NOTE TO USERS

The original manuscript received by UMI contains pages with slanted print. Pages were microfilmed as received.

This reproduction is the best copy available

UMI

**DISCRIMINATIVE FEAR CONDITIONING TO CONTEXT EXPRESSED
BY MULTIPLE MEASURES OF FEAR IN THE RAT**

by

Elena Anna Antoniadis

**A thesis submitted in conformity with the requirements
for the degree of Master of Arts,
Graduate Department of Psychology,
University of Toronto**

© Copyright by Elena Anna Antoniadis 1997

National Library
of Canada

Acquisitions and
Bibliographic Services

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale
du Canada

Acquisitions et
services bibliographiques

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-29181-2

Canada

Discriminative Fear Conditioning to Context Expressed by Multiple Measures of Fear in the Rat. M.A., 1997.

Elena Anna Antoniadis, Department of Psychology, University of Toronto.

Abstract

There has been a renewed interest in the neural basis of fear conditioning to context. These current approaches are accompanied by some limitations including the use of short testing windows, non discriminative paradigms, and unitary fear response assessment. In an attempt to circumvent these limitations, a discriminative context procedure assessing multiple response measures of fear was used in the present study. Conditioning consisted of three training sessions and each session consisted of two days. On day one the animals were placed in the paired context and received three foot shocks. On the other day they were placed in the unpaired chamber in the absence of any aversive event. Animals were tested after each training session and the responses measures of fear recorded included: preference, freezing, heart rate, ultrasonic vocalizations, defecation, body temperature, urination and locomotion. The results suggest that behavioral, as well as physiological changes evoked by fearful stimuli become associated with the context in which the aversive event occurred. In general these findings also suggest that there are different learning rate parameters for the measures of fear examined in this paradigm.

Acknowledgments

My supervisor, Dr Robert James McDonald had a very significant impact on this stage of my graduate studies. I am grateful to him for his dedication to instruct, for his genuine interest in my ideas, and for all the insightful scientific discussions. I thank my subsidiary supervisor Dr Martin Ralph for the advice and discussions on the possible implications of this project. I would be remiss if I didn't acknowledge my primary mentors, Drs Andy Baker, Robin Murphy, Norman White, Eric Hargreaves, Juan Salinas and Brian Devan, for introducing me to the world of animal behaviour and the underlying neural structures of learning and memory. My sincere appreciation to Dave Ellemberg, Marianne Beaudouin, Roberto Salamao, Mac Priebe, Dean Walters and Rick Brown. Their friendship has been inestimable. A heartfelt thanks to my brothers Tasso and Stavros, who despite the distance have always been a primary source of encouragement and support. I am most grateful to my parents for their love. They always have and always will be my greatest inspiration.

.

TABLE OF CONTENTS

Abstract	ii
1.Introduction	1
2.General method	8
3.Results	16
4.Discussion	24

ΓΕΙΡΑΣΚΩ ΑΕΙ ΔΙΔΑΣΚΟΜΕΝΟΣ
Σωκράτης

“I AM GROWING UP AND CONSTANTLY LEARNING”
SOCRATES

To my parents

Introduction

There has been a resurgence of interest in fear conditioning to context, probably because it is a long lasting and rapidly acquired form of learning (Ledoux, 1994). Fear conditioning to context consists of the ability to acquire and retain memories of static stimuli and situations that have been associated with danger, and results in the acquired capacity by these stimuli and situations to elicit natural fear responses. Traditional fear conditioning to context experiments involve the pairings of a neutral stimulus with an aversive event such as a shock and conditioning is subsequently assessed by the ability of these previously neutral stimuli to elicit fear (Estes & Skinner, 1941; Kamin et al, 1963; McAllister & McAllister, 1971). The behavioural expression of fear conditioning to context has been observed in a wide range of organisms ranging from those with primitive nervous systems to the most complex (Tully, 1991; Cohen & Goff, 1978). Throughout the lifespan of human and non-human animals, the ability to use contextual information to select appropriate behaviour must be considered one of the fundamental functions of the nervous system.

Three main views exist concerning the influence that contextual stimuli may exert on behaviour. The first view suggests that the context is functionally equivalent to a conditioned

stimulus(CS) that can be associated with an unconditioned stimulus (US) and compete for associative strength with all other stimuli present during conditioning (Rescorla & Wagner, 1972; Mackintosh, 1975). According to these theorists, since the context is viewed as another CS, then it is natural to conceptualize that context-CS associations would reduce the strength of CS-US associations.

The second view proposes that one function that the context has is to resolve the ambiguity of a stimulus that has had a history of reinforcement and non reinforcement. Context acts as a reference point, in that contextual cues present during training later retrieve a memory of the CS-US association and contextual cues present during extinction later retrieve a memory of the CS alone (Bouton & Bolles, 1979; Bouton & King, 1983). Accordingly, testing in the training context will evoke fear of the CS, and interestingly, so will testing in a novel context(Bouton & Bolles, 1979). This renewal of fear demonstrates that associative strength accrued to the context is not necessary for contextual influence over behaviour (Bouton & King, 1983; Bouton & Bolles, 1979).

The final view is hierarchical according to which the relation between the context and CS should not be viewed as competitive in nature. Accordingly, this theory proposes that the context both contains and predicts CSs (Nadel & Willner, 1980). This view encompasses the notion that environmental context is represented at two levels in the nervous system. Each cue has its

own cortical representation and the entire context is a higher construct elaborated in the hippocampal system.

There is also considerable disagreement in the neurobiology of learning and memory literature with regards to the forebrain learning and memory systems necessary for fear conditioning to context. There are four main views that dominate current thinking about the identification and organization of these forebrain learning and memory systems.

One of the earliest views on the involvement of forebrain learning and memory systems required for fear conditioning to context proposes that the hippocampus directs learning to the most predictive stimuli in a learning situation (Winocur & Olds, 1978; Winocur & Gilbert, 1984). This position stems from the findings that in contrast to controls animals which show little conditioning to context in the presence of an explicit CS, hippocampally lesioned animals exhibit enhanced contextual conditioning to background stimuli of little predictive value (Winocur & Olds, 1978). This set of data suggests that rats with hippocampal lesions show enhanced fear conditioning to context, even in the presence of a phasic CS that reliably signals the occurrence of a shock. This data supports the idea that rats with hippocampal lesions exhibit a learning deficit reflected by their inability to exclude irrelevant stimuli (Winocur & Olds, 1978).

