sex differences were detected in the EPM.

In the open field test there was a Strain [$F(1, 36) = 9.26; p < .01$] and Sex [$F(1, 36) = 16.03; p < .001$] difference, but there was no Strain X Sex interaction for the total

Figure 3: Percent Time Spent in the Center of the Elevated Plus Maze for the Males and Females in the Fast and Slow Strains

Mean \pm Std. Error

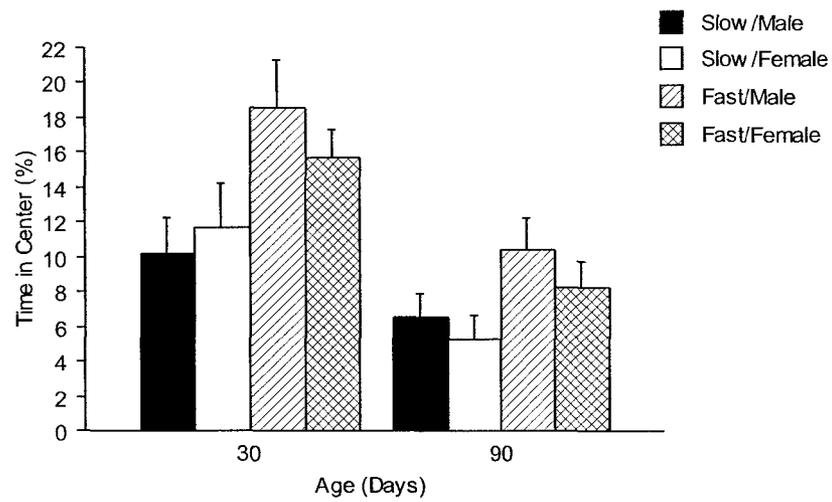


Figure 4: Percent Time spent in the Open Arms of the Elevated Plus Maze for Males and Females in the Fast and Slow Strains

Mean \pm Std. Error

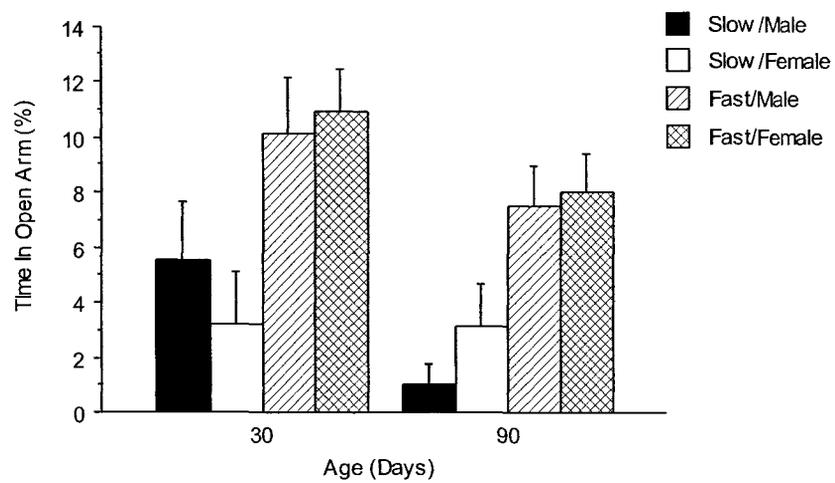
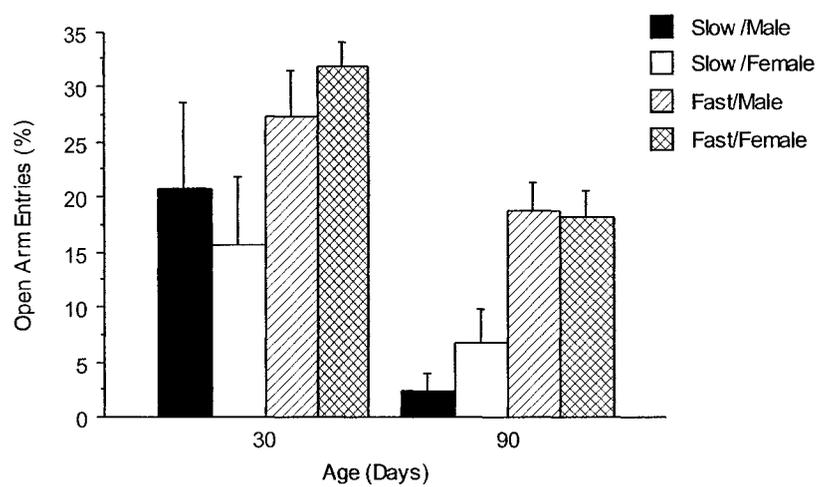


Figure 5: Percent Number of Open Arm Entries in the Elevated Plus Maze for Males and Females in the Fast and Slow Strains

Mean \pm Std. Error



number of lines crossed. The Fast rats made more line crosses than did Slow rats and females more than males (Figure 6). There was no Age difference and no Age X Sex interaction. However, there was an Age X Strain interaction [$F(1, 36) = 5.79; p < .05$] and an Age X Strain X Sex interaction [$F(1, 36) = 4.47; p < .05$]. Two tailed paired t-tests indicated that there was a significant difference between Fast males and Fast females at 32 days of age [$t(1, 9) = 3.93; p = .01$] with the Fast females crossing more lines than Fast males. Furthermore, the Slow males crossed less lines at 92 days of age than did the Slow females [$t(1, 9) = 2.82; p < .05$].

The above results in the open field indicate that the Fast rats made more line crosses than Slow rats suggesting, and in accordance with the total arm entries and closed arm entries in the EPM, that Fast rats were more active. Fast females crossed more lines than Fast males at 30 days of age indicating that they were more active at this age. Conversely, as suggested by the number of lines crossed, Slow males were less active at 92 days of age compared to Slow females.

A near Strain difference was observed for the percent time spent in the center of the open field [$F(1, 36) = 4.13; p = .05$] with Fast animals spending more time in the center than Slow animals (Figure 7). There was no Sex difference, but a Strain X Sex interaction [$F(1, 36) = 11.85; p < .01$] was found. Slow females appeared to spend more time in the center than Slow males at 32 and 92 days of age, whereas Fast males spent more time in the center than did Fast females at 32 and 92 days of age, but paired t-tests did not show any significant differences between these groups. There was an Age difference [$F(1, 36) = 26.95; p < .0001$] indicated by the younger animals spending more

Figure 6: Mean (\pm Std. Error) Number of Lines Crossed in the Open Field for Males and Females in the Fast and Slow Strains

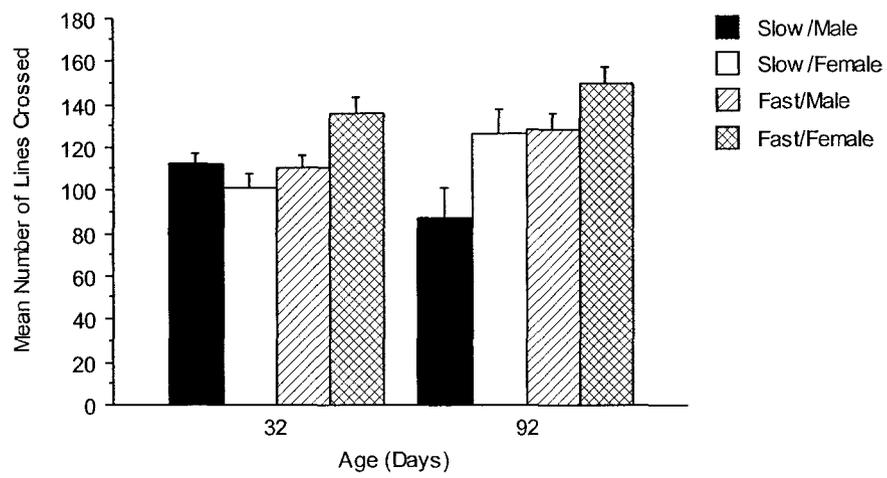
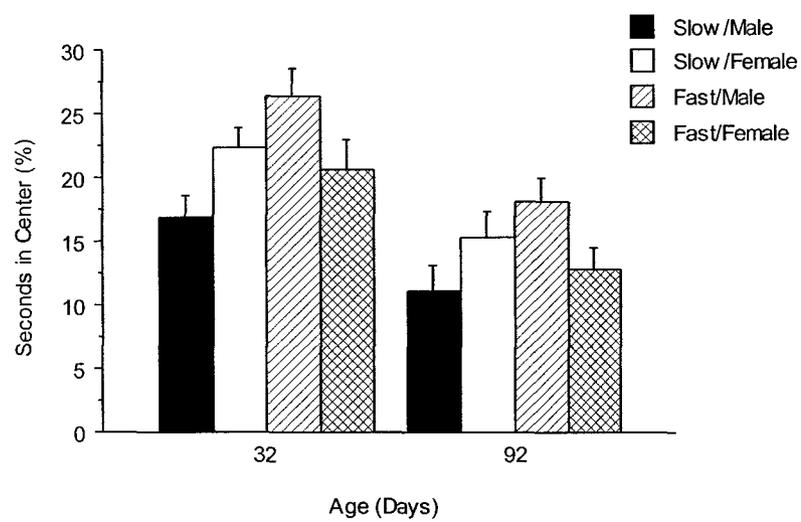


Figure 7: Percent Time spent in the Center of the Open Field for Males and Females in
the Fast and Slow Strains
Mean \pm Std. Error



time in the center of the open field than the older animals. No Age X Strain, Age X Sex, or Age X Strain X Sex interactions were observed.

For the mean number of center entries there was a Strain [$F(1, 36) = 25.29$; $p < .0001$] and a Sex [$F(1, 36) = 4.71$; $p < .05$] difference. Fast rats entered the center of the open field more often than did the Slow rats and female rats entered the center more often than did male rats (Figure 8). There was no Strain X Age interaction. Furthermore, there was no Age difference or Age X Strain and Age X Sex interactions. There was, however, an Age X Strain X Sex interaction [$F(1, 36) = 4.55$; $p < .05$]. There was a tendency for Slow males to enter the center more often at 32 days of age compared to Slow females and this observation was reversed at 92 days of age, although paired t-tests did not reveal any significant differences.

