INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

University of Alberta

Analyzing the immediate shock deficit: A behavioural examination of post-shock activity in rats

by

Sandra L. Ziolkowski ©

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

Department of Psychology

Edmonton, Alberta

Spring, 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 his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced with 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 sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

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

0-612-21664-0

Canada

University of Alberta

Library Release Form

Name of Author: Sandra L. Ziolkowski

Title of Thesis: Analyzing the immediate shock deficit: A behavioural examination of post-shock activity in rats

Degree: Doctor of Philosophy

Year this Degree Granted: 1997

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly, or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Sandra L. Ziolkowski

Sandra L. Ziolkowski
11314 - 104 Street
Edmonton, Alberta
T5G 2K8

April 22, 1997

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Analyzing the immediate shock deficit: A behavioural examination of post-shock activity in rats submitted by Sandra L. Ziolkowski in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Charles Beck

Charles Beck, Supervisor

Dallas Treit

Dallas Treit

Glen Baker

Douglas Grant

Dallas Treit

Dallas Treit for John Pinel

Douglas Wahlsten

Date approved by committee April 22/97

Dedication

This thesis is dedicated to my family whose support throughout the many years of completing this degree has been unfaltering and to Charles Beck who has inspired a sense of professionalism in me that will endure.

Abstract

Five experiments were performed to study the immediate shock deficit, that is, the reduction of freezing observed in rats given a single shock immediately after placement into a box compared to rats shocked after a delay. Rats were administered a brief, single foot-shock on Day 1 and observed for 5 mins. Rats were tested on a subsequent day for 5 mins (20 mins in Experiment 5). The min by min changes in duration of all behaviours throughout each trial were analyzed to provide a complete analysis of activity. In Experiment 1, delayed shock animals tested in the same box on both days froze more than delayed shock animals tested in contextually dissimilar boxes. Both immediately and delayed shock rats froze maximally on Day 2 at the time of shock delivery on Day1. Rats allowed to exit the shock box (Experiment 2) froze less on Day 1 than animals not allowed to exit the shock box. In addition, those animals allowed to escape from the shock box did so, regardless of the time of shock. Rats in Experiment 3 that were handled before a delayed shock froze less than those not handled but more than those immediately shocked. In Experiment 4 shock was delivered contingent upon the rat's body orientation on the bars (parallel vs. perpendicular). the number of bars between its feet (low vs. high), and its behaviour (still vs. locomote). Animals shocked contingent upon their locomotion froze more than those shocked contingent upon being still, regardless of the other factors. This result was further explored in Experiment 5 by shocking animals contingent upon their behaviour either immediately, or after a delay (2 min or 15 min). More freezing among groups shocked contingent upon their locomotion was observed compared to groups shocked contingent upon their being still, regardless of time of shock. It was concluded that both spatial and temporal conditioning effects influence freezing in both immediately and delayed shock animals and that the immediate shock deficit reflects a suppression of different behaviours occurring at the time of shock. These results are discussed in relation to models of animal defensive behaviour.

Acknowledgement

I wish to acknowledge the support and encouragement of numerous people for their perseverance with me in the years to finish this degree. First, I wish to thank my advisor and friend of many years. Charles Beck, whose door was always open to me whenever it was needed. I wish to thank my husband, Josef, and son, Zachary, whose encouragment and help behind the scenes was truly necessary. Third, I wish to thank my parents who taught me to finish what I start and on whom I could turn to at a moments notice for assistance that would allow me to work on this thesis. Finally, I wish to acknowledge Gus Thompson for his enduring faith in me and Linda Weir for her patience awaiting the completion of this degree.

Table of Contents

Table of Contents

Table of Contents

Table of Contents

Heading **Page**

Table of Contents

Heading **Page**

Table of Contents

Heading **Page**

List of Tables

List of Tables

TABLE NUMBER AND DESCRIPTION **PAGE**

LIST OF FIGURES

LIST OF FIGURES

List of Abbreviations and Symbols

C1	first linear contrast defined in Experiment 5
C2	second linear contrast defined in Experiment 5
CR	conditioned response
diff	difference
expt	experiment
f-shk	foot-shock
HP	High-Parallel
HR	High-Perpendicular
imm	immediate
ISD	Immediate Shock Deficit
L	locomotion
LOCO	locomote
LP	Low-Parallel
LR	Low-Perpendicular
M	mean
mA	milliamperes
min	minute
N	noncontingent
pre-exp	pre-exposure
s	second
S	still
SEM	standard error of the mean
sess	session
shk	shock
SSDR	species-specific defense reaction
u	number of factors within a condition minus 1
UR	unconditioned response
↑	increase
↓	decrease
α	criterion of rejection; area under normal curve defining rejection of null hypothesis
β	criterion of acceptance; area under normal curve defining acceptance of null hypothesis
σ	estimate of square root of standard deviation

Analyzing the immediate shock deficit: A behavioural examination of post-shock activity in rats

It is generally accepted that aversive stimulation such as the presence of a predator or the administration of electric shock restricts an animal to defensive behaviours (Crawford & Masterson, 1982). As used here, defensive behaviours refer to those behaviours that an animal engages in as its initial responses to imminent predation. Thus, freezing and fleeing are two examples of defensive behaviour in rats because these responses generally occur as reactions to the presence of predatory threat (Blanchard & Blanchard, 1989; Blanchard, Flannelly, & Blanchard, 1986; Fanselow & Lester, 1987). Using this definition of defensive behaviour, responses such as food hoarding and nest building are not considered as defensive behaviours because these behaviours do not occur in response to actual predatory threat. Rather these behaviours occur well before the presence of a predator. In general, there are two strategies for studying defensive behaviour. First, many investigators study rats in an ethological setting that provides for the most natural examination of defensive behaviour strategies. Researchers working within this framework have identified many behaviours that function to promote survival of the species (Blanchard & Blanchard, 1987, 1989). In addition, ethological study of inter-species behaviour has revealed that many species have similar defensive strategies and many do not (Blanchard, Blanchard, Rodgers, & Weiss, 1990; Edmunds, 1974). A second strategy for examining rat behaviour involves studying animals within a laboratory. Researchers studying behaviour from this approach are better able to manipulate variables that may relate to animal behaviour. In general, the types of behaviour observed in the laboratory are similar to those observed in nature (Blanchard & Blanchard, 1987; Blanchard, Fukunaga, & Blanchard, 1976a, 1976b). Several theories of animal defense within each of these two strategies are discussed below.

First, those theories with an ethological perspective are described. Such theories include an ethoexperimental analysis of behaviour (Blanchard & Blanchard, 1987, 1990) and the predatory imminence theory (Fanselow & Lester, 1988). These two theories describe the importance of the predator-prey distance in the determination of which defensive behaviours are exhibited. Second, the perceptual defensive recuperative model (Fanselow & Lester, 1988) of defensive behaviour is described. Included in this discussion is a description of species specific defense theory (Bolles, 1969, 1970).

Electric shock is a particularly effective stressor for evoking defensive behaviour (Bolles, 1975). Therefore, the behaviours surrounding the administration of electric shock to animals in a confined environment are described, including the apparent lack of defensive behaviour exhibited when a shock is presented simultaneous with entry into a box (Fanselow, 1986, 1990).

Finally, five experiments are presented that focus on examining the post-shock behaviour of rats under conditions of immediate and delayed shock.

Theories of Animal Defense

An Ethoexperimental Analysis of Defensive Behaviour

Ethological research of defensive patterns has typically focused on self-defense responses to attacks by predators in natural or semi-natural environments. This research has identified specific defensive behaviours common to a variety of species (e.g., Blanchard et al., 1990; Edmunds, 1974). Species-typical defensive patterns can also be elicited in a laboratory setting by threatening or painful stimuli. The empirical elucidation of the events which are antecedent to species-typical defenses, and the detailed description of these responses in a laboratory, has been termed the ethoexperimental approach to the study of fear (Blanchard & Blanchard, 1987). The ultimate goal of researchers studying defense responses in this manner is to develop a precise model of the neurobiological and neurochemical determinants of conditioned fear.

Predator-prey distances in a laboratory setting. Typically, there are four major classes of defensive behaviour observed in wild rats confronted by a predator in a laboratory (Blanchard et al., 1986). The behaviour exhibited depends, to a large extent, on the situational features of the environment. Using the experimenter as the predator, Blanchard et al. (1986) have observed that response patterns are influenced by the rat's perception of the magnitude of threat. The predator-prey distances associated with each defensive behaviour in this study are described below.

Distances of 1-5 m evoked flight responses in situations where escape was possible. If escape was impossible, freezing was the dominant response. Freezing has been described as an immobile, crouching posture (Blanchard, Dielman, & Blanchard, 1968) that serves as an index of fear (Blanchard & Blanchard, 1969a). In support of the hypothesis that fear is the underlying motivation of freezing, anxiolytic agents have been observed to decrease the occurrence of this behaviour in aversive situations (Blanchard, Blanchard, & Weiss, 1990; Blanchard, Magee, Veniegas, & Blanchard, 1993; Fanselow & Helmstetter, 1988).

Distances of 0.5-1 m evoked a pattern of defensive threat. Typical behaviours involved in defensive threat include orientation to the predator, sonic vocalizations, and baring of teeth. Such activity has also been observed in laboratory rats (Brudzynski & Ociepa, 1992).

Contact, or near contact, with the predator produced defensive attack. A jump attack, characterized by a flurry of startled activity, was typically evoked if the predator was near contact. Predator contact produced attack (biting and striking) toward the predator's head. Defensive threat and attack behaviours usually resulted in an increase in predator-prey distance, thus allowing for an attempt by the prey to escape. Defensive threat and attack were usually reduced in laboratory rats due to the selective removal of these traits by domestication. However, Blanchard, Kleinschmidt, Fukunaga-Stinson and Blanchard (1980) have observed that defensive biting of a wood dowel, or another rat, can be induced in restrained laboratory rats given a tail-shock. Thus, defensive attack has been observed to occur in response to stress, if an object to strike was available.

In summary, the defensive strategy employed by a rat in response to a predator appears to be modulated by the predator-prey distance and the environmental features available to support the response. It is assumed that fear is the underlying emotion in these predator-prey situations.

Predator-prey distances in a laboratory burrow setting. An elaborate study of rats in a laboratory burrow-surface system revealed that when undisturbed, rats will routinely forage and set up social hierarchies (Blanchard & Blanchard, 1989). However, when exposed to a cat, rats will display a pattern of defensive behaviours (Blanchard & Blanchard, 1989). Similar to the results described above, escape was usually the first mode of defense observed. In this burrow system, escape was observed when rats were in an open area but close to a familiar entrance. Freeze, as a secondary defensive reaction after flee, was observed within the tunnel for long periods after the cat was removed. Furthermore, long latencies to re-enter the surface area and few changes in location after presentation of the cat provided indirect measures of decreased mobility.

Freezing was observed as the primary response if the animal was far from an available exit. Ensuing activity by the rat was dependent on the predator's next response. Freezing could result in the cessation of the attack if the predator 'lost sight' of its prey, resulting in flight to the burrow. Alternatively, if freezing failed to stop the attack, progression to the next stages of defense occurred, i.e., defensive threat and attack. In such situations, predator movement has been shown to be the major factor determining the duration of freezing (Blanchard, Mast & Blanchard, 1975).

An interesting pattern of behaviour occurred during the transition from immobility to foraging. After freezing, initially an animal would briefly poke its head out of the tunnel, sniff, and quickly retreat. This was followed by a stretched posture of some, or most, of the body from the tunnel, and finally, by brief forays onto the surface area. Such progressive scrutinizing was termed risk assessment and has been also observed during the transition from freeze to bury in a test cage with litter on the floor (Pinel, Mana, & Ward, 1989). All of these defensive behaviours occurred to the exclusion of foraging and social behaviours and, in some instances, were observed for days.

In summary, ethoexperimental investigations have led to the conclusion that rats observed in a semi-natural setting typically retreat from a predator encountered on the surface and subsequently remain immobile within the sub-surface burrow for extended periods. Before resuming nondefensive adaptive behaviours, rats typically engage in a pattern of risk assessment which involves a careful investigation of the previously threatening environment. It is important to note that these behaviours are observed for extended periods in the *absence of the predator*. Fear of the predator is assumed to underlie these defensive responses.

Predatory Imminence Theory

Similar to the ethoexperimental approach to the study of defense behaviour, Fanselow and Lester (1988) have described avoidance learning in terms of the importance of predator-prey distance in controlling defense. Because an animal must successfully defend itself from predatory attack before it can engage in more preferred activities, Fanselow and Lester (1988) have described their theory as a *functional* model of defense.

In this theory, a continuum of predatory threat is described ranging from 'no predatory potential' to 'predator makes the kill'. Since both physical distance and the animal's perceived distance contribute to defining predatory threat. the continuum is referred to as predatory imminence.

Each point along the imminence continuum is associated with a class of behaviour. The most 'preferred activities' (foraging. copulation) can be engaged in if the animal perceives 'no predatory potential': obviously. death occurs at the other extreme of the continuum -- 'predator makes the kill'. Defensive behaviours are classed as either 'pre-encounter'. 'post-encounter'. or 'circa-strike'.

The goal of pre-encounter defensive behaviours is to decrease the probability of predation. These behaviours are described as modified preferred behaviours. An example of pre-encounter defensive behaviour is a modification of foraging. Post-encounter defense behaviours include freeze. flight and bury. Freezing is considered the most dominant post-encounter defensive response in the predatory imminence model (Fanselow & Lester. 1988). Examples of circa-strike defensive behaviours include attack. jumping. and tonic immobility (i.e.. an extreme flaccid posture: Menescal-de-Oliveira. & Hoffmann. 1993).

In summary. the predatory imminence theory provides a *functional* view of defensive behaviour. The model predicts that specific behaviours will occur depending on the actual physical predator-prey distance.

Summary of Ethological Assessment of Defense

Ethological and laboratory analyses of rats have indicated that defensive behaviours occur in response to actual predatory presence or to predatory threat. Environmental context appears to be an important factor in determining which defensive strategy will be observed. although a range of defensive behaviours may be exhibited at different times during the predator's presence.

A common factor of both the ethoexperimental and predatory imminence theory is that fear is the underlying motivation of all defense reactions. Because fear is easily conditioned (Blanchard & Blanchard. 1969a: Blanchard et al.. 1986) and can be reliably assessed by freezing (Blanchard & Blanchard. 1968: Bouton

& Bolles, 1980), the detailed analysis of freeze is an important strategy in studying the defense process.

Perceptual-Defensive-Recuperative Model

In the Perceptual-Defensive-Recuperative (PDR) model, fear and pain are considered to be independent and opposing systems that activate different behaviours in response to the presence of a stressful stimulus (Bolles & Fanselow, 1980; Fanselow & Sigmundi, 1987). These behaviours can be broadly classed into three phases: perceptual, defensive, and recuperative. The *perceptual phase* includes detection and encoding of the threatening stimulus. One or more of a variety of behaviours can occur during the *defensive phase*. It is assumed that fear, as the motivational system activated during this phase, limits the animal's behaviour to species-specific defensive reactions (SSDRs: Bolles, 1969). Original descriptions of SSDRs included freeze, flee, and fight (Bolles, 1969, 1970). The *recuperative phase* activates pain-motivated behaviours that function to heal the animal after injury. Therefore, fear and pain are considered to be independent and competing motivational systems which function to limit the animal's behaviour to particular responses at particular times during a dangerous encounter. The ethological advantage of limiting behaviours within a particular range is that it is more adaptive for defense responses to predominate during a life-threatening situation, so as to increase the animal's chance for survival.

Species-Specific Defense Reactions

Bolles (1970) developed species-specific defense reaction (SSDR) theory as an approach to account for avoidance learning. The focus of the theory was to highlight the requirement of recognizing the biological constraints on an animal's ability to rapidly learn avoidance responses. In this manner, Bolles proposed that learning theorists might better explain an animal's laboratory behaviour by examining its natural behaviour.

Bolles (1970) argues that the effect of aversive stimulation on a rat is to restrict its responses to a narrow class of behaviours that generally include flight. freeze, and fight as SSDRs. It is presumed in this theory that these defensive responses are unlearned and minimally vary within a species. However. it is expected that the expression of a particular defensive behaviour might be different between species. For example. flight in response to a predator is different between a rodent and a dog in that the former completely escapes the environment occupied by a predator whereas a dog will only distance itself from a predator. Bolles (1970) contends that an avoidance response could only be rapidly learned if it was one of these SSDRs. In a review of SSDR theory. Crawford & Masterson (1982) concluded that the SSDRs described in Bolles' theory are typically observed in aversive situations.

Within the restricted set of defense responses. Bolles considered the dominant response in rats to be flight. Therefore. if the environment provided the stimulus support for flight. then it was hypothesized that the animal would flee in response to an aversive stimulus. There is substantial empirical evidence in support of this hypothesis (e.g.. Blanchard & Blanchard. 1969b: Bolles. 1971: Masterson. Crawford. & Bartter. 1978). If flight was not possible then it was hypothesized that this response would be suppressed and the animal would freeze. This hypothesis is also supported by the literature (Blanchard et al.. 1976a: Bolles & Collier, 1976; Fanselow. 1990). Bolles (1970. 1972) considered suppression of behaviour as the mechanism determining which one of the SSDRs would actually be observed after an aversive event. For example. under conditions where the environmental features of an aversive situation do not support escape. flight will be suppressed and freezing will emerge as the dominant defensive behaviour.

Initial descriptions of SSDRs included freeze, flee. and fight as the limited set of behaviours elicited by fear in rodents (Bolles. 1969. 1970). Continued research led to the characterization of several other species-typical fear responses.

These included burying, stretched attention, defensive threat, and thigmotaxis.

Burying behaviour is characterized by the rat dispersing bedding material from the floor of the chamber toward an identifiable prod through which it has just received a shock (Pinel & Treit, 1978; Pinel, Treit, Ladak, & MacLennan, 1980; Moser & Tait, 1983). Freezing, however, is usually observed as the first response to a prod-shock (Moser & Tait, 1983; Pinel, Symons, Christensen, & Tees, 1989).

Stretched attention is a term used to describe the elongated posture sometimes observed as a transitory behaviour between freezing and burying (Pinel, Hoyer, & Terlecki, 1980; Blanchard & Blanchard, 1988; Pinel et al., 1989). Defensive threat behaviour occurs in the form of ultrasonic distress cries and the baring of teeth (Blanchard et al., 1990). Thigmotaxis is described as the tendency to stay near walls and away from open areas (Crawford, Masterson, Thomas, & Ellerbrock, 1981; Grossen & Kelley, 1972).

Anxiolytic drugs have been shown to decrease freezing (Conti, Maciver, Ferkany, & Abreu, 1990; Fanselow & Helmstetter, 1988) and to increase the duration of stretched attention postures (Blanchard, Blanchard, & Weiss, 1990), suggesting that fear is the underlying motivation of these behaviours.

In summary, fear is thought to restrict an animal's behaviour to a limited set of hierarchically arranged SSDRs. These behaviours include freeze, flee, fight, bury, defensive threat, and thigmotaxis.

Summary of the Perceptual-Defensive-Recuperative Model

The PDR model of fear and pain assumes that each of these motivational systems activates certain behaviours and inhibits others in order to enhance the animal's chance for survival. This model is particularly suited to providing a framework for evaluating the effects on behaviour of aversive laboratory stimuli that typically evoke both fear and pain (e.g., electric shock and the hot plate test).

Electric Shock and Defensive Behaviour

Electric shock is an aversive stimulus that presents an immediate threat to an animal's welfare (Bolles, 1975). As such, electric shock evokes defensive responses in rats. Painful stimuli, such as electric shock, also produce fear (Bolles & Fanselow, 1980). A brief, single foot-shock is sufficient to evoke fear in rats (Fanselow, 1982, 1990). Other painful stimuli, such as acoustic stimuli (Cranney, 1987; Kiernan & Cranney, 1992) and prod-shock (Blanchard & Blanchard, 1969b; Moser & Tait, 1983) produce defensive responses. Moreover, a comparison of two empirically related studies revealed that the behaviours observed in response to a predator (Blanchard et al., 1976a) were similar to those observed in response to a foot-shock (Blanchard et al., 1976b). This suggests that the behaviours observed in response to either a natural aversive stimulus (e.g., predator) or a laboratory based aversive stimulus (e.g., shock) are examples of the same defensive responses.

Fanselow (1980) has described the rat's reaction to an unsignalled foot-shock as consisting of an unconditioned startle response and a conditioned freezing response. He observed that a rat's initial reaction to a brief foot-shock was frenzied activity. Fanselow (1980, 1982) characterized this startle burst as the unconditioned response to the aversive stimulus. Post-shock freezing was considered to be produced by the fear conditioned cues associated with the shock box rather than as a reaction to the shock itself (see also, Bolles & Collier, 1976; Fanselow, 1990; Fanselow & Helmstetter, 1988).

Post-shock freezing can be increased by presenting explicit cues (e.g., a tone) immediately before shock delivery as compared to not presenting these cues (Fanselow, 1982, 1990; Phillips & LeDoux, 1992). However, post-shock freezing will occur in the absence of all apparent explicit cues (Fanselow, 1986; Kim & Fanselow, 1992; Maier, 1990). Fanselow (1982) has observed that freezing 24 hr after shock can be increased if a continuous tone during shock training is presented, compared to a silent training box. Thus, increasing the contextual complexity of an environment can produce increased freezing in the absence of explicit cues associated with shock.

Under certain circumstances in which no explicit cues are presented, freezing appears not to be conditioned to the contextual features of the chamber when shock is presented. When an animal is given a foot-shock simultaneous with entry into a shock box, an apparent lack of freezing occurs (Blanchard et al., 1976b; Fanselow, 1986, 1990; Fanselow, Landeira-Fernandez, DeCola, & Kim, 1994; Kiernan & Westbrook, 1993; Kiernan, Westbrook, & Cranney, 1995; Landeira-Fernandez, Fanselow, DeCola, & Kim, 1995; Westbrook, Good, & Kiernan, 1994). Fanselow (1986) has termed this effect the immediate shock deficit (ISD). The ISD has also been observed in rats in response to a loud acoustic stimulus (Kiernan & Cranney, 1992). The ISD effect is the focus of the experiments conducted here.

The Immediate Shock Deficit

The study of freezing in the ISD paradigm is important for two reasons. First, the difference in freezing tested after shock (i.e., on Day 1 testing) between immediately and delayed shock animals has been considered a difference in fear (Fanselow, 1986, 1990). Therefore, this paradigm provides an obvious method of studying the processes of fear (e.g., startle and freezing) after a single presentation of a stressor. Secondly, the difference in freezing between immediately and delayed shock groups is consistently observed on a conditioning trial (i.e., on Day 2 testing). Therefore, it is possible to study the effects of contextual conditioning using the ISD paradigm after one presentation of a stressor with a minimal procedural difference between immediately and delayed shock groups.

Ethological Consideration of the ISD

The ISD is a laboratory based empirical effect but the ethological significance of this effect can be reasoned. It is argued in this paper that the ISD paradigm is a laboratory manipulation of predatory threat as described in ethoexperimental analyses of behaviour (Blanchard et al., 1986: Blanchard & Blanchard, 1989). In addition. the ISD effect can also be related to predatory imminence theory (Fanselow & Lester. 1988).

As discussed above. Blanchard and Blanchard (1989) describe the behaviours of rats exposed to a cat in a laboratory burrow-surface system. In general. the presence of a cat had the initial effect of producing flight or freeze in rats. depending on the rat's proximity to a familiar exit. If the rat was close to a familiar exit it fled to that area and froze. Alternatively, if the rat was far from a familiar exit it initially and persistently froze. Therefore. the behaviour observed in this system was based on the proximity of the rat to a familiar exit. The ISD may be a laboratory representation of this effect. That is. the laboratory manipulation of time of shock may parallel the manipulation of proximity in an ethological setting such that an immediate shock in a shock chamber produces similar behaviour as a proximal exit in a burrow setting.

According to the predatory imminence theory. an animal's defensive response is predicted on the basis of the imminence of a threat. Therefore. the freezing of the animal given a delayed shock is consonant with the presentation of an imminent stimulus. However. it is hypothesized in this paper that shock. regardless of time, produces behaviour that is consonant with that produced by an imminent threat.

Theories Pertaining to the ISD

A topographical account.

As the name of this theory suggests. the topographical account attempts to describe the behaviours possible in the ISD paradigm from an ethological perspective. Blanchard et al. (1976a. b) explained the ISD effect by suggesting that the defensive behaviour exhibited by a rat changes as a function of being allowed to explore the box before presentation of an aversive stimulus. That is, without a period to determine whether escape is possible, an animal will freeze when presented with a threatening stimulus because it has not determined if escape would be effective. A primary assumption of this account is that aversive stimulation ubiquitously evokes the rat's defensive system and that fear will consequently control subsequent behaviour.

It is interesting to note that although the topographical account predicts that an immediately shocked animal does not freeze after shock because it has not determined if escape is possible, this hypothesis has not been tested. The lack of freezing by an immediately shocked animal is inferred from the results of ethologically based studies that escape is the prevalent response to an aversive situation (e.g., Blanchard & Blanchard, 1969b; 1971).

In summary, the topographical account of the ISD suggests that an immediately shocked animal will attempt to escape the shock chamber because it has not had the time to determine if the context supports escape. Alternatively, the delayed shock animal will freeze in response to the shock because it has determined that escape is not possible. Fear, as the underlying factor of all defensive behaviours, is presumed to be equivalent in the two groups.

A contextual association account. Fanselow (1986, 1990) explained the ISD in terms of a failure by the immediately shocked animal to associate the memory of the contextual features of the box and the shock. That is, in this account it is hypothesized that the immediately shocked animal fails to form an association between the context and the shock, simply because it has not had the time before being shocked to form a memory of the shock box. This theory, therefore, assumes that backward conditioning does not occur. However, in a review of the literature Spetch, Wilkie and Pinel (1981) discussed the support for backward conditioning especially where a noxious stimulus served as the US. Fanselow (1986) also assumed that lack of freezing in the immediately shocked animal was associated with lack of fear. Therefore, only animals in the delayed shock condition were expected to exhibit fear related behaviours, such as freezing, or physiological correlates of fear, such as increased defecation.

Fanselow (1986) has extensively studied this contextual account of the ISD. In describing the associative account, he also presented evidence to refute the topographical account of Blanchard et al. (1976a). Although Fanselow's conclusions are seemingly convincing, it is argued in this paper that the experiments were not a strict test of the topographical theory.

Fanselow (1986, Experiments 1 & 2) observed a linear increase from 0% freezing in immediately shocked animals to about 15% freezing in 81 s delayed shock animals. This duration of freezing was similar to that observed in the 2 min delayed shock group. Although the increased freeze of the delayed shock group has been generally observed in other experiments, the complete lack of freezing by the immediate group has not been replicated in other studies (e.g., Fanselow, 1990, 1982; Westbrook et al., 1994).

To test the behavioural topography of immediately or delayed shock rats under conditions where escape-like activity was possible, Fanselow (1986) presented a pseudoexit designed to invite such activity. Although the ISD was again observed in the immediately shocked animals, he reported no escape-like behaviour in any of the animals (Experiment 3). Given that the exit consisted of a small, 8 x 8 cm opening and that 3 wires were embedded across the opening, there was no chance for escape. Therefore, this experiment failed to adequately test the effect than an exit may have on either immediately or delayed shock animals.

Fanselow (1986) also tested whether no-shock pre-exposure to the apparatus could strengthen the association formed on subsequent shock sessions (Experiments 4 - 6). He observed that no-shock pre-exposure to the apparatus did not significantly increase freeze above zero on subsequent test days when the animal was given an immediate shock. Therefore, he reasoned that it is the pairing of a familiar environment with an aversive event within a session that is necessary to produce freezing. It is important to note, however, that by analyzing the between-groups differences in freezing with a nonparametric procedure, that pre-exposure to the box did significantly increase freezing compared to animals not pre-exposed to the box. Such freezing is difficult for the contextual theory to explain.

In summary, the contextual account of the ISD suggests that the immediately shocked animal suffers a conditioning deficit between the memory of the context and the shock. Fanselow (1986, 1990) argues that the simultaneous placement of the rat into the box and the shock also fails to evoke fear after shock in these animals.

An alternative theory: activity theory. It has been observed that most animals will orient to a novel box during which time the animal is still (Brudzynski & Ociepa, 1992; Espejo & Mir, 1993). This behaviour is quickly replaced (within about 10 s) by an active exploration of the box lasting at least 10 min before the animal becomes less active (Glickman & Hartz, 1964; Renner, 1990; Walsh & Cummins, 1976). Therefore, it is proposed that suppression of inactivity in the immediately shocked group, and suppression of activity in the delayed shock group, could account for the difference in freezing between these groups. That is, because the probability is greater that an immediately shocked animal will be inactive at the time of shock relative to a delayed shock animal, it is predicted that the animal in the former group will not freeze after shock because it has been punished for immobility.

This theory was inspired by what Bolles (1970, 1975) has defined the SSDR hypothesis and by Bolles and Riley (1973) who demonstrated that freezing can be controlled by a punishment procedure. The SSDR hypothesis predicts that when an animal has been exposed to an aversive stimulus its consequent behaviour is limited to an SSDR. Therefore, it is anticipated that when a rat is shocked its behaviour will necessarily be a defensive response (e.g., flee or freeze). Furthermore, it is predicted by SSDR theory that the particular post-shock response exhibited will be determined by the environment such that all other behaviours are suppressed, including ineffective SSDRs. In addition, it has been observed that freezing can be controlled by shocking an animal contingent on its behaviour (Bolles & Riley, 1973). For example, shocking an animal contingent on its freezing resulted in increased freezing over the trial duration compared to an animal that was shocked contingent on any behaviour other than freezing. It is these hypotheses on which the activity theory is based. That is, it is hypothesized that the behaviour occurring at the time of the shock will be suppressed and the opposite behaviour to that which occurred at the time of shock will be exhibited. Moreover, it is predicted that the design of the ISD paradigm itself affects the probability of the behaviour occurring at the time of shock. These hypotheses were not tested in the Bolles and Riley (1973) study because these investigators were interested in the effects of multiple shocks administered over a 40 min session rather than a single shock delivered contingent on behaviour. Thus, the current study examined the behaviour of animals which had not encountered a previous shock.

The activity theory is based on two assumptions. The first is that the rat's defense system is activated by shock. Therefore, it is hypothesized that both immediately shocked and delayed shock animals perceive and process the pain of the shock similarly. Moreover, it is hypothesized that all animals will startle as the primary response to shock onset and immediately freeze as a subsequent response to shock termination. This assumption is based on SSDR theory (Bolles 1970) that all rats exhibit defensive behaviour in response to aversive stimulation and on the observations of Fanselow (1980) that a startle burst is the rat's first reaction to a shock.

The second assumption of this theory is that the animal's behaviour after the shock will be opposite in form to the behaviour that was occurring at the time of shock. It is presumed, therefore, that any behaviour at the time of shock would be suppressed by the shock. Given the relatively small size of the shock chamber and the fact that the animal receives the shock to its feet, it is hypothesized that locomotor activity would be particularly susceptible to the punishing effects of the shock. That is, a rat is more likely to be reluctant to move its feet if it has just received a painful foot-shock for locomoting than if it was still. Conversely, a rat shocked while its feet are stationary on the bars would be more likely to keep its feet active after shock. These hypotheses were specifically tested in the final two experiments conducted here. However, before these hypotheses were tested, it was necessary to test particular empirical variables specific to the ISD paradigm.

Variables Affecting the ISD

Table 1 provides a summary of all of the literature specifically examining the ISD over the last twenty years. Particular aspects of these studies are summarized below.

Effect of delay of shock. In general, post-shock freezing has been observed to increase with increasing time between entry into the box and shock delivery (Kiernan & Westbrook, 1993; Westbrook et al., 1994). For example, a linear increase in post-shock freezing during a 2 min trial has been observed for delay intervals of 1, 3, 9, and 27 s (Kiernan & Westbrook, 1993).
Similarly, in the Westbrook et al. (1994) study, rats were observed to freeze on Day 2 approximately 40%, 60%, and 80% during a 2 min trial after receiving shock on Day 1 at delay intervals of 4, 16, and 64 s, respectively. This trend, however, was reversed at longer delay intervals. That is, freezing was significantly less at the delay interval of 4 min than at 1 min, and was negligible at the extremely long interval of 17 min. Therefore, from the available evidence, post-shock freezing increased with increasing intervals up to about 1 min, and

Table 1.