Sutherland and McDonald (1990) claim that the hippocampus is

involved in fear conditioning to context and the amygdala is involved in fear conditioning to single cues (Sutherland, & McDonald, 1990). This proposal emerges from their finding that the autonomic response of defecation, conditioned to a context, is disrupted following hippocampal lesions but is not disrupted following amygdala lesions (Sutherland & McDonald, 1990). Accordingly, the hippocampus is responsible in the expression of some conditioned fear responses independent of the amygdala. Further support for this view comes from a study by Selden et al., (1991) in which rats with hippocampal lesions showed normal fear conditioning to explicit cues but were impaired at discriminating environments in which those cues co-occurred. Rats with amygdala lesions were impaired at fear conditioning to explicit cues but not to contextual cues (Selden, et al., 1991).

Phillips and Ledoux (1992), introduced the notion of a functional dependence between the two medial temporal lobe structures in that the hippocampus plays a role in fear conditioning to context by forming a representation of the context and subsequently sending this information to the amygdala where the context-shock association occurs. In line with this proposal, hippocampal lesions interfered with fear conditioning to context but not with phasic CS conditioning. Interestingly, rats with amygdala lesions were unable to acquire either forms of fear conditioning (Phillips & Ledoux, 1992). A similar view has been

proposed by Kim and Fanselow (1992) who also advocate the role of the hippocampus in fear conditioning to context but not to explicit cues. This view stems from their finding that rats with hippocampal lesions were impaired at fear conditioning to context but not to a phasic CS (Kim & Fanselow, 1992).

Finally, a more recent view has developed from the initial proposal put forth by Phillips and Ledoux (1992), suggesting that the hippocampus plays a different role in contextual fear conditioning depending on the training condition (Phillips & Ledoux, 1994). If a phasic CS reliably predicts the occurrence of a shock, conditioned responses are more likely to develop to the CS than to any static cues that form the context. In contrast, if the context is a better predictor of shock onset, conditioned responses are more likely to develop to the static cues that form the environment. The former training procedure is referred to as background fear conditioning to context, whereas the latter is referred to as foreground fear conditioning (Phillips & Ledoux, 1994). Rats with damage to the hippocampus are impaired at background but not foreground fear conditioning.

From this brief review, it seems clear that there is a considerable disagreement about the role of context in fear conditioning as well as the precise roles of the amygdala and hippocampus within the overall organization of the neural circuitry of emotions. Part of the problem in understanding these complex

issues arises from the various limitations found in experiments to date. These limitations include the use of : 1)short testing windows 2)non-discriminative paradigms 3)assessment of single responses of fear. Short testing windows might be insufficient for assessing conditioned fear and may in fact mask conditioning of fear responses that take longer to fully develop. A discriminative fear conditioning to context paradigm may provide insight into which aspects of the context the animal is associating with the aversive event and removes generalized fear and sensitization as possible confounds. The assessment of multiple measures of conditioned fear will give a more sensitive appraisal of conditioning and may be valuable in determining if different forebrain learning and memory systems have differential access to fear responses (Sutherland & McDonald, 1990).

In the present experiment, we modified a discriminative fear conditioning to context paradigm that was originally developed by McDonald et al., 1995. The current discriminative paradigm differed in that we assessed eight measures of fear and used multiple test sessions. The measures of fear we assessed included preference, freezing, heart rate, ultrasonic vocalizations, defecation, body temperature, urination and locomotion. The assessment of multiple measures of fear as well as the expanded testing windows should provide a clearer appraisal of the physiological and behavioural

changes that occur following discriminative fear conditioning to context.

General Method

Subjects

Twelve male Long-Evans rats were used. The animals were individually housed in single Plexiglas cages (24 cm long x 22 cm wide x 20 cm high), and were maintained on a 12:12 hr light-dark cycle. The rats weighed approximately 300-325g at their arrival, and were given free access to food and water.

Apparatus

Two context chambers were used that differed on four dimensions: colour, shape, odour and tactile cues. One context was a black triangle prism measuring 61 cm long x 61 cm wide x 30 cm high. The other context was a white square prism measuring 41 cm long x 41 cm wide x 29 cm high. A pill bottle measuring 1 cm (diameter) protruded from a hole of the same size at the top corner of one of the chamber walls. On a daily basis a drop of each substance, serving as the olfactory cue, was placed on a cotton ball and inserted within the bottle. The odour was present in each chamber during the pre-exposure, conditioning and testing sessions

of the experiment. Isoamyl acetate served as the olfactory cue in the black triangle and eucalyptus served as the olfactory cue in the white square. The third chamber was an alley (16.5 cm long x 11 cm wide x 11 cm high), used to connect the two contexts during pre-exposure and the preference test. The flooring of all chambers consisted of grids, but a Plexiglas platform was placed on top of the grids of the chamber that served as the "unpaired" context. The Plexiglas flooring served as the tactile cue for the unpaired context, and the grids served as the tactile cue for the "paired" context. The three chambers were placed on a Plexiglas table that was 100 cm above the floor. A mirror (91 cm long x 61 cm wide), inclined by 45 degrees, was placed on the floor of the testing room which unobtrusively provided a full view of the chambers. A video camera was placed two feet in front of the mirror. This setup allowed the experimenter to observe and video tape ongoing behaviour throughout all phases of the experiment. The experiment was conducted in two different rooms, located in two different laboratories. One of the rooms served as the conditioning room, where shocks occurred, and the other served as the safe room. The entire apparatus, including the chambers, the computer, the shock generator, the video camera, the mirror and the Dataquest equipment were transported back and forth on a trolley.

Surgery

Rats were first injected with .2mg of atropine to facilitate respiration, and were subsequently anaesthetized with sodium pentobarbital (65 mg/kg ip). Biocompatible and hermetically sealed transmitters (model TA10 CA F40, Data Sciences, St. Paul, MN) were implanted into the peritoneal cavity. These implants are made of a device body (electronics module Battery) with two flexible leads extending from it. The leads are made of stainless steel wires covered with insulating layers of silicone tubing. Following implantation of the transmitter, animals were allowed to recover for 7 days.