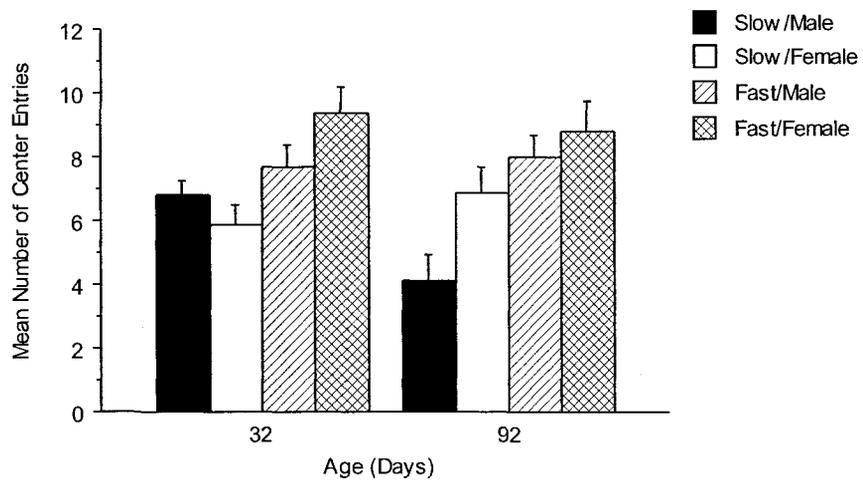
It was of interest to determine if these differences between strains were occurring at ~30 days of age and extended to ~90 days of age. Since weak sex differences were observed the males and females were pooled together in each strain. One-way analysis of variance indicated that Fast rats made more arm entries than did Slow rats at 30 [$F(1, 38) = 45.79$; $p < .001$] and 90 [$F(1, 38) = 47.85$; $p < .001$] days of age (Figure 1). Similarly, Fast rats made more closed arm entries than did the Slow rats (Figure 2) at 30 days [$F(1, 38) = 39.24$; $p < .001$] and 90 days [$F(1, 38) = 35.56$; $p < .001$] of age. One-way analysis found that Slow rats spent less time in the center of the EPM and 30 days [$F(1, 38) = 7.63$; $p < .01$] and 90 days [$F(1, 38) = 5.23$; $p < .05$] of age (Figure 3). Strain differences were found at 30 [$F(1, 38) = 10.64$; $p < .01$] and 90 [$F(1, 38) = 19.12$; $p < .001$] days of age for the percent time spent in the open arms. The Fast rats had spent more time in the

open arm at both age levels (Figure 4). Finally, in the EPM it was found that Fast rats had more percent number of open arm entries compared to the Slow animals at both 30 days [$F(1, 38) = 4.38; p < .05$] and 90 days [$F(1, 38) = 31.79; p < .001$] of age (Figure 5).

For the total number of lines crossed in the open field, one-way analysis found that Fast rats crossed more lines than Slow rats at 32 days [$F(1, 38) = 6.25; p < .05$] and 92 days [$F(1, 38) = 8.05; p < .01$] of age (Figure 6). Since no strain differences were detected for the percent time spent in the center of the open field for either age (Figure 7), this suggests that the Sex X Strain interaction was contributing to the near differences between strains observed above. Finally, there was a difference between strains at both ages for the number of center entries (Figure 8). Fast animals made more center entries at both 30 days [$F(1, 38) = 11.39; p < .01$] and 90 days [$F(1, 38) = 10.88; p < .01$] of age compared to Slow animals.

Although it appeared that Fast rats spent more time in the center of the open field, this effect was sensitive to the interaction with Sex and paired t-tests were not able to detect differences between strains at the level of Age. Younger rats tended to be less fearful than older rats as described by their tendency to spend more time in the center of the open field. Although, there was little Strain differences in the time spent in the center of the open field, Fast rats made more center entries than the Slow rats suggesting that they were less fearful. Females appeared to be less fearful than males as indicated by their proclivity to enter the center more often.

Figure 8: Mean (\pm Std. Error) Number of Center Entries in the Open Field for the Males
and Females in the Fast and Slow Strains



Experiment 2

Methods and Procedures

Animals

Since the animals used in the previous experiment were not 'treated' in any form but merely observed as they explored two different environments (EPM and open field), they were used again in the conditioned emotional response procedure. Rats were singly housed in opaque tubs and placed on a mildly restricted diet in order to reduce weight from their basal weights (i.e., to be maintained at ~80% their original weight). Water was freely available. Rats were weighed every second day rather than every day in order to keep handling stress levels at a minimum. Lights were on a 12 light/12 dark schedule (on at 08:00 and off at 20:00). All experimental manipulations were in accordance with the Canadian Council on Animal Care and a protocol approved by the Carleton University Animal Care Committee.

Apparatus

Conditioned Emotional Response

A room consisted of six conditioned emotional response arenas (H-Series, Coulbourn Instruments, Allentown, PA) contained in sound attenuated white Styrofoam cubicles. The operant units (30 cm high X 25 cm wide X 30 cm long) were constructed of transparent plexiglas on the sides and metal on the ends. The floor consisted of 16 bars that were spaced 2 cm apart. The front end consisted of a lever bar in the middle (5

cm from the grid floor) and a feeder (2 cm from bottom of feeder to grid floor) to the left of the lever bar. A house light (3.5 cm from the roof) was situated in the center of the front panel. The light remained on throughout training, conditioning and extinction. In addition, a tone module (6 cm from center of module to roof) was placed on the back panel opposite the feeder. Graphic State Notation 2 software on a PC (Wave Technology, Inc/128 Mega Bytes) was used to program the CER procedure and recorded a 28 Volt response sensor (i.e., lever) through a Habitest Linc (H02-08) and environmental circuit board (ECB). During training, conditioning and extinction, the operant box was cleaned with a damp cloth and dried, and the bottom slide-out tray was cleaned of any faeces and urine and then rinsed and dried before being placed under the grid floor.

Procedure

Training

Training commenced when animals were 103-106 days of age. Rats, in opaque tubs, were placed on a cart and wheeled to a holding area outside the room to be tested in. Rats were then singly brought into the testing room and placed within the CER unit. Rats were given unassisted training to bar press for food (a 50/50 mixture of vanilla and chocolate pellets for training, conditioning and extinction) once a day for 10 minutes starting at 8:15 am. A 1:1 fixed ratio was used, that is, one 45 milligram pellet (dustless precision pellets by Bio-Serv) was dispensed into the food hopper for each bar press. Training terminated when all animals were lever pressing at a consistent rate (i.e., on

average each Strain/Sex group was pressing the lever at least 40 times in 10 min).

Conditioning

During the conditioning procedure the rats were handled and moved from their housing room to the testing room in the same manner as in the training procedure. After training, rats were introduced to a three minute tone (80 dB) after four minutes from the commencement of a session. After the termination of the tone, rats were left in the unit to lever press for food an additional three minutes. After one trial of acclimatizing to the tone, a 0.8 milliamp scrambled shock (US) delivered by a Precision Regulated Animal Shocker (H13-16, Coulbourn Instruments) set to 8-pole output, was introduced for one second immediately following the termination of the tone (CS). Fear learning (CR) was measured by the conditioned suppression ratio that was obtained by dividing the number of bar presses during the three minute tone (Tone) over the number of bar presses during the three minute period prior to the tone (Pre-Tone) and the number of bar presses during the tone to produce the equation $\text{Tone} / (\text{Pre-Tone} + \text{Tone})$. If there was little suppression during the tone due to fear conditioning then the ratio was closer to 0.5 and if little lever-pressing occurred during the tone due to fear conditioning then a suppression ratio closer to 0.0 was observed. Furthermore, the number of times, rather than the number of fecal bolus, an animal defecated were obtained across sessions. For instance, if there were more than one fecal bolus within a Session it was counted 1. If no fecal boli were observed at the end of a Session it was alternatively numbered 0.

Extinction

After 13 sessions of CS-US pairings, the US was terminated and extinction took place over an additional 13 sessions. Extinction is the presence of the CS (tone) without the US (shock). Otherwise, the animals were treated the same as in the conditioning procedure.

Data Analysis

Behaviours scored (i.e., dependent variables), as described by the suppression ratio, in the CER were analyzed using a repeated measures design. Within factor was Sessions (Days) and between factors were Sex and Strain. The number of times an animal defecated was analyzed using a between-subjects design. All alpha levels were maintained at .05.

Results

Figure 9 indicates that all animals learned the training procedure with the Fast animals clearly learning the procedure more quickly and pressing the lever at a higher rate than Slow animals.

In the conditioning procedure, there was no overall Sex difference or Strain X Sex interaction, but there was an overall Strain [$F(1, 36) = 13.84$; $p < .001$] difference (Figure 10) with Fast rats exhibiting a higher suppression score than Slow rats. There was also a significant decrease in the suppression score across Sessions [$F(12, 432) = 12.64$; $p < .0001$] for the conditioning procedure (Figure 10). This was further supported

by a dramatic decrease in absolute lever responses over 13 sessions for both males and females in the Fast and Slow strains during the tone (Figure 13). There was no Session X Strain interaction, but there was a Session X Sex interaction [$F(12, 432) = 2.48; p < .01$] and a Session X Strain X Sex interaction [$F(12, 432) = 1.95; p < .05$]. Further analysis indicated that Fast females had a higher suppression score (less pressing during the tone) than Slow females [$F(1, 18) = 17.88; p < .001$] in the conditioning procedure, but this Strain difference was not found in males (Figure 10). It appeared that the Fast females did not habituate to the tone on Day 1 (i.e., after the habituation day) compared to the Slow females, but paired t-test indicated that this was not significant.

The above results in the CER indicate that both Fast and Slow strains acquired fear conditioning, evidenced by progressively reducing suppression scores over the first several tone-shock days. In addition, Fast animals were less fearful than Slow animals, or more precisely, the Fast females had higher suppression scores than Slow females, indicating that the strain differences were largely due to the Fast female's reduced fear compared to the Slow females.