Reference	Between Ss variables	Within Ss variables	Freeze effect	Conclusion
Blanchard, Fukunaga, & Blanchard, 1976a	presentation of a cat imm. 2 min	Day 1 cat presentation	↑ freeze in delay vs. imm presentation of cat ↓ locomote delay vs. imm	familiarity of an inescapable chamber promotes freezing in response to a predator
Blanchard, Fukunaga, & Blanchard, 1976b	shock imm. 2 min	Day 1 f-shk 1(3mA.2s)	↑ freeze in delay vs. imm shock ↓ locomote delay vs. imm	familiarity of an inescapable shock chamber promotes freezing in response to shock

Reference	Between Ss variables	Within Ss variables	Freeze effect	Conclusion
Fanselow, 1980	delay (2 min) vs. imm shock after entry at .5 Vs. 1.0mA intensity in same or different context	Day 1 f-shk 4(.75s) Day 2 no shock	Day 2 ↑ freeze in delay group only tested in same box vs. different	post-shock freezing is only a CR (no UR components) to cues associated with the shock itself
Fanselow, 1986 Expt 1	delay (2 min) vs. imm shock vs. no shock	f-shk 1(1mA.2s)	↑ freeze of delay group vs. imm or no shock groups	↑ freeze by delay of shock
Fanselow, 1986 Expt 2	delay of shock 1,3,9,27,81 s	f-shk 1(1mA.2s)	↑ freeze of delay between 27s and 81s	more than 27s needed to ↑ freeze
Fanselow, 1986 Expt 3	no exit vs. pseudo-exit	f-shk 1(1mA.2s)	↑ freeze of delay groups no diffs between exit vs. pseudo-exit	pseudo-exit does not ↓ freeze

Reference	**Between Ss variables**	**Within Ss variables**	**Freeze effect**	**Conclusion**
Fanselow, 1986 Expt 4	context chamber pre-exp (2 min either no exit, or diff context) vs. no pre-exp	Day 1 pre-exp Day 2 f-shk 1(1mA.2s)	↑ freeze in delay group only no diff between pre-exp vs. no pre-exp	pre-exp without shock is insufficient to overcome ISD
Fanselow, 1986 Expt 5	chamber pre-exp (2 min either no exit, exit, or diff context) vs. no pre-exp	Day 1 pre-exp Day 2 f-shk 1(1mA.2s)	Day 2 ↑ freeze in delay group only no diff between 15 min and 180 min pre-exp times	pre-exp without shock is insufficient to overcome ISD even if pre-exp occurs 15 min before shock

Reference	Between Ss variables	Within Ss variables	Freeze effect	Conclusion
Fanselow, 1986 Expt 6	2nd session delay shock on imm and delay groups 1st session	Day 1 f-shk 1(1mA.2s) Day 2 f-shk 1(1mA.2s) 2 min after entry	Day 2 ↑ freeze in delay group pre-shock vs. imm and no shock groups no diff of imm and no shock groups post-shock	imm animal freezes as if given only 1 shock: failure to form shock-context association
Kiernan & Cranney, 1992	auditory startle given imm vs. 60 s delay	Sess 1 auditory startle 1(117dB.50ms) Sess 2 4 hr after sess 1 same context. no startle	↑ freeze in delay vs. imm both sess 1 and sess 2	deficit of freeze in imm group imm not diff from no-startle control

Reference	Between Ss variables	Within Ss variables	Freeze effect	Conclusion
Fanselow, DeCola, & Young, 1993, Expt 5	massed (3s) vs. distributed (20, 60s) pre-exp to shock box vs. no pre-exp	Day 1 pre-exp Day 2 f-shk 4(1mA,1s) imm after entry	↑ freeze of pre-exp 20 s group only vs. no pre-exp 20 s group	pre-exp to shock box has specific effect at 20 s
Kiernan & Westbrook, 1993, Expt 1	# pre-exps to shock box 0,1,4 delay of shock 1,3,9,27 s	Day 1-4 pre-exp to shock box or to wood box Day 5 f-shk 1(.9mA,.5s) Day 6 2 min test either context	Day 5 ↑ freeze in 0.1 pre-exp at 9, 27 s vs. 1,3 s ↑ freeze in 4 pre-exp vs. 0.1 pre-exp	↑ freeze by multiple no-shk pre-exp ↑ freeze by delay shock

Reference	Between Ss variables	Within Ss variables	Freeze effect	Conclusion
Kiernan & Westbrook, 1993, Expt 2	length of pre-exp 0,2,20 min delay of shock 3.9,27.81 s	Day 1-4 pre-expto shock box Day 5 f-shk 1(.9mA,.5s) Day 6 as Expt 1	Day 5 ↑ freeze at 27.81 s vs. 3.9 s ↑ freeze of 2 min pre-exp vs. 0,20 min	↑ freeze by moderate duration of pre-exp to shock box

Reference	Between Ss variables	Within Ss variables	Freeze effect	Conclusion
Kieman & Westbrook. 1993. Expt 3	length of pre-exp 0.2.20 min delay of shock 7.60 s test context shock. novel	Day 1-4 pre-exp to plexiglas/steel or plexiglas/wood Day 5 f-shk 1(.9mA..5s) 4 & 8 hr later 2 min test in either context	Day 5 ↑ freeze of 60 s vs. 7 s ↑ freeze in 7 s of 2 min pre-exp vs. 0.20 min ↓ freeze in 60 s of 20 min pre-exp vs. 0.20 min 4 & 8 hr ↑ freeze in shock box of 60 s vs. 7 s	↑ freeze of delay shock ↑ freeze by brief pre-exp ↓ freeze by prolonged pre-exp

Reference	**Between Ss variables**	**Within Ss variables**	**Freeze effect**	**Conclusion**
Westbrook, Good, & Kiernan, 1994, Expt 1	delay of shock 4,16.64.256. 1024 s	Day 1 f-shk 1(.9mA.1s) Day 2 2 min no-shk	Day 2 ↓ freeze of 1024 s vs. 4.16.64.256 s	↓ freeze by long delay of shock
Westbrook et al., 1994, Expt 2	delay of shock 7.120.480 s	Day 1 f-shk 1(.9mA.1s) Day 2 2 min no-shk in either shock box or novel	Day 2 ↓ freeze of 120 s vs. 7.480 s ↓ freeze in novel of 120s vs. 7.480 s	↑ freeze by intermediate delay of shock ↓ generalization of freezing to novel box by long delay of shock

Reference	**Between Ss variables**	**Within Ss variables**	**Freeze effect**	**Conclusion**
Westbrook et al., 1994, Expt 3	delay of shock 10.30.150.170 s novel or shock context	Day 1 f-shk 1(.9mA.1s) 180 s session regardless of shock time Day 2 as Expt 2	Day 2 ↓ freeze of 10 s vs. 30.150.170 s ↑ freeze in 10 s of novel vs. shock context ↓ freeze in 150. 170 s of novel vs. shock context	↑ freeze by delay of shock regardless of post-shock duration ↓ generalization of freezing to novel box by long delay of shock

Reference	Between Ss variables	Within Ss variables	Freeze effect	Conclusion
Westbrook et al., 1994, Expt 4	delay of shock 0,10.30.150 s novel or shock context	Day 1 f-shk 2(.9mA.1s) separated by 170 s Day 2 as Expt 2	Day 2 ↑ freeze 2 shk vs. 1 shk ↑ freeze of 10.30.150 s vs. 0 ↓ freeze in 150 s of novel vs. shock context	↑ freeze by 2 shk ↓ generalization of freezing to novel box by long delay of shock

Reference	Between Ss variables	Within Ss variables	Freeze effect	Conclusion
Westbrook et al.. 1994, Expt 5	delay of shock 30. 170 s novel or shock context	Day 1 f-shk 1(.9mA.1s) Day 2 f-shk 1(.9mA.1s) reversed delay of shock to Day 1 Days 3-4 2 min no-shk in either shock box or novel box	Day 4 ↓ freeze in novel of 170 s vs. 30 s ↓ freeze in novel in 170 s of Day 4 vs. Day 3	↓ generalization of freezing to novel box by long delay of shock

Reference	Between Ss variables	Within Ss variables	Freeze effect	Conclusion
Landeira-Fernandez, Fanselow, DeCola, & Kim, 1995, Expt 1	handling prior to shock 0.3 min context pre-exp shock box or trapezoidal box	Day 1 f-shk 1(2mA,1s) Day 2 4 min no-shk test in shock box	Day 2 ↓ freeze in handled vs. not handled ↑ freeze 3 min vs. 0 ↓ freeze no pre-exp vs. pre-exp	↓ freeze by imm shock ↓ freeze by handling prior to shock freeze context specific
Landeira-Fernandez et al., 1995, Expt 2	context pre-exp 0.15 min either to shock box or trapezoidal box delay of shock 0.3 min	Day 1 pre-exp 15 min later f-shk 1(2mA,1s) handling before shock Day 2 4 min no-shk test	Day 2 ↓freeze no pre-exp vs. pre-exp ↑ freeze 3 min vs. 0	↓ freeze in imm shock group regardless of context familiarity

Table 1. A summary of the between and within subjects variables, the effect on freezing and the conclusion of the studies examining the immediate shock deficit.

thereafter began to decrease until, at intervals of 17 min, freezing was virtually non-existent.

Context. As mentioned above, Fanselow's (1986) theory of the ISD is based on the association formed between the memory of the contextual features of the box and the shock. Several studies have examined the ISD within this framework. For example, the effects of non-shock pre-exposure to a context generally resulted in increased freezing when the animal was given a delayed shock on the next day in the same box relative to animals who were not pre-exposed to the box (Fanselow, DeCola, & Young, 1993; Kiernan & Westbrook, 1993; Landeira-Fernandez et al., 1995; Westbrook et al., 1994). However, the effects of this pre-exposure on the freezing of immediately shocked animals were only apparent in animals given repeated pre-exposures. For example, Fanselow (1980) and Landeira-Fernandez et al. (1995) found that a single context pre-exposure had little effect on freezing after immediate shock compared to animals that were repeatedly pre-exposed to the shock context. Conversely, Kiernan and Westbrook (1993) and Westbrook et al. (1994) found that pre-exposure to the context on each of four days enhanced the freezing of animals given a 4 s delayed shock (essentially an immediate shock) relative to animals not pre-exposed to the shock box in this manner. Therefore, the effect of increased freezing in immediately shocked animals that occurred as a result of pre-exposure was not observed until the animal had multiple exposures to the shock box.

The effects of shifts in context from a shock day to a subsequent no-shock test day have revealed that freezing was greater 24 hr after shock in the same box where shock was delivered than in a novel box (Westbrook et al., 1994; Kiernan et al., 1995). What is surprising about these results, however, is that freezing was quite substantial in those groups tested in a novel box on Day 2. For example, Westbrook et al. (1994) observed that rats tested in a contextually different box than the one in which shock was delivered, froze for about half of the 2 min trial. Moreover, animals given a 7 s delayed shock on Day 1 froze for a similar duration on Day 2 (about 45%) regardless of context. This level of freezing was even greater in the Kiernan et al. (1995) study where rats were observed to freeze over 80% of a 2 min trial when tested in a contextually different box than the shock box. Therefore, these results provided evidence that there was considerable generalization of freezing between contexts. However, given that spatial associations are necessarily confounded with temporal associations (Baker, Singh, & Bindra, 1985; Church, 1989; Gallistel, 1990), it is unclear how such contextual generalization might be related to the time of shock. Therefore, a min by min analysis of freezing was conducted in Experiment 1.

Effects specific to the ISD paradigm. An obvious implication of studying the effects of delayed and immediate shock is that behaviour at the time of shock would be associated with the time of shock itself. However, there is no published literature that addresses this issue with respect to the ISD paradigm. Therefore, all of the studies conducted here analyzed the min by min differences in freezing across the duration of the trial.

In addition, there is no evidence to support the hypothesis that freezing will occur as the dominant response in the ISD paradigm under conditions where alternative defensive behaviours are permitted. For example, neither the Blanchard et al. (1976b) nor the Fanselow (1986) study adequately tested the effects that the presence of an exit would have under conditions of delayed and immediate shock. Therefore, Experiment 2 tested the hypothesis that escape would occur as the dominant post-shock response under conditions in which escape was permitted.

Another difference between delay and immediate shocked animals is the fact that handling is likely more salient to the latter group. That is, animals shocked immediately after placement into the box have the salient feature of handling associated with shock delivery that may block the effects of the temporal and spatial contextual cues. There are two ISD studies which bear directly on the potential effects of handling on post-shock freezing. First, Blanchard et al. (1976b) equated the difference in the saliency of handling between the delayed and immediately shocked groups by lifting and replacing the delayed shock rat just before shock delivery. They found that animals in the delayed shock group were significantly less active than animals immediately shocked. Unfortunately, the specific effects of handling were not determined because neither an unhandled delayed shock group nor a twice-handled immediately shocked group were included in this study. Therefore, the effects of handling on post-shock freezing have not been adequately tested in studies examining the ISD. Landeira-Fernandez et al. (1995), however, did directly manipulate handling of two delayed shock groups on Day 1 and measured freezing on Day 2. They reported that freezing decreased on Day 2 in those delayed shock animals handled before shock on Day 1 compared to those animals not handled. Unfortunately, these investigators did not measure freezing on Day 1, although it could be argued that the effects of handling would also be apparent on Day 1. This hypothesis was tested in Experiment 3.

Summary

The first three experiments reported here focused on manipulating certain paradigm-specific factors pertinent to the ISD. Experiment 1 tested the effects on freezing of shifts in context between the shock context on Day 1 and a different context on Day 2. Emphasis was placed on the evaluation of both spatial and temporal contextual cues that are necessarily present in every situation. It is hypothesized that animals tested in different contexts on each day will freeze less than animals tested in the same context on each day but more than no-shock control. Furthermore, it is hypothesized that all shocked animals will freeze maximally on Day 2 at the time they were shocked on Day 1. Such results will provide evidence for both contextual and temporal conditioning in the ISD paradigm. In addition these results will support the hypothesis that both immediately shocked and delayed shock animals exhibit defensive behaviours in the ISD paradigm particularly within the first minute of testing after shock. Experiment 2 tested the hypothesis that the hierarchy of defensive behaviour would change from freeze to flee if escape was permitted regardless of the time of shock delivery. These results will provide evidence in support of the hypothesis that flight is possible within the ISD paradigm provided that an exit is provided. In addition, this experiment will further support the hypothesis that defensive behaviours occur in immediately shocked animals as well as delayed shock animals. Experiment 3 tested the hypothesis that contextual conditioning effects could be blocked by providing the salient external cue of handling just before delivering a delayed shock. These results will provide evidence that handling functions to overshadow the contextual conditioning but not the temporal conditioning of immediately shocked animals and delayed shock animals handled before the shock.

Collectively the results of Experiments 1 - 3 will have implications for each of the three theories of the ISD described above. It is expected that a detailed description of defensive behaviour can be outlined in these experiments that will provide evidence in support of the topographical account of the ISD under certain conditions (e.g., when an exit is present). In addition, it is expected that spatial conditioning will be apparent in each of these experiments in support of the contextual association account of the ISD. However, it is hypothesized that contextual associations are formed simultaneous with temporal associations and that both of these are important in the examination of freezing in both immediately and delayed shock animals. Finally, these results are expected to directly support the activity account of the ISD by demonstrating that defensive behaviours occur in both immediately and delayed shock animals. In particular, it is hypothesized that both groups will exhibit the same initial defensive behaviours after shock termination but that the immediately shocked group will suppress these behaviours more rapidly than the delayed shock group.

Experiments 4 and 5 tested the hypothesis that the animal's behaviour at the time of shock is a crucial determinant of the subsequent duration of post-shock freezing. Specifically, Experiment 4 examined the effects of delivering a delayed shock contingent upon the occurrence of several variables observed to correlate with freezing in the previous three experiments. These variables were manipulated so that shock delivery was contingent upon the particular occurrence of these variables. The variables included were number of bars between the animal's feet (high vs. low), its position on the bars relative to the direction of the bars (parallel vs. perpendicular), and its behaviour (still vs. locomote). It was hypothesized that the maximum duration of freezing would be apparent in those animals for which shock was delivered contingent upon the following configuration: a high number of bars between its feet, perpendicular orientation, and locomoting. However, it was hypothesized that the behaviour variable would be the strongest predictor of duration of post-shock freezing. In addition to these variables the differences in the magnitude of startle in response to the shock were also measured in the attempt to further characterize those factors that produced differences in behaviour. It was predicted that animals that startled the most during the shock would subsequently freeze the most during the post-shock interval. The examination of freezing in this manner would have direct implications for the activity account of the ISD, particularly the strength of the behaviour variable in determining differences in freezing between animals shocked while still and those shocked while locomoting.

Experiment 5 examined the effects of delivering shock contingent on the animal's behaviour under conditions of immediate and delayed shock. It was hypothesized that behaviour at the time of shock was a more important determinant of post-shock freezing than time of shock. Therefore, it was hypothesized that immediately shocked animals, shocked contingent on their locomoting at the time of shock would freeze more than those shocked contingent on their being still at the time of shock. A similar effect of behaviour was predicted for those animals given a delayed shock. Moreover, it was hypothesized that the probability of behaviour at the time of shock is different between rats given a immediately and delayed shock such that immediately shocked animals are more likely to be still at the time of shock than delayed shock animals. Therefore, a random control condition was included in this experiment that was shocked contingent on only time (immediate or delay). It was expected that the duration of freezing in the group immediately shocked regardless of behaviour would be similar to those animals shocked contingent upon their being still regardless of time of shock. Further, it was expected that those animals given a delayed shock regardless of behaviour would freeze as long as those animals shocked contingent on their locomotion regardless of time of shock. These results would provide strong evidence that behaviour at the time of shock is the crucial determinant of post-shock freezing and not the time of shock itself. In combination with the results of Experiment 4 this evidence would provide strong support that the ISD is an empirical effect that relates to suppression of activity at the time of shock.

Experiment 1

Rats will typically freeze for several min after receiving a single, unsignalled electric foot-shock (Blanchard & Blanchard, 1969b; Fanselow, 1990; Kim, Rison, & Fanselow, 1993). It is considered that this freezing represents a conditioned response to the contextual stimuli that were present at the time of the shock and continue to be present after the shock (Fanselow, 1980; Phillips & LeDoux, 1992; Rudy, 1993). In support of this contextual hypothesis, animals tested in a box dissimilar from the box in which they were shocked, did not freeze as much as animals tested in the same box twice (Bolles & Collier, 1976; Fanselow, 1980; Fanselow & Helmstetter, 1988). These results have been taken as evidence that freezing can be modulated by shifts in spatial context (Maes & Vossen, 1992).

In further support of the importance of spatial context, animals exposed to the shock box for a few minutes before shock was delivered froze more than animals that were shocked in a novel box (Blanchard et al., 1976a and b; Fanselow, 1986; Westbrook et al., 1994). The reader will recall that the shorter duration of freezing observed in the animal shocked simultaneously with placement into the box compared to those familiar with the box (delayed shock rats) has been termed the immediate shock deficit (ISD; Fanselow, 1986). As discussed in the previous section, Fanselow (1986) interpreted these results to mean that immediately shocked animals had insufficient time to form an integrated representation of the box before shock, so a deficit in freezing arises as a result of the failure of these animals to associate this representation of the box with the shock.

As with all behavioural measurements, however, there are two fundamental components of learning that must be considered: space and time (Gallistel, 1990, p. 221). Animals use spatial and temporal information to recall specific learned events (Baker et al., 1985; Church, 1989; Gibbon, 1977). For example, freezing measured 24 hrs after shock, has been observed to peak at the time of shock delivery on the previous day (Fanselow. 1982. Experiment 2; Maes & Vossen, 1992). However. the changes in freezing after an immediate shock have not been explored over the course of a session. That is. all of the studies exploring the ISD have measured freezing by averaging over the entire 5 min trial duration, obscuring min by min changes in the time course of freezing.

An important observation in comparing ISD studies is that where the trial duration was shorter (i.e.. 2 min). more freezing was observed on Day 2 testing than in those studies where a longer trial duration was used (i.e.. 5 min). For example, Fanselow (Experiments 2. 1990; Experiment 2. 1985) reported the mean duration of freezing over 5 min was less than 5% (i.e.. 15 s) for rats given a 9 s delayed shock 24 hr previously. Similarly treated rats observed over a 2 min period spent a mean duration of almost 20% (i.e.. 24 s) freezing (Kiernan & Westbrook, 1993; Westbrook et al.. 1994). These comparisons suggest that the duration of conditioned freezing of animals immediately shocked may be attenuated by longer trial durations. Therefore. potentially significant differences in freezing may be rendered insignificant by averaging freezing durations across a 5 min trial, instead of averaging over a shorter period.

Attenuated levels of freezing as a function of time have been also observed in response to the shock itself (i.e.. Day 1 testing). For example. freezing in mice significantly decreased after the first min of a three min trial regardless if the shock was delivered after a 15 s. or 3 hr delay (Hammond. 1995). There are no available data, however. from those studies exploring the ISD to demonstrate a similar effect. However. it would be reasonable to expect that significant levels of freezing by immediately shocked animals in the first min may be rendered nonsignificant by averaging freezing duration over a 5 min period.

In summary, despite the evidence that post-shock freezing decreases over time within a session investigators studying the ISD have not analyzed freezing min by min over the session.

The main objective of the present experiment was to examine the effects of spatial context on conditioned freezing assessed 24 hr after shock. Specifically, one group of rats received a single shock immediately and two groups were shocked 2 min after entry into the shock chamber and were subsequently observed for 5 min on Day 2. A fourth group was observed for 5 min on each day, without shock. To adequately assess the effects of shock over the course of a session, the average amount of freezing during each min of the trial was analyzed. One group of rats was tested in a dissimilar conditioning box to reduce the spatial conditioning effects on freezing. This allowed for examination of the effect of spatial conditioning.

Several of the procedural details typical of most ISD studies were also changed in the present experiment. First, in this study freezing was defined as an immobile crouched posture rather than simply a cessation of movement (Blanchard et al., 1976) that was continuously measured, rather than time-sampled (Fanselow, 1986). Continuous coding of specific behaviours relative to sampling at fixed time intervals provides more accurate observations (Rodger & Rosebrugh, 1979; Van Hooff, 1982; Bressers, Meelis, Haccou, & Kruk, 1991). In particular, continuous coding relative to time sampling is better suited to measuring behaviours, such as locomotion and sniff, that occur frequently for short durations (Van Hooff, 1982).

Second, this experiment measured the occurrence of all behaviours during the trial, not only freezing, to determine the general post-shock behavioural topography of rats. Such an analysis has proven to be important for understanding the general motivational state of the animal after shock. For example, rats that are fearful are likely to spend less time engaged in active locomotor behaviour and more time in brief bursts of sniffing, than are less fearful rats (File & Vellucci, 1979; Hall, 1938; Whimbey & Denenberg, 1967). Detailed recording of the changes in behaviour over the course of the first 20 s after shock helped to define the sequence of defensive behaviours that occur during that time (Fanselow, 1982; Moser & Tait, 1983). One of the assumptions of the contextual account of the ISD is that an immediate shock fails to evoke the animal's defense system (Fanselow, 1986; 1990). However, Fanselow (1986) has only provided evidence in the form of reduced defecation in immediately shocked animals to support this claim. In an earlier investigation of post-shock activity Fanselow (1982, Experiment 4) described the behaviours of animals during the initial 20 s post-shock. He observed that animals given a single, 3 min delayed shock froze for a mean of 2.2 s and did not move their bodies for an additional mean of 8.8 s during the 20 s after shock termination. Because this brief freezing was significantly less than the duration of freezing observed during the same period on a subsequent no shock test session, Fanselow concluded that post-shock freezing represented a conditioned response to the contextual cues associated with shock. He did not, however, test whether this initial freezing was significantly different than the duration of freezing observed prior to shock (i.e., 0 s). He also failed to report when during the 20 s this freezing occurred. Moreover, because no immediately shocked group was included in this study, the hypothesis that an immediate shock does not evoke the animal's defensive system could not be tested. Consonant with Fanselow's (1982) procedure, the present experiment investigated the rat's responses to the shock during the 1 s of shock administration and during the 20 s following shock termination. However, in this experiment, both immediately and delayed shock groups were evaluated. This allowed for differences in fear between the groups to be revealed.

In summary, it was expected that these procedural changes would provide a more detailed assessment of the rat's behaviour in response to shock, during a 5 min post-shock trial, and 24 hr after shock. Moreover, it was hypothesized that the min by min analysis of freezing would reveal differences in freezing across the session, including immediate shock animals. Consequently, it was hypothesized that the immediately shocked animal does not suffer a conditioning deficit.

Method

Subjects

The subjects were 40 naive, male Sprague-Dawley rats (Ellerslie, Alberta) weighing between 210-310 g (M=287 g, SEM=2.86). Each subject was individually housed with ad libitum access to laboratory chow and water in a 23 °C colony room. The experiment was conducted in the dark portion of a 12-h-on/12-h-off light/dark cycle. Testing was carried out in the dark phase to ensure more overall activity and a better approximation of the rat's normal range of nocturnal behaviour (Evans, 1988; Prescott, 1967; Wollnik, 1989).

Apparatus

Two separate chambers were used in this experiment. A standard 24 x 20 x 22 cm shock box was used to deliver shock to all groups on Day 1. The ceiling, as well as the front and back walls (24 cm) were clear plexiglas. The side walls (20 cm) were stainless steel. The floor consisted of 18 stainless steel bars, 2.5 mm in diameter and spaced 1.0 cm apart. Each rod was wired to a standard shock generator/scrambler which provided a 0.8 mA shock for 1 s. The second compartment was constructed to approximate a home cage (43 x 20 x 26 cm). The walls and floor of this compartment were clear plexiglas with a standard home cage wire lid.

The experimental room was dark with the exception of a 7.5 W red bulb mounted 20 cm above the chamber. Each trial was recorded on video tape for subsequent assessment of the rat at the time of shock. Behaviour was coded throughout the trial by computer.

Procedure

Rats were handled once each day for 1 min. for 5 days prior to the beginning of the experiment to minimize the likelihood of freezing on the test day from handling alone (Hammond, 1995). Equal numbers of subjects were randomly assigned to one of four groups, as described below. On the first day of experimentation (Day 1), each rat was individually carried in its home cage to the test room. Rats in the standard delayed shock (DEL-CNTRL) and the context delayed shock (DEL-CNTXT) groups were placed in the shock compartment through the ceiling. After 2 min they were given a .8 mA foot-shock for 1 s. Rats in the standard immediately shocked (IMM-CNTRL) group were given the same shock as soon as the roof was closed. The NO-SHK group was observed in the box for 7 min without shock.

Each of the shocked groups was observed for 5 min after shock termination. Behaviour was continuously coded in the shock box throughout the pre- and post-shock period. The frequency and duration of each behaviour were recorded on disk. Behaviour categories were mutually exclusive and exhaustive. A definition of each of these categories is discussed below.

Freeze. Freezing was defined as the absence of all visible movement, except for that required by respiration (Blanchard & Blanchard, 1976). Freeze is differentiated from resting by a crouched posture that is usually accompanied by rapid, shallow respiratory movement of the rat's rib cage. Resting was not observed during either the pre- or post-shock period. Interobserver reliability, using this definition of freezing, was high (r= .98).

Sniff. Sniffing was defined as active vibrissal movement while still. Therefore, sniffing in conjunction with another behaviour (e.g., locomoting) was coded as belonging to the latter category.

Locomote. Locomoting was defined as the traversing of at least one bar, accompanied with or without another behaviour.

Rear. An animal was considered to be rearing when its head was higher than the horizontal plane of its body. This was most frequently observed when the animal was standing on its hind paws.

Autogroom. Autogrooming was most frequently observed as washing of the body but was also classified when the animal scratched itself.

Startle. Startle was defined as the frenzied activity of the animal during shock.

Indeterminate. This category was used when the rat's behaviour could not be adequately classified as one of the above. There were few instances of indeterminate behaviour in this experiment.

The definitions of these behaviours are standard (Beck & Chow, 1984). In this experiment, test-retest reliability was determined by comparing the author's coding of each behaviour occurrence of a complete session on two occasions. Interobserver reliability was determined by comparing the author's coding of behaviour occurrences of a session with another person's coding of the same session. A total of 20 sessions were included in these reliability assessments. Test-retest and interobserver reliabilities of these behaviour categories were between 90-100%.

At the end of the trial on Day 1, the animal was removed to its home cage and returned to the colony room. The apparatus was cleaned with an acetic acid solution (5% in tap water) between trials.

On Day 2, one delay group (DEL-CNTRL), the immediately shocked group (IMM-CNTRL), and the NO-SHK group were observed in the shock box. The other delay group (DEL-CNTXT) was tested in the home cage apparatus. All groups were observed for 5 min, without shock.

In addition to the continuous coding of behaviour throughout the 5 min trial outlined above, behaviours were coded specifically during the 1 s of shock and during each second of the 20 s following shock. This provided a detailed assessment of the rat's initial reactions to the shock, similar to Fanselow's (1982) procedure.

Primary analyses were performed on the dependent measure of post-shock freezing during the 5 min trial. To assess differences among the groups, freezing was subjected to one-way Analysis of Variance followed by Bonferroni post-hoc comparisons, where appropriate. The min by min changes in freezing, were assessed by calculating the mean duration of freezing during each of the five 1 min bins. A two-way ANOVA, with groups and bins (repeated measure) as factors, was used to assess differences in freezing across bins. Because group differences were assessed in the one-way ANOVA, statistics relating to the main effect of group were redundant and are not discussed. To statistically correct for the within-groups measure, all degrees of freedom associated with bins were multiplied by the calculated Greenhouse-Geisser Epsilon (Greenhouse & Geisser, 1959).

The durations of the remaining exploratory behavioural categories were analyzed by between-groups ANOVA followed by Bonferroni pair-wise comparisons.

Results

Initial post-shock reactions

All 30 of the shocked rats exhibited a startle reaction during the 1 s of shock. Startle activity was followed by a brief period of locomotion (1-2 s) in 20 out of 30 rats (11 out of 20 rats in the DEL groups; 9 out of 10 rats in the IMM-CNTRL group). The remaining 10 rats were observed to freeze after startling. Within 3 s after shock, 100% of the rats were observed to be freezing. This freezing lasted for a minimum of 1 s and for a M±SEM of 5.61±.02 for DEL groups and 2.43±.04 for the IMM-CNTRL group. A one-way ANOVA, followed by Bonferroni tests on the measure of freezing, revealed that each of the shocked groups froze significantly longer than the NO-SHK group during the 20 s period [$F(3,36) = 12.19$; $p < .0001$]. Freezing either continued for the remainder of the 20 s period (16 out of 20 rats in the DEL groups; 1 out of 20 in the IMM-CNTRL group) or was replaced by sniffing in 3 out of the 4 rats not freezing in the DEL groups; and in 6 out of the 10 rats not freezing in the IMM-CNTRL group. The remaining rats (1 in the DEL groups; 3 in the IMM-CNTRL group) were observed to locomote following the initial freezing. Once a post-shock exploratory behaviour was observed, freezing did not typically re-occur during the 20 s period (4 out of 4 rats in the DEL groups; 8 out of 10 rats in the IMM-CNTRL group). By contrast, none of the rats in the NO-SHK group exhibited startle. However, these rats remained motionless for a M±SEM duration of 1.26±.03 after being placed in the box. Following this brief period of immobility, the unshocked rats typically alternated between sniff, rear, and locomote with a M±SEM for each of these behaviours of 9.26±.34 s; 4.12±.42 s and: 6.81±.23 s, respectively.

In summary, the typical behaviour sequence observed during shock and the 20 s following shock in the IMM-CNTRL group was an initial burst of activity, including startle (1 s), and locomote (1-2 s). This activity was promptly replaced by freezing (approximately 2 s). An alternating pattern of sniff, rear, and locomote ensued following the freezing. The typical behaviour sequence of the DEL group during this period was the same as for the IMM-CNTRL group except that freezing lasted longer in the DEL group (80% of these animals froze for the entire 20 s period).

Post-shock reactions throughout the trial

Freezing on Day 1. Mean percent time of post-shock freezing averaged over the full 5 min by rats in each of the groups on Day 1 is shown in the left graph of Figure 1. A between groups ANOVA on mean percent time freeze on Day 1 revealed a significant difference for groups [F(3.36) = 21.39; p<.0001). As expected, significant Bonferroni pair-wise comparisons confirmed that the DEL groups froze more than the IMM-CNTRL and NO-SHK groups. In addition, the IMM-CNTRL group froze more than the NO-SHK group.