Data collection

Heart rate and body temperature: Implanted transmitters produced a temperature and electrocardiogram (ECG) signal (10 mv peak-to-peak biopotential) that was telemetrically transmitted via radio-frequency signals to a receiver (model RLA1020, Data Sciences) placed close to the cage. The receiver converted these signals into a form accessible to the Dataquest PC-based data acquisition system which processed the data into digital form. ECG and body temperature were sampled for 5 sec every 10 sec, and heart rate was extracted from the ECG.

Ultrasonic vocalizations: A bat detector (Mini-3 bat detector, Hockley, Birmingham) equipped with a high frequency microphone served as the ultrasonic signal device and transduced ultrasounds into the audible range. This bat detector, placed non intrusively underneath the chamber grids, was set at 22-KHz because of previously reported findings that rats emit vocalizations in the 20-28-KHz range in reaction to noxious stimuli such as footshock (Tonoue et al., 1984; Cuomo et al., 1988; van der Poel et al., 1989). The bat detector was in turn connected to a CTR-102 tape recorder. All vocalizations were recorded and kept as part of the permanent data set. Headphones were connected to the bat detector, allowing the experimenter to audit and count the number of occurrences during the experiment.

Freezing: Freezing was defined as a total absence of body or head movement except that associated with breathing (Blanchard & Blanchard, 1969). Video tapes containing all the test sessions were scored by the experimenter at the end of the experiment, and this measure of fear was quantified in amount of time spent freezing in seconds.

Locomotion: Equi distant lines (2 inches) perpendicular to the chamber grids were drawn on the Plexiglas table were used to quantify locomotor behaviour. The number of times that the animal crossed any of the lines served as an index of the amount of locomotion occurring in the paired and unpaired context. This

measure was also assessed by re-scoring the video tapes.

Urination: During the testing sessions the experimenter counted the number of emissions by each animal in each context, and confirmed the initial assessment by scoring the video tapes .

Defecation: On a daily basis faecal matter was collected and weighed on a Mettler scale.

Preference test: Time spent in each chamber was assessed by the experimenter during the testing phase and confirmed during the video scoring. Dwell time was recorded when both forepaws were past the threshold of the doorway into one of the chambers. Dwell time was not recorded when both forepaws were past the threshold of the doorway into the alley.

We have the a-priori assumption that freezing, heart rate, ultrasonic vocalizations, defecation, body temperature, urination and locomotion will be higher in the paired context. We also hypothesize that dwell time will be higher in the unpaired context during the preference test.

Procedure

The entire experiment consisted of four phases: 1) baseline recordings; 2) pre-exposure; 3) training; 4) testing.

Baseline. The first phase consisted of baseline recordings which lasted 4 days. Rats' home cages were individually placed on

top of the receiver (model RLA1020) while ECG and body temperature were recorded for a total period of 2 min. This procedure was used to get an assessment of baseline heart rate and body temperature. For the next three minutes, rats were individually handled by the experimenter in an attempt to render the room and the experimenter neutral. On each of the four days rats had two baseline sessions, one in each room. The order in which the animals experienced these two rooms during the baseline procedures was counterbalanced. One of the rooms was designated the shock room because all of the shocks occurred there. The other room was designated the no shock room because no shocks ever occurred there during conditioning.

Pre-exposure. The second phase lasted two days and consisted of pre-exposure to the entire apparatus. This pre-exposure occurred in the no shock room for both days. Animals were placed in the middle alley and were given free access to the experimental apparatus for a total period of 10 min each day.

Training. During the training phase of the experiment, each daily trial lasted 5 minutes. On day one of each training session, half the animals were confined in their assigned paired context and received one set of 3 footshocks (1 mA) in the shock room at the 2, 3, and 4 minute mark of the training session. The other half of the rats were confined in their assigned unpaired context and received no foot shock in the safe room. The order in which the animals experienced each context was counterbalanced so that half

the animals were confined in the "paired" context on day one of each session and confined in the "unpaired" context on day 2. The chamber that served as the paired context was also counterbalanced so that half the animals experienced the aversive event in the black triangle and the other half experienced the aversive event in the white square. Animals always experienced their paired context in the shock room and their unpaired context in the safe room. During the training sessions, behaviour via the video camera was recorded and measures that required specialized equipment were also recorded. Following each session, faecal matter was collected, and weighed. Each training trial lasted for a total of five minutes.

Testing: All testing occurred in the safe room to assess the amount of fear conditioning to the chambers versus the room. Half of the animals were confined individually in the paired context and experienced no shock on day 1 and the other half were confined in their paired context on day 2 of the session. The behavioural measures, available to the naked eye were freezing, defecation, urination locomotion and preference. The physiological measures recorded with specialized equipment were heart rate, body temperature and ultrasonic vocalizations. The conditioning and training sequence described above was repeated three times, and on the last session the testing period was expanded to 20 min as opposed to 10 min.

Preference test: For the last phase of the experiment animals

were given free access to the entire apparatus by being individually placed in the alley. Animals were expected to spend more time in the unpaired context and express an active avoidance of the paired context.

Results

Pre-exposure

Figure 1(A) shows the mean amount of time spent by a normal group of rats in each chamber during the pre-exposure phase. The graph shows that rats spent an equal amount of time in the two chambers. A matched sample t-test, used to compare the average time spent by the rats in the black triangle prism and in the white square prism indicates that the difference was not statistically different ($t_{11} = .73, p > .05$).

Preference Test

Figure 1 (B) shows the mean amount of time that the rats spent in each chamber during the preference phase. The graph illustrates that the animals spent considerably more time in the unpaired context. A matched sample t-test, used to compare the average time spent by the rats in their respective paired and unpaired contexts, indicates a significant difference, ($t_{11} = 2.91, p < .05$). These results suggest that subsequent to conditioning, the initial lack of preference for any of the chambers turned into an active

avoidance of the paired context and a preference for the unpaired context. The combination of the behaviour exerted on the pre-exposure day and on the preference test supports the notion that this is an unbiased method.