In the extinction procedure, there was a Strain [$F(1, 36) = 10.76; p < .01$] difference and a Strain X Sex interaction [$F(1, 36) = 5.59; p < .05$], but there was no Sex difference (Figure 11). There was also a within factor Session [$F(12, 432) = 7.53; p < .0001$] difference and a Session X Strain X Sex interaction [$F(12, 432) = 4.25; p < .0001$], but there were no Session X Strain or Session X Sex interactions. Statistical comparison between the Fast and Slow males did not show any differences in suppression ratio for extinction, but there was a Strain difference between Fast and Slow females. Fast

Figure 9: Mean (\pm Std. Error) Bar Pressing during Training for Males and Females of the Fast and Slow Strains

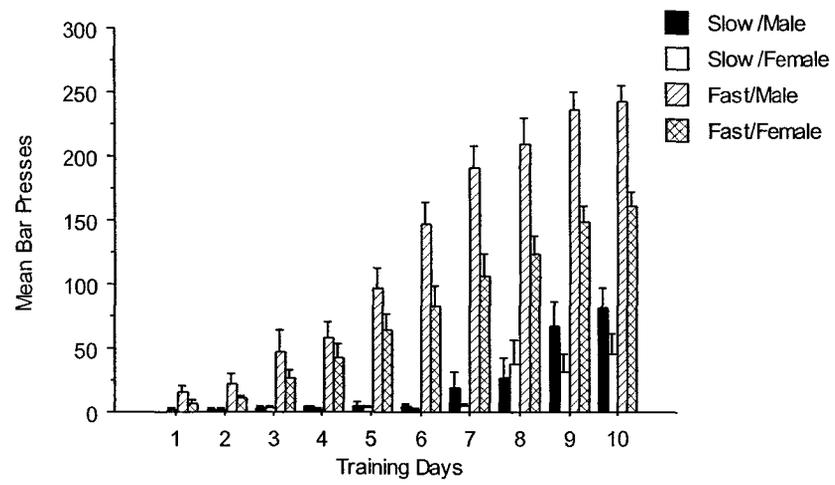
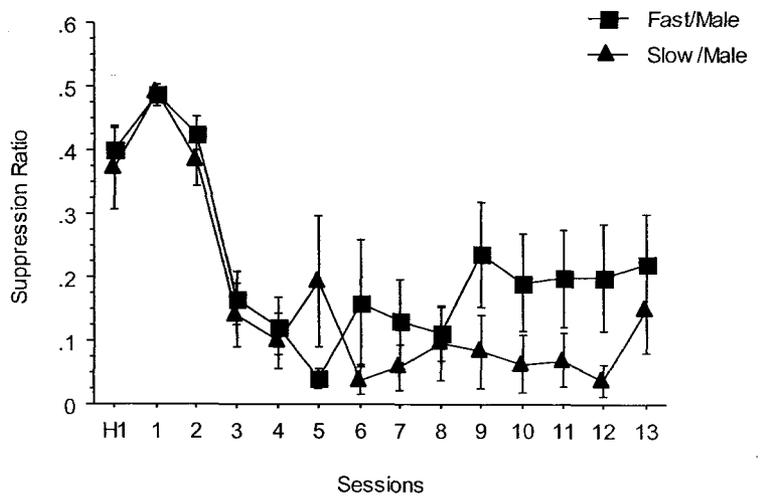
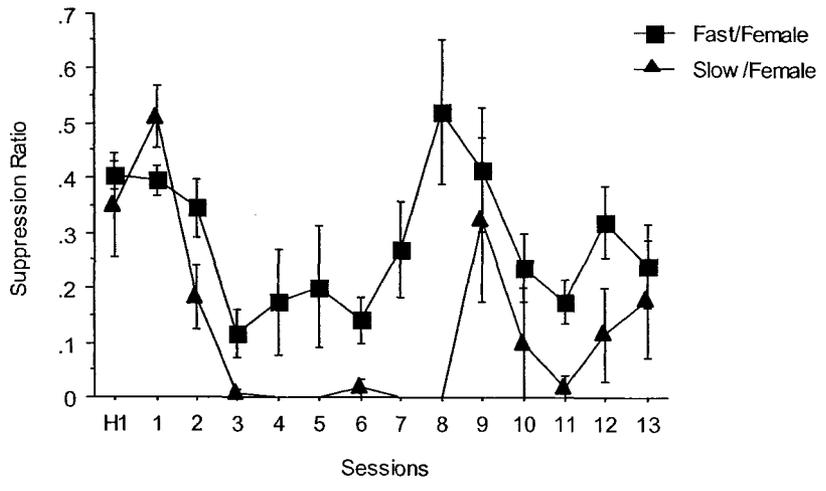


Figure 10: Suppression Ratio for Females and Males in the Fast and Slow
Strains across Sessions in the CER Apparatus during Conditioning

Mean \pm Std. Error



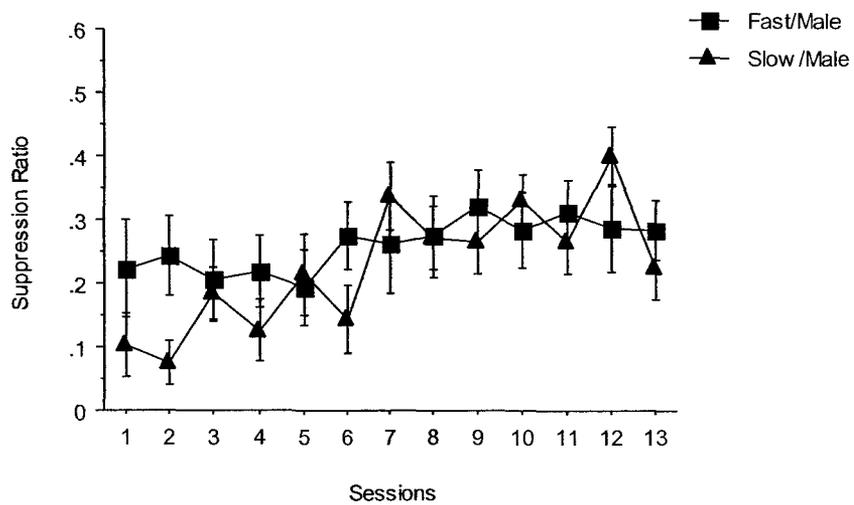
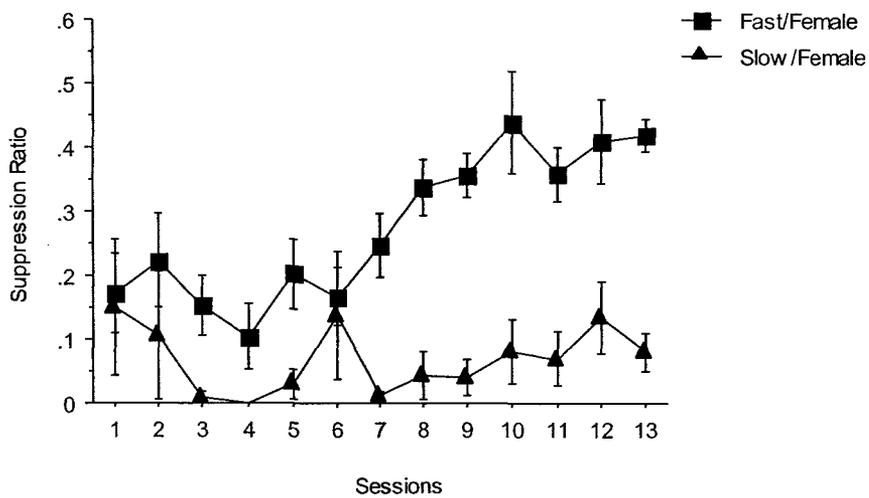
Females had substantially higher suppression scores as sessions increased compared to Slow females [$F(1, 18) = 23.17; p < .001$]. These results indicate that the Slow rats were most negatively affected by the conditioning procedure and as a result had lower suppression scores during extinction. This was particularly true in the females.

It was also of interest to determine if there were any differences in the behaviours exhibited between strains and sexes in absolute lever pressing rates throughout each of the conditioning trials, including the period a) prior to the tone (Pre-Tone) onset, b) during the tone (Tone) itself and c) after the tone (Post-Tone) offset. It was noticed that across all sessions there was a proclivity for responses *within a session* to decrease across 30 second blocks (Figure 12) during the 4 min Pre-Tone period. Indeed, as animals neared the tone onset, they exhibited a significant decrease [$F(7, 273) = 7.76; p < .0001$] in responding (Figure 15). In addition, there were Sex [$F(1, 36) = 38.21; p < .0001$] and Strain [$F(1, 36) = 15.33; p < .001$] differences during the Pre-Tone period in absolute lever responses averaged across all 13 daily sessions of 4 min each [in 8 blocks of 30 seconds] (Figure 15). Males lever pressed more than females and Fast animals lever pressed more than Slow animals during the 4-minute Pre-Tone.

During the Tone, there was a significant tendency to increase lever responses over blocks [$F(5, 195) = 5.04; p < .001$] (Figure 16). Finally, there was a significant Sex [$F(1, 36) = 8.89; p < .005$] and Strain [$F(1, 36) = 12.39; p < .01$] difference in lever presses during the Tone time frame. Males made more lever presses than females and Fast rats made more lever responses than Slow rats (Figure 16). During the Post-Tone, as expected, there was a significant difference in responding across blocks [$F(5, 195) =$

Figure 11: Suppression Ratio for Male and Females of the Fast and Slow Strains across Sessions in the CER Apparatus during Extinction