Mean percentages of post-shock freezing for each of the 1 min bins on Day 1 are shown in Figure 2, left panel. A significant main effect of bins was revealed [F(1.88,144) = 14.95; p<.0001]. As indicated by the Bonferroni tests, a significant group x bin interaction [F(5.64,144) = 2.99; p<.001] was caused by the decline in freezing of the IMM-CNTRL group after the first min, but an increase in freezing in the DEL groups during the same interval. As can be seen in this figure, the DEL groups differed in their pattern of within session freezing from the IMM-CNTRL and NO-SHK groups. The DEL groups increased freezing after shock from 1 min to 2 min, maintained a consistent level of freezing through min 3 and 4, and thereafter decreased freezing. The IMM-CNTRL group

Figure 1.

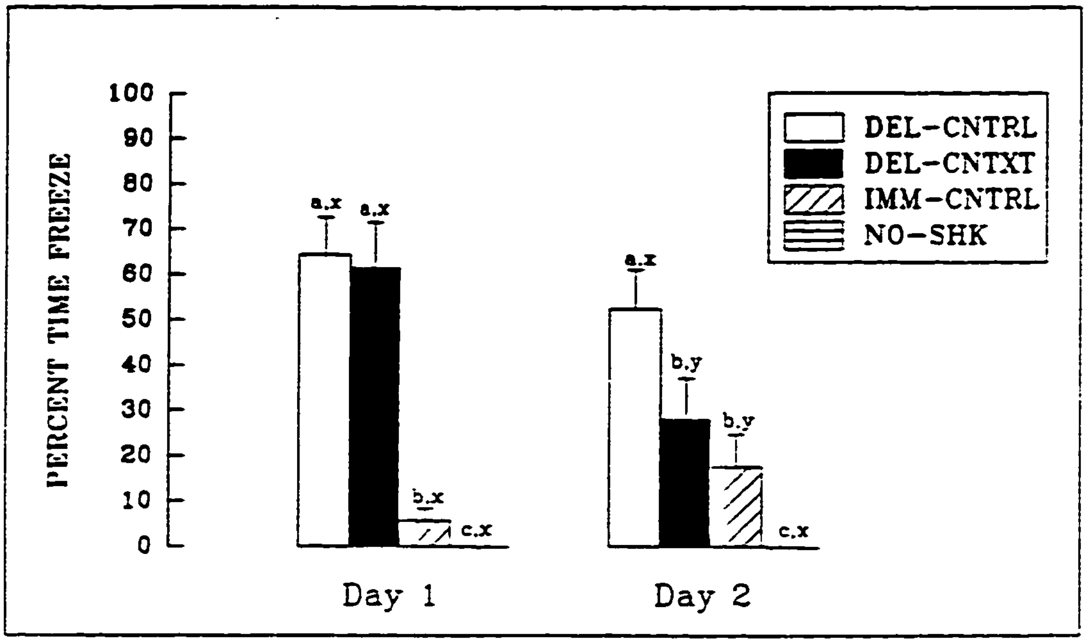

Figure 1. Mean (±SEM) percentage time spent freezing during the 5 min trial (**left:** Day 1 = post-shock: **right:** Day 2 = no-shock) by each of the three groups of rats given a 1s, .8mA foot-shock and the no-shock control rats in Experiment 1. DEL-CNTRL (open bars) = Delay Control group (n = 10), rats were shocked 2 min after placement in the box on Day 1 and tested in the same box on Day 2; DEL-CNTXT (filled bars) = Delay Context group (n = 10), rats were shocked 2 min after placement in the box on Day 1 and tested in a different box on Day 2. IMM-CNTRL (diagonal-hatched bars) = Immediate Control group (n = 10), rats were shocked immediately after placement on Day 1 and tested in the same box on Day 2; NO-SHK (horizontal-hatched bars) = No Shock Control group (n = 10), rats observed without shock in the same box on both days.

a,b,c - bars with different letters are significantly different ($p < .05$) within Days

x,y - bars with different letters are significantly different ($p < .05$) between Days

Figure 2.

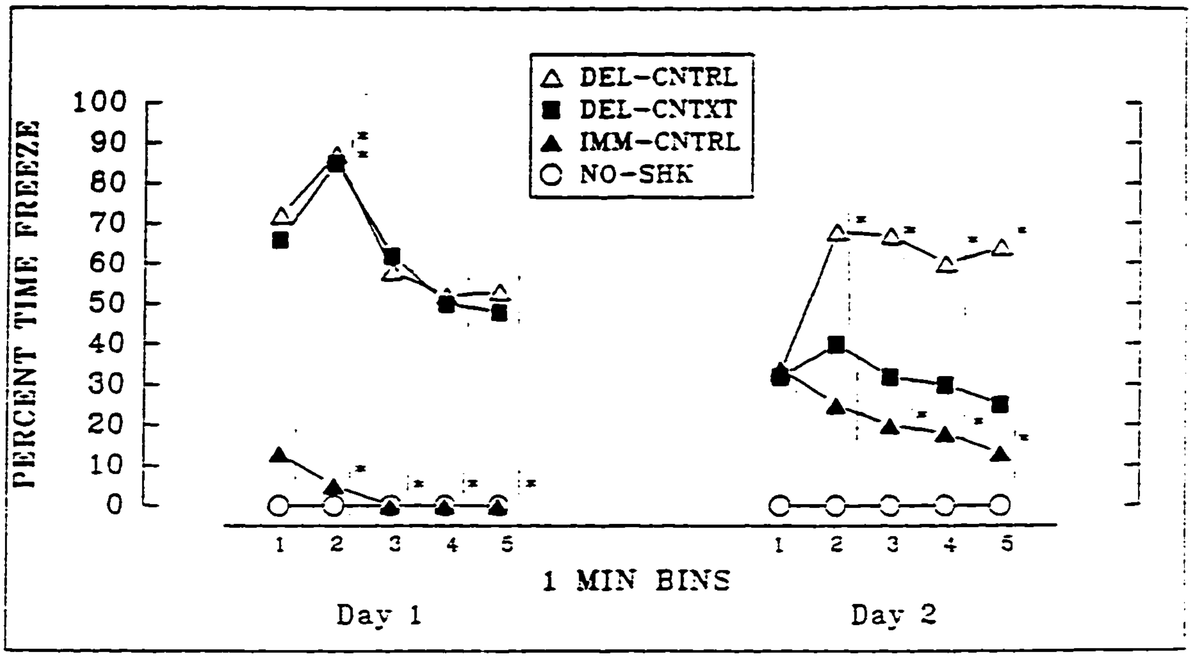

Figure 2. Mean percentage time spent freezing during each of the five, 1 min periods of the trial (**left:** Day 1 = post-shock; **right:** Day 2 = no-shock) by each of the three groups of rats given a 1s, .8mA foot-shock and the no-shock control rats in Experiment 1. DEL-CNTRL (open triangles) = Delay Control group (n = 10), rats were shocked 2 min after placement in the box on Day 1 and tested in the same box on Day 2; DEL-CNTXT (closed squares) = Delay Context group (n = 10), rats were shocked 2 min after placement in the box on Day 1 and tested in a different box on Day 2; IMM-CNTRL (closed triangles) = Immediate Control group (n = 10), rats were shocked immediately after placement on Day 1, and tested in the same box on Day 2; NO-SHK (open circles) = No-Shock Control group (n = 10), rats observed without shock in the same box on both days.
Points marked with an adjacent asterisk are significantly different ($p < .05$) within groups from the mean of freezing during the first min.
Means not joined by the adjacent vertical line are significantly different ($p < .05$) between groups.

showed a steady decrease in freezing after the first min post-shock. The amount of freezing during the first min in all shocked groups was significantly more than that observed in the NO-SHK control group during this time.

Exploration on Day 1. Analysis of the other behavioural categories revealed that most behaviour could be classified into one of three categories of exploration: sniff, rear, and locomote. That is, there were very few instances of autogroom and indeterminate behaviours. Because the NO-SHK group and the DEL groups did not significantly differ in the pattern of exploratory behaviour prior to shock [F(2,27) = .67; p>.10], these data are not included here. The pattern was typical of exploratory behavior in non-shocked rats (Evans, 1988; Ley, 1975; Walsh & Cummins, 1976).

The mean percent time (SEM) of the post-shock duration of each of the three exploratory behaviours on Day 1 is presented in Table 2.

Sniff. Between groups ANOVA revealed a significant difference among groups on duration of post-shock sniff on Day 1 [F(3,36) = 13.3; p<.0001; Table 2]. As indicated by the appropriate Bonferroni tests, the NO-SHK group sniffed significantly more than the DEL groups; the IMM-CNTRL group did not significantly differ from the NO-SHK group on this variable.

Rear. On Day 1, a similar pattern of results was found in the duration of rear as reflected in the sniff effect [F(3,36) = 8.9; p<.0002; Table 2]. That is, rearing was significantly decreased in both delayed shock groups, relative to the other two groups which did not significantly differ.

Locomote. Duration of locomote on Day 1 was the only post-shock behaviour that was significantly different in the NO-SHK group relative to all other groups [F(3,36) = 61.5; p<.0001; Table 2].

Summary of Behaviour on Day 1

In summary, similar reactions to shock onset and in the 20 s following shock termination were observed in all shocked groups. This pattern of initial activity included startle, locomote, freeze, and explore.

Table 2.

DAY	GROUP	SNIFF	REAR	LOCO
Day 1				
	NO-SHK	57.2(3.0)**a**	20.5(1.8)**a**	15.3(.72)**a**
	DEL-CNTRL	27.2(6.8)**b**	4.20(1.7)**b**	2.00(.70)**b**
	DEL-CNTXT	28.6(6.9)**b**	7.20(2.8)**b**	2.60(.88)**b**
	IMM-CNTRL	63.2(2.9)**a**	27.1(6.2)**a**	9.20(.89)**c**
Day 2				
	NO-SHK	52.9(4.6)**a**	9.51(1.1)**a**	9.91(1.1)**a**
	DEL-CNTRL	25.5(3.0)**b**	10.7(3.0)**a**	3.02(1.1)**b**
	DEL-CNTXT	31.6(4.9)**b**	41.0(6.2)**b**	10.1(1.7)**a**
	IMM-CNTRL	67.4(5.0)**c**	24.0(4.7)**c**	8.81(1.2)**a**

Table 2. Mean (±SEM) percentage time spent sniffing, rearing, and locomoting during the 5 min trial (Day 1 = post-shock; Day 2 = no-shock) by each of the three groups of rats given a 1s, .8mA foot-shock and the no-shock control rats in Experiment 1.

a,b - values with different letters are significantly different ($p < .05$) within Days.

Rats in each of the DEL groups froze significantly longer than rats in either the IMM-CNTRL group or the NO-SHK group. However, rats in the IMM-CNTRL group froze longer than rats in the NO-SHK group during the first min. Freezing in the IMM-CNTRL group decreased after the first min of the trial. Freezing in the DEL groups increased from the first to the second min post-shock and thereafter decreased.

Analysis of the behavioural topography of the DEL shocked rats on Day 1 demonstrated that freezing was related to all three exploratory behaviours in these groups. In the IMM-CNTRL group, the increased freezing was offset only by a decrease in locomotion.

Behaviour on Day 2

Freezing on Day 2. The proportion of time spent freezing on Day 2 by each of the four groups is displayed in the right graph of Figure 1. Statistical analysis failed to reveal a significant difference among the groups on percent time freezing on Day 2 compared to Day 1 [$\underline{t}(78) = 1.11$; $\underline{p}>.05$].

Within sessions ANOVA followed by Bonferroni pair-wise comparisons, confirmed that rats in the DEL-CNTRL group froze longer than rats in the other three groups [$\underline{F}(3,36) = 9.05$; $\underline{p}<.0001$). Furthermore, rats in the each of the three shocked groups froze significantly more than rats in the NO-SHK group.

The percent time of freezing across bins on Day 2 is shown in Figure 2, right graph. Repeated measures ANOVA confirmed a significant main effect of bins [$\underline{F}(2.8,144) = 3.09$; $\underline{p}<.018$) and a significant group x bin interaction effect [$\underline{F}(8.4,144) = 3.11$; $\underline{p}<.001$]. The interaction effect was caused by the nonsignificant difference of freezing among shocked groups during the first min and the significant difference between the DEL-CNTRL group and the IMM-CNTRL group during the second min, as indicated by the Bonferroni tests. A significant Bonferroni test revealed that the DEL-CNTRL group increased freezing from the first to the second min of the trial and was thereafter maintained at a consistent level. The DEL-CNTXT group maintained a consistent level of freezing throughout the trial. Conversely, rats in the IMM-CNTRL group showed a decline in freezing after the first min in the box.

Exploration on Day 2. The mean percent time (SEM) of each of the exploratory behaviours on Day 2 is presented in Table 2.

Sniff. Between groups ANOVA, followed by Bonferroni pair-wise tests revealed that on Day 2, significantly less sniff was observed in the DEL groups than the IMM-CNTRL and NO-SHK groups which did not statistically differ from each other [F(3,26) = 5.36; p<.003; Table 2].

Rear. Duration of rearing on Day 2 was longer in the DEL-CNTXT and IMM-CNTRL group relative to the NO-SHK and DEL-CNTRL groups which did not statistically differ from each other, as indicated by the group effect [F(3,36) = 28.3; p<.0001; Table 2].

Locomote. The DEL-CNTRL group locomoted significantly less on Day 2 than the other three groups which did not significantly differ from each other [F(3,36) = 6.7; p<.001; Table 2].

Summary of Behaviours on Day 2

In summary, the duration of freezing on Day 2 was significantly different among the three shocked groups and the NO-SHK control group. However, percent time freezing by the shocked groups during the first min of the test session was equivalent. In the DEL-CNTRL group, freezing increased during the second min and was then maintained. Freezing was relatively consistent throughout the trial in the DEL-CNTXT group although maximal freezing occurred 2 min into the trial. In the IMM-CNTRL group, freezing decreased after the first min.

Relative to the NO-SHK group, the DEL-CNTRL group spent significantly less time sniffing and locomoting during the 5 min trial on Day 2. The DEL-CNTRL group, however, spent less time engaged in all three exploratory behaviours relative to the IMM-CNTRL group. Therefore, delayed shock relative to immediate shock on Day 1, had the effect of decreasing exploration on Day 2.

Discussion

The results of the present experiment replicated several effects described in the literature. First, these results replicated the immediate shock deficit (e.g., Blanchard et al., 1976b; Fanselow, 1986; Kiernan & Westbrook, 1993; Westbrook et al., 1994; Landeira-Fernandez et al., 1995). Although rats shocked immediately after placement into the apparatus did not freeze more over the entire trial duration than did no-shock controls, rats shocked after a 2 min delay froze significantly longer on Day 1. Second, the present results replicated the effect reported by Hammond (1995) that freezing decreases over time within a session. In the IMM-CNTRL group, freezing in the third post-shock min was significantly reduced compared to the first post-shock min. In both of the DEL-SHK groups, freezing in the fourth post-shock min was significantly reduced compared to the first post-shock min. Third, the present results replicated the spatial conditioning effects reported in the ISD literature (Fanselow, 1986; Kiernan & Westbrook, 1993; Landeira-Fernandez et al., 1995; Westbrook et al., 1994). Rats tested on Day 2 in the same box that they were shocked in on Day 1 froze more than those tested in a contextually different box than the one they were shocked in. Finally, the present results replicated the post-shock behaviour sequence of startle, freeze, and explore observed by other investigators (Blanchard & Blanchard, 1968; Fanselow, 1982).

Analysis of behaviour during the 20 s post-shock showed that shocked rats exhibited a similar pattern of initial activity. This pattern of initial activity included startle, locomote, freeze, and explore. Freezing was observed in every animal within 3 s after the startle suggesting that freezing may be the unconditioned reaction to shock termination. An alternative explanation is that initial post-shock freezing may serve as an alerting response to pain (Espejo & Mir, 1993). Fanselow (1982) has reported a similar finding that rats exhibited a mean percent time freeze of 2 s during the 20 s following shock. These results extend Fanselow's (1982) observations of post-shock activity to include the precise sequence of events after shock rather than an overall description of those behaviours observed during the 20 s following shock.

The finding that IMM-CNTRL animals froze significantly less than both DEL groups on Day 1 is a replication of the ISD. However, approximately two times more overall freezing was observed on Day 1 in all the shocked groups of the present study than what has been typically reported by Fanselow (1986, 1990, 1994). Furthermore, approximately three times as much overall freezing on Day 2 was observed in the DEL-CNTRL group in the present study than in the delayed control groups that Fanselow (1986, 1990) has reported (60% vs. 20%, respectively).

There are several subject, pre-experimental, and procedural differences between the Fanselow (1986) study and the present experiment that may account for this discrepancy (see Table 3). Although the potential contribution to freezing of several of these factors is unknown, the literature suggests that three differences could account for the increased level of freezing observed in the present experiment (these are marked by an asterisk in Table 3). First, the literature suggests that males freeze longer than females in response to foot-shock (Fanselow et al., 1993). Second, it has been found that presenting foot-shock during the dark portion of the animal's cycle results in more freezing than if the shock was presented during the lighted portion (Lester & Fanselow, 1992). Finally, behavioural assessment is more accurate if continuously coded than if sampled at fixed time intervals (Bressers et al., 1991; Rodger & Rosebrugh, 1979; Van Hooff, 1982). Because short bout freezing occurred in the present experiment it could be argued that some freezing in the Fanselow (1986) study was overlooked. Thus, the increased level of freezing observed in this experiment could have been due to the fact that males were tested instead of females, animals were shocked in the dark portion of their cycle, and behaviour was coded continuously rather than time sampled.

An important finding revealed by the min by min time bin analysis done in

Table 3.

Variable	Experiment 1	Fanselow (1986)
sex*	male	female
weight	210-310 g	adult (weight unspecified)
species	Sprague Dawley (Ellerslie)	Sprague Dawley (source unknown)
cycle	12:12 hr light:dark	14:10 hr light:dark
housing	individual	individual
pre-handling	5 days	10 days
transport exposure	not adapted	adapted
access to food and water	ad libitum	ad libitum
tested*	dark portion of cycle	light portion of cycle
shock	0.8mA,1s	1.0mA,2s
delay	2 min	2 min
observed	5 min	5 min
scored*	continuous	time sampled
testing	dark overhead; room dark	light overhead; room dark

Table 3. A list of the subject, pre-experimental, and procedural variables in the Fanselow (1986) study and Experiment 1. Those variables for which the literature supports the increased baseline of freezing observed in the present experiment are marked by an asterisk.

this experiment was that all shocked rats, including those shocked immediately, froze significantly more than NO-SHK control rats during the first post-shock min on Day 1. Therefore, shock had the effect of increasing freezing in all groups during the first min after shock, regardless of the time of shock delivery. The second by second analysis of the 20 s post-shock period provides additional evidence for this conclusion because freezing was observed in all rats during this time. However, rats in the IMM-CNTRL group did not maintain a consistent level of freezing after the first post-shock min, as did the DEL shocked rats. This suggests that a delayed shock had a more enduring effect on the disruption of ongoing behaviour (i.e., freezing) than did immediate shock.

Analysis of the exploratory behaviours during the post-shock period on Day 1 supports the conclusion that all ongoing exploratory behaviours were disrupted in the DEL shock group relative to the NO-SHK group. That is, post-shock trial durations of sniff, rear, and locomote were significantly reduced in the DEL-SHK groups on Day 1 relative to the NO-SHK group. Furthermore, the duration of sniff and rear in the DEL-SHK groups was significantly less than in the IMM-SHK group. Shock, however, decreased the duration of locomotion in all shocked groups relative to the NO-SHK group. This suggests that shock, regardless of time of delivery, decreases the probability of subsequent locomotion.

Another important conclusion from this experiment is that IMM-SHK animals were fearful after shock. This conclusion is supported by the finding that animals in the IMM shock condition exhibited significantly more freezing than animals in the NO-SHK group during both Day 1 and Day 2. Furthermore, IMM shocked animals froze equally as long as DEL shocked animals during the first min of the Day 2 session. Additional support for the fear evoking effects of immediate shock was evident in that they, along with the other shocked animals, exhibited a similar pattern of initial (20 s) post-shock activity. These findings clearly support the hypothesis that IMM shocked animals were fearful after shock, and oppose Fanselow's (1986, 1990) hypothesis that a rat's defense system is not evoked by an immediate shock.

In summary, the results of this experiment replicated the ISD and extended Fanselow's (1986) spatial contextual account of the effect. The results of this experiment support the hypothesis that all animals exposed to a foot-shock are fearful, regardless of the time of shock. In particular, it was revealed that IMM-SHK animals, like DEL-SHK animals exibited some freeze during the first 20 s after shock.

This experiment, however, did not allow the animal to engage in any defensive behaviour other than freezing. For example, had the animal been allowed to escape from the shock, it is possible that a different pattern of results would have been observed. Therefore, to further test the hypotheses that shocked animals are fearful after shock, the next experiment examined post-shock behaviour in an environment that the animal could escape from after shock.

Experiment 2

The foundation of the topographical theory of the ISD (Blanchard et al., 1976b) is that post-shock behaviour is organized into a hierarchy with flight as the dominant response, followed by freezing. As mentioned previously, flight occurs in response to a variety of fear provoking stimuli (Anisman, deCatanzaro, & Remington, 1978; Baron, 1963; Blanchard & Blanchard, 1968, 1989).

According to the topographical theory, immediately shocked animals would flee as the primary response to the shock. Further, delayed shocked animals having had time to discover that flight is not likely to be a successful response because there is no exit, freeze after shock (see also Blanchard & Blanchard, 1987). However, these hypotheses have not been adequately tested in studies of the ISD.

For example, in the Blanchard et al. (1976b) study, suppression of the rat's movement (presumably suppression of locomotion) was taken as an inferential measure of escape. Comparison of all groups in this experiment revealed that immediately shocked rats and no-shock control rats had similar mean movement times which were significantly higher than the delayed shock rats. The authors concluded that the reduced mean movement time of the delayed shock rats indicated that these rats learned that escape was impossible. However, this conclusion is conjectural because the opportunity to escape was not actually manipulated.

Fanselow (1986, Experiment 3) attempted to test the hypothesis that flight would prevail as the dominant post-shock response by administering shock in a box that had an opening blocked by steel wires. He found that the rats did not attempt to escape, nor did they spend more time near the blocked exit. However, it is possible that the animals perceived this pseudo-exit as just that, rather than as a genuine route for escape. Therefore, this study also failed to empirically manipulate the opportunity of a shocked rat to escape from the shock context.

Because escape necessarily involves a physical change in the animal's environment, there are two possible factors mediating exit from the box: fearfulness of the shock box, or exploration of the escape box. The motivation for fleeing from a shock box has generally been considered to be escape from fear rather than exploration of the safe box (Blanchard, Kelley, & Blanchard, 1974; Lorenzini, Bucherelli, Giachetti & Tassoni, 1990). In these experiments, shocked animals exhibited a reduced latency to exit from the shock box relative to no shock control animals suggesting that fear was the motivating factor underlying the early escape from the shock box. However, in those studies where more than one type of defensive behaviour was measured, escape from the stressful environment most often occurred as a secondary response to freezing (Blanchard et al., 1986; 1989; Moser & Tait, 1983). Therefore, in order to accurately determine the post-shock sequence of behaviours, it is essential to measure each of the potential defensive strategies.

The present experiment investigated the effects of presenting an unimpeded opening through which the animal was allowed to escape from the shock box. Delayed shock animals not allowed to escape were compared with delayed and immediately shocked animals allowed to escape from the shock box. As in Experiment 1, the mean percent time of freezing was the primary measure of fear in this experiment. The design of the apparatus in Experiment 2, however, allowed for latency to exit as an additional index of fear (Blanchard & Blanchard, 1971). These two measures combined for a more complete assessment of the fearfulness of both delayed and immediately shocked animals. In particular, each second of the first 20 s of the post-shock period was examined to delineate the specific pattern of defensive behaviour.

Escapable shock, relative to inescapable shock, has been observed to produce less fear conditioning of contextual cues (Mineka, Cook, & Miller, 1984; Williams, 1987; Williams & Lierle, 1986). Therefore, it was expected that less post-shock freezing would occur in groups allowed to escape compared to groups confined to the shock box. Similarly, reduced freezing in the escape groups, relative to the confined groups, was expected on Day 2.

Because it was expected that animals would escape from the shock chamber, the amount of post-shock time spent in this box was anticipated to be minimal. Although delayed shock animals would have spent 2 min in this chamber pre-shock, immediately shocked animals would be essentially unfamiliar with the box at the time of shock. Therefore, it was expected that the immediately shocked animal would spend less time in the shock chamber than its delayed shock counterpart and consequently its amount of exposure to the spatial context cues would be limited.

In summary, the objectives of this experiment were to determine if immediately and delayed shocked animals would escape from the shock box, and to study the behavioural sequence that occurred following shock. The duration of freezing and the latency to exit were variables measuring the animal's fearfulness.

Method

Subjects and Apparatus

Experiment 2 had 32 subjects that weighed similar (M = 272 g, SEM = 3.98) to those used in the first experiment.

A chamber consisting of two compartments was constructed. The shock compartment consisted of the same shock box as in Experiment 1 except that one metal side wall was removed. The safe compartment was the same as the mock home cage used in Experiment 1 except that one side wall was removed. The two compartments were joined by a plexiglas wall that had a centered 8 x 8 cm opening level with the floor of the shock box. A vertically sliding plexiglas door covered the opening. A portion of the sawdust from the home cage of the rat was scattered on the floor to facilitate familiarity of the safe compartment.

Procedure

Rats were handled for 5 days prior to experimentation, as in Experiment 1. On the first day of experimentation (Day 1), each rat was randomly assigned to one of four groups. The rats in the standard delayed shock group (DEL-CNTRL) were placed in the shock compartment through the ceiling. After 2 min they were given a .8 mA foot-shock for 1 s. The opening between compartments remained closed throughout the 5 min post-shock period. To provide rats with a fixed brief period of exposure to the safe box before entry into the shock box, rats in the delayed shock group (DEL-EXIT1) were initially placed in the safe compartment through the ceiling. After 10 s. the rat was manually pushed through the opening into the shock box and the plexiglas sheet lowered. The same shock was delivered 2 min post-entry into the shock compartment. Following shock termination the plexiglas sheet was raised, revealing the opening. Rats in the exit immediate group (IMM-EXIT) were treated the same as the DEL-EXIT1 group except that the shock was delivered immediately after entry into the shock compartment via the ceiling.

Baron (1963) has suggested that a familiar exit environment will suppress activity more than a novel exit environment. Therefore, to test the effect of an unfamiliar exit, rats in the DEL-EXIT2 group were treated the same as the DEL-CNTRL group except that the opening was revealed after shock.

The same observational procedure was used as in the first experiment. The latency of the animal to place its two front paws in the safe box compartment. from the onset of shock was also recorded as an additional measure of fear.

On Day 2, each rat was placed in the shock compartment. with the opening closed and observed for 5 min. without shock.

Results

Initial post-shock reactions

The proportion of shocked rats who exhibited a startle reaction during the 1 s of shock was 40 out of 4. Startle activity was followed by a brief period of locomotion (1-2 s) in 27 out of 40 rats (8 in the DEL-CNTRL group; 6 in the DEL-EXIT1 group; 7 in the DEL-EXIT2 group; 6 in the IMM-EXIT group). The remaining 13 rats were observed to freeze after startling. Within 3 s after shock. 100% of the shocked rats were observed to be freezing. This freezing lasted for a minimum of 1 s and a M±SEM of 6.2±.03 s for the DEL-CTRL group: 7.1±.04 s for the DEL-EXIT1 group: 2.4±.03 s for DEL-EXIT2 group and: 3.5±.05 s for the IMM-EXIT group. Freezing continued for the remaining 20 s period in 14 rats (7 in the DEL-CNTRL group: 4 in the DEL-EXIT1 group; 1 in the DEL-EXIT2 group; 2 in the IMM-EXIT group).

During this 20 s period 15 rats escaped (2 in the DEL-EXIT1 group; 7 in the DEL-EXIT2 group: 6 in the IMM-EXIT group). The remaining 11 rats (3 in the DEL-CNTRL group: 4 in the DEL-EXIT1 group; 2 in the DEL-EXIT 2 group: 2 in the IMM-EXIT group) were observed to either sniff or locomote following the initial freezing.

In summary, the typical behaviour sequence observed during shock and the 20 s following shock in the DEL-CNTRL group was an initial burst of activity, including startle (1 s). and locomote (1-2 s). This activity was promptly replaced by freezing (approximately 2 s). In 70% of the control animals. the freezing continued for the remaining duration of the 20 s period. In the remaining 30% of control animals. an alternating pattern of sniff, rear. and locomote followed the initial 2 s of freezing.

The typical behaviour sequence of the EXIT groups during this 20 s period was similar to the DEL-CNTRL group except that escape occurred in 50% of the animals. Those animals in the EXIT groups remaining in the shock box. including the majority of the DEL-EXIT1 animals. were observed to either freeze (23%) or engage in exploratory activity (27%) during this 20 s period.

Post-shock reactions throughout the trial

Escape. The mean latency for each group to escape from the shock box after shock is displayed in Figure 3. Oneway ANOVA followed by Bonferroni pair-wise comparisons, indicated that animals in the IMM-EXIT group had a longer latency to escape than the DEL-EXIT2 group and that both of these groups escaped faster than rats in the DEL-EXIT1 group [F(2,27) = 5.9: p<.008]. As discussed in the previous section. 50% of the animals exited the shock box within

Figure 3.

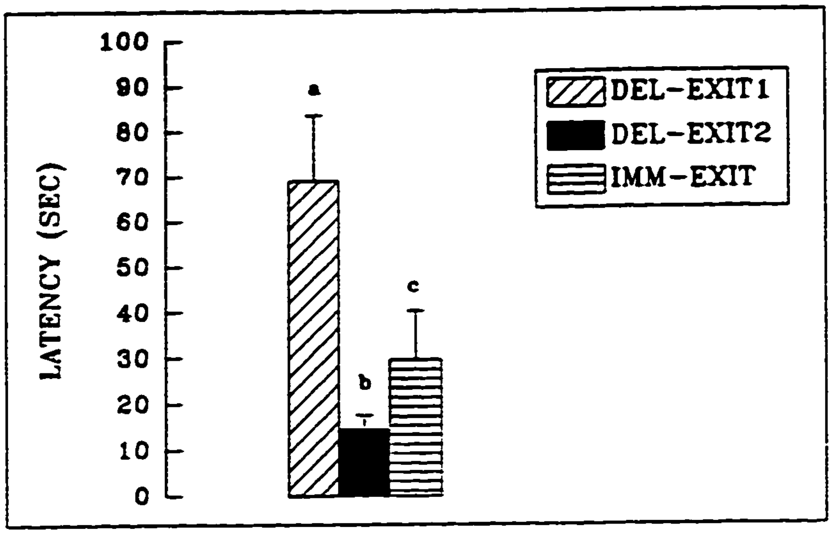

Figure 3. Mean (±SEM) latency in seconds for those rats that were allowed to exit, to place their forepaws into the exit chamber after a 1s, .8mA foot-shock in Experiment 2. DEL-EXIT 1 (diagonal-hatched bars) = Delay Exit-1 group (n = 10), rats were entered into the shock chamber via the safe compartment, shocked 2 min after placement, and allowed to exit. DEL-EXIT2 (solid bars) = Delay Exit-2 group (n = 10) rats were entered into the shock chamber via the roof of the shock chamber, shocked 2 min after placement, and allowed to exit. IMM-EXIT (horizontal bars) = Immediate Exit group (n = 10), rats were entered into the shock chamber via the safe compartment, shocked immediately after being entered, and allowed to exit.

a,b,c - bars with different letters are significantly different ($p < .05$)

20 s following shock termination. however, most animals in the DEL-EXIT1 group did not exit quickly (see Figure 3).

Because there was no relevant comparison to determine if rats in this experiment were escaping the shock box more quickly than unshocked rats. two additional groups were tested in another experiment. This second experiment was procedurally indentical. in all critical respects, to the main experiment. In this second experiment, the latency to exit of an unshocked group was compared with that of a standard delayed shock group (i.e.. rat entered through roof of shock box). Although statistical comparisons can not be made between the two experiments, it was revealed in the second experiment that the latency to exit of the delayed shock group (M = 15.6. SEM = 3.2) was significantly less [t(14) = 6.19. p<.0001] than the unshocked group (M = 38.6. SEM = 2.9). This latency to exit is consistent with that observed by Lorenzini. Bucherelli. Giachetti and Tassoni (1990) in similarly treated unshocked animals. Furthermore. the latency to exit of the unshocked group was longer than that of the immediately shocked group of the main experiment.