Freezing

Figure 2 shows the mean amount of freezing elicited by a group of normal rats in their paired and unpaired contexts during the three testing sessions. Clearly, rats spent more time freezing in the paired than in the unpaired context in all three testing sessions. A matched sample t-test was used to compute the difference between the average amount of time freezing in the paired and unpaired context at sessions 1, 2 and 3. Results indicate that there was a statistically significant difference at session 1, ($t_{11} = 2.88$, $p < .05$), session 2, ($t_{11} = 4.33$, $p < .05$) and session 3 ($t_{11} = 6.69$, $p < .05$). These results suggest that freezing is a fear response that conditions to context with a rapid acquisition rate.

Heart Rate

Figure 3 shows the mean difference in heart rate in a normal

group of rats in the paired and unpaired context for test sessions 1, 2 and 3. As can be seen in the graph, heart rate was higher in the unpaired context in session 1 given the negative mean difference. A one tailed matched sample t-test was used to compute the difference between the two contexts and revealed a non-significant difference $t_{11} = 1.01$, $p > .05$. For test session 2 heart rate was higher in the paired context given the positive mean difference, however, this difference was not statistically significant, $t_{11} = 1.00$, $p > .05$. For test session 3 mean heart rate was also higher in the paired context however this difference did not reach statistical significance, $t_{11} = 1.74$, $p > .05$.

Figure 4 shows the mean difference in heart rate for the first 10 minute block and the last 10 minute block of test session 3. As depicted in the graph, heart rate was higher in the paired context during the first and last 10 minute block, but the mean difference was greater during the last 10 minute block. A one tailed matched sample t-test used to compute the difference in heart rate between the two contexts did not reveal a significant difference in the first block, $t_{11} = .63$, $p > .05$, but revealed a significant difference in the second 10 minute block, $t_{11} = 3.90$, $p < .05$. This analysis shows that discriminative heart rate occurred starting at the 10 minute mark of session 3. An analysis of variance (ANOVA) with time block of 2 minutes as the repeated factor, indicated a significant session effect, $F(9,99) = 2.50$, $p < .05$. The same

analysis with time block of 10 minutes as the repeated factor also revealed a significant session effect, $F(1,11) = .12$, $p < .05$. These results support the idea that the significant difference emerges across time within the session.

Ultrasonic Vocalizations

Figure 5 shows the mean amount of ultrasonic vocalizations emitted by the group of normal animals in their paired and unpaired chambers. For test session 1, although some vocalizations occurred in the unpaired context, the greatest amount of vocalizations occurred in the paired context. A one tailed matched sample t-test was used to compute the difference between the two means and revealed a non-significant difference, ($t_{11} = 1.54$, $p > .05$) For test session 2 there was a decrease in the amount of vocalizations that occurred in the paired context, and there was a total absence of vocalizations in the unpaired context. The same test was used to compute the difference between the two means and indicated a non significant difference, ($t_{11} = 1.65$, $p > .05$). For test session 3, there was an increase in the amount of vocalizations emitted in the paired context and a total absence of vocalizations in the unpaired context. A one tailed matched sample t-test used to compute the difference between the two means indicated a significant difference, $t_{11} = 2.02$, $p < .05$. This result indicates that the

emission of ultrasonic vocalizations is a response of fear that conditions to context, in a discriminative manner. In addition, these results suggest that similar to heart rate, discriminative emission of ultrasonic vocalizations in the paired context has a slower learning rate parameter since three sessions are required for a significant discrimination to surface.

Defecation

Figure 6 shows the mean amount of defecation emitted by a group of normal animals, expressed as grams of faecal material, for the three test sessions. For test session 1 there was no significant difference between the amount of faecal matter produced in the paired and unpaired contexts. A one tailed matched sample t-test indicated that this difference was not significant, ($t_{11} = .25$, $p > .05$). For test session 2 and 3, more faecal matter was produced in the paired context. A one tailed matched sample t-test for session 2 indicated a non-significant difference between the two means, ($t_{11} = 1.35$, $p > .05$), and the same test for session 3 indicated a significant difference between the mean defecation in the paired and unpaired context, ($t_{11} = 2.32$, $p < .05$). These results suggest that this measure of fear conditions to context and requires at least 3 training trials for the discriminative difference between the paired and unpaired context to emerge.

Body temperature

Figure 7 shows the mean difference in body temperature in a group of normal rats in the paired and unpaired context for test sessions 1, 2 and 3. As can be seen in the graph, body temperature was higher in the unpaired context in session 1, given the negative mean difference. A one tailed matched sample t-test revealed a non-significant effect, $t_{11} = .70$, $p > .05$ for session 1. For test session 2, body temperature was higher in the paired context, however this difference was not significant, $t_{11} = .55$, $p > .05$. For test session 3, mean body temperature was also higher in the paired context but the difference did not reach a statistically significant level, $t_{11} = 1.05$, $p > .05$.

Figure 8 represents the difference in body temperature for the first and last 10 minute block of session 3. As depicted in the graph body temperature was higher in the unpaired context during the first block, and it was higher in the paired context for the second block. One tailed matched sample t-tests used to compute the mean differences between the paired and unpaired context for each block, revealed no significant difference for block 1, $t_{11} = .87$, $p > .05$, or block 2, $t_{11} = .32$, $p > .05$. These results suggest that body temperature is not a measure of conditioned fear.

Urination

Figure 9 shows the mean level of urination, expressed in number of emissions, of a group of normal rats in their paired and unpaired contexts. Across the three testing sessions, number of emissions is consistently greater in the paired context. A matched sample t-test used to compute this difference indicated a statistically significant difference between the two means at test session 1, ($t_{11} = 3.45$, $p < .05$), at test session 2, ($t_{11} = 3.80$, $p < .05$), and at test session 3, ($t_{11} = 3.32$, $p < .05$). These results suggest that like conditioned freezing, conditioned urination to context, is a rapidly acquired fear response.

Locomotion

Figure 10 shows the mean level of locomotion for a normal group of rats in both contexts. As can be seen in Figure 10, there is more locomotion in the unpaired than in the paired context at test sessions 1, 2, and 3. A matched sample t-test was used to compute the difference in locomotion in the paired and unpaired context and indicated a significant difference at test session 1, ($t_{11} = 3.02$, $p < .05$), at test session 2 ($t_{11} = 3.53$, $p < .05$), and at test session 3, ($t_{11} = 5.09$, $p < .05$). This data suggests that the

lack of locomotion in the paired relative to the unpaired context occurs following a single training trial and that this difference between the paired and the unpaired context is consistent in test session 2 and test session 3.