Mean \pm Std. Error

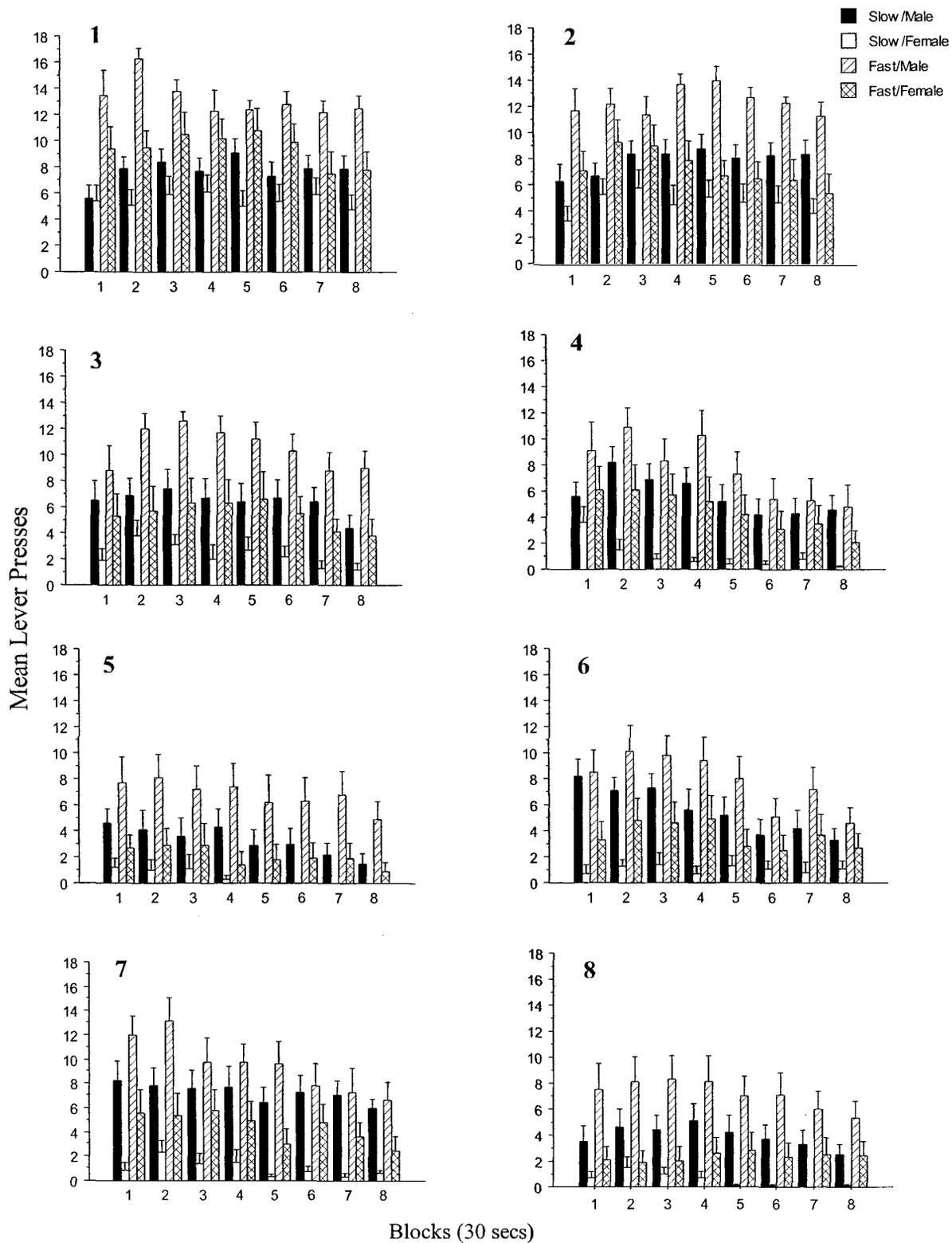


13.82; $p < .0001$] with the least number of responses made in the first 30 second block after the shock for both sexes and strains (Figure 17). In addition, there was a significant difference in the mean lever presses made for Sex [$F(1, 36) = 31.00$; $p < .0001$] and Strain [$F(1, 36) = 33.34$; $p < .0001$] during the Post-Tone period. Males made more lever presses than females and Fast rats made more lever presses than did Slow rats in the CER during the Post-Tone (Figure 17) period.

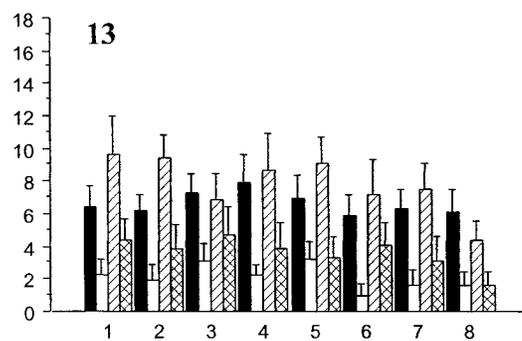
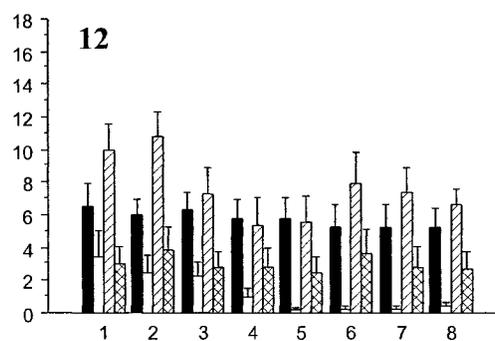
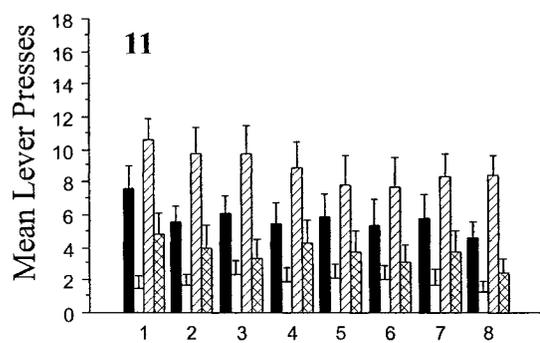
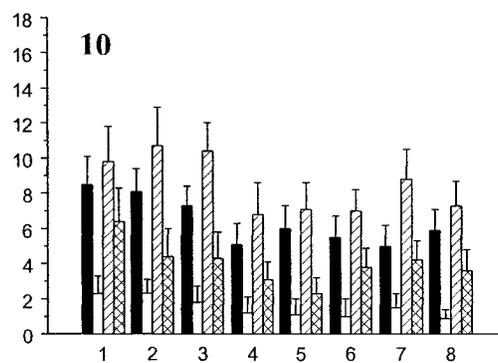
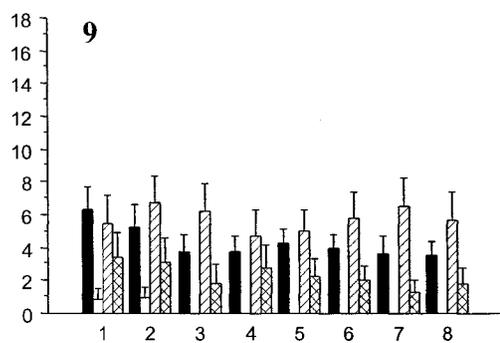
The number of times an animal defecated were also tallied, and analyzed over all sessions during conditioning in the CER. ANOVA indicated a significant difference in Sex [$F(1, 36) = 41.11$; $p < .001$] and Strain [$F(1, 36) = 17.03$; $p < .001$] with males defecating less than females and Fast defecating less than Slow rats. Fast ($\underline{M} = .40$, $\underline{S.E.M} = .22$) and Slow ($\underline{M} = 2.60$, $\underline{S.E.M.} = 1.02$) males had the lowest number of excrements with Fast ($\underline{M} = 4.40$, $\underline{S.E.M.} = .83$) and Slow ($\underline{M} = 8.70$, $\underline{S.E.M.} = .77$) females having a higher number of excrements in the CER during conditioning. If defecation is indicative of fear, then the Fast animals are less fearful than the Slow animals, and the males are less fearful than the females. The absolute values provide a more detailed analysis of the behaviours produced in the CER. Across all sessions during the Pre-Tone period (Figure 12), Fast rats had higher bar responding than Slow animals. By Session 8 and 9, Slow females had nearly no bar pressing during the Pre-Tone, but seem to recuperate in Sessions 10 through 13. All other groups also show this tendency, that is, an increase in bar pressing by the end of the Sessions.

The absolute values of pressing during the conditioning Tone show a drastic reduction by Session 3 (Figure 13) for all groups, and they remain this way to Session 8,

Figure 12: Mean (\pm Std. Error) Number of Bar Presses during the 4 min Pre-Tone Period (in Blocks of 30 sec) during all 13 Conditioning Sessions for Male and Female Animals in the Fast and Slow Strains

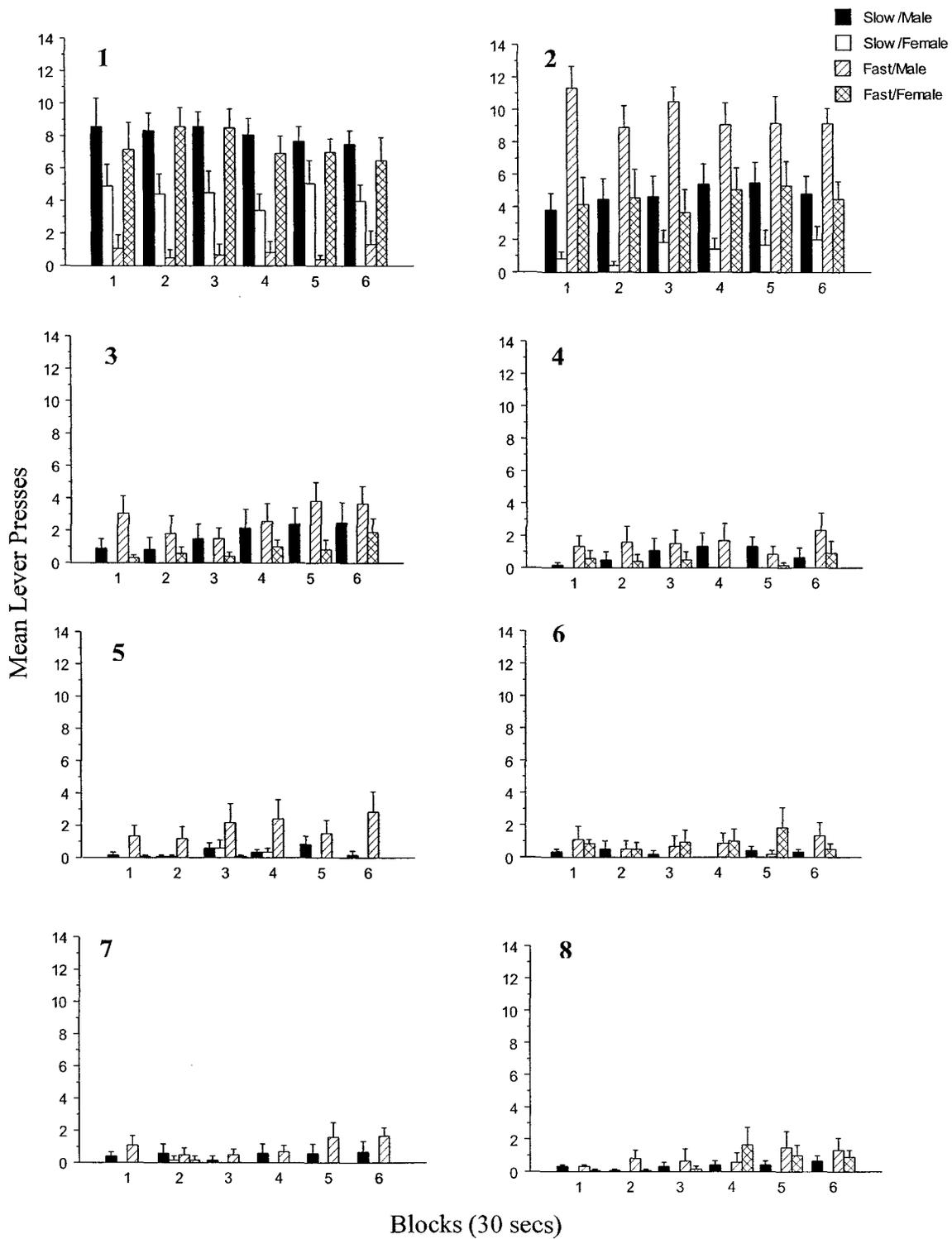


Blocks (30 secs)