In summary, rats presented with an exit were observed to escape as part of the overall pattern of defensive behaviour. regardless of the time of shock. Escape behaviour was not observed as the first defensive response: rather escape occurred as a subsequent behaviour to the initial freezing that was observed following shock termination.

Freezing on Day 1. The left graph of Figure 4 depicts the mean post-shock percentage of time spent freezing in either cage by rats in each group on Day 1. A between-groups ANOVA confirmed that freezing was significantly different among the groups [F(3.36) = 18.0; p<.0001]. Bonferroni pair-wise comparisons revealed that the DEL-CNTRL group froze significantly more than the three groups allowed to exit (p<.001). That is. rats allowed to exit the shock chamber froze for shorter durations than rats not allowed to leave. No other

Figure 4.

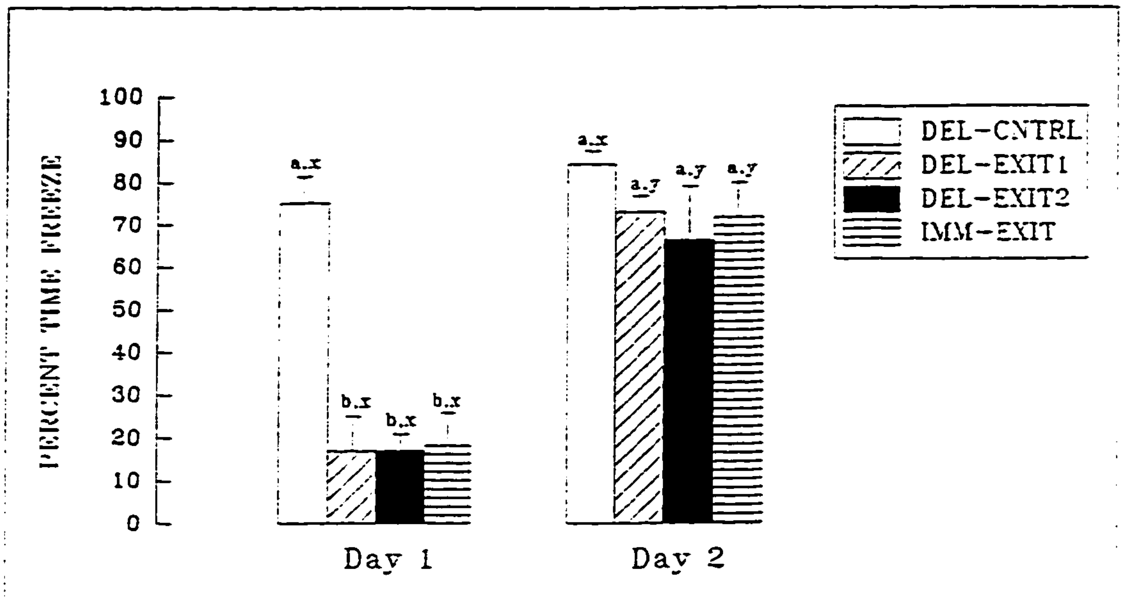

Figure 4. Mean (±SEM) percentage time spent freezing during the 5 min trial (**left:** Day 1 = post-shock: **right:** Day 2 = no-shock) by each of the four groups of rats given a 1s, .8mA foot-shock in Experiment 2. DEL-CNTRL (open bars) = Delay Control group (n = 10). rats were shocked 2 min after placement in the box on Day 1 and tested in the same box on Day 2; DEL-EXIT1 (diagonal-hatched bars) = Delay Exit-1 group (n = 10). rats entered into the shock chamber via the safe compartment, were shocked 2 min after placement, and allowed to exit. DEL-EXIT2 (solid bars) = Delay Exit-2 group (n = 10), rats entered into the shock chamber via the roof of the shock chamber, were shocked 2 min after placement, and allowed to exit. IMM-EXIT (horizontal bars) = Immediate Exit group (n = 10), rats entered into the shock chamber via the safe compartment, were shocked immediately after being entered, and allowed to exit.
a,b - bars with different letters are significantly different ($p < .05$) within Days
x,y - bars with different letters are significantly different ($p < .05$) between Days

significant between group differences in freezing were found.

It is important to note that there were no significant differences in freezing between the IMM-EXIT group and the two DEL-EXIT groups even though the IMM-EXT group spent little time in the shock chamber (see Figure 3). Consequently, animals in the IMM-EXIT condition froze mostly in the safe box and, therefore, after little contextual exposure to the shock box. Subsequent analysis of the location of freezing revealed that animals were observed to freeze in either box. Statistical analysis revealed no difference in post-shock freezing between the DEL-EXIT1 and DEL-EXIT2 groups on Day 1, so the data of these two groups were pooled for the bin analysis. Mean percentages of post-shock freezing according to bins on Day 1 are displayed in the left graph of Figure 5. While the group by bin interaction was not significant, a significant main effect of bins [F(2.6,108) = 3.71, p<.007) was found. As revealed by the subsequent Bonferroni tests, freezing was maintained at a constant level throughout the trial in the EXIT groups and only significantly increased in the DEL-CNTRL group during the second min.

In summary, rats allowed to exit spent significantly less time freezing than the control group not allowed to exit. Among the EXIT groups, the location of freezing was different. Rats in the DEL-EXIT2 and IMM-EXIT groups froze mostly in the safe chamber, whereas rats in the DEL-EXIT1 group froze mostly in the shock box.

Exploration on Day 1. The mean percent time (SEM) of the post-shock duration of each of the three exploratory behaviours on Day 1 is presented in Table 4.

Relative to the DEL-CNTRL group, the durations of sniff, rear, and locomote were significantly increased in the EXIT groups [Fs(3,36)>6.0; p<.001, see Table 4]. Rats allowed to exit showed a similar pattern of exploratory behaviour to the NO-SHK group of the previous experiment, except that locomotion was decreased in the EXIT groups (M = 15.3% vs. 5.92%)

Figure 5.

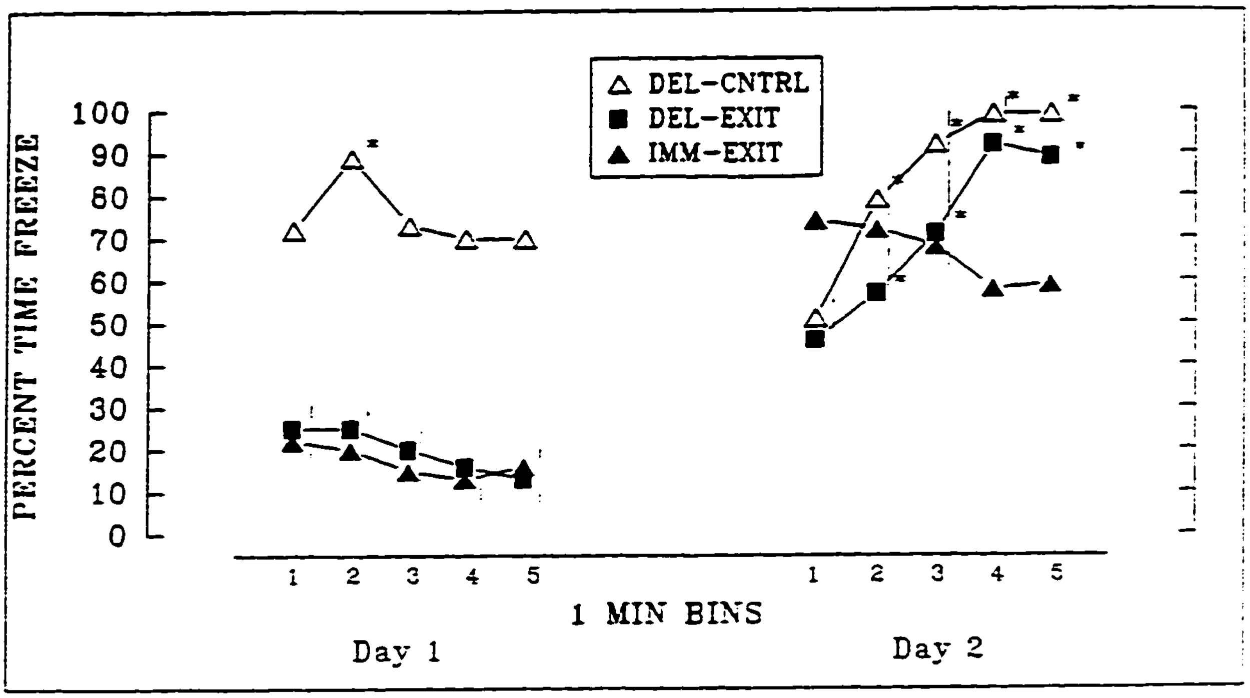

Figure 5. Mean percentage time spent freezing during each of the five, 1 min periods of the trial (**left:** Day 1 = post-shock; **right:** Day 2 = no-shock) by each of the three groups of rats given a 1s. .8mA foot-shock in Experiment 2. DEL-CNTRL (open triangles) = Delay Control group (n = 10), rats were shocked 2 min after placement in the box on Day 1 and tested in the same box on Day 2; DEL-EXIT (closed squares) = Delay Exit groups (n = 20), rats were shocked 2 min after placement in the box on Day 1 and allowed to exit, and tested in the same box on Day 2 with the exit closed; IMM-EXIT (closed triangle) = Immediate Exit group (n = 10), rats were shocked immediately after placement on Day 1 and allowed to exit, and tested in the same box on Day 2 with the exit closed.
Points marked with an adjacent asterisk are significantly different ($p < .05$) within groups from the mean of freezing during the first min.
Means not joined by the adjacent vertical line are significantly different ($p < .05$) between groups.

Table 4.

DAY	GROUP	SNIFF	REAR	LOCO
Day 1				
	DEL-CNTRL	20.4(5.5)**a**	2.32(1.1)**a**	1.31(.41)**a**
	DEL-EXIT1	38.5(7.4)**b**	22.6(4.2)**b**	7.71(1.7)**b**
	DEL-EXIT2	53.4(4.5)**c**	12.6(2.0)**c**	4.01(.63)**c**
	IMM-EXIT	55.4(5.5)**c**	27.4(6.4)**b**	5.92(1.1)**c**
Day 2				
	DEL-CNTRL	2.92(2.1)**a**	10.7(3.0)**a**	3.02(1.1)**a**
	DEL-EXIT1	3.01(.87)**a**	41.0(6.2)**b**	10.1(1.7)**a**
	DEL-EXIT2	3.22(.55)**a**	24.0(4.7)**b**	8.81(1.2)**a**
	IMM-EXIT	21.2(8.9)**b**	9.23(3.7)**a**	1.74(.81)**a**

Table 4. Mean (±SEM) percentage time spent sniffing, rearing, and locomoting during the 5 min trial (Day 1 = post-shock: Day 2 = no-shock) by each of the four groups of rats given a 1s. .8mA foot-shock in Experiment 2.
a,b,c - values with different letters are significantly different ($p < .05$) within Days.

Summary of Behaviours on Day 1

In general, rats in this experiment were observed to exhibit a similar pattern of initial post-shock responses as rats in Experiment 1. That is, all rats startled in response to the shock and subsequently froze within a few seconds after shock termination. All rats in the EXIT groups escaped from the shock chamber: 50% of rats escaped within a few seconds after the initial freezing. Therefore, the typical pattern of behaviour was startle, freeze, and escape.

Freezing was decreased in the EXIT groups compared to the control group although the location of freezing varied in the EXIT groups. Rats in the DEL-EXIT1 group froze more in the shock box than the safe box: this group also had the longest latency to escape. The DEL-EXIT2 and IMM-EXIT groups froze more in the safe box than in the shock box.

Rats in the EXIT groups also engaged in more exploratory behavior than rats in the DEL-CNTRL group.

Behaviour on Day 2

Freezing on Day 2. Group means of freezing on Day 2 are depicted in the right panel of Figure 4. The one-way between groups ANOVA did not reveal a statistically significant difference in freezing among the groups. However, a one-way between sessions ANOVA followed by pair-wise Bonferroni tests, did reveal that freezing was significantly longer on Day 2 than Day 1 for the exit groups [$F(1,59) = 37.0$; $p<.0001$).

The very high level of freezing observed in the exit groups may be attributable to a procedural detail, namely that these animals, like the DEL-CNTRL group, were not allowed to leave the shock chamber on Day 2. Furthermore, the IMM-EXIT group froze about two times more on Day 2 than the corresponding IMM-CNTRL group of the previous experiment. Thus, it appears that simply restricting the animal to the shock chamber on Day 2 is sufficient to increase freezing on this day compared to Day 1.

The right graph of Figure 5 displays the mean percentage of freezing across bins observed for each group on Day 2. The statistically similar data of groups DEL-EXIT1 and DEL-EXIT2 were combined. Repeated measures ANOVA revealed significant effects of bin [F(2.5.108) = 12.82. p<.0001) and group x bin interaction [F(5.2.108) = 12.82, p<.0001). As revealed by the Bonferroni tests, the significant interaction effect was caused by the overall increase in freezing across the trial in the DEL groups and a maintenance of freezing in the IMM group. Increased freezing in the DEL-CNTRL group was observed between the first and second min of the trial and thereafter maintained. These results are consistent with the results of the DEL-CNTRL group tested in Experiment 1. The IMM-EXIT group also exhibited substantial freezing in the first min of the trial on Day 2 similar to that observed in the IMM-CNTRL group of Experiment 1. Unlike the IMM-CNTRL group. however. rats in the IMM-EXIT group did not decrease freezing after the first min on Day 2.

Exploration on Day 2. The mean percent time (SEM) of each of the exploratory behaviours on Day 2 is presented in Table 4. Between groups ANOVA on each of these behaviours revealed a significant difference among groups only in duration of rearing [F(2.27) = 4.29; p<.05]. As indicated by the Bonferroni tests, the DEL-EXIT groups spent significantly less time rearing than either the DEL-CNTRL group or the IMM-EXIT group.

Discussion

The duration of freezing. as well as the durations of the exploratory behaviours, observed in the IMM-EXIT group were similar to those observed in the IMM-CNTRL group of Experiment 1. Therefore. the results reported for the two immediately shocked groups in the two experiments are similar.

All rats in this study exited from the shock box after shock. Therefore. the results of this experiment clearly support the findings of earlier studies that escape is a primary defensive response to an aversive stimulus (Baron. 1963; Blanchard & Blanchard. 1968. 1987).

One of the primary objectives of this study. however. was to examine the potential difference in the latency to escape between immediately shocked and delayed shock animals. Escape was observed in this experiment as one of the primary defensive responses to shock in both immediately shocked. and delayed shock animals. Therefore. regardless of the time of shock delivery. all animals fled from the shock box to the safe box.

The latency to exit was taken as a measure of the animal's fearfulness. Therefore. the shorter latency to exit by the IMM-EXIT group. relative to the unshocked group (of the second exit experiment). supports the hypothesis that immediately shocked animals were fearful after shock. This result further supports the results of Experiment 1 that immediately shocked animals were fearful after shock. Moreover. because animals not shocked remained in the shock box longer than shocked animals. it was concluded that all shocked animals were indeed fearful of the shock box. and that the motivation for exiting the shock was not simply exploratory. However. the difference between the DEL-EXIT1 and DEL-EXIT2 groups in latency to exit. suggests that forcing the DEL-EXIT1 animal through the opening may have been an aversive experience causing the rat to be reluctant to re-enter the safe box.

Overall freezing on Day 1 was reduced in the delayed shock animals allowed to exit compared to the animals confined to the shock box. This result is consistent with the finding that reduced freezing is a typical consequence of escapable shock compared to inescapable shock (Mineka. Cook. & Miller. 1984; Williams. 1987; Williams & Lierle. 1986). An increase in overall exploration was observed in the EXIT groups relative to the control group. Therefore. these findings support the hypothesis that post-shock fear can be reduced by allowing the delayed shock animal to exit. relative to confining it in the shock environment. However. the similar durations of freezing among all the groups allowed to exit indicated that freezing occurs as a typical defensive behaviour. regardless of the time of shock. This finding further supports the conclusion of Experiment 1 that all animals were fearful after shock.

In summary, delayed shock rats, if allowed, will exit the box in which they were shocked, and freeze less than delayed shock animals not allowed to exit. Moreover, these rats will escape more quickly if they do not have prior experience with the escape route, than if they have been exposed to the route before the shock. Similarly, the immediately shocked rats exited the shock box quicker than unshocked rats. These findings support the hypothesis that both delayed and immediately shocked rats were fearful after shock. In addition, these results provide evidence that delayed shock rats allowed to escape from the shock box, will escape, and will subsequently freeze less than delayed shock rats not allowed to escape.

The post-shock sequence of defensive behaviours observed here was similar to the pattern of post-stress activity observed in other studies (e.g., Blanchard & Blanchard, 1989; Moser & Tait, 1983). In the present experiment, the sequence of post-shock behaviour typically involved a period of brief freezing that was quickly followed by escape and subsequent freezing in the safe box. Because the safe box was constructed to be dissimilar from the shock box, the additional freezing in the safe box is evidence against the hypothesis that post-shock freezing is in response to the contextual spatial cues associated with shock (Fanselow, 1986). Rather, the present results suggest that freezing can occur in the absence of spatial cues directly related to shock. It may be that freezing in the safe box serves as a strategy for assessing the potential safety of a new environment similar to the gathering of information through stretched approach behaviours observed following prod-shock (Blanchard & Blanchard, 1988; Pinel, Hoyer, & Terlecki, 1980; Pinel et al., 1989).

It was predicted that immediately shocked rats would spend relatively little time in the shock box on Day 1 because they were expected to escape to the safe box after shock. This was found to be the case, as discussed above. Thus, rats in the IMM-EXIT group spent less time in the shock chamber post-shock than rats in a standard immediate condition (mean of 30 s vs. 300 s, respectively).

Hence, animals in the IMM-EXIT group had very limited exposure to spatial contextual conditioning effects. However, rats in the IMM-EXIT group froze considerably more on Day 2 than on Day 1. It is difficult to see how this finding. can be explained as a spatial conditioning effect. The present data suggest that considerable freezing may be observed in shocked rats in a shock chamber with minimal prior exposure to that environment.

Unlike the IMM-CNTRL group of Experiment 1, rats in the IMM-EXIT group maintained freezing at a consistently high level throughout the trial on Day 2. Therefore, preventing the animal from escaping on Day 2 had the effect of maintaining freezing for a longer period than would be expected if no escape had been offered on Day 1. This finding provides evidence that confining an animal to a previously escapable situation in the absence of shock is sufficient to increase fear within that environment.

In summary, the results of this experiment suggest that post-shock freezing can be reduced by allowing an animal to escape the shock chamber. Escape from the shock box did not, however, entirely prevent the animal from freezing. The fact that the majority of rats froze in both the shock box and the safe box suggests that the effect of conditioning of cues directly related to the shock environment is insufficient to explain freezing.

In this experiment it was possible to decrease freezing by changing the stimulus features of the box to include an exit. In the next experiment, the hypothesis that post-shock freezing can be decreased by providing a salient cue (i.e., handling) before shock was tested.

Experiment 3

One of the necessary aspects of shocking an animal immediately after placement into the apparatus is that handling directly precedes the shock. By contrast, in the typical delayed shock condition, animals are not typically handled during the period of 2 min or so between placement into the box and shock delivery. Therefore, handling in the delayed shock condition is not contiguous with shock delivery, as it is in the immediately shocked condition.

Only one published experiment attempted to equate handling between delayed and immediately shocked animals during the first post-shock session. Blanchard et al. (1976b) found that delayed shock animals handled briefly before the shock moved significantly less, and presumably froze more, than animals immediately shocked. Unfortunately, the specific effects of handling can not be determined from this study because neither an unhandled delayed shock group nor a twice-handled immediately shocked group was included. Furthermore, freezing was not directly measured, but was inferred from decreased movement times. Therefore, the effects of handling on post-shock freezing (i.e., Day 1) have not been adequately tested in the ISD paradigm.

One study bears directly on the effects of handling during Day 2 testing. Landeira-Fernandez et al. (1995) compared the freezing on Day 2 of rats handled and not handled before receiving a delayed shock on Day 1. To equate for the extra handling of some of the delayed shock rats, immediately shocked animals were handled twice before shock. Freezing was not measured until 24 hr after shock, so the effects of handling specifically related to contextual conditioning were observed. The immediately shocked rats froze less on Day 2 than the delayed shocked rats. However, the extra handling of the delayed shock rats on Day 1 reduced the median percent freezing on Day 2 in the same box by 50 percent as compared to rats not handled twice. That is, handling the rat just before the shock substantially reduced the contextual conditioning apparent on Day 2, relative to not handling the rat. Handling was insufficient to reduce the freezing to the level of the immediately shocked groups suggesting that some contextual conditioning occurred in the delayed shock group that was handled twice.

Freezing, however, was measured in the Landeira-Fernandez et al. (1995) study by averaging duration of freezing over the 4 min trial rather than by analyzing min by min. The bin results of Experiments 1 and 2 of the present study showed that freezing decreased over time within a session in both delayed and immediately shocked rats. Moreover, the design of the Landeira-Fernandez et al. (1995) study did not include observation of freezing on Day 1. If handling functions as an explicit cue of shock, then the effects of handling would be most evident during the first min after shock on Day 1. Therefore, the effects of the handling in the Landeira-Fernandez et al. (1995) study, may have been obscured by averaging freezing across the trial and by only measuring freezing on Day 2.

Handling has been observed to facilitate food reinforced discrimination as well as avoidance learning. For example, brief handling just before an event presented to an animal during testing in a discrimination paradigm was found to serve as a cue for the subsequent identification of an event (Thomas & Lieberman, 1990; Thomas, Lieberman, McIntosh, & Ronaldson, 1983; Urcuioli & Kasprow, 1988). That is, a rat's identification of a particular event may be based on the occurrence of a salient stimulus either directly preceding the event, or after a brief delay (<5 s). Thus, handling an animal just before shock, as occurs in the ISD paradigm, may serve as a cue for the occurrence of shock that obscures the effects of contextual conditioning.

Handling has also been observed to facilitate avoidance learning. For example, Wahlsten and colleagues (Wahlsten, Cole, Sharp, & Fantino, 1968; Wahlsten & Sharp, 1969) observed that handling rats either prior to a test session or between test sessions reduced subsequent freezing and facilitated avoidance learning. Bar-press avoidance was similarly improved by handling the animal just before the start of the trial relative to that of the unhandled animal which also resulted in less freezing (Wahlsten et al., 1968). Therefore, the procedural difference of handling immediately shocked rats just before shock may serve to reduce the associative strength of the context in this group. Handling would be less likely to serve as an explicit cue signalling shock in the delayed shock condition because the period between the handling and the shock is too long for the animal to associate the two events. Because the effect of handling would be closely associated with the shock onset, the min by min analysis of freezing is crucial to demonstrating this effect. The two handling studies reviewed above, however, averaged freezing across the entire duration of the post-shock period rather than assessing freezing across several intervals.

Experiment 3 had three objectives. The primary objective of this experiment was to determine the effects of handling on freezing during Day 1. The duration of freezing of delayed shock animals briefly removed from and replaced into the shock box just before shock was compared with the duration of freezing of control animals not handled before shock. To control for the extra handling rats of the delayed-handle group received, immediately shocked animals were picked up and replaced into their home cages 2 min before being placed in the shock box and the shock administered. It was expected that less freezing would occur in the delayed-handle group relative to the delayed-control group due to the reduced associative strength of the context. A second, related objective of this experiment was to examine the sequence of behaviours during the initial 20 s post-shock. It was hypothesized that the effects of the handling would be apparent during the initial 20 s post-shock period. The final objective of this experiment was to examine the effects of handling on Day 2. If handling disrupted contextual conditioning, then reduced freezing in the delayed-handle group would be expected on Day 2.

Method

Subjects and Apparatus

The 30 subjects weighed similar (M = 267 g, SEM = 3.96) to the rats of the previous experiments. All other details were the same as in Experiment 1.

Procedure

The rats were randomly assigned to one of three groups after being handled once per day for five days. Rats in the delayed control group (DEL-CNTRL) were brought to the testing room in their home cages and placed individually in the shock chamber. After 2 min each rat received a .8 mA foot-shock for 1 s. Rats in the delayed handle group (DEL-HNDL) were treated similarly to rats in the DEL-CNTRL group except that they were picked up from the shock box and returned to it prior to being shocked. Subsequent to being brought to the testing room via their home cage, rats in the immediately handled group (IMM-HNDL) were picked up and returned to the home cage. Two min following this handling, each of these rats was placed in the shock box and given the same shock as the delayed shock animals, as soon as the roof was closed. The extra handling of the IMM-HNDL group was done to equate the number of handling occurrences to the DEL-HNDL group. The same observation procedure was used as in the first experiment. On Day 2, all rats were tested in the shock compartment for 5 min, without shock.

Results

Initial post-shock reactions

All 30 of the shocked rats exhibited a startle reaction during the 1 s of shock. Startle activity was followed by a brief period of locomotion (1-2 s) in 22 out of 30 rats (6 out of 10 rats in the DEL-CNTRL group; 8 out of 10 rats in the DEL-HDNL group; 8 out of 10 rats in the IMM-HNDL group). The remaining 8 rats were observed to freeze after startling. Within 3 s after shock, 100% of the rats were observed to be freezing. This freezing lasted for a minimum of 1 s and for a M±SEM of 5.37±.04 s for the DEL-CNTRL group; 3.12±.03 s for the DEL-HNDL group; and 2.56±.04 for the IMM-HNDL group. Freezing either continued for the remaining 20 s period (6 out of 10 rats in the DEL-CNTRL group; 3 out of 10 rats in the DEL-HNDL group; 1 out of 10 rats in the IMM-

HNDL group) or was replaced by sniffing in 7 out of the 11 rats not freezing in the DEL groups; and in 6 out of the 10 rats not freezing in the IMM-HNDL group. The remaining rats (4 in the DEL groups: 4 in the IMM-HNDL group) were observed to locomote following the initial freezing. A one-way ANOVA. followed by Bonferroni tests on the measure of freezing. revealed that rats in the DEL-CNTRL group spent significantly more time freezing than rats in either of the DEL-HNDL or IMM-HNDL groups which did not significantly differ from each other. Once a post-shock exploratory behaviour was observed. freezing did not typically re-occur during the initial 20 s period (4 out of 4 rats in the DEL-CNTRL group: 7 out of 7 rats in the DEL-HNDL group: 8 out of 9 rats in the IMM-HNDL group).

In summary. the typical behaviour sequence observed during shock and the 20 s following shock in the IMM-HNDL group was an initial burst of activity. including startle (1 s). and locomote (1-2 s). This activity was promptly replaced by freezing (approximately 2 s). An alternating pattern of sniff. rear. and locomote ensued following the freezing. The DEL-HNDL group exhibited a similar pattern of behaviour as the IMM-HNDL group during this 20 s period. The behaviour sequence of the DEL-CNTRL group during this period was similar to the other groups except that freezing lasted longer in this group (60% of these animals froze for the entire 20 s period) than in the other two groups (30% of the DEL-HNDL and 10% of the IMM-HNDL groups).

Post-shock reactions throughout the trial

Freezing on Day 1. Mean percent time of overall post-shock freezing by rats in each of the groups on Day 1 is shown in the left graph of Figure 6. Oneway ANOVA revealed an overall difference in freezing among groups [$F(2,27) = 18.52$; $p<.0001$]. Pair-wise comparisons showed that rats in the DEL-HNDL group froze significantly less than the DEL-CNTRL group but significantly more than the IMM-HNDL group.

The mean percentages of freezing on Day 1 for each 1 min bin are shown

Figure 6.

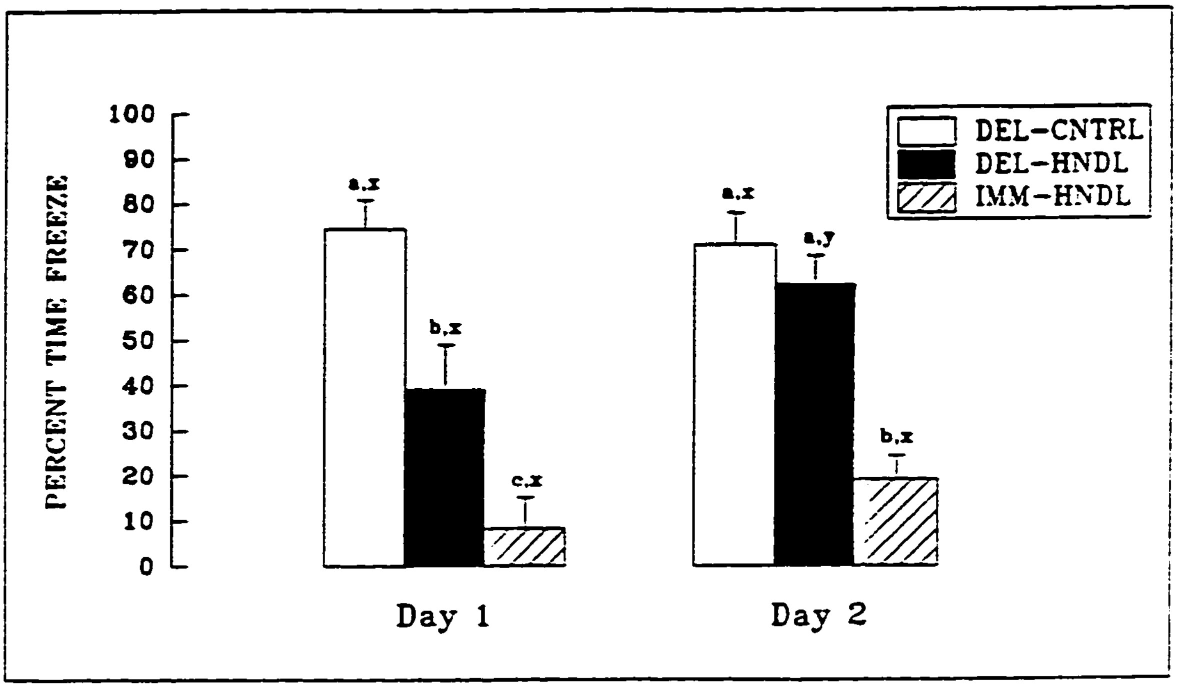

Figure 6. Mean (±SEM) percentage time spent freezing during the 5 min trial (**left:** Day 1 = post-shock: **right:** Day 2 = no-shock) by each of the three groups of rats given a 1s, .8mA foot-shock in Experiment 3. DEL-CNTRL (open bars) = Delay Control group (n = 10). rats were shocked 2 min after placement in the box on Day 1 and tested in the same box on Day 2; DEL-HNDL (solid bars) = Delay Handle group (n = 10), rats handled after 2 min in the shock box, replaced, and were shocked on Day 1 and tested in a different box on Day 2; IMM-HNDL (diagonal-hatched bars) = Immediate Handle group (n = 10), rats handled 2 min before being placed in the shock chamber, and were shocked immediately after placement on Day 1 and tested in the same box on Day 2.
a,b,c - bars with different letters are significantly different ($p < .05$) within Days
x,y - bars with different letters are significantly different ($p < .05$) between Days

in the left panel of Figure 7. These data were analyzed in a 3 (between groups) x 5 bins (repeated measures) ANOVA. Although the main effect of bins was not significant [F(4,108) = 2.36; p>.05] the group x bin interaction was significant [F(4,108) = 2.86; p<.001]. As revealed by the Bonferroni tests, the interaction effect was caused by the increased freezing observed in the DEL groups from min 1 to min 2 and the corresponding decrease in freezing in the IMM group.

One-way ANOVA revealed that freezing within the first min was significantly different among the groups [F(2,27) = 26.82; p<.0001]. The Bonferroni tests showed that all three groups were significantly different from each other during this 1 min period. Freezing in the DEL groups occurred throughout the trial; whereas, freezing in the IMM group occurred during only the first min.

Exploration on Day 1. Analysis of the exploratory behaviours on Day 1 revealed that the IMM-HNDL group engaged in significantly more active behaviour than either DEL group [Fs(2,27)>8.9; p<.001, see Table 5]. The reduced freezing in the DEL-HNDL group relative to the DEL-CNTRL group was compensated for by an increase only in sniffing.