Discussion

Using a discriminative fear conditioning to context paradigm several interesting findings emerged from multiple testing sessions. This experiment clearly shows evidence for discriminative fear conditioning to context expressed by multiple measures of fear in the rodent. The experiment also shows that subsets of these fear responses have different learning rate parameters. The latter finding suggests that these different fear responses might be controlled, or influenced, by different forebrain learning and memory systems (Sutherland and McDonald, 1990). Conditioned fear responses such as freezing, urination, locomotion showed fast acquisition rates compared to the other fear responses assessed in this study. The other fear responses including heart rate, ultrasonic vocalizations and defecation did not show discriminative conditioning to context until the third testing session. Interestingly, although the preference test was conducted following the third testing session we have a reason to believe that the behaviour observed on this test is the most rapidly acquired conditioned fear response to context assessed in the present experiment. In preliminary experiments when steel grid floors served as the tactile cues in both the paired and unpaired contexts, rendering the two chambers more similar and probably more ambiguous, the preference for the unpaired context surfaced

following a single conditioning session even though the amount of freezing was similar in both contexts (unpublished data). With the introduction of different tactile cues characteristic to each context in the present experiment, discriminative freezing emerged following a single conditioning session. Based on this pattern of data, we are prompted to believe that conditioned preference is the most rapidly acquired fear response to context. Lastly, given the present data, there is no indication that body temperature is a reliable measure of discriminative fear conditioning to context, as there were no differences in body temperature between the paired and the unpaired contexts at any of the testing sessions.

The present data are consistent with previous reports suggesting that freezing and defecation are good measures of unconditioned and conditioned fear (Blanchard & Blanchard, 1969; Vanderwolf, 1962; Sutherland & McDonald, 1990). Also, some of these measures have been shown to emerge following a single conditioning trial, did so consistently in our experiment (Blanchard & Blanchard, 1969; Fanselow, 1990; Kim & Fanselow, 1992). The results reported here are consistent with current research showing that rats demonstrate fear conditioning to context (Blanchard & Blanchard, 1969; Winocur et al., 1987; Sutherland & McDonald, 1990; Fanselow, 1990; Selden et al., 1991; Phillips & Ledoux, 1992; McDonald et al., 1995). However, an important aspect of the experiment we conducted was the use of a discriminative paradigm

(McDonald et al., 1995). For example, our demonstration of an initial lack of preference for any chamber during the pre-exposure phase turned into an active avoidance of the chamber previously paired with shock and a preference for the context that was previously associated with safety. This pattern of behaviour strongly suggests that these contexts only acquired biological significance for the animals following conditioning and illustrates that this paradigm is an unbiased procedure (Carr, et al., 1989). The use of the discriminative paradigm also enables us to reject the possibility of context conditioning to the room in which the chambers were contained, to the experimenter, or to any aspect of the procedure. The discriminative paradigm also allows us to reject the possibility that the animals are showing sensitized fear to the entire apparatus and procedure. Sensitization is a process that is fundamental to environmental adjustment, and occurs in nearly all species and response systems (Peeke & Petrinovich, 1984). It develops with environmental stimuli that are effective in eliciting responses, and results in an increased general responsiveness. For instance, rats exposed to a loud noise showed a sensitized response to a tone (Davis, 1974). In a non-discriminative paradigm, sensitization to the entire apparatus and/or procedure may occur given the lack of equal experience in the unpaired context and the safety associated with it. Our procedure allows us to affirm, given that the animals had an equal amount of experience in both

contexts, and clearly demonstrated a discrimination expressed by different fear responses, that the fear exhibited in one context can only be attributed to an experience in that environment. In the future, the discriminative paradigm will also allow us to systematically manipulate contextual properties and to assess which attributes of the context the animal is associating with the fearful experience. In previously reported procedures (Blanchard & Blanchard, 1969; Winocur et al., 1987), the animals were solely confined to the paired context and responses indicative of fear were taken to illustrate fear conditioning to context. However, other interpretations of these data are possible because confounds such as sensitized fear and fear conditioning to the room are overlooked. Attempts to overcome such limitations, resulted in the development of discriminative paradigms whereby an animal experiences during testing, the context previously paired with shock and a novel context (Helmstetter, 1992; Kim & Fanselow, 1992; Penick & Solomon, 1991). Responses indicative of fear are assessed in both contexts, and lack of freezing in the novel context is taken to indicate an absence of fear relative to the paired context. Unfortunately, this interpretation may be misleading because locomotion in the novel context may just be an instance of novelty-induced exploration (Sutherland, 1985; Poucet, 1989), and the motivation to explore a novel environment may override fear-induced freezing. Rats with hippocampal lesions were tested in such

a discriminative paradigm, as they were placed in the chamber previously paired with shock and in a novel chamber. Although their freezing behaviour was non-discriminative since the level was high in both chambers, their level of locomotion was elevated in the novel context (McDonald et al., 1995). These findings suggest that novelty induces exploration, and represents a serious confound in such discriminative paradigms.

In another discriminative procedure, animals were given free access to both chambers, but poor counterbalancing techniques render this paradigm flawed for the understanding of contextual fear conditioning. (Selden et al, 1991). In this conditioning procedure, all of the rats experienced the same shock context. This absence of counterbalancing for the shock context and the lack of equal experience with the safe chamber limits conclusive interpretations about associative learning to context. Another problem with this procedure occurred during testing when animals were individually placed into the safe chamber and the time spent within it was considered an index of fear acquired to the shock context. It is possible that an increased dwell time may be due to generalized fear which results in freezing in the safe chamber and consequently spending more time within it. Taken together, it seems clear that these different types of fear conditioning to context paradigms should be avoided because of their limited interpretive value.