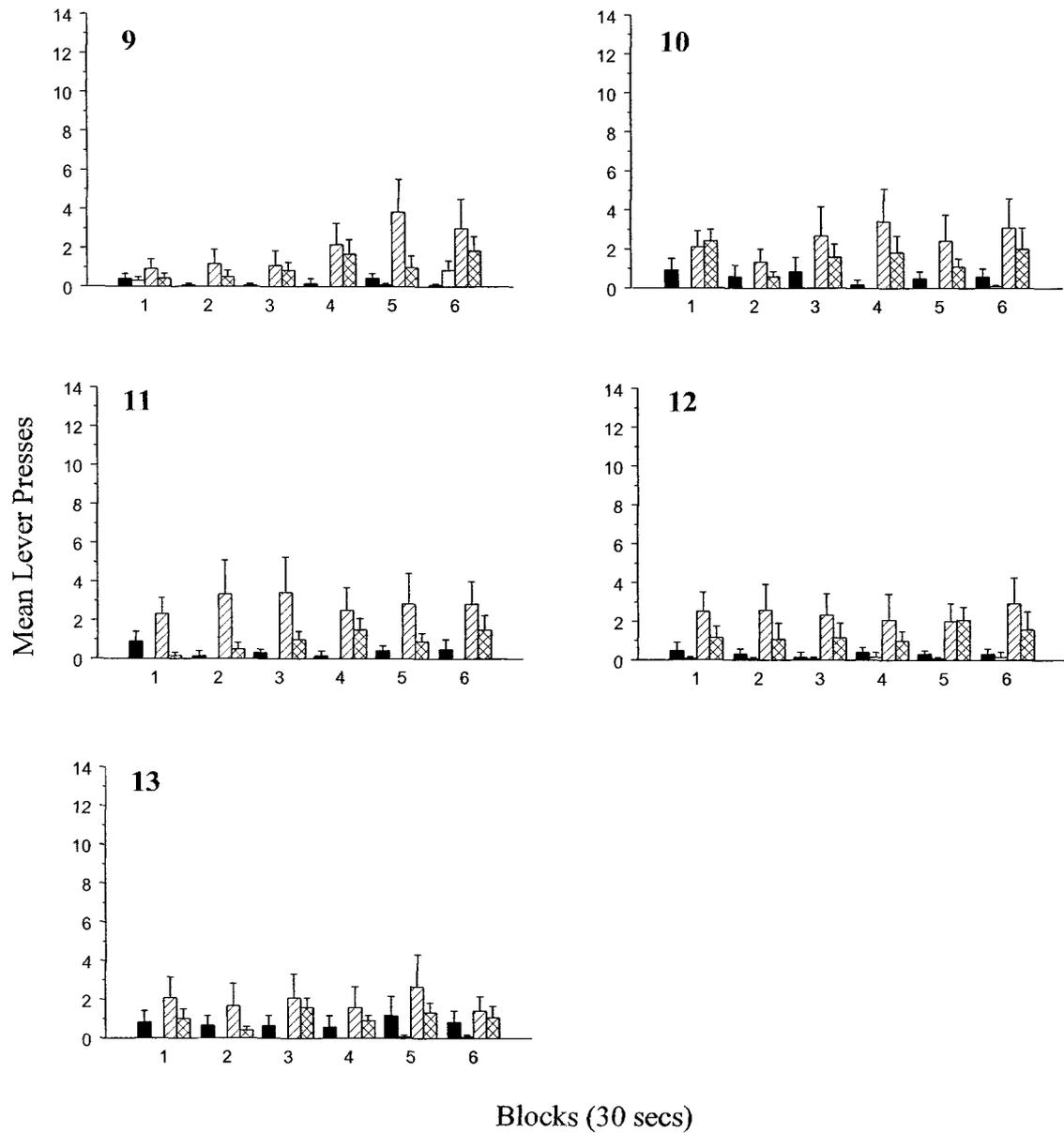


Blocks (30 secs)

Figure 13: Mean (\pm Std. Error) Number of Bar Presses during the 3 min Tone (CS) Period (in Blocks of 30 secs) during all 13 Conditioning Sessions for Male and Female Animals in the Fast and Slow Strains

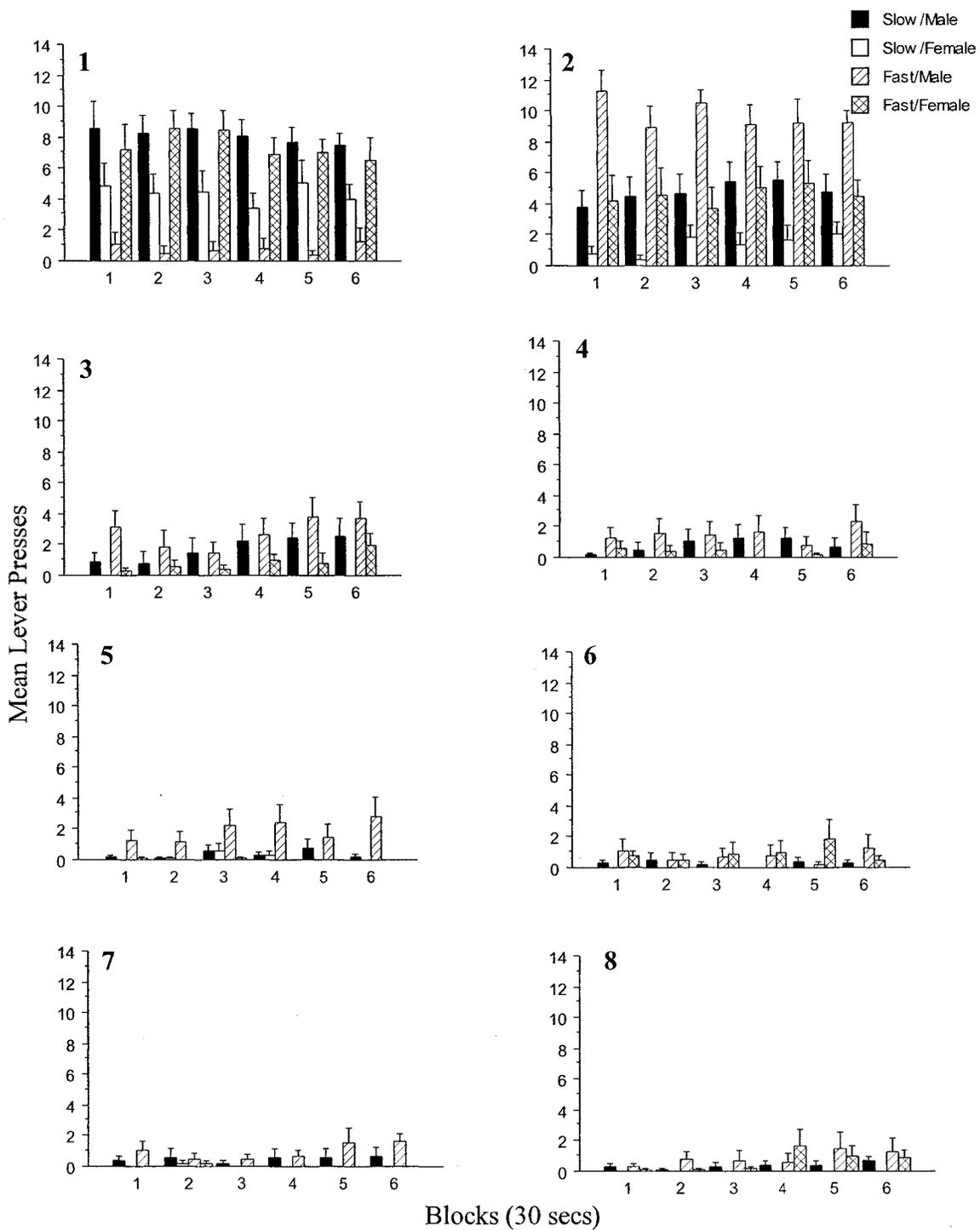


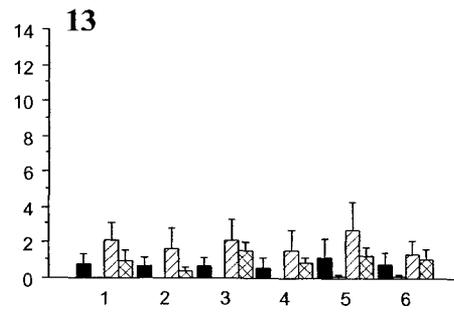
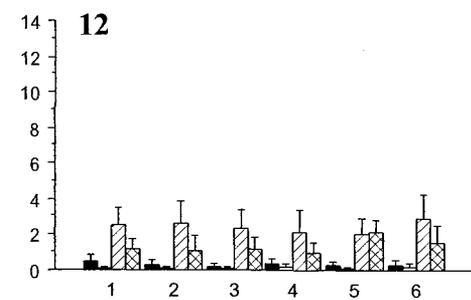
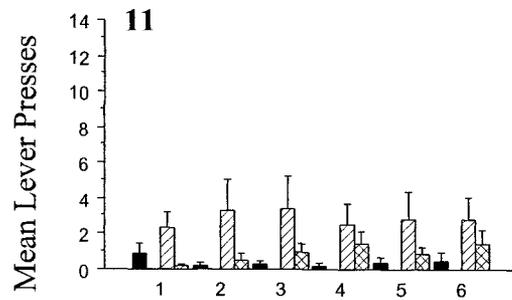
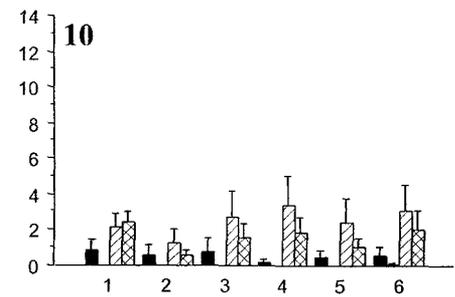
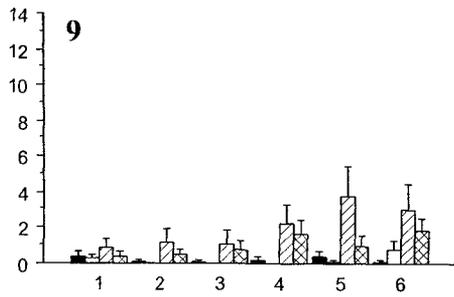
Blocks (30 secs)



Blocks (30 secs)