Summary of Behaviour on Day 1

In summary, similar reactions to the shock onset and in the 20 s following shock termination were observed in all groups on Day 1. This pattern of initial activity included startle, locomote, freeze, and explore. Freezing during this 20 s period was significantly different among the three groups. Rats in the DEL-HNDL group froze significantly less than rats in the DEL-CNTRL group but significantly more than rats in the IMM-HNDL group. These group differences were maintained throughout the first min of the trial.

Freezing in the IMM-HNDL group decreased after the first min of the trial whereas freezing in the DEL groups increased from the first to the second min post-shock. After the second min, freezing in the DEL groups was maintained at a level not significantly different than that observed during the first min in these

Figure 7.

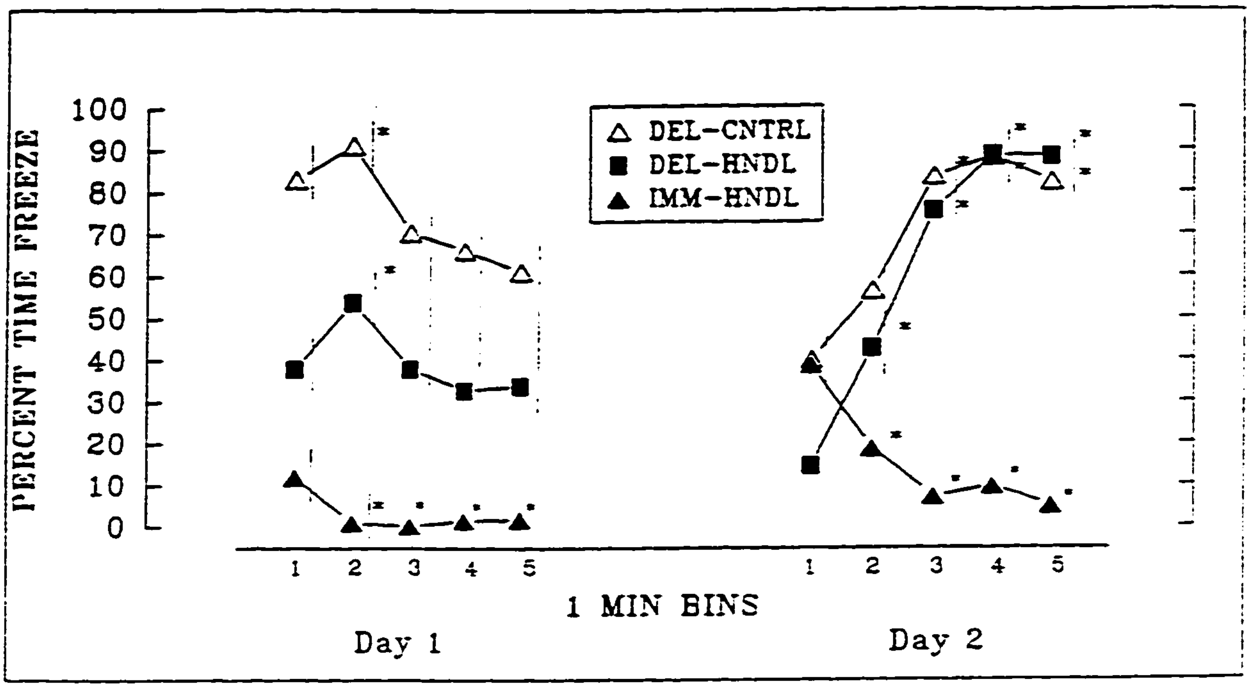

Figure 7. Mean percentage time spent freezing during each of the five, 1 min periods of the trial (**left:** Day 1 = post-shock; **right:** Day 2 = no-shock) by each of the three groups of rats given a 1s, .8mA foot-shock in Experiment 3. DEL-CNTRL (open triangles) = Delay Control group (n = 10), rats were shocked 2 min after placement in the box on Day 1 and tested in the same box on Day 2; DEL-HNDL (closed squares) = Delay Handle group (n = 10), rats handled after 2 min in the shock box, replaced, and were shocked on Day 1 and tested in the same box on Day 2; IMM-HNDL (closed triangle) = Immediate Handle group (n = 10), rats handled 2 min before being placed in the shock box, and were shocked immediately after placement on Day 1, and tested in the same box on Day 2.

Points marked with an adjacent asterisk are significantly different ($p < .05$) within groups from the mean of freezing during the first min.

Means not joined by the adjacent vertical line are significantly different ($p < .05$) between groups.

Table 5.

DAY	GROUP	SNIFF	REAR	LOCO
Day 1				
	DEL-CNTRL	19.6(4.8)**a**	3.72(3.0)**a**	1.00(.42)**a**
	DEL-HNDL	35.6(6.5)**b**	7.51(2.0)**a**	4.00(1.5)**a**
	IMM-HNDL	53.4(4.5)**c**	23.8(2.9)**b**	7.85(.73)**b**
Day 2				
	DEL-CNTRL	13.7(6.8)**a**	13.8(1.0)**a**	3.42(.43)**a**
	DEL-HNDL	8.72(3.9)**a**	12.7(2.9)**a**	3.21(.28)**a**
	IMM-HNDL	51.0(3.6)**b**	29.5(3.7)**b**	6.35(.68)**b**

Table 5. Mean (±SEM) percentage time spent sniffing, rearing, and locomoting during the 5 min trial (Day 1 = post-shock; Day 2 = no-shock) by each of the three groups of rats given a 1s, .8mA foot-shock in Experiment 3.
a,b,c - values with different letters are significantly different ($p < .05$) within Days.

groups.

Analysis of the behavioural topography of the DEL-HNDL group relative to the DEL-CNTRL group on Day 1 demonstrated that sniffing was the only behaviour which compensated for the decreased freezing observed in this group. All three exploratory behaviours were increased in the IMM-HNDL group relative to the DEL shocked groups. Therefore, delayed shock relative to immediate shock had the effect of decreasing all exploratory behaviours irrespective of the handling procedure.

Behaviour on Day 2

Freezing on Day 2. The proportion of time spent freezing on Day 2 by each of the three groups is displayed in the right graph of Figure 6. Within-sessions ANOVA of groups followed by Bonferroni pair-wise comparisons, confirmed that rats in the DEL groups froze longer than rats in the IMM group on Day 2 [$F(2,27) = 27.25$; $p<.0001$]. The two delay groups did not differ in the mean percent time freeze on Day 2.

A between sessions ANOVA, followed by Bonferroni tests, revealed that the DEL-HNDL group spent significantly more time freezing on Day 2 than on Day 1 [$F(1,78) = 4.59$; $p<.05$]. No other group differences in freezing between days were observed.

The percent time of freezing across bins on Day 2 is shown in Figure 7, right graph. Repeated measures ANOVA confirmed a nonsignificant main effect of bins [$F(4,108) = 1.92$; $p>.10$]. However, a significant group x bin interaction effect was found [$F(4,108) = 3.80$; $p<.001$]. As indicated by the Bonferroni tests, the interaction effect was caused, in part, by the difference in freezing between the DEL-CNTRL and DEL-HNDL groups during the first min of the trial and the similarity in freezing between these groups thereafter. Both DEL-CNTRL and DEL-HNDL groups increased freezing from the beginning of the trial to about the third min and maintained a constant level of freezing thereafter. Conversely, rats in the IMM-HNDL group showed a sharp decline in freezing after the first min in the box.

Exploration on Day 2. The mean percent time (SEM) of each of the exploratory behaviours on Day 2 is presented in Table 5.

No significant differences in exploratory behaviour were observed between the DEL groups. The IMM group, however, engaged in significantly more of each of the exploratory behaviours than the DEL groups.

Summary of Behaviours on Day 2

In summary, the overall duration of freezing on Day 2 was significantly different between the DEL groups and the IMM group. The DEL-HNDL group did not significantly differ from the DEL-CNTRL in percent time freezing across the 5 min trial of Day 2. However, percent time freezing by the DEL-HNDL group was significantly decreased relative to the DEL-CNTRL and IMM-HNDL groups during the first min of the trial. Freezing in both DEL groups increased across the trial but decreased in the IMM group after the first minute. Relative to the IMM group, the DEL groups spent significantly less time engaged in all three exploratory behaviours during the 5 min trial on Day 2. Therefore, delayed shock relative to immediate shock on Day 1, had the effect of decreasing exploration on Day 2.

Discussion

It is important to note that the duration of freezing observed in the IMM-HNDL group was similar to that observed in the IMM-CNTRL group of Experiment 1. Furthermore, the amount of freezing observed in the DEL-CNTRL group of this experiment was comparable to that observed in the DEL-CNTRL groups of the previous two experiments. The mean duration of freezing (approximately 75%) in the DEL-CNTRL group is consistent with recent reports in the ISD literature (e.g., Kiernan et al., 1995; Landeira-Fernandez et al., 1995; Westbrook et al., 1994). Therefore, the results reported in this study are consistent with the durations of freezing described in the previous two experiments, as well as with the ISD literature.

The present results provided evidence that handling the rat just before a delayed shock decreased freezing on Day 1 relative to not handling the rat before shock. This finding has not been reported in the ISD literature.

The bin analysis of Day 1 freezing revealed that the two DEL groups were significantly different only in the first min of the session. Furthermore, analysis of the behaviours during the first 20 s revealed that the DEL-HNDL and the IMM-HNDL groups froze significantly less than the DEL-CNTRL group during this period. Thus, handling caused only a transient decrease in freezing during the initial 1 min of the trial on Day 1. The sessional time course of freezing in the DEL-CNTRL and DEL-HNDL groups was essentially identical after this first min. These findings are consistent with the hypothesis that handling serves as an explicit cue associated with shock that reduces the associative strength of the context.

It should be noted that the nature of the disruption that decreased freezing on Day 1 was simply a brief removal of the animal followed by replacement into the shock box. The extra handling of the DEL-HNDL group, however, was insufficient to reduce freezing to that of the IMM-HNDL group suggesting that some contextual associative conditioning occurred in the DEL-HNDL group.

The reduced freezing observed on Day 1 in the DEL-HNDL group was compensated for by an increase only in sniffing. Because sniffing does not involve the use of the animal's feet, as do rear and locomote, it can be assumed that the shock suppressed activity that required the animal to use its feet.

In summary, brief removal of rats in the DEL-HNDL group caused a decrease in freezing relative to DEL-CNTRL rats that was evident during only the first min of the trial on Day 1. This finding supports the hypothesis that handling was able to reduce the duration of freezing although the effect did not continue throughout the trial.

The associative conditioning that occurred on Day 1 was evident on Day 2 in that the DEL-HNDL group did not significantly differ from the DEL-CNTRL group in the overall duration of freezing. However, as on Day 1, handling did cause a decrease in freezing during the first min of the trial on Day 2. Impressively, the effect of the handling was of sufficient magnitude to reduce freezing below that of the IMM-CNTRL group during this period. Because the reduced freezing was observed at the beginning of the trial (i.e., first min after shock), this finding is consistent with the hypothesis that handling served as a cue signalling the occurrence of shock (Thomas et al., 1983; Thomas & Lieberman, 1990; Urcuioli & Kasprow, 1988). That is, the handle served as a temporally explicit cue that reduced the associative strength of the spatial contextual cues present at the time of shock delivery.

Although the difference in freezing between the DEL-CNTRL and DEL-HNDL groups on Day 2 in the present experiment was not statistically significant, the effect is in the same direction as that reported by Landeira-Fernandez et al. (1995). That is, in both experiments, handling the animal before a delayed shock on Day 1 had the effect of reducing freezing on Day 2, relative to the control animals which were not handled. However, the Landeira-Fernandez et al. (1995) results should be considered with caution because the level of freezing reported in the control group in their experiment was several fold greater than previously reported results from the same laboratory (Fanselow, 1986; 1990) and other laboratories (Kiernan & Westbrook, 1993;Kiernan et al., 1995). The results of the Landeira-Fernandez et al. (1995) study warrant replication because the effect would have been similar to that reported here, if they had obtained more typical durations of freeze in the control animals.

In summary, handling the animal just before delayed shock decreased overall freezing on both Day 1 and Day 2 testing, relative to control. This decrease in overall freezing was significant only on Day 1; however, the time bin analysis revealed that the DEL-HNDL group froze significantly less than the DEL-CNTRL and IMM-CNTRL groups during the first 2 min on Day 2. These findings support the hypothesis that handling serves as an explicit cue for the occurrence of shock and that the associative strength of the context is reduced by the extra handling.

Experiment 4

Post-hoc analysis of within group variation

Multiple Regression

Throughout the previous experiments, it became apparent that there were large within-group individual variations in the duration of post-shock freezing. Several investigators have noted that there is considerable genetic variability among inbred and outbred strains of rats (File & Vellucci, 1979; Harrington, 1981; Maier, Vandenhoff, & Crowne, 1988). In order to identify potential sources of individual variability which may influence within group durations of freezing, the Day 1 videotaped performances of the delayed shock control rats of the three previous experiments (n=30) were examined. It was discovered that there were consistent differences among the rats which froze for long periods and those rats which scarcely froze at all. A post-hoc investigation revealed that several variables differentiated high- from low-duration freezing rats. These variables and their relationship to post-shock freezing are described below.

Number of bars. A *numerical count* of the number of the grid-floor bars between the front and rear paws, at the time of shock. The number of bars between the closest front and back paws was counted. Because the range of values was large (1-9), the number of bars were grouped as follows: 1-3 bars (coded as 1); 4-6 bars (coded as 2); 7-9 bars (coded as 3). The number of bars was positively correlated with the percent time spent freezing (Pearson r=.49; p<.001). That is, the more bars spanned by the rat's feet, the greater the duration of post-shock freezing.

Body position on the bars. Body position was defined as the orientation of the body of the rat to the bars of the grid floor, at the time of shock. Position was rated as *parallel* (coded as 1); *diagonal* (coded as 2); or *perpendicular* (coded as 3). The coding of the body position on the bars was positively correlated with the percent time spent freezing (Pearson r=.35; p<.01). That is, the more perpendicular the rat's body was to the orientation of the bars, the more the rat froze in the post-shock period.

Behaviour. A rating of the rat's behaviour immediately preceding the shock was determined as follows: *still* (defined as inactivity; coded as 1); *rear* (defined as the two front paws off the floor; coded as 2); *sniff down* - without locomoting (defined as active sniffing anywhere in the box but with all 4 paws stationary on the floor; coded as 3); or *locomote* (defined as the traversing of at least 1-bar, with or without sniffing; coded as 4). The coding of the behaviour was positively correlated with freezing (Pearson r=.60; p<.001). That is, the more likely it was that the rat was locomoting at the time of shock, the greater the duration of post-shock freezing.

Startle. A subjective ranking of the magnitude of the activity burst during the shock as determined as follows: *weak* (coded as 1); *average* (coded as 2); or *strong* (coded as 3). The rating of the startle was positively correlated with freezing (Pearson r=.60; p<.001). That is, the greater the startle magnitude in response to the shock, the more the rat froze during the post-shock period.

Independent observer agreement with these definitions was in the 90-100% range.

A step-wise linear regression analysis of these 4 independent variables and the dependent variable of post-shock freeze, revealed that the predictor variables of number of bars, startle and behaviour, significantly contributed to the variance associated with freeze (see below). Body position on the bars, although significantly correlated with freezing (r=.35), did not contribute to the overall variance of freeze (R^2=.11; p>.05).

The two strongest predictors of post-shock freezing were behaviour (R^2=.36) and startle (R^2=.36). The inter-correlation of these variables was weaker (r=.32), suggesting that each variable significantly contributed to freezing. The high tolerance values of these variables confirms that behaviour and startle independently contribute information to the linear equation predicting freeze (.83 and .87, respectively).

Position was more strongly correlated with startle (r=.47) than with freeze

(r=.35) suggesting an indirect contribution of position to increased freeze by increasing startle magnitude. A subsequent step-wise linear regression analysis, confirmed that only the position variable was a significant contributor to the variance of startle (R^2=.47).

Because the shock source was intended to provide a scrambled shock, it was assumed that the electrical current was constant across all bars, regardless of the number of bars between the rat's feet. However, casual observations relating bars and postion to startle suggested that these variables may have affected the rat's response to shock intensity. This is reasonable because startle magnitude increases with shock intensity (Falls & Davis, 1994; Davis & Astrachan, 1978). Accordingly, a human observer subjectively rated the shock intensity over a range of number of bars and position of the contacts. It was observed that as the number of bars between contact points (i.e., the thumb and forefinger) increased, the subjective rating of shock magnitude increased. Similarly, measurement of electrical current revealed increased current readings across increased numbers of bars. Thus, the large positive correlation found between startle magnitude and freezing duration may, in part, be mediated by a relationship between shock current intensity and the number of bars between the two points of contact.

The other major factor related to freezing was the pre-shock behaviour. It was observed that animals which were locomoting at the time of shock, froze the longest; whereas, animals which were still at the time of shock, froze the least.

In summary, it appears that post-shock activity can be predicted on the basis of certain shock contingencies. However, these relationships are not necessarily causal because the factors were not empirically manipulated to produce susbsequent changes in freezing and startle. There are no published reports that directly relate to the manipulation of the rat's position, or number of bars between the rat's paws and the consequent duration of post-shock freezing. Similarly, there is no literature which directly supports the enhancement of freezing by shocking the animal contingent on its locomotion. Therefore, Experiment 4 attempted to alter the duration of freezing by shocking the animal dependent upon the occurrence of these particular behaviours.

Empirical Manipulation of Pre-shock Factors

The multiple regression analyses of the control data discussed above raised the possibility of controlling post-shock behaviour at the time of shock delivery. Three hypotheses are offerred to explain the high correlation between the duration of post-shock freezing and the pre-shock factors.

First, post-shock freezing, although typically interpreted as a species-typical response to fear, may actually be explained as an associative response between the animal's behaviour at the time of shock and the shock itself. For example, it is widely accepted that electric shock suppresses appetitive behaviours ongoing at the time of shock (Dunham, 1971; Mackintosh, 1983; Shettleworth, 1993). Several reports demonstrated the ability of electric shock to suppress ongoing defensive behaviour, two on fleeing in chicks (Broom, 1980; Forrester & Broom, 1980), and two on fleeing in voles (Fentress, 1968a & b). However, a similar effect has not been demonstrated in rats, nor has it been demonstrated for the defensive behaviour of freezing. However, the hypothesis could be tested by manipulating the animal's behaviour at the time of the shock. If pre-shock behaviour is an important predictor of post-shock freezing, as described above, then it should be possible to increase freezing by shocking the animal contingent on its locomotion relative to shocking it contingent on its not locomoting.

A second hypothesis to explain the apparent controllability of post-shock behaviour by the pre-shock factors is that the intensity of the shock actually varies according to the animal's position and the number of bars between its feet. Although the design of the shock generator is presumed to produce a scrambled shock of uniform intensity, it may be that small changes in the rat's position may cause minimal changes in the current delivered. Freezing has been shown to vary by as much as 20 % with changes in mA of 0.02 (Van Hooff, 1982). This hypothesis can be tested by delivering shock contingent on the animal's position and the number of bars between its feet and measuring the resultant changes in the intensity of shock. Two measures of shock intensity are proposed. An objective electronic measurement (e.g., ammeter), and an inferential behavioural measurement (e.g., duration of post-shock freezing). The latter measurement of intensity of shock is possible because duration of freezing has been shown to linearly increase with increasing shock intensities up to 1.0mA (Phillips & LeDoux, 1992). However, given the results of the multiple regression described above, it is likely that position and number of bars are weaker predictors of post-shock freezing than is the rat's behaviour at the time of shock. It may be possible, however, that the factors of position and bar number contribute to the within groups variance of freezing among animals shocked contingent on their behaviour.

A final hypothesis explaining the strong correlation between these pre-shock factors and post-shock freezing is that these contingencies indirectly affect freezing by increasing startle magnitude. In general, duration of freezing and magnitude of startle in response to shock have been shown to be positively correlated (Fanselow, 1980; Kiernan et al., 1995; Leaton & Cranney, 1990). However, there is limited literature that describes the specific factors which affect startle magnitude. Plappert, Pilz and Schnitzler (1993) have suggested that differences in startle magnitude are related to constitutional differences in anxiety among rats. In the regression analyses described here, the Pearson correlation between position and startle was .47. Therefore, one variable which may directly affect startle magnitude is the rat's position. This hypothesis can be tested by relating the rat's position at the time of shock with the rating of its startle in response to the shock.

The basic design of this experiment was a 2 x 2 x 2 factorial with behaviour (still or locomote), number of bars (low or high), and body position (parallel or perpendicular) as the factors. A delayed shock was delivered in order to maximize the amount of freezing observed. This was done so as to adequately test the effects of these factors on post-shock freezing. Commensurate with the results of the regression analyses reported in the previous section, it was expected that the behaviour factor would be the strongest predictor of post-shock freezing. That is, those animals which were shocked contingent upon locomotion were expected to freeze significantly longer than those animals which were shocked contingent upon non-locomotion.

The contribution of the number of bars and body position factors to freezing was expected to be weaker than the contribution of the behaviour factor. It was hypothesized that within the groups shocked contingent on behaviour, these factors would contribute to duration of freezing. For example, it was expected that within the group shocked contingent upon locomotion, a high number of bars between the animal's feet and a perpendicular body position would produce maximal freezing. Conversely, it was hypothesized that within the group shocked contingent upon being still, a low number of bars between the animal's feet and a parallel body position would produce minimal freezing.

Procedural Modifications

Several procedural modifications were made to those used in the previous experiments discussed here. First, a 1 min delayed shock was used in this study because research has demonstrated that this interval produced similar durations in post-shock freezing to a 2 min delayed shock (Fanselow et al., 1994; Kiernan et al., 1995). Second, an electronic method to measure the intensity of the shock current received by the rat was developed. Using this method, it was possible to relate an accurate measure of current intensity with the pre-shock factors. Third, fecal boli were counted as a measure of anxiety (Pare, 1964). In studies where both freezing and defecation have been measured in an open field test, it has been found that more boli were deposited in response to a shock than if no shock was delivered (Shettleworth, 1978). Fanselow (1986) observed that immediately shocked animals deposited fewer feces than delayed shock animals. He suggested that this reduced defecation was commensurate with a reduced level of fear in the immediately shocked animals. Fifth, behaviours were coded by an observer who was blind with respect to condition. That is, the observer did not know what behaviour shock was contingent upon. Finally, the animal was subjected to a loud acoustic startle stimulus following Day 2 testing in the shock box. There is evidence that an animal's emotional state can be represented by its response to loud acoustic stimuli, thereby providing a measure of within group variation (Plappert et al., 1993). That is, it was hypothesized that if an animal was constitutionally more anxious than its cohorts then it would freeze substantially more than the other members of its group. In the present experiment, it was hypothesized that a weaker acoustic startle reaction in the startle chamber would be correlated with less freezing in the shock box.

In summary, Experiment 4 tested three hypotheses. First, that animals shocked contingent upon locomotion would freeze longer than animals shocked contingent upon non-locomotion. Second, that increased current intensity associated with an increased number of bars and perpendicular position would differentiate duration of freezing among animals within groups shocked contingent on behaviour. And finally, that freezing is indirectly influenced by an increased startle magnitude as a result of the rat being perpendicular and with a large number of bars between its feet at the time of shock.

Method

Subjects and Apparatus

A total of 80 rats that had similar weights ($\underline{M}$ = 266 g, $\underline{SEM}$ = 3.81) to those in the previous experiments were used in this experiment.

The shock box was the same as used in Experiment 1. The amount of current delivered at the time of shock was electrically measured and recorded with a peak-hold meter (Fluke, 8024 B Multimeter) connected to the shock generator. A function was defined to permit the conversion of the readings from the ammeter to an estimate of the current intensity. The function was developed by plotting the calibrated measurement of the actual current delivered (as measured at the shock source) against the ammeter reading of the current delivered (see Figure 8).

Subsequent to the shock box testing, the animal was tested in the acoustic startle box. This chamber was constructed of wire mesh (1 cm square, .5 mm in diameter) and measured 19 x 9 x 9 cm. This chamber was placed on a weigh scale (Sartorius) that provided a digital read out of the changes in weight as the animal moved. This scale was accurate to the nearest .1 g and provided a reliable measure of startle magnitude (Plappert et al., 1993).

Procedure

The design was a 2 x 2 x 2 factorial with behaviour (Still or Locomote), number of bars (Low or High), and body position (Parallel or Perpendicular) as factors. Rats were randomly assigned to each of the 8 cells (see Table 6). Delivery of shock was contingent upon the occurrence of the factors which were designated by the animal's group assignment. Sample size was estimated according to the procedure described by Cohen (1988). Using this formula, a sample size of 10 was calculated as the minimum number of rats necessary to reject the null hypothesis with .01 probability and power of .80.

On Day 1, after 1 min had elapsed from the time of entry into the shock box, the experimenter began observing the rat for the occurrence of the factors assigned to that rat. As soon as the rat was observed to be in its assigned condition, the same shock as in the previous experiments was delivered. After shock, the reading of the current was recorded from the peak-hold meter and the time of shock was noted. The ammeter reading was converted to mA using the function curve (see Figure 8). Data were collected for 5 min post-shock as previously described. At the end of the trial, the number of fecal boluses were counted. The following day, rats were observed in the same apparatus for 5 min without shock. All other details of the trial were as in Experiment 1.

Following the shock box testing, the rat was tested in the acoustic startle apparatus. The rat was placed into the wire chamber and the apparatus placed on

Figure 8.

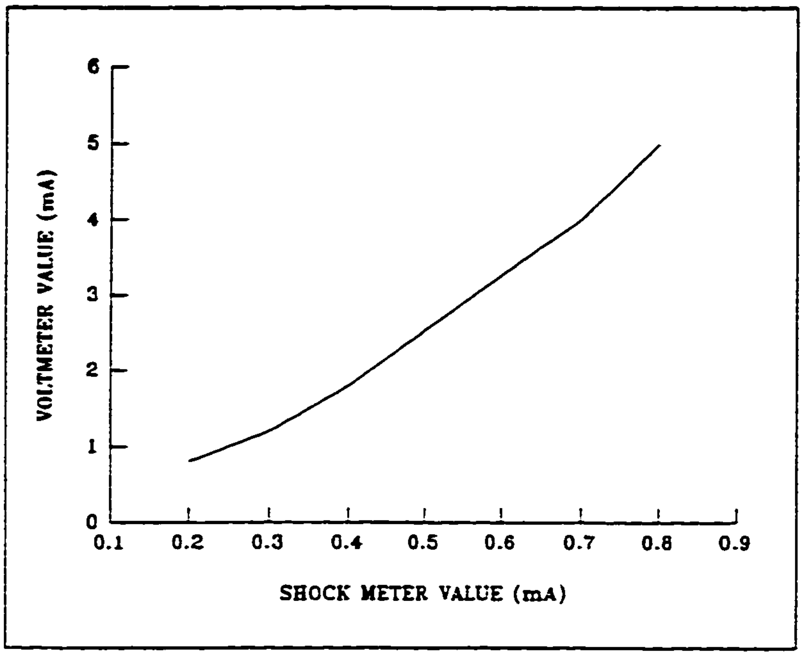

Figure 8. A plot of the reading of the generator (mA) as a function of the ammeter reading (mA).

Table 6.

n	group name	group description	experimental manipulation
12	STILL LP	STILL-LOW-PARALLEL	Shock contingent on: Still - ≤ 3 bars - Parallel position
9	STILL LR	STILL-LOW-PERPENDICULAR	Shock contingent on: Still - ≤ 3 bars - Perpendicular position
9	STILL HP	STILL-HIGH-PARALLEL	Shock contingent on: Still - ≥ 4 bars - Parallel position
10	STILL HR	STILL-HIGH-PERPENDICULAR	Shock contingent on: Still - ≥ 4 bars - Perpendicular position
9	LOCO LP	LOCO-LOW-PARALLEL	Shock contingent on: Loco - ≤ 3 bars - Parallel position
10	LOCO LR	LOCO-LOW-PERPENDICULAR	Shock contingent on: Loco - ≤ 3 bars - Perpendicular position
10	LOCO HP	LOCO-HIGH-PARALLEL	Shock contingent on: Loco - ≥ 4 bars - Parallel position
11	LOCO HR	LOCO-HIGH-PERPENDICULAR	Shock contingent on: Loco - ≥ 4 bars - Perpendicular position

Table 6. Random assignment of the rats to each of 8 cells defined by the 2 x 2 x 2 factorial design of Experiment 4. The factors were behaviour (Still of Locomote), number of bars (Low or High), and body position (Parallel or Perpendicular). The experimental manipulation defines the shock contingency. STILL = Still; LOCO = Locomote; LP = Low-Parallel; LR = Low-Perpendicular; HP = High-Parallel; HR = High-Perpendicular.

the weigh scale. After 1 min. a single acoustic startle stimulus (110dB, 10kHz) was delivered for 30 ms. The trial continued for another 5 min. Each trial was videotaped for subsequent analysis. At the end of the trial the animal was returned to the colony room. An estimate of startle amplitude was calculated by averaging the scale readings 100 ms prior to the startle stimulus and subtracting the average reading 100 ms after the occurrence of the stimulus.

Each animal's videotaped performance was viewed to determine the accuracy of shocking the animal congruent with its assigned contingency and its startle magnitude. That is, the number of bars between the animal's feet were counted, the position of the rat's body was determined, the behaviour just before shock was assessed, and the startle magnitude during shock was rated. Startle magnitude was rated by two independent observers as minimal, moderate, or maximal by subjectively assessing the height and duration of the jumping and/or the animal's rapid body shaking during the shock. Interobserver reliability using these criteria was high (r = .94).

Results

Measures on Day 1

Time of Shock. The design of this experiment meant that the precise time of shock relative to the placement of the rat in the box was variable due to the delivery of shock being contingent on the occurrence of the designated factors. However, most animals were shocked within the second min of the trial (M = 79.23 s, SEM=5.78; range=64-234 s). Between groups ANOVA indicated that the time of shock was not different among the groups [$F(7,72) = 1.28$; $p>.10$].

Accuracy of Behaviour Contingent Delivery of Shock. The delivery of the shock according to assigned condition was another potential source of error because of the difficulty in judging the particular factors present in real time. However, 82% of the animals were accurately assigned and appropriately shocked as revealed by subsequent videotape assessment. Some groups were particularly difficult to shock because the condition occurred infrequently (e.g., groups

LOCOMOTE-LOW-PARALLEL and LOCOMOTE-HIGH-PARALLEL each had less than 10 subjects, see Table 6). The remaining 18% of rats were reassigned to the group to which they accurately belonged.

Shock Intensity. There were no significant differences in the shock current delivered to the eight groups [$F(7,72) = 2.10$; $p>.10$). In order to increase statistical power, the eight groups were combined into two groups according to the shock contingent behaviour (i.e., locomote and still). Subsequent statistical analysis failed to reveal a significant difference in shock current between groups shocked contingent on behaviour [$t(78) = .64$; $p>.05$]. The correlation between ammeter reading and freezing was $r = .10$ ($p>.05$).

The lack of a significant difference in shock current between groups as measured above led the investigator to believe that the ammeter was an insufficient means of assessing the magnitude of the shock. Therefore, a post-hoc inquiry to determine the range of ammeter readings was done. Although, there was little variance among the converted readings ($M = .7738$ mA, $SEM = .0012$, range = .71-.80) it was determined by a post-hoc inquiry that the change in current intensity was likely discernible by the rat. That is, when a sample of 21 animals was tested under conditions designed to minimize or maximize shock intensity (e.g., dry vs. wet feet, respectively), a significant difference in the ammeter reading between dry and wet feet was obtained [$t(20) = 2.79$; $p<.01$). The average change in ammeter reading between dry and wet feet was .05. Furthermore, there was a positive correlation between the ammeter reading and the rating of startle magnitude by a human observer who was blind with respect to condition ($r = .55$). Finally, the minimum change in ammeter reading required to result in a change in an experimenter's subjective rating of shock intensity suggested that an ammeter change of .04 was sufficient to produce a noticeable difference in subjective shock intensity. In summary, it is likely that the range of ammeter readings was adequate to measure discernible differences in the shock intensity.

Startle. The $M \pm SEM$ magnitude of startle among all of the groups was

1.8±.08. Startle magnitude of the groups shocked contingent upon the same behaviour (i.e., locomote or still), did not differ [Fs(1,16)<.5; p>.10]. Therefore, these groups were combined for subsequent analysis of startle magnitude. Animals shocked contingent upon locomotion were revealed to have a significantly greater startle magnitude than animals shocked contingent upon non-locomotion [t(78) = 72.9; p<.0001]. The correlation between startle magnitude and freezing was r=.75 (p<.0001).