The existence of different learning rate parameters for subsets of the fear responses assessed in the present study suggests that different learning mechanisms and systems may contribute to the acquisition and expression of a variety of responses indicative of conditioned fear. These different learning and neural systems may function in enhancing information processing to motivationally significant stimuli and the surroundings in which they occur. The assessment of eight measures of fear, to date, gives us the opportunity to examine the role that each structure may play in the mediation of one, or many responses, and allows us to attain a better understanding of the phenomenon of fear conditioning to context.

The subset of fear responses that take longer to develop include heart rate, ultrasonic vocalization, and defecation. Conditioned heart rate is interesting because it not only requires three training sessions to show discriminative responding but also an extended window. The data also shows that there is no difference in heart rate between the paired and unpaired context in the first 10 min of session 3, suggesting that there is substantial generalization on this measure. Stimulus generalization, as first described by Pavlov, is the opposite of differential responding and it refers to a failure of the subject to respond differentially to various stimuli (Pavlov, 1927). In our experiment generalized fear

would refer to an inability to discriminate between the two conceptually distinct chambers and to exert similar behaviour of fear in both contexts. This pattern of data suggest that the learning rate parameter of this conditioned fear response is not solely determined by the amount of training but rather is an outcome of the interaction between the amount of training and the size of the testing window. Further support for this idea is illustrated in recent experiments in our laboratory, using the same procedure except that the testing period was extended to 30 min for each session. Normal animals did not show differentially higher heart rate in the paired context at test session 1 or 2 but did in test session 3, beginning at the 10 min mark of the test (unpublished data). This outcome is in line with the results reported in the present experiment and provides evidence in support of the idea that during the first 10 min in the paired and unpaired context, generalized fear dominates the heart rate response. If the effect was only due to the testing window size, then we would expect differentially higher heart rate in the paired context to occur at test session 1 and 2. The fact that it only appears at test session 3 supports the idea that a large testing window is not the only factor influencing conditioned heart rate, but rather the interaction of the testing window size and the testing session.

We firmly believe that the expanded length of our testing window is an important aspect of the present experimental design.

High impact reports of the forebrain learning and memory systems necessary for fear conditioning to context are based on research by Phillips and Ledoux (Phillips & Ledoux, 1992) using a short testing window of 20 sec. Based on the data reported in the present paper, such short testing sessions should be avoided because they do not provide a clear picture of fear conditioning to context. For example, a longer test session enabled us to assess heart rate and its development within the testing window, a conditioned fear response that would have passed unnoticed if we had used short testing windows.

Ultrasonic vocalizations showed a similar learning rate parameter as conditioned heart rate. The vocalizations examined in this experiment are referred to as the 22-KHz ultrasounds and have been shown to occur in different behavioural situations (Anderson, 1954; Blanchard & Blanchard, 1977; Fryszak et al., 1988). The production of ultrasonic vocalizations emerges in situations in which highly significant behavioural events occur. The initial appearance of ultrasonic vocalizations in the rat occurs as early as the first postnatal day and subsequently increases in number and intensity in response to isolation, unfamiliar cold environments or rough handling (Hard et al., 1985; Hofer & Shair, 1978). In the adult rat, ultrasonic vocalizations have been reported to occur during and after copulation (Barfield & Geyer, 1972: 1975; Barfield & Thomas, 1986; Floody, Lisk & Vomachka, 1986), in defense and

submission (Sewell, 1967; Blanchard & Blanchard, 1977), in response to pain (Levine et al., 1984) and footshock (Tonoue et al., 1986; van der Poel et al., 1989), in conjunction with the startle reflex (Kaltwasser, 1990), in response to brain stimulation (Yajima et al., 1980), and during opiate withdrawal (Vivian & Miczek, 1991). Embedded within the overlapping function that this behaviour has for different biologically significant events in the rat's life span, there exists individual differences in ultrasonic production that begin at an early age. Genetic differences have been reported to influence the occurrence of vocalizations produced in pups (Broadhurst, 1975; Insel & Hill, 1987; Shapiro & Insel, 1990) as observed between pups of the prairie vole, *Microtus Ochrogaster* and the closely related montane vole *Microtus Montanus*. In adult rats, individual differences have been observed in treatments that evoke vocalizations in some animals but not others. For instance, some reports suggest that even a light touch by a human hand, in some areas of the rat body, can evoke ultrasonic vocalizations similar to those reported to occur in other situations and referred to as the 22-KHz ultrasounds (Brudzynski & Ociepa, 1991). Brudzynski reported that 67.6% of a group of rats vocalized to this treatment, substantiating the idea of individual differences in ultrasonic production. Studies examining ultrasonic vocalizations in response to footshock have also reported a similar variability in emissions. Tonoue et al., (1986) observed that 33% of their animals emitted

pain related calls in reaction to footshock, whereas Cuomo et al., (1988) observed 77% of their animals to produce similar vocalizations . In our study 50% of the animals vocalized during footshock, and emitted substantially more ultrasounds in the paired context during testing. Despite the variability in number of emissions observed in our study, the results of the analysis, support the idea that the emission of ultrasonic vocalizations is a fear response that conditions to context, and that it has the same acquisition rate as conditioned heart rate and defecation. To our knowledge this is the first report of discriminative fear conditioning to context using this response measure, although there are reports of classically conditioned ultrasonic vocalization to a tone (Fryszak et al., 1988).

The locomotion measure assesses total body motion in the chamber and might be informative in future experiments because of its sensitivity to lower levels of fear undetected by the more conservative freezing response. That is, rats with a medium level of fear associated with a particular context may not freeze but they may limit their locomotor activity in the chamber. The general point is that we feel that the combined assessment of freezing and locomotor activity might be more sensitive to the wide range of levels of fear experienced by an organism.

Conditioned defecation has been previously examined in relation to the effects of amygdala and hippocampus lesions on the

conditioning of this autonomic response to context (Vanderwolf, 1962; Sutherland & McDonald , 1990). In our present experiment we examined the development of this conditioned response to context across multiple testing sessions. The novel finding reported here is that, in a discriminative paradigm, the conditioning of this autonomic response requires at least three conditioning sessions for a differentially higher level of defecation to appear in the paired context.

The demonstration of conditioned urination in the present discriminative paradigm is also a novel finding and it emerged during the first test session suggesting that it is a rapidly acquired fear response.