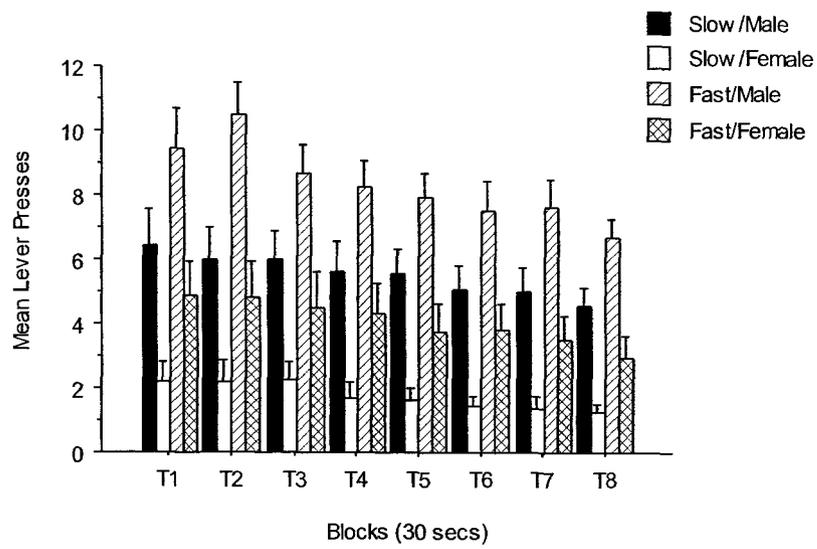
Figure 14: Mean (\pm Std. Error) Number of Bar Presses during the 3 min Post-Tone
Period (in Blocks of 30 secs) during all 13 Sessions for Male and Female
Animals in the Fast and Slow Strains





Blocks (30 secs)

Figure 15: Mean (\pm Std. Error) Lever Presses by Female and Male Animals for Fast and Slow Strains during a 4-Minute Pre-Tone Period. Responses are Split between 30 second Blocks (T1-8) Averaged over 13 Sessions for Conditioning.



but by Session 9 through 13, it appears that the Fast rats recovered and, perhaps, even the Slow males show this tendency as well, but the Slow females had very little absolute responding, which was reflected in their lower suppression ratios (Figure 10). The values in Figure 14 reveal that animals increased their responding after the shock, but the Slow females were the least to rebound, as indicated by their lower responses during the Post-Tone period.

When all the scores were collapsed and averaged over Strain, Sex, and Session, bar pressing was reduced as animals near the tone, suggesting that they are able to predict the tone, and this anticipation appeared in all groups (Figure 15). During the tone, bar pressing increased as animals near the US (shock/Figure 16), but Fast rats, especially Fast males, seem to contribute most to this behaviour. During the Post-Tone, it was noticed that when all responses were collapsed over Sex, Strain, and Session there was an initial decline in responding within the first block (Figure 17) due to the preceding shock, but immediately thereafter an increase in bar pressing occurred.

Discussion

In this study, we were interested in investigating the contribution of age and sex in two lines (strains) of rats selectively bred for their susceptibility in amygdala kindling. Generally, strain and age differences were prevalent and little sex differences were noticed. In particular, the Fast rats tended to be less fearful than the Slow rats, and the younger animals were less fearful than the older animals.

In a previous study, Fast adult rats were shown to have more total arm entries

Figure 16: Mean (\pm Std. Error) Lever Presses made by Female and Male Animals for Fast and Slow Strains during the 3-Minute Tone Period. Responses are Split between 30 sec Blocks (T1-6) Averaged over 13 Sessions for Conditioning.

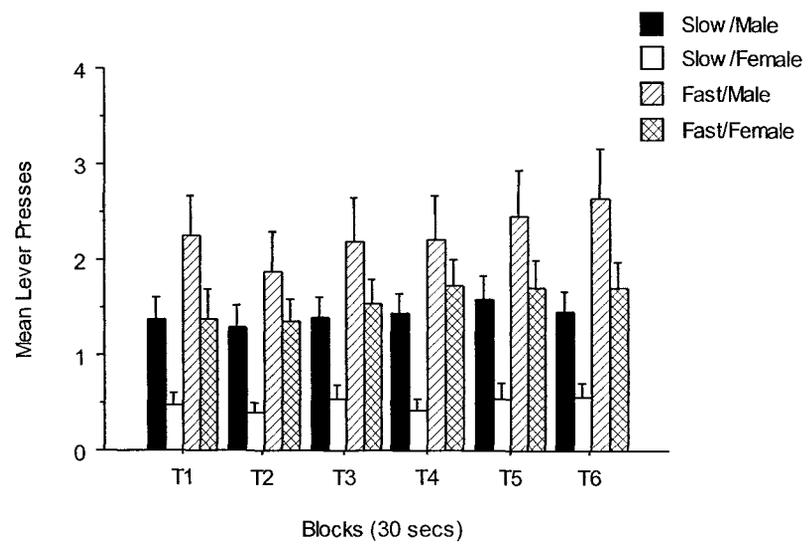
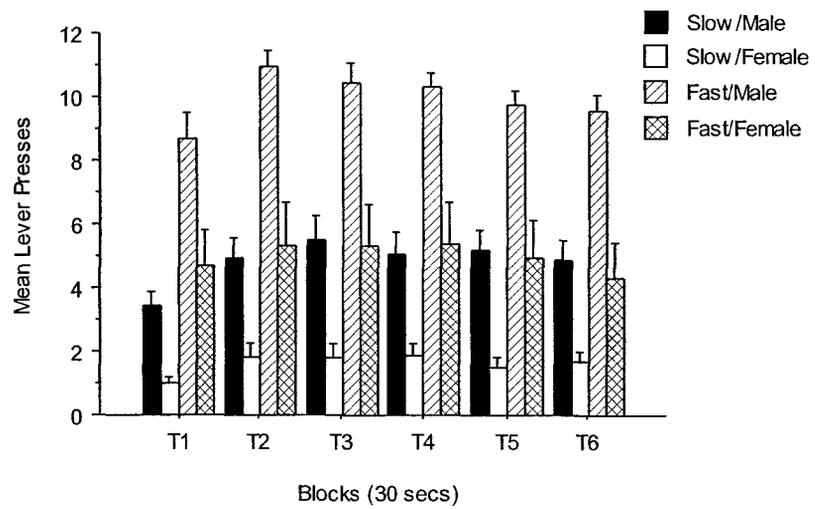


Figure 17: Mean (\pm Std. Error) Lever Presses made by Female and Male Animals for Fast and Slow Strains during the 3-Minute Post-Tone Period. Responses are Split between 30 second Blocks (T1-6) Averaged over 13 Sessions for Conditioning.



in the EPM compared to the Slow rats (Mohapel & McIntyre, 1998), and this was replicated in the current study. The results here also found that the Fast strain made more closed arm entries than did the Slow strain. The total number of arm entries and the total closed arm entries are indicative of locomotion and the total closed arm entries tend to be a superior measure of activity because it is a measure independent of the percent number of open arms and the percent time spent in the open arms (Hogg, 1996). The Fast strain is clearly more active in the EPM. This is further supported by the increased number of lines crossed in the open field made by the Fast compared to the Slow rats. Cumulatively, these observations suggest that the Fast rats were more active than the Slow rats. The intense activity of the Fast animals was also observed in the absolute number of bar presses made during the Pre-Tone, Tone, and Post-Tone time periods. The Fast rats were superior in their bar pressing behaviour compared to the Slow rats. It has been suggested previously that the Fast rats showed certain symptoms of attention-deficit/hyperactivity disorder (ADHD), such as impulsivity and attention disturbances (Anisman & McIntyre, 2002; McIntyre et al., 2002). The Fast strain tends to be more impulsive in their sexual performance, having difficulty inhibiting mounting behaviour even when confronted with a non-estrous female (Michaud, McIntyre, Anisman, & Merali, 1999). In addition, Fast rats tend to vigorously struggle more than Slow rats when restrained (Anisman, Lu, Song, Kent, McIntyre et al., 1997; McIntyre, Kent, Hayley, Merali, & Anisman, 1999) most notably due to the genetic and neurological differences between the strains. Furthermore, when tested in the T-maze, the Fast strain was noticed to lack behavioural hesitation, indicative of reduced decision making, but

opted instead to maintain movement in the direction in which they were oriented (McLeod & McIntyre, 1995; McIntyre, McLeod & Anisman, 2004), suggesting that these animals were impulsive in their behaviours compared to the Slow animals. Paradoxically, time spent in the center of the EPM is thought to reflect decision making (Boguszewski & Zagrodzka, 2002; Cruz, Frei, & Graeff, 1994) and the Fast strain spent more time in the center area of the EPM compared to the Slow strain, apparently indicating the Fast rats tendency to be hesitant of arm choice. It may be that these differences in decision making behaviour in the Fast strain between the T-maze and EPM has to do with their differences in motivation. In the T-maze animal behaviour is motivated by hunger, whereas in the EPM fear motivates behaviour. Since Fast animals tend to be less fearful than Slow animals they may be more likely to spend more time in the center portion of the EPM. If this is the case, the center may not reflect decision making *per se*, but may indicate how fearful the animal is instead. Furthermore, Fast rats had difficulty performing in the Morris water maze when a cued stimulus indicating a submerged platform was adjacent to distracting and irrelevant stimuli (Anisman & McIntyre, 2002). The increased scores in locomotion, measured by the EPM and open field, and the heightened bar pressing behaviour in the CER may reflect hyperactive characteristics within the Fast strain, enhancing the idea that these animals are a relevant model of ADHD.

Strain differences were also observed in fear when rats were tested in the EPM and open field. The Fast rats spent more time in the open arms of the EPM and made more open arm entries compared to the Slow rats, the latter result being reported

previously (Mohapel & McIntyre, 1998). It may be suggested that the differences between the strains in the percent time spent in the open arms as well as the percent number of open arm entries may be due to differences in strain activity. More specifically, since the Fast rats are more active in the EPM this may contribute to their increased time spent in the open arms and their higher open arm entry scores. However, as noted above, total closed arm entries, a measure of rodent activity, has been shown to be a measure independent of percent time spent in the open arms and percent number of open arm entries. In addition, the total arm entries increased with age in both the Fast and Slow rats. If activity in the EPM contributes to percent time spent in the open arms and the percent number of arm entries, then logically these measures should increase with an increase in total arm entries. However, the opposite was observed, that is, the percent time spent in the open arms and percent number of open arm entries decreased with age as total number of open arm entries and closed arm entries increased. The additional arm entries were made into the closed arms with age, further suggesting that activity is an independent measure of fear in the EPM. The results in the EPM, i.e., that Fast rats are less fearful in the maze than Slow rats, was also confirmed in the open field.

In a previous study, it was noticed that the Fast rats crossed more lines in the open field compared to Slow rats over the course of 4 days (Mohapel & McIntyre, 1998). The results in this study also support this observation. In addition, in that study, it was found that Slow rats had made more line crosses on day 1 and significantly reduced the number of lines crossed over 3 subsequent days. The Fast animals, however, had crossed fewer lines on day 1, but maintained that level over the 3 following days. There are two

alternative views that may explain the reduction of lines crossed made by the Slow rats and the maintenance of lines crossed by the Fast rats over days in the open field.