Defecation. The M±SEM of boli counted on Day 1 was 5.7±.29. A between groups ANOVA on defecation failed to show a significant difference in number of boli between groups [F(7,72) = .31; p>.10]. Subsequent analysis of number of boli within groups shocked contingent on behaviour also failed to reach statistical significance (t(78) = .31; p>.10). In addition there was no correlation between number of boli and the ammeter reading (r=.01; p>.10).

Post-shock Freezing on Day 1. Figure 9, left graph displays the mean durations of post-shock freezing exhibited by each of the eight groups on Day 1. These data were analyzed in a 2 x 2 x 2 ANOVA. As expected, the main effect of behaviour was reliable [F(1,71) = 102.36, p<.0001]. However, the main effects of position [F(1,71) = .221; p>.10] and bar number [F(1,71) = 3.01; p>.05] did not approach statistical significance. Only the position x bar interaction was statistically significant [F(1,71) = 4.8; p<.05].

As can be seen in Figure 9, behaviour at the time of shock was a highly significant factor between groups. For example, group LOCOMOTE-HIGH-PARALLEL froze more than group STILL-HIGH-PARALLEL. This comparison was reliable for every group pair differing only by behaviour (p<.01).

Because position and bar number did not significantly differ among the groups, these data were collapsed for the bin analysis. The mean durations of post-shock freezing exibited by each of the two groups shocked contingent on behaviour during each bin on Day 1 are presented in the left graph of Figure 10. A repeated measures ANOVA revealed a significant main effect of bin

Figure 9.

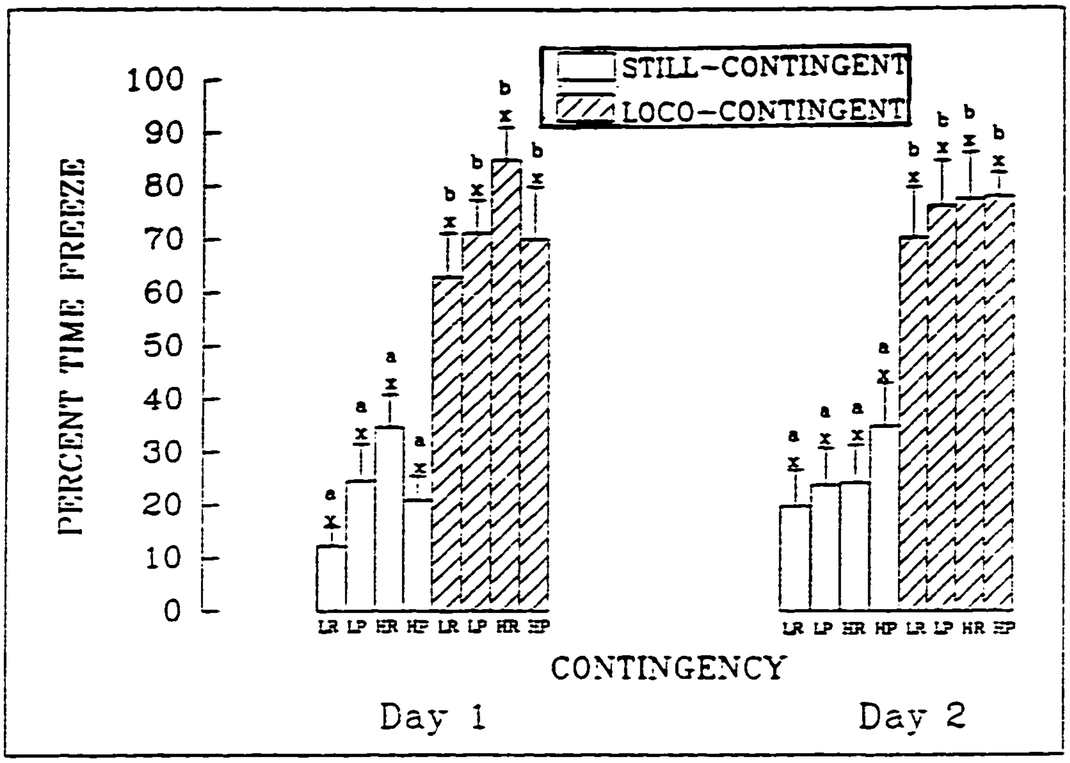

Figure 9. Mean (±SEM) percentage time spent freezing during the 5 min trial (**left:** Day 1 = post-shock; **right:** Day 2 = no-shock) by each of the eight groups of rats given a 1s, .8mA foot-shock in Experiment 4. STILL-CONTINGENT groups (open bars) = Still-Contingent groups (n = 40), rats were shocked 2 min after placement in the box contingent on being still on Day 1 and tested in the same box on Day 2; LOCO-CONTINGENT (diagonal-hatched bars) = Locomote-Contingent groups (n = 40), rats were shocked 2 min after placement in the box contingent on locomotion on Day 1 and tested in the same box on Day 2. All groups were shocked according to number of bars (Low vs. High) and position on the bars (Parallel vs. Perpendicular). LR = Low-Perpendicular; LP = Low-Parallel; HR = High-Perpendicular; HL = High-Parallel

a,b - bars with different letters are significantly different ($p < .05$) within Days

x - bars are not significantly different ($p < .05$) between Days

Figure 10.

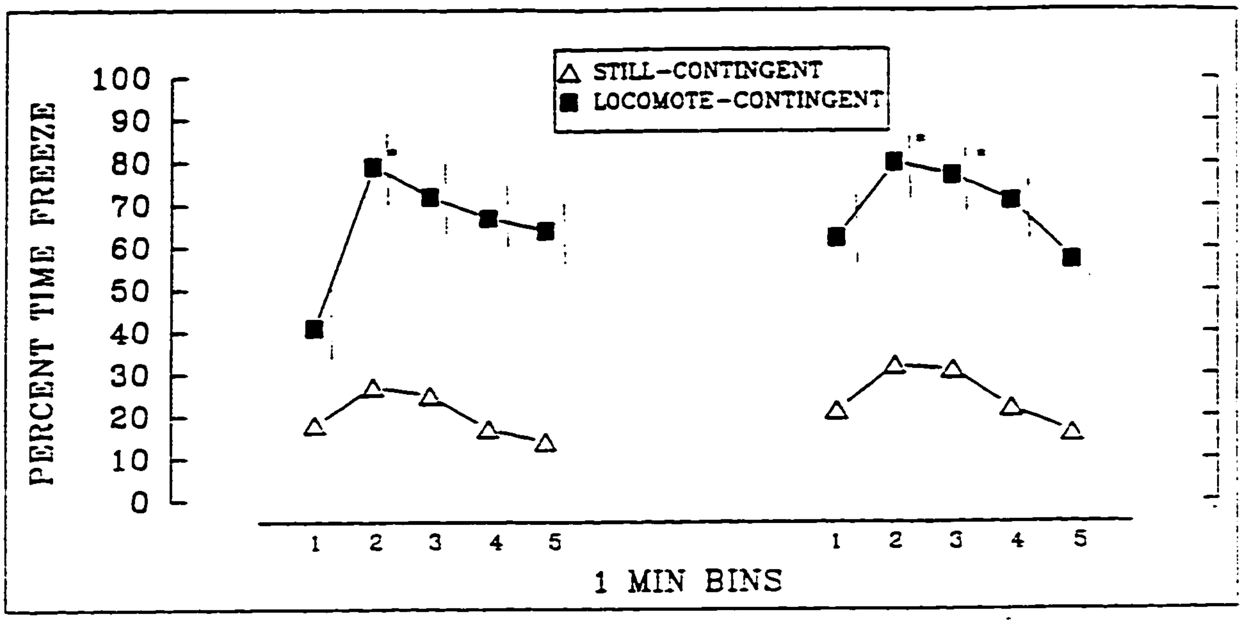

Figure 10. Mean percentage time spent freezing during each of the five, 1 min periods of the trial (**left:** Day 1 = post-shock; **right:** Day 2 = no-shock) by each of the eight groups of rats (data collapsed by behaviour) given a 1s, .8mA foot-shock in Experiment 4. STILL-CONTINGENT (open triangles) = Still-Contingent groups (n = 40), rats were shocked 2 min after placement in the box contingent upon being still on Day 1 and tested in the same box on Day 2; LOCOMOTE-CONTINGENT (closed squares) = Locomote-Contingent groups (n = 40), rats were shocked 2 min after placement in the box contingent upon locomotion on Day 1 and tested in the same box on Day 2.
Points marked with an adjacent asterisk are significantly different ($p < .05$) within groups from the mean of freezing during the first min.
Means not joined by the adjacent vertical line are significantly different ($p < .05$) between groups.

[F(2.28,308) = 14.9; $p<.0001$] and significant group x bin interaction effect [F(2.28,308) = 4.65; $p<.05$]. As revealed by the Bonferroni tests, the interaction was caused by a significant increase in freezing in the LOCO groups during the second min of the trial followed by a constant level of freezing, relative to the non significant changes in freezing in the STILL groups throughout the trial.

Exploration on Day 1. Table 7 presents the mean percentage time spent on Day 1 by rats in each of the three exploratory behaviours. As revealed by the Bonferroni tests, the only significant difference to emerge between the LOCO and STILL groups was that animals in the former group locomoted less.

Multiple Regression Analysis on Day 1

Results of a stepwise linear regression analysis indicated that two variables signficantly contributed to the variance of post-shock freezing. As expected, these variables were behaviour and startle. Behaviour was the first factor entered into the regression equation and was found to significantly contribute to post-shock freezing ($R^2=.58$; F(1,78) = 106.8: $p<.0001$). When startle was added to the regression equation as a second factor the variance of post-shock freezing accounted for increased ($R^2=.63$; F(2,77) = 65.3; $p<.0001$). Position and number of bars were removed from the regression equation. These results partially replicate the regression findings of the previous experiments in that behaviour and startle were again found to be significant predictors of freezing.

Summary of Behaviour on Day 1

Animals shocked contingent upon locomotion froze significantly longer and locomoted less than those animals shocked contingent upon being still. The significant contribution of behavioural contingency and startle magnitude were revealed by the regression analyses. The other factors hypothesized to influence freezing (i.e., number of bars and position) did not significantly contribute to the duration of freezing. These differences in freezing were not related to the number of boli deposited during the trial nor did they relate to the current intensity calculated from the ammeter reading.

Behaviour on Day 2

Freezing on Day 2. Mean durations of freezing for the groups on Day 2 are depicted in the right graph of Figure 9. A 2 x 2 x 2 factor ANOVA confirmed

Table 7.

DAY	GROUP	SNIFF	REAR	LOCO
Day 1				
	STILL-CONTINGENT	37.3(5.2)**a**	19.0(3.2)**a**	9.18(2.0)**a**
	LOCO-CONTINGENT	23.3(7.4)**a**	11.5(2.4)**a**	4.82(1.4)**b**
Day 2				
	STILL-CONTINGENT	53.6(4.2)**a**	12.2(1.6)**a**	11.7(.88)**a**
	LOCO-CONTINGENT	20.8(4.2)**b**	3.92(1.3)**b**	1.50(.88)**b**

Table 7. Mean (±SEM) percentage time spent sniffing, rearing, and locomoting during the 5 min trial (Day 1 = post-shock; Day 2 = no-shock) by each of the groups of rats given a 1s, .8mA foot-shock contingent upon being still or upon locomoting in Experiment 4.
a,b - values with different letters are significantly different ($p < .05$) within Days.

that the behaviour factor was significant [F(1,79) = 58.2; p<.0001]. The significance of the behaviour factor is readily apparent in that the groups shocked contingent on locomotion froze substantially more than the groups shocked contingent upon being still. Significant differences were calculated to occur between every group pair differing only by behaviour (.01<p<.05, see Figure 9). No other significant between groups effects were found [Fs(1.79)<1.0; p>.10].

For the bin analysis on Day 2, groups were collapsed across position and bar number as for Day 1. The right graph of Figure 10 displays the mean duration of freezing for each 1 min bin of the trial on Day 2. Repeated measures ANOVA confirmed a significant main effect of bin [F(2.24.308) = 3.0; p<.0001]. The Bonferroni tests revealed that the groups shocked contingent upon locomotion froze more during the second and third min of the trial than during the first min. No significant differences were observed across bins in the groups shocked contingent upon being still. The group x bin interaction was not significant [F(2.24,308) = .79; p>.10].

Exploration on Day 2. Table 7 presents the mean duration of sniff, rear and locomote on Day 2. Rats in the LOCO groups spent significantly less time engaged in each of sniff, rear and locomote than rats in the STILL groups.

Summary of Behaviour on Day 2

The animals shocked contingent upon locomotion on Day 1 froze significantly more on Day 2 and explored significantly less than those animals shocked contingent upon being still. Freezing in the LOCO groups during the final 2 min of the trial was not significantly different from the duration of freezing observed in these animals during the first min. The animals shocked contingent upon being still on Day 1 froze consistently throughout the trial.

Acoustic Startle Testing

Analysis of the acoustic startle data focused on identifying more fearful individual animals. It was hypothesized that animals with greater acoustic startle magnitudes would freeze more than rats with smaller amplitude startle responses.

Durations of post-shock freezing were examined to identify individuals that exhibited a percent time freeze which was a minimum of ±1.5 times the standard deviation for their group. A total of six animals (7.5% of the sample) were observed to freeze disproportionately for their group. Of these animals, five froze longer than their cohorts but only one was observed to have a greater mean reaction to the acoustic startle (M = 21.9 g, SEM = 3.26). Congruently, weak correlations were obtained between duration of post-shock freezing and acoustic startle magnitude ($r = .03$; $p > .10$): and between shock startle magnitude and acoustic startle magnitude ($r = .02$; $p > .10$). As would be expected from these results, the locomote and still contingent groups did not differ in acoustic startle magnitudes (M(SEMs) = 19.97(3.88): 23.40(4.97), respectively). Therefore, it appears that the intensity of the reaction to a single acoustic startle was insufficient to distinguish animals that disproportionately froze in the shock box.

Discussion

The present results provided evidence that post-shock freezing can be empirically manipulated by shocking an animal contingent upon its behaviour. Specifically, freezing was significantly increased by shocking an animal contingent upon its locomotion relative to shocking it contingent upon its non-locomotion. Therefore, these results clearly indicate that freezing can be increased based on the behavioural contingency surrounding the delivery of shock under conditions designed to produce maximum freezing (i.e., delayed shock). However, the hypothesis that number of bars and position would differentiate freezing among the groups shocked contingent on behaviour was not supported by the present data. That is, shocking the animal contingent on the number of bars between its feet, and its position on the bars did not influence freezing beyond that of shocking the animal contingent on its behaviour. The effect of the removal of position and number of bars in the present statistical regression is inconsistent with the results of the prior multiple regression performed on animals in the control groups of Experiments 1 - 3. Given that the original regression analysis was based on correlational data. the present results are a more valid assessment of the factors affecting post-shock freezing.

There are several possible alternative explanations that require discussion before it can be concluded that the behaviour contingency was the critical factor causing the difference in freezing. First. it could be argued that some contingency other than shocking the rat according to its behaviour could account for this effect. One possibility is that the other contingency factors (bar number and position) had a greater effect on freezing than did behaviour. As discussed above, this hypthesis can be ruled out because these variables did not significantly contribute to duration of freezing either between or within groups shocked contingent on behaviour. A second possibility is that the behaviour contingency was confounded with either the time of shock. or with individual differences. In this experiment, constitutional differences were defined by the number of boli deposited during the post-shock period. and by the startle magnitude in response to the acoustic startle stimulus. This hypothesis was not supported by the data because neither of these measures was significantly correlated with duration of freezing. A final possibility is that the post-hoc re-assignment of some rats to the alternative behavioural category resulted in a significant source of error. However, the experimenter re-assigned rats blind with respect to the duration of freezing, and without knowledge of behaviour category. Furthermore. the very small SEMs in this experiment compared to the other experiments described here. confirm that individual differences were not a large contributing factor of within group variations. Thus. consideration of these alternative explanations does not discount the effect of the behaviour contingency. Therefore, it can be reasonably concluded that shocking the animal contingent on its behaviour is the critical factor determining the duration of post-shock freezing in this experiment.

There are few reports in the literature that behaviour at the time of predatory threat can influence subsequent defensive behaviour. Fentress (1968a. 1968b) demonstrated that a vole will react to a moving overhead cloth differently according to its behaviour at the time when the stimulus was presented. Those animals that were walking at the time of the stimulus fled from the stimulus before freezing; those animals that were grooming when the stimulus was presented instantly and persistently froze. Given that the animal was stationary as it groomed, Fentress concluded that behaviour at the time of stimulus presentation was potentiated after termination of the stimulus.

Forrester and Broom (1980) have extended Fentress' findings (1968a, 1968b) to include chicks. These investigators found that the behaviour of a chick was potentiated in response to illumination of a bulb. They observed that if a bird was immobile at the time of the stimulus presentation then it remained in a crouched position for most of the trial. Conversely, if a bird was active when the light was turned on, it subsequently spent less time crouching than those birds which were in the still condition. Thus, these results suggested that ongoing behaviour was easily potentiated by presenting a brief overhead stressor to an animal.

The results of the above studies are contradictory to the results of the present experiment. That is, ongoing behaviour was potentiated by the sudden delivery of a stressor, whereas, the results of the present experiment showed that ongoing behaviour was suppressed by a stressful stimulus. There are two distinctive features related to the nature of the stimulus that may explain the contradiction. First, the aversive stimulus was presented differently in the previous studies than that delivered here. That is, the overhead cloth and the lightbulb were presented above the animal. Second, the cloth and the lightbulb were localizable stimuli as suggested by the fact that all animals in these studies oriented to the stimulus. Foot-shock, however, is a diffuse stimulus that occurs beneath the animal. The behavioural topography of rats has been shown to differ in response to localizable, versus nonlocalizable stimuli (e.g., Bevins & Ayres, 1992; Pinel et al., 1989; Pinel & Treit, 1978). For example, Pinel and colleagues have consistently reported that rats will briefly freeze in response to shock from a localizable shock probe before actively engaging in burying behaviour. but that burying does not occur in response to nonlocalizable stimuli. Similarly. Bevins and Ayres (1992) found that rats will freeze more to a less localizable stimulus (e.g., overhead auditory tone) than to a clearly localizable stimulus (e.g.. visual cue). Therefore, the discrepancy between the results found by Fentress (1968a, 1968b) and Forrester and Broom (1980) and those described here may have been due to the different method of presenting the stressor. If the stressor was presented in a more diffuse manner. as was the case with foot-shock in the present experiment, it is possible that freezing would have been observed as the primary defensive response in those earlier investigations.

One of the hypotheses generated by the finding that post-shock freezing is controlled by shocking the animal contingent on its behaviour. is that the duration of this behaviour should also be suppressed after shock. For example. the animal shocked contingent on its locomotion should subsequently locomote less after shock. The large decrease in duration of locomote by the LOCO groups is evidence that these animals did not move their feet after shock as much as animals in the STILL condition. Moreover. the reduced duration in the LOCO groups of all three exploratory behaviours on Day 2 as compared to the STILL groups is further evidence of this effect. Therefore, ongoing locomotory activity was suppressed in the LOCO groups compared to the STILL groups. This finding is consistent with the general consensus that aversive stimulation can suppress certain types of ongoing activity (e.g., Domjan & Galef, 1983; Mast, Blanchard, & Blanchard, 1982; Shettleworth. 1978). For example, Shettleworth (1978) found that rearing and walking in hamsters were more effectively suppressed by shock than was grooming. These suppressed behaviours were generally not replaced by freezing but by other active behaviours that were not associated with the shock.

In summary, the results of the present study confirm the hypothesis that duration of freezing can be predicted on the basis of shocking the animal contingent upon its locomotion or non-locomotion. Animals shocked contingent on locomotion froze more. and locomoted less after shock than animals shocked contingent on non-locomotion.

Because the minimum delay of shock was held constant in this experiment (i.e., all animals were given at least a 1 min delayed shock), these results support the hypothesis that the behavioural contingency associated with the delivery of shock is an important contributor to post-shock freezing. The fact that the animals shocked contingent upon being immobile froze approximately the same amount as immediately shocked animals in Experiments 1-3 suggests that behaviour at the time of shock rather than time of shock delivery may be the crucial factor involved in limiting post-shock freezing. However, the time of shock delivery was not manipulated in this study. In particular, the effects of delayed vs. immediate shock were not tested in this experiment. Therefore, the next experiment focused on manipulating both the time of shock and the behaviour upon which shock was contingent.

Experiment 5

The results of Experiment 4 showed that shocking an animal contingent upon its behaviour affected the amount of post-shock freezing. Because all animals in Experiment 4 were shocked approximately 1 min after entry into the box, no conclusions regarding the effects of delivering shock, contingent upon behaviour, at different intervals post-entry into the box could be made. The present experiment manipulated both the time of shock and the behaviour upon which shock was contingent. In this manner, the hypothesis that it is the behaviour at the time of shock, and not the time at which shock is delivered, that is crucial to the subsequent duration of post-shock freezing could be tested. The testing of this hypothesis has direct implications for the activity theory.

It is generally accepted that the behaviour of an animal introduced to a novel chamber changes over the course of a session (Broom, 1980; Evans & Hammond, 1983; Fentress, 1968a). For example, the initial behaviour exhibited by rats is a brief period of immobility (orientation) either in response to a novel stimulus in a familiar location (Broom, 1980; Evans & Hammond, 1983; Melzack, 1961), or as the initial response to a new environment (Brudzynski & Ociepa, 1992; Espejo & Mir, 1993; present study Experiment 1). This initial freezing is replaced within a few seconds with an active pattern of exploration which includes sniffing, rearing, and locomoting (Glickman & Hartz, 1964; Renner, 1990; Walsh & Cummins, 1976; present study Experiment 1). In general, the frequency and duration of each of these behaviours decreases with time spent in the box, but the occurrence of locomotion decreases considerably throughout the trial such that most animals are resting after about 6 min in an open field (Glickman & Hartz, 1964; Howarth, 1962). Therefore, although it is difficult to predict which specific behaviour will occur at any time during the trial (due to the large number of possible behaviour categories), it is possible to predict the likelihood of locomotion. These intervals are: when the animal is placed into the apparatus, a few seconds after placement, and about 6 min after placement. The behaviours associated with these intervals are freezing, locomotion, and resting, respectively. Thus, the probability that an animal is still is greater immediately after being placed into a novel environment than after a few seconds. The probability of locomotion peaks in the first few min and then decreases as the trial progresses.

The results of Experiment 4 demonstrated that post-shock freezing was affected by shocking the animal contingent on its locomotor behaviour. In the typical ISD experiment, the shock is not delivered contingent upon the animal's behaviour. However, it could be argued that the design of the ISD paradigm typically results in a high probability of shocking the animal during non-locomotion in the case of the immediately shocked animal, and while the rat is locomoting in the case of the delayed shock animal. Thus, little freezing would be expected in immediately shocked animals, whereas considerable freezing would be expected in delay shocked rats because of the interaction of changes in the probability of orienting and exploration over the session with the experimenter's choice of shock delay interval.

The assumptions discussed above provide the foundation of the activity theory of the ISD. Experiment 5 tested these assumptions in a 3 x 3 factorial with time of shock post-entry into the box (immediate, 1 min, 15 min) and behaviour at the time of shock (still, locomote, noncontingent) as factors. Congruent with the results of the previous experiment, it was hypothesized that animals shocked contingent upon locomotion would freeze more than animals shocked contingent upon non-locomotion, regardless of the time of shock. The noncontingent conditions were included as control groups.

There is only one report in the ISD literature that specifically examined the effects of a longer than 2 min delayed foot-shock (Westbrook et al., 1994). In this study, the amount of freezing during a 2 min trial on Day 2 among groups shocked at varying intervals on Day 1 was investigated. The intervals chosen were 4, 16, 64, 256, and 1024 s. The experimenters observed that animals shocked 64 s after being placed in the apparatus froze significantly longer on Day 2 than animals which were shocked at either the shorter or the longer intervals. In particular, animals shocked at 1024 s exhibited virtually no freezing. Applying an associative account to these results. the investigators concluded that an intermediate amount of exposure (about 1 min) was required for the animal to establish an excitatory link between the apparatus cues and the shock. Intervals less than 1 min were assumed to be insufficient to produce a satisfactory network of cues that would enable the animal to associate the shock with those cues. Extremely long intervals were assumed to produce such a strong network of cues that the animal's ability to associate any element with the shock would be extremely low. Therefore. at extremely short or extremely long intervals freezing would be expected to be substantially reduced due to the weak associability of the apparatus cues with the shock.

Applying the activity theory to the results obtained by Westbrook et al. (1994), freezing should be reduced in the 1024 s group because the probability of locomotion in this group would have been substantially less than the probability of locomotion in the groups shocked at the intermediate interval (i.e.. 64 s). Similarly, the reduced freezing in the groups shocked at the extremely short interval would be accounted for because the probability of locomotion in these animals would also be less than that at an intermediate interval. Thus. according to the Activity theory, animals shocked at extremely long or extremely short intervals froze minimally because the probability that these animals were shocked while locomoting was low. compared to those animals shocked at the intermediate interval. However, this conclusion is conjectural because Westbrook et al. (1994) did not report the animal's behaviour at the time of shock.

In summary, this experiment tested the hypothesis that behaviour at the time of shock is a more crucial determinant of post-shock freezing than the time of shock itself. It was predicted that shocking an animal contingent upon its locomotion would increase freezing significantly above that of shocking an animal contingent upon its non-locomotion, regardless of the time of shock. In addition, it was predicted that the ISD effect would be evident in animals not shocked contingent upon their behaviour, that is, those animals that were shocked regardless of their behaviour. In other words, immediately and 1-min delayed shock animals shocked regardless of their behaviour were predicted to exhibit a similar duration of freezing as the control groups of Experiment 1. It was also predicted that the probability of locomotion in the 15-min delayed shock animals would be low, and consequently that the duration of freezing in animals shocked at this interval regardless of their behaviour would also be low. Furthermore, it was hypothesized that the duration of freezing in the groups shocked at the extremely short or extremely long intervals (immediate or 15-min, respectively) irrespective of their behaviour would freeze for a similar duration as those animals shocked contingent on non-locomotion. It was also hypothesized that the group given a delayed shock irrespective of their behaviour would freeze for a similar duration as the group shocked contingent upon locomotion. This hypothesis was based on evidence that the probability of locomotion changes throughout a session. Finally, this experiment tested the hypothesis that the greatest duration of freezing on Day 2 would be observed during the interval inclusive of the time of shock on Day 1.

Method

Subjects and Apparatus

Experiment 5 had 72 rats that had similar weights (M = 277 g. SEM = 1.87) to those used in the previous experiments.

The shock box, peak-hold meter and startle box were the same as used in Experiment 4.

Procedure

The design was a 3 x 3 factorial with behavioural contingency (Still. Locomote. Noncontingent) and time of shock (Immediate. 1 min. 15 min) as factors. Rats were randomly assigned to each of the 9 cells (see Table 8).

Table 8.

n	**group name**	**experimental manipulation**
8	IMM-NONCONTINGENT	shock delivered imm, not contingent on particular behaviour
8	IMM-LOCO	shock delivered imm, contingent on first instance of loco after placement into chamber
8	IMM-STILL	shock delivered imm, contingent on first instance of still after placement into chamber
8	1 MIN-NONCONTINGENT	shock delivered at 1 min, not contingent on particular behaviour
8	1 MIN-LOCO	shock delivered after 1 min, contingent on first instance of loco
8	1 MIN-STILL	shock delivered after 1 min, contingent on first instance of still
8	15 MIN-NONCONTINGENT	shock delivered at 15 min, not contingent on particular behaviour
8	15 MIN-LOCO	shock delivered after 15 min, contingent on first instance of loco
8	15 MIN-STILL	shock delivered after 15 min, contingent on first instance of still

Table 8. Random assignment of the rats to each of 9 cells defined by the 3 x 3 factorial design of Experiment 5. The factors were behaviour (Noncontingent, Still, or Locomote) and time of shock (Immediate, 1 min, or 15 min). The experimental manipulation defines the shock contingency and the time of shock. IMM = Immediate; 1 MIN = 1 min: 15 MIN = 15 min: NONCONTINGENT = Noncontingent; STILL = Still: LOCO = Locomote.

Sample size was estimated according to the procedures described by Cohen (1988) and Wahlsten (1991). The hypothesized means (expressed as percent time freezing) used in the sample size calculations are listed in Table 9. The interaction values for this 3 x 3 table of means were calculated using Formula 8.3.4 from Cohen (1988) and are listed in Table 10. The effect size for the interaction effect was calculated using Formulas 8.3.6 and 8.3.7 from Cohen (1988) using σ=.20. The effect size was calculated to be .42. Inserting .42 into Formula 8.4.1 with α=.05; u=4; power=.80; and the value for $n_{.05}$ determined to be 956 from Table 8.4.4 a value of 14.55 was calculated for n'. Inserting 14.55 into formula 8.4.4 with u=4 and number of cells=9 a value of 8.53 (round to 9) was calculated for the number of subjects needed per cell. That is, using the method of Cohen (1988) it was calculated that in order to have power of .80 and alpha of .05 a sample size of 9 per cell would be required to detect a significant interaction of behaviour at time of shock x time of shock.

It was reasoned that the most important hypothesis tested in this experiment is that behaviour at the time of shock would affect duration of post-shock freezing, regardless of time of shock delivery. Therefore, a linear contrast was developed to test the hypothesis that there is a difference among animals shocked noncontingent upon their behaviour and animals shocked contingent upon their behaviour when shock is delivered immediately or at 1 min delay. This contrast served as the principal comparison for determining sample size using the method developed by Wahlsten (1991).

Using Equation 5 described in Wahlsten (1991) and the following values: z_{α}=1.645 ; $z_{1-\beta}$= -.842 where α=.05 and β=.80 for a 1-tailed test of significance; sum of squared contrast coeffecients of 4; sample contrast sum of 40; and a pooled variance estimate of .04, a sample size of 8.19 (round to 8) per cell was calculated. Thus, the estimate of sample size using the Cohen (1988) method was slightly higher than that calculated using the Wahlsten (1991) method, 9 versus 8, respectively for the same power and alpha levels. A sample size of 72 (n=8 per 9 groups) was used in this study.

Table 9.

	IMM	1-MIN	15-MIN	**M of row**
LOCO	50***	70**	70***	**63**
STILL	5***	20**	20***	**15**
NON-CONTINGENT	5*	60*	60***	**42**
M of column	**20**	**50**	**50**	

* data from Experiment 1
** data from Experiment 4
*** estimated data

Table 9. A table of hypothesized means of freezing (expressed as percent time) for 9 groups in the 3 x 3 design in Experiment 5 with time of shock (immediate, 1 min, 15 min) and behaviour at time of shock (locomote, still, noncontingent) as factors. The mean of each row and each column are shown in **bold.** The global mean was calculated to be 40. These values were used to calculate estimates of sample size by using the methods of Cohen (1988) and Wahlsten (1991).

Table 10.

	IMM	1-MIN	15-MIN
LOCO	7	-3	-3
STILL	10	-5	-5
NON-CONTINGENT	-17	8	8

Table 10. Calculated interaction values for the 3 x 3 table of hypothesized means used to estimate sample size in Experiment 5 (see Table 9). These values were calculated using the Formula 8.3.4 from Cohen (1988) by subtracting the row mean and column mean from the hypothesized cell mean and adding the global mean. For example, the interaction value for the IMM-LOCO group (7) was equal to 50 (cell mean) minus 63 (row mean) minus 20 (column mean) plus 40 (global mean).

As soon as the rat was observed to be in its assigned condition, the same shock as in the previous experiments was delivered. Upon termination of the shock, the reading of the current was recorded from the peak-hold meter. Behavioural data were collected for 5 min post-shock as described in Experiment 4. Freezing in the 15 min group was not observed during the pre-shock interval. A bolus count was recorded for each rat after it was removed from the box. Post-hoc determination of the rat's postural position, the number of bars spanned by the rat's feet, and the magnitude of the rat's startle in response to the shock was completed by examining the performance of each rat on videotape.

The following day, rats were observed for 20 min in the same box. To maximize the probability of observing freezing on Day 2, the freezing of all groups was analyzed using the 5 min period inclusive of the time of shock on Day 1. Therefore, freezing in the IMM, 1 min, and 15 min groups were analyzed during min 1-5, 1-5, and 16-20 on Day 2, respectively. Animals in the 15 min group were not observed to freeze for long periods of time during min 1-15. The average amount of freezing during this time did not exceed 15% in any 1 min interval. All other details were as in Experiment 1.