In conclusion, the present results show that multiple measures of fear can be shown to condition to context in a discriminative manner and subsets of these fear responses have different learning rate parameters. Our short term goal is to use this paradigm to reassess contributions of forebrain long term memory systems necessary for fear conditioning to context in the rat.

Anderson, J.W.(1954). The production of ultrasonic sounds by laboratory rats and other mammals. *Science*, 119, 808-809.

Barfield, R.J., Geyer, L.A.(1972). Sexual behavior: Ultrasonic postejaculatory song of the male rat. *Science*, 176(41), 1349-1350.

Barfield, R.J., Geyer, L.A.(1975). The ultrasonic postejaculatory vocalization and the postejaculatory refractory period of the male rat. *Journal of Comparative and Physiological Psychology*, 88(2), 723-734.

Barfield, R.J., & Thomas, D.A.(1986). The role of ultrasonic vocalizations in the regulation of reproduction in rats. *Annals of the New York Academy of Sciences*, 474, 33-43.

Blanchard, R.J., & Blanchard, D.C.(1969). Crouching as an index of fear. *Journal of Comparative and Physiological Psychology*, 67(3), 370-375.

Blanchard, R.J., & Blanchard, D.C.(1977). Aggressive behavior in the rat. *Behavioral Biology*, 21(2), 197-224.

Blanchard, R.J., Blanchard, D.C., Agullana R., & Weiss, S.M. (1991). Twenty-two KHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. *Physiology and Behavior*, 50(5), 967-972.

Bouton, M. E., & Bolles, R.C.(1979).Contextual control of the extinction of conditioned fear. *Learning and Motivation*, 10, 445-466.

Bouton, M.E., & king, D.A.(1983). Contextual control of the extinction of conditioned fear: Tests for the associative value of the context. *Journal of Experimental Psychology: Animal Behavior Processes*, 9(3), 248-265.

Broadhurst, P.L.(1975). The Maudsley reactive and nonreactive strains of rats: A survey. *Behavior Genetics*, 5(4), 299-319.

Brudzynski, S.M., & Ociepa, D.(1992). Ultrasonic vocalization of laboratory rats in response to handling and touch. *Physiology and Behavior*, 52(4), 665-660.

Carr, G.D., Fibiger, H.C., & Phillips, A.G.(1989). Conditioned place preference as a measure of drug reward. In J.M. Leibman & S.J. Cooper(Eds.), *The Neuropharmacological Basis of Reward* (pp 264-319). Oxford: Oxford University Press.

Cohen, D.H., & Goff, D.M.(1978). Effect of avian basal forebrain lesions, including septum, on heart rate conditioning. *Brain Research Bulletin*, 3(4), 311-318.

Cuomo, V., Cagiano, R., DeSalvia M.A., Restani P., Galimberti R., Colonna S., Racagni G., & Galli, C.L.(1988). Ultrasonic vocalization in rat pups as a marker of behavioral development: An investigation of the effects of drugs influencing brain opioid system. *Neurotoxicology and Teratology*, 10(5), 465-469.

Davis, M.(1974). Sensitization of the rat startle response by noise. *Journal of Comparative and Physiological Psychology*, 87(3),1-581.

Davis, M.(1992). The role of the amygdala in conditioned fear. In J.P. Aggleton(Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp 255-305). New York : Wiley-Liss.

Estes, W.K., & Skinner, B.F.(1941). *Some quantitative properties of anxiety. Journal of Experimental Psychology, 29*, 390-400.

Fanselow, M.S.(1990). Factors governing one-trial contextual conditioning. *Animal Learning and Behavior, 18*(3), 264-270.

Floody O.R., Lisk, R.D., & Vomachka A.J.(1986). Facilitation of lordosis by estradiol in the mesencephalic central gray. *Physiology and Behavior, 37*(4), 587-595.

Fryszak, R.J., Plopa, L., & Neafsey, E.J.(1988). Ultrasonic vocalizations are part of the rat's conditioned emotional response. *Society for Neuroscience Abstract. 179.10.*

Hard, E., Engel, J., Larsson, K., Liljequist, S., & Musi, B.(1985). Effects of maternal ethanol consumption on the offspring sensory-motor development, ultrasonic vocalization, audiogenic immobility reaction and brain monoamine synthesis. *Acta Pharmacologica et Toxicologica, 56*(5), 354-363.

HelmeStetter, F.J.(1992). Contribution of the amygdala to learning and performance of conditioned fear. *Physiology and Behavior, 51*(6), 1271-1276.

Hofer, M.A., & Shair H.(1978). Ultrasonic vocalization during social interaction and isolation in 2-week-old rats. *Developmental Psychobiology*, 11(5), 495-504.

Insel,T.R., & Hill, J.L.(1987). Infant seperation distress in genetically fearful rats. *Biological Psychiatry*, 22(6), 786-789.

Kaltwasser, M.T.(1990). Startle-inducing acoustic stimuli evoke ultrasonic vocalization in the rat. *Physiology and Behaviour*, 48(1), 13-17.

Kamin, L.J., Brimer, C.J., Black, A.H.(1963). Conditioned suppression as a monitor of fear of the CS in the course of avoidance learning. *Journal of Comparative and Physiological Psychology*, 56, 497-501.

Kim, J.J., & Fanselow, M.S.(1992). Modality-specific retrograde amnesia of fear. *Science*, 256(5057), 675-677.

Ledoux, J.E.(1994). Emotion, memory and the brain. *Scientific American*. 270(6), 50-57.

Levine, J.D., Feldmesser, M., Tecott, L., Gordon, N.C., & Izdebski, K. (1984). Pain-induced vocalization in the rat and its modification by pharmacological agents. *Brain Research*, 296(1), 121-127.

Mackintosh, N.J.A. (1975). A theory of attention: Variations in the associability of stimuli with reinforcement. *Psychological Review*, 82(4), 276-298.

McAllister, W.R., & McAllister, D.E. (1971). Behavioral measurement of conditioned fear. In: F.R. Brush (Ed.), *Aversive conditioning and learning* (pp 105-179). New-York: Academic Press.

McDonald, R.J., Koerner, A., & Sutherland, R.J. (1995). Contextual conditioning and hippocampus, *Neuroscience Abstract*, 20.