It could be said that the Slow rats initially explore the open field and subsequently reduce exploratory behaviour due to familiarity with the environment, whereas the Fast rats failed to undergo this habituation. However, ambulation in the open field may be inversely related to defecation, an index of fear. That is, there is a tendency for animals to reduce locomotion with increased fear. However, the opposite has been noticed; a decrease in ambulation with a decrease in defecation in the open field. Furthermore, a rodent may initially increase its locomotion in attempting to escape the open field and later take on a passive role (Ramos & Mormède, 1998). An alternative view is that the Slow rats, with their more fearful characteristic, learn to avoid the fear evoking center of the open field and thus remain in the periphery. It has been noticed that rats, when tested in the open field, will establish a home base and subsequently make exploratory excursions from this area and later return to the home base (Eilam & Golani, 1989). It could be conjectured that the Slow rats, once they develop a home base in the open field, are less likely to leave this area, where Fast rats are inclined to show the opposite pattern and thus emit exploratory behaviour. If so, then Slow rats would reduce the number of lines crossed indicating fearfulness. In order to further evaluate the differences in fearfulness in the open field, the present study measured the percent time spent in the center of the open field and the number of center entries. The Fast rats tended to spend more time in the center of the open field and the Fast rats entered the center of the open field more often than the Slow strain. Since the Fast rats tended to enter the center more

often and spent more time in the center of the open field, this would suggest that the Slow rats made most of their line crosses in the peripheral zone, although it is possible that most of their locomotion scores could have been in the center zone if they established a home base nearby and only exited this area to enter the center of the open field. The results in the open field indicate that the Slow animals are indeed more fearful than the Fast rats, and this is congruent with the results in the EPM. Whether the strains develop a home base that is preferred across days in the open field has not yet been investigated, but from the above results, it would be predicted that the Slow rats would be less likely to leave the more familiar spot over time. In addition, in neither this study or the Mohapel and McIntyre (1998) study were the number of lines crossed tabulated as a function of center and peripheral zone. It may be that the heightened score in lines crossed on day 1 made by the Slow animals were made in the peripheral zone, and if so this may reflect an attempt to escape the open field rather than explore it. Further studies on the differences in fearfulness between the strains will need to be addressed in the open field and EPM.

Substantial strain differences occurred in the CER. Initially there was little difference between strains on day 1 of the pre-conditioning tone exposure; although the Slow rats after a single habituation to the tone only, had a higher suppression score compared to Fast strain on the first conditioning day (i.e., first tone-shock pairing). However, on subsequent days the Slow animal's suppression scores remained significantly below that of the Fast animals, further confirming that the Slow rats were more susceptible to the aversive learning paradigm. A closer look at the strain

differences at the level of sexes indicated that the strain differences were between the Slow and Fast females, and generally not the males. Although, it appears that the Fast males become less fearful at session 8 and beyond compared to the Slow males. It should be noted that the Fast females appeared not to have increased their responses on day 1 after habituation, where the Fast males, Slow females, and Slow males all increased their scores on day1 of tone-shock compared to the habituation day. It could be reasoned that the differences between the strain suppression scores may be due to their differences in shock sensitivity. That is, perhaps the Fast rats are less sensitive to the US/shock, thus their suppression score would be higher compared to the more sensitive Slow animals. However, it was found that sensitivity to foot-shock runs in the opposite direction between strains. Slow animals were noticed to need a slightly larger electrical current to produce a flinch and jump response than did the Fast rats (Mohapel & McIntyre, 1998). So it is unlikely the results in the CER are due to nociceptive/reflexive response properties.

The Slow rats tend to adopt an immobility style in other aversive tasks such as the active avoidance and inhibitory avoidance tasks (Mohapel & McIntyre, 1998). In addition, the Slow animals tend to become more immobile across days in the open field. It would be expected that the Slow rats would take longer to become fearful in the open field because of its less intense fearful properties compared to the more aversive learning paradigms. This tendency to become immobile appears to be the case in the CER apparatus, although this study did not measure the amount of time freezing. However, there tends to be a positive correlation between freezing behaviour and suppression

scores (Bouton & Bolles, 1980; Mast, Blanchard, & Blanchard, 1982). Yet, freezing is not necessary for conditioned suppression (Amorapanth, Nader, & LeDoux, 1999), and so it cannot be ruled out that the Slow animals show similar behavioural profiles in the CER. From previous studies on the Fast and Slow rats, it would be hypothesized that Slow animals would be inclined to freeze more in the CER compared to Fast rats, and future studies will be needed to determine if this is the case (it will be recalled that the animals' movement in the CER apparatus could not be seen because of the apparatus placement in a styrofoam box, which was designed for sound and vision attenuation). In addition, future studies that free Slow rats from immobilization either through electrolytic lesions or reversible intracranial pharmacological injections and observing their suppression scores will shed some light on the neuroanatomical and neurochemical mechanisms that contribute to the differences between the strains.

The proclivity of the fearful Slow animals toward heightened anxiety in aversive tasks is further supported by their slower extinction rates compared to the Fast rats in the CER. Extinction is thought to be a relearning that the CS/tone is no longer followed by the US/shock, and not simply a forgetting that the CS/tone was once paired with the US/shock. Although the Slow animals have been shown to be superior in learning in the Morris water maze and the choice behaviours in the T-maze, clearly their suppression scores indicate that they are inferior compared to the Fast rats in associating a CS with no shock. However, it may be their heightened sensitivity to aversive situations as was shown by their rapid decline in suppression scores during the conditioning procedure that later hindered their ability to learn the extinction procedure.

A closer investigation of the strain differences at the level of the sexes indicated that the Slow and Fast females differed in their suppression scores during extinction, whereas the males did not differ, paralleling the results in the conditioning procedure. It appears that the Fast females are the sex/strain group to benefit most from the extinction process. There could be a number of explanations for the differential suppression scores during conditioning and extinction produced by the Fast versus Slow animals.

The Fast rats may be less affected by the previous conditioning and are quickly able to rebound in the extinction procedure. The Fast animals tend to have higher suppression scores during conditioning compared to the Slow animals, suggesting that they are indeed not as emotionally affected by the aversive conditioning compared to the Slow strain. However, it should be remembered that the Fast rats tend to have a slightly lower threshold for aversive stimuli such as shocks compared to the Slow strain, and from these results it would suggest that the Fast strain could be more affected by the shock than the Slow rats. Further studies will need to be conducted to clear up these contradictory interpretations.

An alternative explanation for the heightened suppression scores in the Fast animals may be that food tends to be a higher motivational incentive for these animals, especially for the Fast females, and this may contribute to their increased bar pressing during the tone across the extinction days. Indeed, we have repeatedly observed naïve Fast rats to consume more food and water than Slow rats. Further, in a previous study, it was observed that the Fast females reached the end of the T-maze more quickly than the

Slow females and the Fast animal's behaviour was more impulsive at the choice point, which contributed to their increased errors compared to the Slow rats who hesitated at the choice point and 'chose', more likely than not, the correct arm of the maze (McIntyre et al., 2004; McLeod & McIntyre, 1995). A third, and equally weighted explanation may be that the Fast females are more impulsive compared to the other groups and this contributes to their increased suppression scores in the CER at both extinction and conditioning procedures. Since the operant task required simple bar pressing behaviour the animals did not need to rely heavily on attention faculties compared to the T-maze task. Further studies may be interested in assessing the Fast strain's suppression scores when a more attention taxing procedure is applied in the operant box. It would be hypothesized that their scores would drop in a task that would require more complex conceptual rules. In fact, behavioral differences like that described above are being seen in another bar pressing task that requires more cue interpretation, and thus, is of greater task complexity (N. Farrell, unpublished observations).

An interesting phenomenon observed in the Fast females was they appeared not to habituate to the tone, as expressed by their lower suppression scores 1 day after the tone alone (i.e., habituation) exposure. Yet these animals appeared to be little phased at the extinction procedure, suggesting that the Fast female's behaviour reflect an underlying dissociation between two different types of learning - non-associative tone alone learning and associative tone-no shock learning.

There are several lines of evidence that suggest that the amygdala may be the

neural structure responsible for the differences in fear behaviour between the two strains. First of all, even though these animals were not selected for their differences in emotionality, they were selected for their divergent rates in amygdala kindling from cross breeding two parental stocks of Long-Evans Hooded and Wistar rats, and the amygdala is an important contributor to fear like behaviour. The amygdala nuclei that were most likely to be kindled during the selection process were the basolateral, accessory basal and medial nuclei (Racine, Steingart, & McIntyre, 1999). In addition, kindling of the amygdala produces changes in fear learning in the CER (McIntyre & Molino, 1972) and changes in emotional behaviour in the EPM and open field (Kalynchuk & Meaney, 2003; Adamec & Morgan, 1994). One of the marked differences in the Fast and Slow rats is in their immobility/mobility behaviour. For instance, the Slow animals were less mobile in the fear relevant tasks such as the active and inhibitory avoidance tasks, the open field test, and presumably in the CER apparatus, whereas the Fast rats tended to be more active in such tasks. An amygdala nucleus that may be contributing to these differences in mobility is the central amygdala (CeA). The CeA has multiple connections with brainstem and diencephalon structures that are responsible for the autonomic responses produced in aversive conditions such as Pavlovian conditioning. One particular structure that is important for conditioned freezing and receives projections from the CeA is the periaqueductal gray (PAG), and lesions to the PAG disrupt freezing (Hopkins & Holstege, 1978; Leibman, Mayer, & Liebeskind, 1970; LeDoux, Iwata, Cicchetti, & Reis, 1988; Vianna, Landeira-Fernandez, & Brandao, 2001). It is thought that the place of CS-US associations is performed in the lateral amygdala (LA) and axons from the LA do not

directly project to the CeA, but rather the LA disinhibits the CeA neurons through the GABAergic intercalated cells (ITC) that lie between the basolateral amygdala (BLA) and the CeA (Paré, Quirk, & LeDoux, 2004) during fear conditioning. Lesions to the CeA tend to disrupt conditioned suppression, whereas lesions to the BLA do not (Killcross, Robbins & Everitt, 1997). It may be that the Slow animals, with their reduced suppression scores (higher fear) in the CER, are inclined to rely on the CeA, whereas the Fast animals tend to rely less on the CeA but more on the LA, which is another site where the CS-US association takes place (Paré et al., 2004). Furthermore, it has been suggested that the BLA is involved in voluntary choice behaviour for instrumental responses, and the CeA is involved with producing CS-US associations, and this nucleus is less involved with active avoidance tasks compared to the BLA. For instance, lesions to the BLA disrupt conditioned suppression, a procedure where two levers are made available to a rodent and one of the levers terminate the onset of the shock (Killcross, Robbins, & Everitt, 1997). Perhaps the Fast rats, with their higher instrumental responses, rely on the BLA and its circuitry. It has been shown that lesions to the BLA produce impulsivity in rats (Winstanley, Theobald, Cardinal, & Robbins, 2004) further suggesting that through bi-directional amygdala kindling selection, these two strains may have differentially dissociated CeA and BLA systems, and this maybe accentuated in the females in the CER.