Results

Variables Associated with Shock Delivery on Day 1

Time of Shock. For those groups shocked contingent on their behaviour, the precise time of shock was variable. All animals in the immediate shock condition were shocked within 30 s after entrance to the chamber (M = 13.5 s, SEM = 1.16, range = 1-23 s). Animals in the 1 min and 15 min delayed shock conditions were shocked within 2 min after the appropriate delay had elapsed (M = 71 s, SEM = 2.13, range = 57-93 s; M = 914 s, SEM = 2.23, range = 873-960 s, respectively). ANOVAs indicated that the time of shock was not different among temporally similar groups [$Fs(2,21) < 2.5$ $p > .10$].

Behaviour at the Time of Shock Delivery. Most animals (92%) shocked contingent on behaviour were shocked according to condition. However, 4 out of

48 animals were re-assigned post-hoc to the opposite behavioural category. This resulted in equal subjects among the groups.

Analysis of the behaviour at the time of shock of those rats shocked noncontingent on their behaviour immediately after placement revealed that only 1 out of the 8 animals was locomoting at the time of shock. Conversely, 6 out of the 8 rats in the 1 min NONCONTINGENT condition were observed to be locomoting at the time of shock. Similarly, 5 out of the 8 rats in the 15 min NONCONTINGENT condition were observed to be locomoting at the time of shock.

Shock Intensity. There were no significant differences in the shock current delivered to the groups [$F(8.63) = 1.10$; $p>.10$). As in Experiment 4, there was little variance among the converted readings in this experiment ($M = .7738$ mA, $SEM = .0012$, range = .72-.80). However, the similar range of readings between these two experiments is evidence that the ammeter readings were a reliable estimate of shock current.

Startle. The M±SEM magnitude of startle among all of the groups was 1.9±.03. As in Experiment 4, the startle data of the groups shocked contingent on the same behaviour were combined for statistical analysis of startle magnitude. The M±SEM magnitude of startle for the LOCO and STILL groups were 2.5±.05 and 1.56±.04, respectively. Animals shocked contingent upon locomotion were revealed to have a significantly greater startle magnitude than animals shocked contingent upon non-locomotion [$t(70) = 45.14$; $p<.0001$]. The correlation between startle magnitude and freezing was $r = .76$ ($p<.0001$).

Behaviour on Day 1

Defecation. For all animals, the M±SEM of boli counted on Day 1 was 7.5±.46. As in Experiment 4, a between groups ANOVA did not reveal a reliable difference in the number of boli between groups [$Fs(8,63)<1.1$; $p>.10$] nor within groups shocked contingent on the same behaviour [$t(70) = .45$;$p>.10$].

Post-shock Freezing. Mean duration of freezing on Day 1 by each of the groups is displayed in the left graph of Figure 11. These data were analyzed by a 3 (behaviour) x 3 (time) factor ANOVA. As expected, the main effect of behavioural contingency was significant [F(2,62) = 11.98, p<.0001]. The main effect of time was also significant [F(2,62) = 17.89; p<.001]. However, the behavioural contingency x time interaction effect as analyzed by the ANOVA was not significant [F(4,62) = 2.06: p>.05]. In order to more adequately test for an interaction effect between behavioural contingency and time of shock two linear contrast were developed (see Table 11). These contrasts tested the hypotheses that significant differences existed among NON-CONTINGENT and STILL groups at IMM and 1MIN and among NON-CONTINGENT and LOCO groups at IMM and 1 MIN. For the purposes of testing these hypotheses of interaction. the 15 MIN groups were not included because animals in these groups froze similarly to animals in the 1 MIN condition. That is. the contrast coeffecients were 0 for all 15 MIN groups. The first contrast (C1) revealed a significant interaction among NON-CONTINGENT and STILL groups at IMM and 1MIN [t(63) = 2.89: p<.01]. Therefore, it appears that behaviour is more important than time of shock in determining post-shock duration of freezing. The second contrast (C2) did not reveal a significant interaction among STILL and LOCO groups at IMM and 1MIN [t(63) = 1.03; p>.10].

A oneway ANOVA on all 9 groups indicated a significant overall difference among the groups in mean percent freezing on Day 1 [F(8.63) = 13.4. p<.0001]. Subsequent Bonferroni tests using an experimentwise error rate revealed that the IMM-LOCO group froze significantly longer than each of the IMM-RAND and IMM-STILL groups. The 1 MIN-LOCO and the 15 MIN-LOCO groups each froze significantly longer than all STILL-CONTINGENT groups and the IMM-RAND group.

Because both behavioural contingency and time were significantly different among the groups. the data were not collapsed for the bin analysis. Figure 12 displays mean durations of post-shock freezing for each 1 min bin

Figure 11.

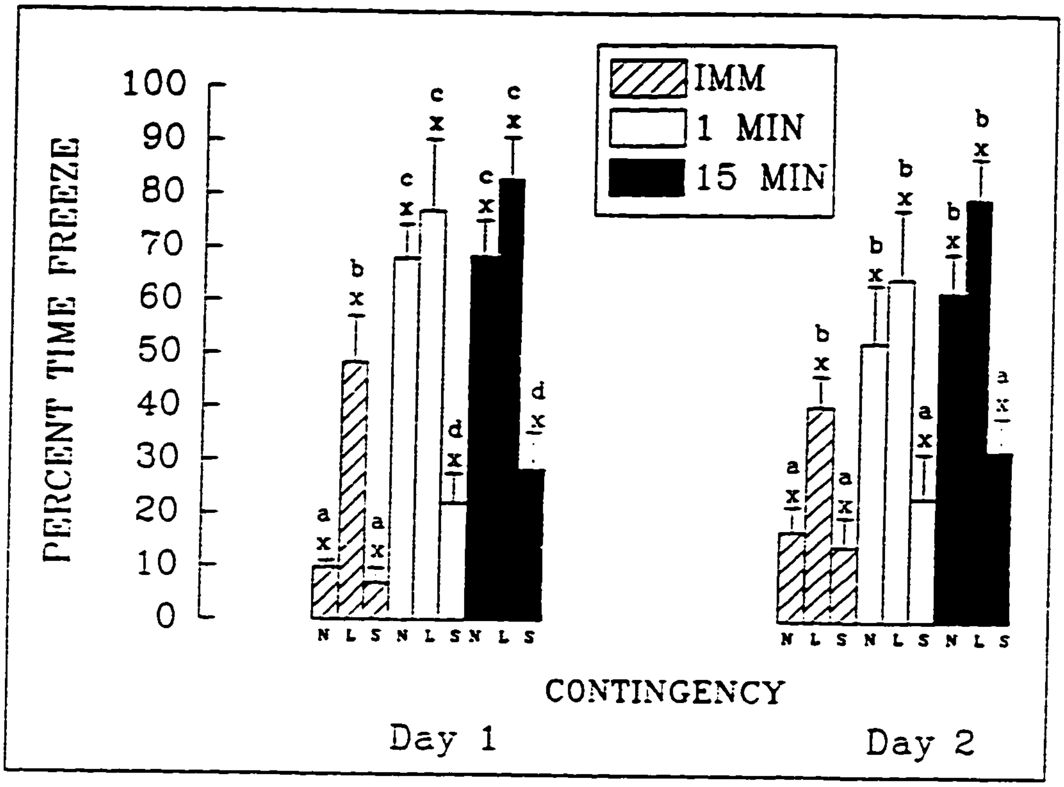

Figure 11. Mean (±SEM) percentage time spent freezing during the 5 min trial (**left:** Day 1 = post-shock; **right:** Day 2 = no-shock) by each of the nine groups of rats given a 1s, .8mA foot-shock in Experiment 5. Rats in the IMM groups (diagonal-hatched bars) were shocked immediately after placement in the shock chamber. Rats in the 1 MIN groups (open bars) were shocked following 1 min after placement in the shock chamber. Rats in the 15 MIN groups (solid bars) were shocked following 15 min after placement in the shock chamber. Groups were either shocked noncontingent on their behaviour (N), or were shocked contingent upon their locomotion (L), or upon being still (S).
a,b,c,d - bars with different letters are significantly different ($p < .05$) within Days
x - bars are not significantly different ($p < .05$) between Days

Table 11.

Group	Observed M (percent time)	C1	C2
IMM-NONCONTINGENT	8.1	1	0
IMM-STILL	7.4	-1	1
IMM-LOCO	48.3	0	-1
1 MIN-NONCONTINGENT	66.3	-1	0
1 MIN-STILL	22.5	1	-1
1 MIN-LOCO	80.0	0	1

Table 11. Observed means of freezing for each of the IMM and 1 MIN groups of Experiment 5 analyzed by orthogonal contrasts (C1 and C2).

Figure 12.

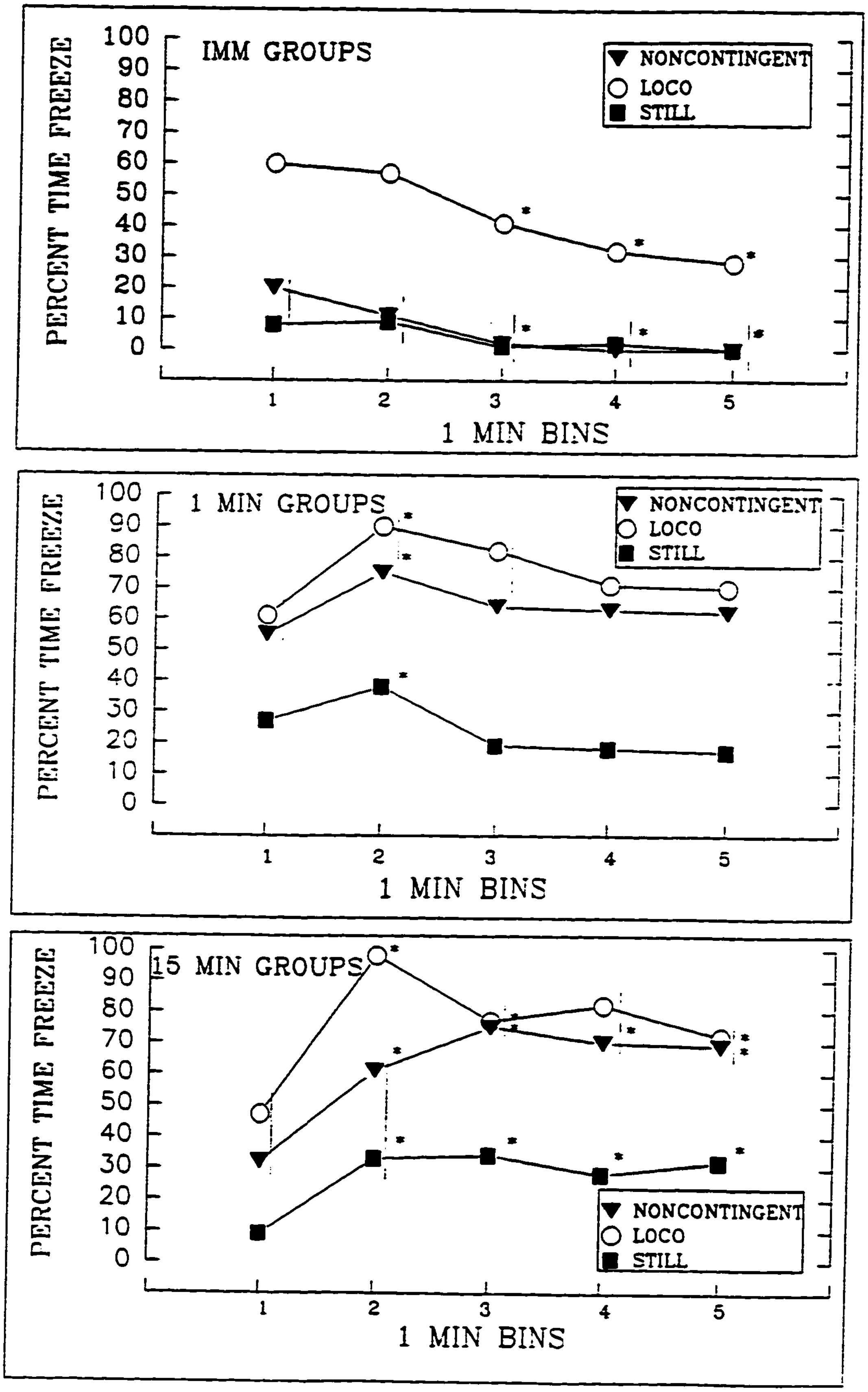

Figure 12. Mean percentage time spent freezing during each of the five, 1 min

periods of the post-shock trial on Day 1 by the 72 rats given a 1s, .8mA foot-shock in Experiment 5. Freezing was measured during min 1-5, 2-6, and 16-20 in groups IMM, 1 MIN, and 15 MIN. respectively. Freezing was not observed in any group prior to shock. Rats in the NONCONTINGENT groups (closed triangles) were shocked according to time (IMM. $n = 8$; 1 MIN, $n = 8$; 15 MIN, $n = 8$) and not contingent upon their behaviour. Rats in the LOCO groups (open circles) were shocked according to time (IMM. $n = 8$; 1 MIN, $n = 8$; 15 MIN, $n = 8$) and contingent upon their locomotion. Rats in the STILL groups (closed squares) were shocked according to time (IMM. $n = 8$; 1 MIN, $n = 8$; 15 MIN, $n = 8$) and contingent upon their being still.

Points marked with an adjacent asterisk are significantly different ($p < .05$) within groups from the mean of freezing during the first min.

Means not joined by the adjacent vertical line are significantly different ($p < .05$) between groups.

during Day 1. These data were analyzed by a 9 (between groups) x 5 (repeated measures) ANOVA. A significant main effect of bin [F(2.5,252) = 9.27; p<.001] was revealed as well as a significant interaction effect [F(20.0,252) = 3.18; p<.001]. The interaction effect was due to the increased freezing from in 1 to min 2 in the delay NONCONTINGENT and delay LOCO groups compared to the nonsignificant difference in the immediate groups during this time.

Exploration on Day 1. The mean durations of sniff, rear, and locomote on Day 1 are presented in Table 12. As revealed by Bonferroni tests, the LOCO-CONTINGENT groups spent significantly less time than the NONCONTINGENT and STILL-CONTINGENT groups engaged in locomote and rear and significantly less time sniffing than the STILL-CONTINGENT groups. The STILL-CONTINGENT groups spent significantly more time locomoting and sniffing than the NONCONTINGENT groups.

Multiple Regression on Day 1

Results of a stepwise linear regression analysis indicated that two variables signficantly contributed to the variance of post-shock freezing. As expected, these variables were behaviour and startle. Behaviour was the first factor entered into the regression equation and was found to significantly contribute to post-shock freezing ($R^2 = .66$; F(1,70) = 136.9; p<.0001). When startle was added to the regression equation as a second factor the variance of post-shock freezing accounted for increased ($R^2 = .75$; F(2,69) = 105.4; p<.0001). Position and number of bars were removed from the regression equation. These results replicate the regression findings of the previous experiment and provide further evidence for the accurate prediction of post-shock freezing based on the behaviour upon which shock was contingent and on the startle magnitude.

Summary of Behaviour on Day 1

Animals shocked contingent upon locomotion froze significantly longer than rats shocked contingent upon non-locomotion. Rats in the IMM-LOCO group also froze more than rats in the IMM-NONCONTINGENT group.

Table 12.

DAY	GROUP	SNIFF	REAR	LOCO
Day 1				
	NON-CONTINGENT	26.0(8.2)**a**	8.83(3.9)**a**	5.00(1.8)**a**
	STILL-CONTINGENT	47.8(3.2)**b**	9.21(1.7)**a**	13.7(1.8)**b**
	LOCO-CONTINGENT	19.5(3.9)**a**	3.40(1.7)**b**	1.30(1.1)**c**
Day 2				
	NON-CONTINGENT	40.6(6.3)**a**	24.0(2.3)**a**	7.35(.65)**a**
	STILL-CONTINGENT	30.8(4.9)**a**	21.1(3.2)**a**	8.75(.91)**a**
	LOCO-CONTINGENT	20.0(2.2)**a**	15.9(2.7)**a**	2.62(1.5)**b**

Table 12. Mean (±SEM) percentage time spent sniffing, rearing, and locomoting during the 5 min trial (Day 1 = post-shock; Day 2 = no-shock) by each of the three groups of rats given a 1s, .8mA foot-shock non-contingent upon their behaviour, or contingent upon being still, or upon locomoting in Experiment 5.
a,b,c - values with different letters are significantly different ($p < .05$) within Days.

However, rats in the LOCO-CONTINGENT delay groups did not significantly differ from the amount of freezing observed in the NONCONTINGENT delay groups. Less exploratory behaviour was also observed in all groups shocked contingent on locomotion than shocked contingent on non-locomotion. Results of the regression analysis confirmed the significant contribution of the behaviour contingency on the variance of freezing and extended the finding to include the magnitude of the startle response as an additional significant contributor to the freeze variance. Neither time of shock, shock intensity, nor number of boli were found to significantly differ among groups shocked at similar times.

Behaviour on Day 2

Freezing on Day 2. The mean durations of freezing for each of the groups on Day 2 are depicted in the right graph of Figure 11. A 3 x 3 factor ANOVA confirmed that the behaviour factor was significant [$F(2,62) = 3.2$; $p<.01$]. The main effect of time was also significant [$F(2,62) = 8.7$; $p<.001$]. However, the behavioural contingency x time interaction effect was not significant [$F(4,62) = .61$; $p>.10$]. In order to more adequately test for an interaction effect between behavioural contingency and time of shock a linear contrast was developed, as discussed above (see Table 11). The first contrast (C1) revealed a significant interaction among NON-CONTINGENT and STILL groups at IMM and 1MIN [$t(63) = 2.33$; $p<.05$]. The second contrast (C2) did not reveal a significant interaction among STILL and LOCO groups at IMM and 1MIN [$t(63) = 1.01$; $p>.10$]. The significance of the behaviour factor is readily apparent in that the groups shocked contingent on locomotion froze substantially more than the groups shocked contingent upon being still.

A oneway ANOVA on all 9 groups indicated a significant overall difference among the groups in mean percent freezing on Day 1 [$F(8,63) = 5.95$, $p<.0001$]. Subsequent Bonferroni tests using an experimentwise error rate revealed that the 1 MIN-LOCO group froze significantly longer than each of the IMM-RAND and the IMM-STILL groups. The 15 MIN-LOCO group froze significantly longer than each of the 1 MIN-STILL, IMM-STILL and IMM-RAND groups.

Figure 13 depicts the mean durations of post-shock freezing for each 1 min bin during Day 2 for each condition. These data were analyzed by a 9 (between groups) x 5 (repeated measures) ANOVA. A significant main effect of bin [$F(2.56,252) = 3.80$; $p<.001$] was revealed, as well as, a significant interaction effect [$F(2.0,252) = 3.18$; $p<.001$]. The group x bin interaction effect was due to the significant increase in freezing in the delay NONCONTINGENT and delay LOCO groups from min 1 to min 2 and the corresponding significant decrease in freezing in the immediate groups.

Exploration on Day 2. Table 12 presents the mean duration time spent on Day 2 by animals engaged in each of the three exploratory behaviours. The only between groups difference to emerge as a result of the Bonferroni analysis was a decrease in the duration of locomotion in the LOCO-CONTINGENT groups compared to the STILL-CONTINGENT and NONCONTINGENT groups.

Summary of Behaviour on Day 2

Freezing on Day 2 increased from min 1 to min 2 in the groups given a DEL shock on Day 1 in the LOCO-CONTINGENT and NONCONTINGENT conditions but decreased during this interval in the STILL-CONTINGENT condition. Freezing of all rats on Day 2 given an IMM shock on Day 1 was maximal during the first min: rats in the IMM-LOCO group froze the most during this time. The only difference in exploratory behaviours among the groups shocked according to behavioural contingency was that rats in the LOCO-CONTINGENT condition locomoted significantly less on Day 2 than rats in the STILL-CONTINGENT or NONCONTINGENT conditions.

Discussion

The duration of freezing observed in the NONCONTINGENT groups was similar to that observed in the CNTRL groups of Experiment 1, and to that reported in the ISD literature (e.g., Kiernan et al., 1995; Landeira-Fernandez et al.,

Figure 13.

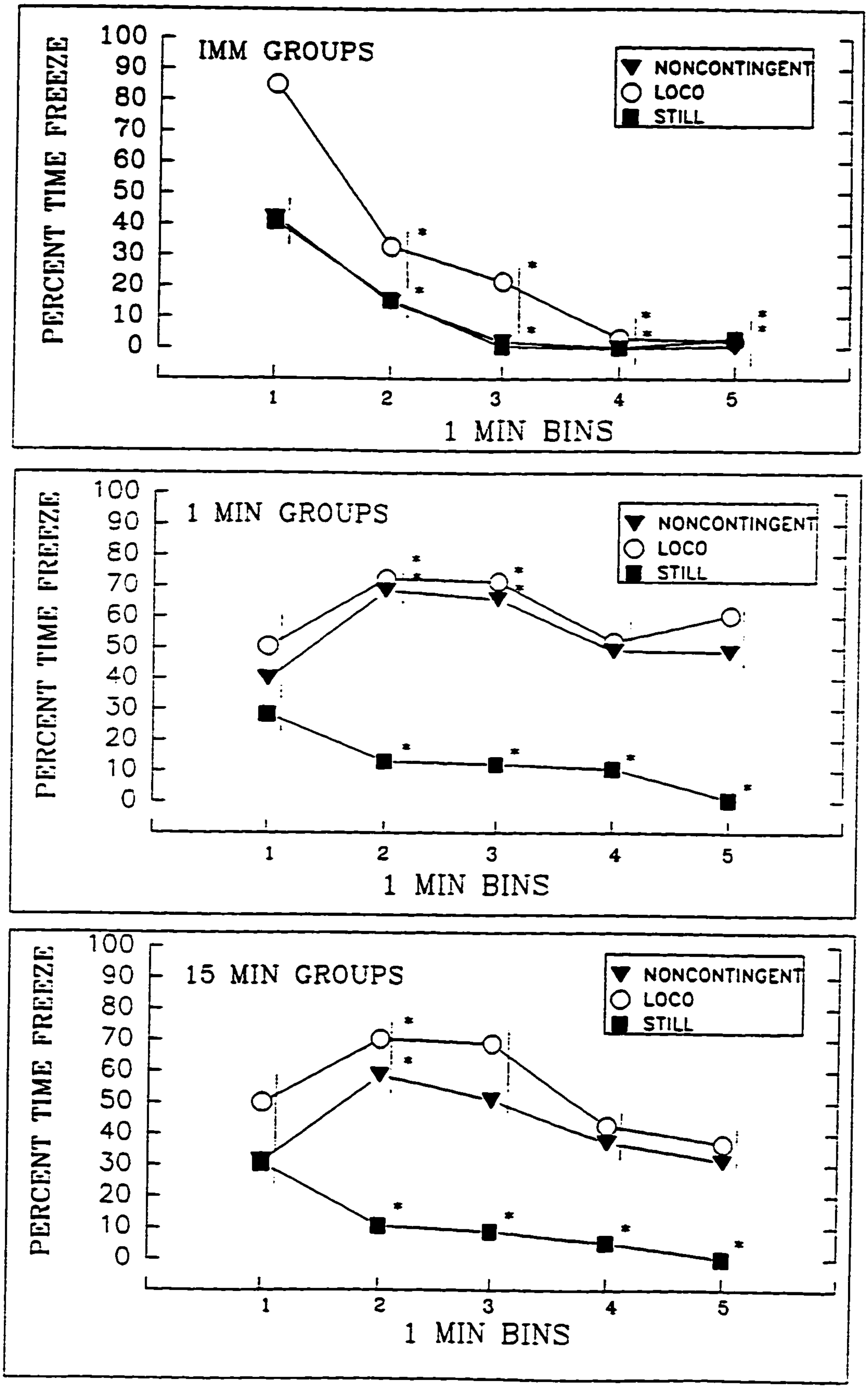

Figure 13. Mean percentage time spent freezing during each of the five. 1 min

periods of the no-shock trial on Day 2 by the 72 rats tested in the same box as on Day 1 in Experiment 5. Freezing was measured during min 1-5, 2-6, and 16-20 in groups IMM, 1 MIN, and 15 MIN, respectively. Freezing in the 15 MIN group did not exceed 15% in any 1 min interval during min 1-15. Rats in the NONCONTINGENT groups (closed triangles) were shocked according to time (IMM, $n = 8$; 1 MIN, $n = 8$: 15 MIN. $n = 8$) and not contingent upon their behaviour. Rats in the LOCO groups (open circles) were shocked according to time (IMM, $n = 8$; 1 MIN, $n = 8$; 15 MIN. $n = 8$) and contingent upon their locomotion. Rats in the STILL groups (closed squares) were shocked according to time (IMM, $n = 8$; 1 MIN, $n = 8$; 15 MIN. $n = 8$) and contingent upon their being still.
Points marked with an adjacent asterisk are significantly different ($p < .05$) within groups from the mean of freezing during the first min.
Means not joined by the adjacent vertical line are significantly different ($p < .05$) between groups.

1995; Westbrook et al., 1994). The 1 MIN-NONCONTINGENT and 15 MIN-NONCONTINGENT groups froze for a similar duration as did the DEL-CNTRL group and the IMM-NONCONTINGENT group froze about as long as the IMM-CNTRL group of Experiment 1. Therefore, the results reported in this study are consistent with the durations of freezing described in the previous experiments, as well as with the ISD literature.

The results of this experiment support the hypothesis that post-shock freezing can be increased by shocking the rat contingent upon its locomotion relative to shocking it contingent upon its non-movement, regardless of when the shock was administered. These results are contrary to Fanselow's (1986) spatial contextual conditioning account of the ISD. That is, this account can not explain why rats in the DEL-STILL group did not freeze as much as DEL-LOCO rats even though both groups had equal time to condition to the context cues. Moreover, this account can not explain why rats in the IMM-LOCO group froze more than the other IMM groups even though none of the groups, according to Fanselow (1986), would have had enough time to have conditioned to the context cues. Therefore, these results do not support the spatial contextual conditioning account of the ISD.

The results of this study, however, provide powerful support for the activity account of the ISD. That is, shocking the animal contingent on its being still significantly decreased the duration of freezing compared to shocking the animal contingent upon its locomotion. The significant contribution of behaviour to duration of freezing as revealed by the results of the multiple regression supported this conclusion. This effect was obtained regardless of whether the shock was delivered immediately after the rat was placed in the box, or after a delay (1 min or 15 min) post-entry into the box. Thus, behaviour at the time of shock was revealed to be the most important factor controlling the subsequent duration of post-shock freezing.

The groups shocked noncontingent on their behaviour, however, did show a difference in the duration of freezing dependent upon the time of shock delivery. That is, the IMM-NONCONTINGENT animals froze as long as the IMM-STILL animals, and the 1 MIN- and 15 MIN-NONCONTINGENT animals froze as long as the rats in the respective LOCO groups. Because the time of shock did not statistically differ between these groups, this effect was not the result of the IMM-LOCO animals being shocked later in the trial, or of the IMM-STILL animals being shocked earlier in the trial. It was also observed that the probability of locomotion in the IMM-NONCONTINGENT condition and in the 1 MIN- and 15 MIN-NONCONTINGENT conditions was different. Therefore, these results account for the ISD as dependent on shock delay not because of the time required to form a spatial contextual association but because the delay alters the probability of the behaviour at the time of shock.

The mean durations of sniff, rear, and locomote were also different between the groups shocked contingent versus noncontingent, on their behaviour. The LOCO-CONTINGENT groups spent less time than the STILL-CONTINGENT groups on Day 1 engaged in behaviours requiring movement of the feet (i.e., locomote and rear), regardless of the time of shock. This result is consistent with the hypothesis that shock will suppress the behaviour that was on-going at the time when the shock was delivered (Domjan & Galef, 1983; Mast et al., 1982; Shettleworth, 1978). Furthermore, the LOCO-CONTINGENT groups spent less time locomoting than both the STILL-CONTINGENT and NONCONTINGENT groups on Day 2. Therefore, the suppressive effects of the shock specifically on locomotion extended to the second day of testing.

The duration of freezing observed in the 15 MIN-LOCO and 15 MIN-NONCONTINGENT groups was substantially longer than that reported by Westbrook et al. (1994). However, given the substantial freezing during min 15-20 of rats in the 15 min group, an alternative explanation could be offered to explain the lack of freezing in the 17 min group in the Westbrook et al. (1994) study. It could be argued that quite simply, the animal in the 17 min group was not observed to freeze because the 2 min duration was too short a trial for freezing to be observed. Had the trial duration been long enough to include the time of shock on Day 1, it is likely that substantial freezing would have been observed, as it was in the present experiment.

In summary, the results of this study provide strong evidence that behaviour at the time of shock, and not the time of shock itself, is crucial to the subsequent duration of freezing. This effect, together with the knowledge that the probability of locomotion changes throughout a trial, can adequately account for the ISD effect. That is, the ISD can be accounted for as a time-related effect in that most animals shocked immediately after entry would likely have been shocked while immobile and therefore would be expected to suppress this behaviour during the post-shock trial. The result would be that immediately shocked animals would not freeze as much as animals shocked while locomoting, as would likely be the case under conditions of a delayed shock.

GENERAL DISCUSSION

The purposes of the present series of experiments were twofold. First, Experiments 1, 2, and 3 empirically examined certain variables pertinent to the ISD paradigm. Second, Experiments 4 and 5 tested the hypothesis that the duration of post-shock freezing could be modified on the basis of shocking the animal contingent upon the occurrence of particular factors. In general, the results of these studies replicated the freeze deficit observed in control animals given an immediate shock compared to control animals given a delayed shock (Blanchard et al., 1976b; Fanselow, 1986, 1990; Kiernan & Westbrook, 1993; Westbrook et al., 1994; Fanselow et al., 1994; Kiernan et al., 1995; Landeira-Fernandez et al., 1995). A discussion of how the results of each of these studies extend the basic interpretations of the ISD and theories of animal defensive behaviour follows.

In Experiment 1, the min by min analysis of freezing on Day 2 revealed that freezing decreased over the course of the session. Both DEL and IMM groups showed a maximum duration of freezing during the min that they were shocked on Day 1. The spatial conditioning effects were evident in that the animals tested in the same box on Day 2 as they were shocked in on Day 1 froze longer overall than the rats tested in two spatially distinct boxes. The results of Experiment 2 supported the hypothesis that animals would not only exit the shock box, if allowed, but that these animals would freeze less than rats confined to the shock box after shock. In Experiment 3, those animals that were handled just before a delayed shock was given froze less than animals given a delayed shock and not handled after being placed in the shock box.

The results of Experiment 4 provided evidence that it was possible to increase freezing by delivering a delayed shock contingent upon the animal's locomotion. In Experiment 5, this finding was extended to include animals immediately shocked.

The results from each of these experiments have implications for the contextual and the topographical accounts of the ISD. These implications will be explored below, along with the support for the activity account proposed as an alternative theory of the ISD.

Activation of the Defense System by Foot-shock

A basic theoretical distinction between the contextual and the topographical accounts of the ISD is the role of fear in mediating post-shock behaviour. Whereas the topographical (Blanchard et al., 1976a, 1976b) and activity theories assume that shock activates the defense system in all animals, the contextual theory (Fanselow, 1986) contends that the defense system is only activated in delayed shock animals. Analysis of the behaviour during the 20 s post shock in Experiments 1 - 3 revealed that all shocked rats exhibited a pattern of initial activity similar to what Fanselow (1982) observed during the same interval. In the present experiments, it was revealed that freezing during this period was observed in every animal within 3 s after the startle, suggesting that freezing may be the unconditioned reaction to shock termination. This finding is consistent with both the topographical and activity theories but not with the contextual account of the ISD and is evidence that immediately shocked animals, in addition to the delayed shock animals, are fearful after shock.

In addition, it was observed that the ubiquitous reaction to shock onset was vigorous startle activity and that the reaction to shock termination was freezing. There are two reasons why freezing might be expected as a universal response to foot-shock. First, given that foot-shock has been characterized as a valid means of mimicking a predator (Fanselow & Lester, 1988) and that predatory threat evokes freezing, it is logical to assume that foot-shock should also evoke freezing. Second, freezing is consistently the first response to unnatural aversive stimuli such as prod-shock (Moser & Tait, 1983; Pinel et al., 1980). Given that foot-shock applied to all four feet simultaneously is a diffuse stimulus, and that more freezing occurs in response to unlocalizable stimuli (Bevins & Ayres, 1991), freezing should be expected as a response to foot-shock.