Nadel, L., & Willner, J. (1980). Context and conditioning: A place for space. *Physiological Psychology*, 8(2), 218-228.

Pascoe J.P., & Kapp, B.S. (1987). Responses of amygdaloid central nucleus neurons to stimulation of the insular cortex in awake rabbits. *Neuroscience*, 21(2), 471-485.

Pavlov, I.P.(1927). The analysing and synthesizing activity of the cerebral hemispheres: A) The initial generalization of conditioned stimuli. In G. V. Anrep (Ed.), *Conditioned reflexes* (pp 110-130). London: Oxford University Press.

Peeke, H.V.S., & Petrinovich, L.(1984). Approaches, constructs, and terminology for the study of response change in the intact organism. In H.V.S. Peeke & L. Petrinovich (Eds.), *Habituation, sensitization, and behavior* (pp 1-14). New York: Academic press.

Penick, S., & Solomon, P.R.(1991). Hippocampus, context, and conditioning. *Behavioral Neuroscience*, 105(5), 611-617.

Phillips, R.G., & Ledoux, J.E.(1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience*, 106(2), 274-285.

Phillips, R.G., & Ledoux, J.E.(1994). Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. *Learning & Memory*, 1, 34-44.

Poucet, B.(1989). Object exploration, habituation, and response to a spatial change following septal or medial frontal cortical damage. *Behavioral Neuroscience*, 103(5), 1009-1016.

Rescorla, R.A., & Wagner, A.R.(1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A.H. Black & W.F. Prokasy (Eds.), *Classical conditioning: II. Current research and theory* (pp 64-99). New York: Appleton-Century-Crofts.

Selden, N.R.W., Everitt, B.J., Jarrard, L.E., & Robbins, T.W.(1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. *Neuroscience*, 42(2), 335-350.

Sewell, G.D.(1967). Ultrasound in adult rodents. *Nature*, 215(100), 512.

Shapiro, L.E., & Insel, T.R.(1990). Infant's response to social separation reflects adult differences in affiliative behavior: a comparative developmental study in prairie and montane voles. *Developmental Psychobiology*, 23(5), 375-393.

Sutherland, R.J.(1985). The navigating hippocampus: An individual medley of movement, space, and memory. In G. Buzaski & C.H. Vanderwolf (Eds.), *Electrical activity of the archicortex* (pp 255-279). Budapest: Akademiai Kiado.

Sutherland, R.J., & McDonald, R.J.(1990). Hippocampus amygdala and memory deficits. *Behavioural Brain Research*, 34, 57-79.

Tonoue, T., Ashida, Y., Makino, H., & Hata, H.(1986). Inhibition of shock-elicited ultrasonic vocalization by opioid peptides in the rat: A psychotropic effect. *Psychoneuroendocrinology*, 11(2), 177-184.

Tully, T.(1991). Physiology of mutations affecting learning and memory in *Drosophila*-- the missing link between gene product and behavior. *Trends in Neurosciences*, 14(5), 163-164.

van der Poel, AM., Noach,E.J. & Miczek, K.A.(1989). Temporal patterning of ultrasonic distress calls in the adult rat: Effects of morphine and benzodiazepines. *Psychopharmacology*, 97(2), 147-148.

Vanderwolf, C.H.(1962). Medial thalamic functions in voluntary behavior. *Canadian Journal of Psychology*, 16, 318-330.

Vivian, J.A., Miczek, K.A.(1991). Ultrasounds during morphine withdrawal in rats. *Psychopharmacology*, 104(2), 187-193.

Winocur, G., & Gilbert, M.(1984). The hippocampus, context and information processing. *Behavioral and Neural Biology*, 40(1), 27-43.

Winocur, G., & Olds J.(1978). Effects of context manipulation on memory and reversal learning in rats with hippocampal lesions. *Journal of Comparative and Physiological Psychology*, 92(2), 312-321.

Winocur, G., Rawlins, J.N., & Gray, J.A.(1987). The hippocampus and conditioning to contextual cues. *Behavioral Neuroscience*, 101(5), 617-625.

Yajima, Y., Hayashi, Y., Yoshii, N.(1980). The midbrain central gray substance as a highly sensitive neural structure for the production of ultrasonic vocalization in the rat. *Brain Research*, 198(2), 446-452.

Figure captions

Figure 1 (A): Mean amount of time (sec) spent by a normal group of animals in the black triangle prism and the white square prism during the pre-exposure phase. **(B)** Mean amount of time spent by a normal group of animals in their paired and unpaired context during the preference test, expressed in seconds.

Figure 2: Mean amount of time (sec) spent freezing by a group of normal animals in the paired and unpaired context during test sessions 1,2 and 3.

Figure 3: Mean difference (BPM: beats/min) in heart rate between the paired and unpaired context in a normal group of animals, during test sessions 1, 2 & 3.

Figure 4: Mean difference (BPM: beats/minute) in heart rate between the paired and unpaired context, in a normal group of animals during the first and last 10 minute block of session 3.

Figure 5: Mean amount of ultrasonic vocalizations emitted by a group of normal animals in their paired and unpaired context during test sessions 1, 2, and 3.

Figure 6: Mean amount of faecal matter (grams) emitted by a group of normal animals in their paired and unpaired context during test sessions 1, 2 and 3.

Figure 7: Mean difference in body temperature (degrees Celsius), between the paired and unpaired context in a normal group of animals during test sessions 1, 2, and 3.

Figure 8: Mean difference in body temperature (degrees Celsius), between the paired and unpaired context in a normal group of animals during the first and last 10 minute block of session 3.

Figure 9: Mean level of urination (number of emissions) by a group of normal rats in their paired and unpaired context during test session 1, 2 and 3.

Figure 10: Mean level of locomotion (number of crossings), for a normal group of rats in their paired and unpaired context during test sessions 1,2 and 3.

FIGURE 1

FREEZING

FIGURE 2

HEART RATE

FIGURE 3

HEART RATE

FIGURE 4

VOCALIZATIONS

FIGURE 5

DEFECATION

FIGURE 6

BODY TEMPERATURE

FIGURE 7

BODY TEMPERATURE

FIGURE 8

URINATION

FIGURE 9

LOCOMOTION

FIGURE 10

© 1993, Applied Image, Inc., All Rights Reserved