The strain differences in extinction may be supported by amygdala and medial prefrontal cortex (mPFC) connections (McDonald, Mascagni, & Guo, 1996). There is evidence that this pathway is important in forming new memories about the CS-no US

procedure, which inhibits the responses acquired during the CS-US exposure (Morgan, Romanski, & LeDoux, 1993; Quirk, Russo, Barron, & Lebron, 2000; Milad & Quirk, 2002). In addition, this pathway commencing in the mPFC terminates on the GABAergic intercalated (ITC) cells of the amygdala (Quirk, Likhtik, Pelletier, & Paré, 2003) and that this pathway mediates the neuronal transmission from the BLA to the CeA in order to inhibit the fear conditioning responses. The Fast rats, especially the females of this strain, could have a superior mPFC-amygdala pathway compared to the Slow strain since their suppression scores increased during extinction, that is, their fear responses were inhibited much more quickly than that of the Slow strain. It may be that a combination of impulsivity, which is associated with the BLA, and the inhibitory mechanisms of the mPFC on the ITC cells of the amygdala enhance the Fast strains extinction of fear conditioning. Further studies on the neurophysiology, neuropharmacology and neuroanatomy of fear conditioning and extinction, especially those connections between the nuclei within the amygdala will need to be addressed in order to further understand the differences between the two strains in aversive environments.

The differences between the strains are relatively similar at both juvenile and adult ages with Slow rats being more fearful in the EPM and open field, and the differences in activity between the strains also remained similar at both ages with Fast rats being more active than Slow rats. The early divergence in fear between the strains indicate that the Fast rats are consistently less fearful than the Slow animals throughout the developmental process compared to the Slow animals, suggesting that the selection of strain differences in amygdala kindling have also genetically altered the two strain's

responses in fear evoking tests. Although the Fast and Slow rats were not selected for their differences in fear, they do have some similarities to strains that were bidirectionally selected for emotionality. For instance, the high-activity behaviour (H line) and low-activity behaviour (L line) rats were selected for their differences in open field center zone locomotion. The H line was noticed to be more active in the fear evoking open field center compared to the L line of rats. The H strain also spent more time in the open arms of EPM and also entered more open arms in the EPM compared to the L strain (Ramos, Correia, Izídio, & Brüske, 2003).

Other rat strains bred for their differences in emotionality and show similar characteristics to the Fast and Slow strains are the high-anxiety behaviour (HAB) rats and low-anxiety behaviour (LAB) rats. The HAB strain was noticed to spend less time in the open arms and enter the open arms less often than do the LAB rats. In addition, the LAB rats spent more time in the center of the open field compared to the HAB strain. Also the HAB rats tended to adopt a floating style in the forced swim test, whereas the LAB rats were more active, suggesting the HAB rats engage in a passive coping style (see Langraf & Wigger, 2002). These results are similar to those found in the current study. Also it was observed in an earlier study that the Slow rats, when tested in a forced swim paradigm, were initially as active as the Fast rats on trial 1 but on trials 2 and 3 the Slow rats engaged in floating behaviour. The Fast rats in contrast maintained an active behavioural profile throughout the three trials in the forced swim test (Anisman & McIntyre, 2002). The differences in the HAB and LAB rats have been contributed to by a vasopressin gene. Administration of vasopressin injected into the CeA of rats has been

shown to engage a passive coping style (Rooszendaal, Wiersma, Driscoll, Koolhaas, & Bohus, 1992), and providing a vasopressin antagonist to HAB reduced their levels of anxiety (Langraf & Wigger, 2002). It could be conjectured that the Slow rats, with their proclivity to freeze in aversive tasks and adopt a passive style over days in the open field, have a copious supply of vasopressin. However, this might be incongruous with their kindling rates compared to Fast rats, since vasopressin is thought to reduce the number of stimulations to induce convulsions (Gillis & Cain, 1983).

A developmentally related molecular component that may contribute to the continual differences at both age groups between the two strains is that the Fast and Slow rats have differences in their expression of GABA_A receptor subunits. It was found that the Slow animals in adulthood have an over-expression of the adult subunit α_1 , while the Fast animals in adulthood over-express the developmentally early subunits α_2 , α_3 , and α_5 , and the amygdala was one of several structures where this difference was prevalent (Poulter et al., 1999). In addition, the α_2 , α_3 , and α_5 subtypes tend to be copious in young animals, and with aging, these receptors deplete in number as the α_1 subunit increases (Poulter, Barker, O'Corroll, Lolait, & Mahan, 1992). It also appears that the α_2 and α_3 subunits are involved with anxiety like behaviours (Atack, 2005). It has also been noticed that the anxiolytic effects of anti-anxiety benzodiazepines is on the α_2 and α_3 and not on the α_1 subunit (Rowlett, Platt, Lehas, Atack, & Dawson, 2005). The over-expression of α_2 and α_3 subunits may contribute to the lower levels of fear in the Fast rats at both ages. If these receptor subunits do play a role in the strain differences for fear related behaviour, then it would appear that these differences are already prevalent at 30

and 32 days of age, since Fast rats are significantly less fearful especially in the EPM, compared to the Slow rats. Although the temporal transition from the juvenile α_2 , α_3 , and α_5 subtypes to adult α_1 subtype are unknown in the Fast and Slow strains, it could be hypothesized that the GABA_A receptor subunits differences are already observant at a pre-pubertal age. The α_1 subunit is thought to play a role in the sedative effects of benzodiazepines (McKernan, Rasahl, Reynolds, Sur, Wafford et al., 2000), and it is interesting to note that the Slow rats with their copious expression of α_1 subunits become sedated at relatively low doses of diazepam compared to Fast rats (McIntyre et al., 2002).

It has been shown that play behaviour is a time most marked with impulsivity and distractibility (Adriani & Laviola, 2003; Laviola, Adriani, Terranova, & Gerra, 1999; Laviola, Macri, Morley-Fletcher, & Adriani, 2003). It appears that play in the Fast and Slow rats develops normally, where both strains tend to reduce play behaviour (Reinhart, Pellis, & McIntyre, 2004) and increase fear-like behaviour with increasing age; the Slow animals tend to be sided on the adult end of the developmental continuum, whereas the Fast rats, with their increased play behaviour, impulsivity, distractibility and lower fearfulness scores, are positioned nearer to the juvenile end of the developmental continuum. Furthermore, as mentioned above, both fear and play behaviours in rodents tend to have a similar neural mechanism and this appears to involve the amygdala (Daenen, Wolterink, Gerrits, & Van Ree, 2002; Panksepp, 1998). It also appears that genetic constraints on the neurodevelopment of the amygdala contribute to the differences in behaviour between the two strains. Although this study pursued the strain differences in fear at juvenile ages 30 and 32 days in the EPM and open field,

respectively, and since these strain differences are significantly noticeable especially in the EPM, it would be of interest to investigate if these differences are also apparent at even younger ages, for example, infancy. From the results in the present study, it would appear that the two strains, with their genetically different backgrounds, would show differences in fear even at infancy, with Slow rats being more fearful than Fast rats. For instance, since it is thought that the number of vocalizations made by a pup, when separated from its mother, is reflective of anxiety (Tornatzky & Miczek, 1994), it would be hypothesized that the Slow animals, when separated from their litter, would initially produce more vocalizations than the Fast rats, and subsequently the Slow animals may adopt a passive coping style. The increase in fear with age is consistent with similar studies (Boguszewski & Zagrodzka, 2002; Imhof, Coelho, Schmitt, Morato, & Carobrez, 1993). However, a number of cases showed that as an animal increases in age, there is a decline in locomotion (Lamberty & Gower, 1993; Sprott & Eleftheriou, 1974), whereas the Fast and Slow rats tend to increase their activity with age, as indicated by their increase in total arm entries and their number of closed arm entries in the EPM. As mentioned earlier, this activity tends to be independent of the fear related behaviour. Even though the activity increased, it was weighted more on the closed arms and less on the open arms in the EPM as age increased reflecting elevated fear.

A number of studies have found sex differences in rodents in the EPM and open field with males being more fearful than females (Masur, Shutz, & Boerngen, 1980; Johnston & File, 1991). No sex differences were noticed in the Fast and Slow strains in the EPM and CER at conditioning and extinction. Interestingly, however, the female Fast

and Slow rats remarkably differed from each other in their conditioning and extinction scores, whereas the male strains did not. The Fast females tended to have higher bar pressing during the tone compared to the Slow females.

Another interesting result that differentiated the males from the females is the oscillatory or cyclic bar pressing during conditioning observed in the females. The Fast females started increasing their bar pressing during the tone at session 7 and peak on session 8 and subsequently bar pressing during the tone declined from session 8 to session 11 only to start increasing at session 12 (see Figure 10). The Slow females more or less parallel the pattern of the Fast females in this behaviour. The Slow females increase their bar pressing during the tone at session 9 and this is the session at which they peak and then bar pressing decreases between sessions 9 and 11 and starts to rise again thereafter. Since males did not show this type of pattern in their bar pressing during conditioning, it may be that female gonadal hormones (a 4 day cycle) were affecting the motivated pressing behaviours in the female strains. Indeed, a number of studies have shown females are less fearful when in estrous (Frye, Petralia & Rhodes, 2000; Gray & Levine, 1964). In addition, male gonadal hormones (which do not cycle) have little affect on anxiety-like behaviours (Zimmerberg & Farley, 1993). Surprisingly, the fluctuating behaviours noticed in the females during conditioning was not apparent during extinction, suggesting that the neurological pathway for extinction (i.e., amygdala-mPFC) is not influenced by female hormones compared to the apparent neurological pathway involved with conditioning (e.g., BLA or CeA). Also a sex difference was observed in the number of center entries in the open field, but apparently this difference

was not observed in time spent in the center, suggesting that the female rats perform increased risk behaviour by entering the center of the open field more often than males, but quickly revert to the safe peripheral zone.

In summary, it can be said that the Fast rats are less fearful than Slow rats, and this is probably due to differences in the functioning of limbic structures, and in differences in either the neural connections, neurophysiology or neurochemistry of the systems involved with fear. Since the amygdala is especially important in fear like behaviours, and was the structure kindled in the selection process, it would appear that these animals would have comorbid differences particularly related to this structure.

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