A possible interpretation of the startle-freeze behaviour set observed as the initial reactions to shock is that this response combination is an example of a biphasic reaction to a US. The sometimes-opponent-process model (Wagner, 1981) and the affective extension of this model (Wagner & Brandon, 1989) maintain that shock is processed in two phases. In the former model, it is assumed that in some cases a CR will be opposite to the UR. Therefore, applying this model to shock it would be predicted that the primary reaction to shock would be activation (e.g. startle) which would then gradually decay to an inactive state (e.g. freeze). The extended version of this model assumes that there is an affective component to the processing of aversive stimuli such that the initial reaction is related to sensory properties of the US and the second reaction is related to emotional properties of the US. Applying this model to the behaviours observed initially after shock in the present studies the startle could be interpreted as a reaction to the pain of shock and the freeze could be interpreted as a reaction to the fear associated with the context in which shock occurred.

In summary, the present results strongly suggest that foot-shock does evoke the defense system of rats and that freezing is one of the initial post-shock reactions. The startle-freeze behaviour set is observed in both immediate and delayed shock animals.

Foot-shock-Induced Activity and Freezing

Initial post-shock freezing has been observed to be replaced by foot-shock induced activity (Pinel, Corcoran, & Malsbury, 1971; Pinel & Mucha, 1973a, 1973b). In Experiments 1 - 3, analysis of exploratory behaviours revealed that freezing replaced ongoing behaviours of sniff and rear in animals given a delayed shock more often than those given an immediate shock. All shocked animals, however, locomoted less than rats not shocked. These findings suggest that foot-shock significantly suppresses ongoing locomotion but that the initial suppressive effects of the shock are less in immediately shocked animals than in delayed shock animals. The suppression of locomotion was particularly evident in those animals which were shocked contingent upon their locomoting (Experiments 4 and 5). Conversely, locomotion was heightened by the delivery of shock contingent upon the animal's being still. Therefore, delivering shock contingent upon a particular behaviour has the effect of suppressing that behaviour.

This second phase of activity has been referred to as the defensive stage of aversively learned behaviour (Bolles & Fanselow, 1980). It is during this phase that the activity theory is most relevant to predicting the behaviour of the rat. A discussion of the topography of behaviours observed in Experiments 2 and 3 follows as evidence in support of the first hypothesis of the activity theory that shock regardless of time of delivery evokes defensive behaviour.

The Influence of an Exit. Experiment 2 provided evidence that escape is likely to occur after the initial period of post-shock freezing subsides. That is, 50% of the rats escaped during the initial 20 s post-shock but none of the rats escaped without first freezing briefly during this period. Two important findings relevant to the topographical theory of the ISD (Blanchard et al., 1976a, 1976b) were revealed in this experiment. First, most animals, regardless of when shock was delivered, escaped from the shock context if given the opportunity. As mentioned previously, freezing occurred as the initial response to shock; escape, therefore, was delayed. This finding is inconsistent with the topographical theory. Furthermore, the finding that the delayed shock group unfamiliar with the exit escaped from the shock chamber is inconsistent with the topographical theory of the ISD because the theory assumes that these animals, having had time to explore when the door was closed, do not realize that an exit is now present. The fact that most animals, including immediate shock rats, escaped the shock chamber is also incompatible with the contextual theory (Fanselow, 1986) because it is assumed that the defensive system in these animals is not evoked, so, escape from a fearful context is not a likely behaviour.

These findings further support the notion that the defense system is activated by shock in all animals. This interpretation assumes that escape and freeze are valid indicators of fear (Blanchard et al., 1974; Lorenzini et al., 1990). The equal levels of defecation in the groups in Experiments 4 and 5 further supports the conclusion that all animals were fearful after shock.

The typical post-shock sequence of behaviour consisted of freezing in the shock box, escaping to the mock home chamber, and subsequent freezing in this second box. Similar behaviour patterns have been observed in response to a prod shock (Moser & Tait, 1983) and in situations involving confrontation with a predator (Blanchard et al., 1986; Blanchard & Blanchard, 1971).

In Experiment 2, the latency to escape was determined by the rat's proximity to the exit after startle, and its familiarity with the exit. Those animals which were closest to, and unfamiliar with, the exit escaped the quickest. If the animal did not escape rapidly, it was observed to continue freezing in the shock box. This finding is important because it supports the view outlined in the introduction that a rat will be hesitant to move its feet after shock, even though escape is possible.

In summary, the sequela of post-shock behaviours involving freezing and escape has generally been considered to occur in response to a discriminable stimulus and the present results extend this finding to include foot-shock. Furthermore, these results were observed in both delayed and immediate shock rats. Such results are inconsistent with both the topographical and the contextual theories of the ISD because all animals exited the shock box. The topographical theory would not have predicted the delay shock animals to have exited. Further, the contextual theory would not have predicted the immediately shocked animals to have exited.

The Influence of Handling. The results of Experiment 3 demonstrated that post-shock freezing was attenuated by handling an animal just before it received a delayed shock. This finding is consistent with the hypothesis that handling serves as an explicit cue which becomes associated with shock and reduces the associative strength of the context. Therefore, the results of Experiment 2, on the exit, and Experiment 3, on handling, strongly suggest that post-shock behaviour can be modified either by allowing the animal to escape from the shock chamber or by providing an explicit cue that reduces the associative strength of the context. These findings are inconsistent with the contextual theory (Fanselow, 1986) which is based on a differential level of fear between immediately and delayed shock animals but are consistent with the activity theory which is based on all animals being fearful after shock. These findings are consistent with both the activity approach and the topographical theory (Blanchard et al., 1976a, 1976b) in that both theories assume that the environment will determine which defensive behaviour will occur after shock.

The next section describes how the results of the experiments conducted here support the hypothesis that post-shock behaviour can be manipulated by shocking the animal contingent upon its behaviour.

The Influence of Behaviour-Contingent Shock. The most convincing evidence in support of the activity theory was provided by Experiments 4 and 5 where shock was delivered contingent on behaviour. By shocking a still animal it was possible to produce a large reduction in freezing, relative to shocking an animal while it was locomoting. Related differences between the STILL and LOCO groups were evident in the greater percentage of active behaviours (e.g., locomote) observed in the STILL groups. The duration of locomote in the STILL groups does not appear to be different from that observed in the NO-SHK group in Experiment 1. Collectively, these results suggest that shock delivered contingent on behaviour suppresses that behaviour for some period of time following the shock.

Of particular importance for the activity theory is the independence of this effect from the time of shock delivery (Experiment 5). That is, it was possible to entirely eliminate the freeze deficit in immediate shock animals by presenting shock contingent on movement. Conversely, it was possible to produce a reduction in freezing in delayed shock animals by presenting shock contingent on nonmovement. This reduced freezing was similar to the duration of freezing observed after immediate shock. These findings are important because they are contradictory to both the contextual (Fanselow, 1986) and the topographical (Blanchard et al., 1976a, 1976b) theories. They are inconsistent with the contextual account because freezing occurred in immediate shock animals which are presumed by Fanselow (1986) not to be fearful. The freezing in immediate shock animals is also inconsistent with the topographical account because it is assumed that these animals engage in fleeing, and not freezing, as the defensive behaviour in response to shock.

There are some reports that behaviour at the time of predatory threat can influence subsequent defensive behaviour. Fentress (1968a, 1968b) demonstrated that a vole reacts to a moving overhead cloth differently according to its behaviour at the time when the stimulus was presented. Those animals that were walking at the time of the stimulus fled from the stimulus before freezing; those animals that were grooming when the stimulus was presented, immediately and persistently froze. Given that the animal was stationary as it groomed, Fentress concluded that behaviour at the time of stimulus presentation was potentiated upon termination of the stimulus.

Forrester and Broom (1980) have extended Fentress' (1968a, 1968b) findings to include chicks. These investigators found that the behaviour of a chick was potentiated in response to illumination of a bulb. They observed that if a bird was immobile at the time of stimulus presentation, then it remained in a crouched position for most of the trial. Conversely, if a bird was active when the light was turned on, it subsequently spent less time crouching than those birds which were in the still condition. Culshaw and Broom (1980) observed that this behaviour suppression was more apparent in chicks startled near the end of a session than those startled at the beginning of a session. These investigators attributed the difference between the groups to the magnitude of the startle response. Because the adapted animals were more likely to be startled by the light than their unhabituated counterparts, the ongoing behaviour of these animals was more likely to have been suppressed. These results suggest that ongoing behaviour can be easily suppressed by presenting a brief overhead stressor to an animal.

The findings of the above studies appear to be contradictory to the activity theory which states that shock will subsequently attenuate the behaviour occurring at the time of shock. However, there are two distinctive features related to the nature of the stimulus that may explain the disagreement. First, the aversive stimulus was presented differently in the reported studies than was the case in the present studies. That is, the overhead cloth and the lightbulb were examples of a stimulus presented above the animal. In particular, one study demonstrated that presenting a threatening stimulus above the animal will produce more freezing than will providing the stimulus from below (Westby, Keay, Redgrave, Dean, & Bannister, 1990). Second, the cloth and the lightbulb were stimuli that were easily localized, as suggested by the fact that all animals in these studies oriented to the stimulus. Foot-shock, however, is a diffuse stimulus that occurs beneath the animal. As discussed in Experiment 4, the behavioural topography of rats differs in response to the localizability of the stimulus (see also, Bevins & Ayres, 1991; Pinel & Treit, 1978). Thus, it may be that suppression of ongoing behaviour is a characteristic reaction to stimuli that are not readily localizable. Whereas localizable stimuli signalling an aversive event potentiate ongoing behaviour.

In summary, the present results of Day 1 testing confirmed that post-shock behaviour can be both predicted and altered based on pre-shock factors. The results of these experiments demonstrated that the duration of post-shock freezing is modifiable either by changes in the shock context, or by making the delivery of shock contingent on the animal's behaviour. That is, it is possible to accurately predict the duration of post-shock freezing based on the animal's behaviour at the time of shock and its startle magnitude in reaction to the shock. Such results provide evidence for the activity theory and against both the topographical and contextual theories of the ISD.

Spatial Conditioning Effects on Day 2

The results observed on Day 2 of the present experiments are particularly relevant to the contextual theory of the ISD. This is because Day 2 testing is not confounded by the direct effects of the shock, as on Day 1. Thus, Day 2 testing allows for the specific examination of spatial contextual conditioning.

Effects of Context. The effects of spatial conditioning were apparent in Experiment 1 when the animal was tested in a simulated home cage that was dissimilar to the shock box. The present results demonstrated that information can be learned after a single US exposure and in the absence of explicit conditioning cues or strong contextual cues.

The equivalent duration of freezing exhibited by all groups in the first minute of the trial provides evidence that immediate shock animals do not suffer a conditioning deficit. Rather, it appears that the assessment of freezing over the standard 5 min length of the Day 2 session is too long to adequately assess conditioning in immediate shocked animals.

Time of Shock. The influence of the time of shock on freezing was evident in Day 2 of Experiment 5, in that the 15 min delayed shock group did not freeze until the last half of the trial. The lack of freezing in the first 10 min of the trial is consistent with the results reported by Westbrook et al. (1994). These investigators observed that virtually no freezing was observed during the 2 min trial of Day 2 in animals shocked 17 min after entry on Day 1. Although these authors concluded that the associability of the context was reduced in these animals, the present results suggest that had the session been long enough, freezing would have been observed. That is, freezing on Day 2 is expected to be observed within a specific time range that corresponds to the time of shock on Day 1. Although the ISD is a temporally-based empirical effect, such results have not been previously reported in the ISD literature.

Experiment 2 revealed that placing an animal in a previously escapable environment on Day 2 produced substantial freezing. Excessive freezing was observed in all groups, including immediate shock animals. This is of particular importance to the contextual associative account because most animals spent relatively little time in the shock box. Given that animals in the IMM-EXIT group spent less time in the shock chamber than immediate control animals, Fanselow (1986) would predict virtually no freezing in this group. The fact the opposite effect occurred in immediate shock rats, that is, substantially more freezing on Day 2 than on Day 1, cannot be explained by a contextual association theory.

It is apparent that restricting access to only the shock chamber on Day 2 produced decreased activity in animals previously allowed to escape. This procedure is the reverse of the learned helplessness procedure where a performance deficit is observed when a previously restrained animal is subsequently free to escape (Maier, 1990, 1991). Learned helplessness is thought to be mediated by increased levels of fear and occurs after repeated inescapable exposures to stress (Maier, 1990). The present finding was apparent after a single shock exposure, suggesting that fear can be readily induced.

Collectively, these findings fail to support the contextual account (Fanselow, 1986) of the ISD as the primary mechanism responsible for freezing on Day 2.

The Incubation Effect

The long duration of freezing observed at the beginning of the trial on Day 2 in those animals given an immediate shock on Day 1 of the present experiments may be an instance of the incubation effect (Pinel, 1970). The basic paradigm used to demonstrate the incubation effect involves an initial 2 min pre-exposure to the shock chamber followed 24 hrs later by an immediate 5 sec, 1 mA shock. Following the shock, the animal is promptly returned home and placed into the box some time later. During this re-exposure, the number of line crossings are counted for 2 min as a measure of activity. The incubation effect is the gradual increase in suppression of movement in animals re-exposed to the chamber at increasing intervals between the shock session and the re-exposure session (Pinel, 1970; Pinel, Corcoran, & Malsbury, 1971; Pinel & Mucha, 1973a, 1973b). For example, Pinel (1970) demonstrated that activity was lower at shock-test intervals of 2 hrs, 12 hrs, and 24 hrs than at intervals of 1 min and 30 min. Thus, the tendency for a rat to be immobile in an apparatus where it was previously given an immediate shock increases as the interval between shock and time of replacement increases. Given that in the current experiments Day 2 testing occurred 24 hrs after shock, the present result of increased freezing of immediately shocked rats on Day 2 testing relative to Day 1 may be an instance of the incubation effect. However, this hypothesis would require additional testing by varying the shock-test time in the current ISD paradigm.

Ethological Implications of Activity Theory

In addition to providing evidence for the activity theory of the ISD, the present results are also relevant to an ethological assessment of freezing. In this section, implications arising from the current results will be discussed within the predatory imminence and the ethoexperimental theoretical framework.

Predatory Imminence Theory. As mentioned previously, Fanselow and Lester (1988) have described the use of foot-shock as a valid means of evoking predatory threat. Specifically, foot-shock is thought to be roughly equivalent to detection of a predator. The continuum of defensive behaviours defined in this model are, in order of increasing predatory threat, "pre-encounter", "post-encounter", and "circa-strike". Predator detection is considered to evoke post-encounter defensive responses, such as freezing. According to this theory, post-encounter defensive behaviours eventually give way to preferred patterns of activity, such as foraging. Therefore, a typical sequence of post-shock behaviour predicted by this theory could consist of freezing followed by an increasing range of normal movements.

However, in the present experiments, the immediate reaction to shock was consistently observed to be a burst of activity (see also, Fanselow & Bolles, 1979; Kiernan & Cranney, 1992). According to the imminence theory, such startle activity is congruent with circa-strike defensive behaviour. Furthermore, the present studies revealed that freezing always occurred after startle. If possible, escape then ensued. Stretched attention postures were also evident in most animals between changes in behaviour, particularly after the animal had escaped. Therefore, a typical post-shock behaviour sequence, if escape was allowed, consisted of: startle (activity burst), freeze (during the 20 s period), escape, freeze (during the 5 min trial), stretched attention, and normal movement. Such a pattern of activity according to terminology of the predatory imminence theory would be described as: circa-strike (activity burst), post-encounter (freeze, and/or flee, stretched attention), and preferred activity (normal movement). In inescapable situations, this sequence was similar except that escape was not observed. This pattern of behaviour is congruent with a pattern of circa-strike activity (Fanselow & Lester, 1988).

It is argued in this paper that foot-shock produces a pattern of behaviour consistent with predatory capture (circa-strike defense) and not predatory detection (post-encounter defense) as described by Fanselow and Lester (1988). Congruent with a circa-strike model, the first reaction to capture is startle. Freezing immediately follows as a recuperative behaviour from the pain of the shock and subsequently as a strategy to assess the potential degree of further threat. Logically, this is reasonable given that foot-shock is a painful, yet poorly localized, stimulus. Fleeing, if possible, would then occur as a means to escape the threat followed by ensuing freeze as the animal continues to recuperate from the shock. Stretched attention responses would occur as a strategy to gain information regarding the shock source (Pinel et al., 1986; Pinel et al., 1989) or simply as a transition behaviour between defensive behaviours (Moser & Tait, 1983; Blanchard & Blanchard, 1987). Finally, the animal would return to exploration as the preferred activity.

The activity theory of post-shock behaviour is also consistent with a circa-strike model of defense. As discussed previously, shock activates a rat's defense system regardless of the familiarity of the surroundings. However, the experiments discussed here demonstrated that an animal will spend less time freezing if shock is delivered when it is still compared to when it is moving. It is argued that this difference relates to the animal's strategy to reduce the possibility of future predatory strike. For example, an animal shocked while it is still will freeze briefly as a recuperative response to the pain of the shock but will not freeze thereafter. Facilitation of movement, therefore, is an avoidance response that reduces future predatory threat. Similarly, an animal shocked while it is moving will freeze as both an initial recuperative response and as an avoidance response of a potential recurring strike. The two tendencies summate in a final common behavioural output to produce a longer duration of freezing in the animals shocked while moving than in the animals shocked while still. That is, suppression of movement would serve as an avoidance response to future threat. Therefore, increased freezing and locomotion are both equally important to the ultimate survival of the animal. Such a pattern of activity would be expected to occur in natural settings.

In summary, behaviour induced by foot-shock is consonant with a circa-strike pattern of defensive behaviour as proposed by Fanselow and Lester (1988). It is proposed that freezing is both a recuperative defensive behaviour in response to the immediate pain of shock and an avoidance response to the potential danger of future predatory strike.

Ethoexperimental Approach. The present findings also have implications for the ethoexperimental study of defensive behaviour proposed by Blanchard and Blanchard (1987, 1989). Investigators using this approach have typically observed the laboratory rat under conditions of predatory threat, such as the appearance of a cat. Foot-shock has not been generally used as a stressor in this type of study because it is thought that freezing will occur as the single most dominant post-shock response (Blanchard et al., 1986). Furthermore, predatory defense is thought to only occur in response to highly localizable stimuli. The present experiments, however, suggest that foot-shock (a poorly localized stimulus) is a valid elicitor of a range of defensive behaviours that are similar to that evoked by predatory threat, regardless of the time of shock delivery.

According to the ethoexperimental approach of studying defensive behaviour (Blanchard et al., 1986; Blanchard & Blanchard, 1987, 1989), the prepotent defensive behaviour observed is a joint function of the localizability of the threatening stimulus and the predator-prey distance. In order to evoke the defense system, the stimulus must be highly discriminable. Once the system is active, the perceived predator-prey distance determines which defensive behaviour will be observed. As the predator-prey distance approaches zero, the rat orients to the predator and physically readies itself to attack. If contact with the predator follows, the rat will jump toward and bite its attacker. The rat will then flee, if possible, or freeze. A period of reassessment follows, where the animal attempts to assess the risk of predatory threat. In time, the animal returns to a nondefensive pattern of behaviour. It is argued in this paper that a similar pattern of behaviour is observed in response to both a foot-shock and a predatory threat. This implies that the sequence of defensive responses is not entirely dependent on the proximity of a predator but may be generated by the prey itself as a post-strike cascade of behaviour.

In summary, foot-shock appears to evoke a similar pattern of defensive behaviour to that elicited by predatory encounter. From an ethological viewpoint of defense, the use of a readily controllable, unnatural stimulus to evoke defensive behaviours is important to the future empirical investigation of animal defense systems in the laboratory.

REFERENCES

Anisman, H., deCatanzaro, D., & Remington, G. (1978). Escape performance following exposure to inescapable shock: Deficits in motor response maintenance. Journal of Experimental Psychology: Animal Behavior Processes, 4, 197-235.

Baker, A. G., Singh. M., & Bindra, D. (1985). Some effects of contextual conditioning and US predictability on Pavlovian conditioning. In P. D. Balsam & A. Tomie (Eds.), Context and Learning (pp. 73-104). Hillsdale, NJ: Erlbaum.

Baron, A. (1963). Differential effects of fear on activity in novel and familiar environments. Psychological Reports, 13, 251-257.

Beck, C. H. M., & Chow, H. L. (1984). Solitary and social behavior of male rats in the open-field. Physiology & Behavior, 32, 941-944.

Bevins, R. A., & Ayres, J. L. (1992). Behaviour Research and Therapy, 30, 551-554.

Blanchard, R. J., & Blanchard, D. C. (1969a). Crouching as an index of fear. Journal of

Comparative and Physiological Psychology, 67, 370-375.

Blanchard, R. J., & Blanchard, D. C. (1969b). Passive and active avoidance reactions to fear-eliciting stimuli. Journal of Comparative and Physiological Psychology, 68, 129-135.

Blanchard, R. J., & Blanchard, D. C. (1971). Defensive reactions in the albino rat. Learning and Motivation, 2, 351-362.

Blanchard, R. J., & Blanchard, D. C. (1987). An ethoexperimental approach to the study of fear. The Psychological Record, 37, 305-316.

Blanchard, R. J., & Blanchard, D. C. (1989). Antipredator defensive behaviors in a visible burrow system. Journal of Comparative Psychology, 103, 70-82.

Blanchard, R. J., Blanchard, D. C., & Weiss, S. M. (1990). Ethanol effects in an Anxiety/Defense Test Battery. Alcohol, 7, 375-381.

Blanchard, R. J., Blanchard, D. C., Rodgers, J., & Weiss, S. M. (1990). The characterization and modelling of antipredator defensive behavior. Neuroscience and Biobehavioral Reviews, 14, 463-472.

Blanchard, R. J., Dielman, T. E., & Blanchard, D. C. (1968). Postshock crouching: Familiarity with the shock situation. Psychonomic Science, 10, 371-372.

Blanchard, R. J., Flannelly, K. J., & Blanchard, D. C. (1986). Defensive behaviors of laboratory and wild *Rattus norvegicus*. Journal of Comparative Psychology, 100, 101-107.

Blanchard, R. J., Fukunaga, K. K., & Blanchard, D. C. (1976a). Environmental control of defensive reactions to a cat. Bulletin of the Psychonomic Society, 8, 179-181.

Blanchard, R. J., Fukunaga, K. K., & Blanchard, D. C. (1976b). Environmental control of defensive reactions to footshock. Bulletin of the Psychonomic Society, 8, 129-130.

Blanchard, R. J., Kelley, M. J., & Blanchard, D. C. (1974). Defensive reactions and exploratory behavior in rats. Journal of Comparative and Physiological Psychology, 6, 1129-1133.

Blanchard, R. J., Kleinschmidt, C. F., Fukunaga-Stinson, C., & Blanchard, D. C. (1980).

Defensive attack behavior in male and female rats. Animal Learning & Behavior, 8, 177-183.

Blanchard, R. J., Magee, L. K., Veniegas, R., & Blanchard, D. C. (1993). Alcohol and anxiety: Ethopharmacology approaches. Progress in Neuro-psychopharmacology and Biological Psychiatry, 17, 171-182.

Blanchard, R. J., Mast, M., & Blanchard, D. C. (1975). Stimulus control of defensive reactions in the albino rat. Journal of Comparative and Physiological Psychology, 88, 81-88.

Bolles, R. C. (1969). Avoidance and escape learning: Simultaneous acquisition of different responses. Journal of Comparative and Physiological Psychology, 68, 355-358.

Bolles, R. C. (1970). Species-specific defensive reactions and avoidance learning. Psychological Review, 71, 32-48.

Bolles, R. C. (1972). Reinforcement, expectancy, and learning. Psychological Review, 79, 394-409.

Bolles, R. C. (1975). Theory of Motivation (2nd edition). New York, NY: Harper & Row.

Bolles, R. C., & Collier, A. C. (1976). Effect of predictive cues of freezing in rats. Animal Learning and Behavior, 4, 6-8.

Bolles, R. C., & Fanselow, M. S. (1980). A perceptual-defensive-recuperative model of fear and pain. Behavioral and Brain Sciences, 3, 291-301.

Bolles, R. C., & Riley, A. L. (1973). Freezing as an avoidance response: Another look at the operant-respondent distinction. Learning and Motivation, 4, 268-275.

Bouton, M. E., & Bolles, R. C. (1980). Conditioned fear assessed by freezing and by the

suppression of three different baselines. Animal Learning & Behavior, 8, 429-434.

Bressers, M., Meelis, E., Haccou, P., & Kruk, M. (1991). When did it really start or stop: the impact of censored observations on the analysis of duration. Behavioural Processes, 23, 1-20.

Brewster, J., & Leon, M. (1980). Relocation of the site of mother-young contact: Maternal transport behaviour in Norway rats. Journal of Comparative and Physiological Psychology, 94, 69-79.

Broom, D. M. (1980). Activity rhythms and position preferences of domestic chicks which can see a moving object. Animal Behavior, 28, 201-211.

Brudzynski, S. M., & Ociepa, D. (1992). Ultrasonic vocalization of laboratory rats in response to handling and touch. Physiology & Behavior, 52, 655-660.

Church, R. M. (1989). Theories of timing behavior. In S. B. Klein & R. R. Mowrer (Eds.), Contemporary learning theories: Instrumental conditioning theory and the impact of biological constraints on learning (pp. 41-71). Hillsdale, NJ: Erlbaum.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences (2nd edition). Hillsdale, NJ: Lawrence Erlbaum Associates Inc.

Conti, L. H., Maciver. C. R., Ferkany, J. W., & Abreu, M. E. (1990). Footshock-induced freezing behavior in rats as a model for assessing anxiolytics. Psychopharmacology, 102, 492-497.

Cranney, J. (1987). Startle responding and context conditioning: Naloxone pretreatment and stimulus intensity. Pavlovian Journal of Biological Sciences, 22, 47-51.

Crawford, M. & Masterson. F. A. (1982). Species-specific defense reactions and avoidance learning: An evaluative review. Pavlovian Journal of Biological Sciences, 17, 204-214.

Crawford, M., Masterson. F. A., Thomas, L. A., & Ellerbrock, G. (1981). Defensive behavior and passive avoidance learning in rats and gerbils. Bulletin of the Psychonomic Society, 18, 121-124.

Davis, M., & Astrachan. D. I. (1978). Conditioned fear and startle magnitude: Effects of different footshock or backshock intensities used in training. Journal of Experimental Psychology: Animal Behavior Processes, 4, 95-103.

Domjan, M., & Galef. B. G. (1983). Biological constraints on instrumental and classical

conditioning: Retrospect and prospect. Animal Learning & Behavior, 11, 151-161.

Dunham, P. J. (1971). Punishment: method and theory. Psychological Review, 78, 58-70.

Edmunds, M. (1974). Defense in animals. Longman, NY: McGraw-Hill .

Espejo, E. F., & Mir. D. (1993). Structure of the rat's behaviour in the hot plate test. Behavioural Brain Research, 56, 171-176.

Evans, H. L. (1988). Quantitation of naturalistic behaviors. Toxicology Letters, 43, 345-359.

Falls, W. A., & Davis, M. (1994). Fear-potentiated startle using three conditioned stimulus modalities. Animal Learning & Behavior, 22, 379-383.

Fanselow, M. S. (1980). Conditional and unconditional components of post-shock freezing. Pavlovian Journal of Biological Science, 15, 177-182.

Fanselow, M. S. (1982). The postshock activity burst. Animal Learning & Behavior, 10, 448-454.

Fanselow, M. S. (1986). Associative vs. topographical accounts of the immediate shock-freezing deficit in rats: Implications for the response selection rules governing species-specific defensive reactions. Learning and Motivation, 17, 16-39.

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

Fanselow, M. S., & Bolles, R. C. (1979). Naloxone and shock-elicited freezing in the rat. Journal of Comparative and Physiological Psychology, 93, 736-744.

Fanselow, M. S., DeCola, J. P., & Young, S. L. (1993). Mechanisms responsible for reduced contextual conditioning with massed unsignaled unconditional stimuli. Journal of Experimental Psychology: Animal Behavior Processes, 19, 121-137.

Fanselow, M. S., & Helmstetter, F. J. (1988). Conditional analgesia, defensive freezing, and benzodiazepines. Behavioral Neuroscience, 102, 233-243.

Fanselow, M. S., Landeira-Fernandez, J., DeCola, J. P., & Kim, J. J. (1994). The immediate-shock deficit and post-shock analgesia: Implications for the relationship between the analgesic CR and UR. Animal Learning & Behavior, 22, 72-76.

Fanselow, M. S., & Lester, L. S. (1988). A functional behavioristic approach to aversively motivated behavior: Predatory imminence as a determinant of the topography of defensive behavior. In R. C. Bolles & M. D. Beecher (Eds.), Evolution and Learning. Hillsdale, NJ: Erlbaum.

Fanselow, M. S., & Sigmundi, R. A. (1987). Functional behaviorism and aversively motivated behavior: A role for endogenous opioids in the defensive behavior of the rat. The Psychological Record, 37, 317-334.

Fentress, J. C. (1968a). Interrupted ongoing behavior in two species of vole (*Microtus agestis*) and (*Clethrionomys britannicus*). I. Response as a function of preceding activity and the "context" of an apparently irrelevant motor pattern. Animal Behavior, 16, 135-153.

Fentress, J. C. (1968b). Interrupted ongoing behavior in two species of vole (*Microtus agestis*) and (*Clethrionomys britannicus*). II. Extended analysis of motivational variables underlying fleeing and grooming behavior. Animal Behavior, 16, 154-167.

File, S. E., & Vellucci, S. V. (1979). Behavioral and biochemical measures of stress in hooded rats from different sources. Physiology & Behavior, 22, 31-35.

Forrester, R. C., & Broom, D. M. (1980). Ongoing behaviour and startle responses in chicks. Behaviour, 73, 51-63.

Gallistel, C. R. (1990). The organization of learning. Cambridge, MA: The MIT Press.

Gibbon, J. (1977). Scalar expectancy theory and Weber's law in animal timing.

Psychological Review, 84, 279-325.

Greenhouse, B., & Geisser, R. (1959). Psychometrika, 24, 95-112.

Grossen, N. W., & Kelley, M. J. (1972). Species-specific behavior and acquisition of avoidance behavior in rats. Journal of Comparative and Physiological Psychology, 81, 307-310.

Hall, C. S. (1938). Emotional behavior in the rat. III. The relationship between emotionality and ambulatory activity. Journal of Comparative Psychology, 22, 345-352.

Hammond, L. J. (1995). Pavlovian conditioning to contexts that precede the place where shock occurs as measured by freezing and activity suppression in mice. Animal Learning & Behavior, 23, 349-356.

Harrington, G. M. (1981). The Har strains off rats: Origins and characteristics. Behavior Genetics, 11, 445-468.

Heynen, A. J., Sainsbury, R. S., & Montoya, C. P. (1989). Cross-species responses in the defensive burying paradigm: A comparison between Long-Evans rats (*Rattus norvegicus*), Richardson's ground squirrels (*Spermophilus richardsonii*), and Thirteen-Lined ground squirrels (*Catellus tridecemlineatus*). Journal of Comparative Psychology, 103, 184-190.

Jackson, N. W., & Elwood, R. W. (1990). Interrupting an assessment process to probe changes in the motivational state. Animal Behavior, 39, 1068-1077.

Kiernan, M., & Cranney, J. (1992). Immediate-startle stimulus presentation fails to condition freezing responses to contextual cues. Behavioral Neuroscience, 106, 121-124.

Kiernan, M. J., & Westbrook, R. F. (1993). Effects of exposure to a to-be-shocked environment upon the rat's freezing response: Evidence for facilitation, latent inhibition, and perceptual learning. The Quarterly Journal of Experimental Psychology, 46B, 271-288.

Kiernan, M. J., Westbrook, R. F., & Cranney, J. (1995). Immediate shock, passive avoidance, and potentiated startle: Implications for the unconditioned response to shock. Animal Learning & Behavior, 23, 22-30.

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

Landeira-Fernandez, J., Fanselow, M. S., DeCola, J. P., & Kim, J. J. (1995). Effects of handling and context preexposure on the immediate shock deficit. Animal Learning & Behavior, 23, 335-339.

Leaton, R. N., & Cranney, J. (1990). Potentiation of the acoustic startle response by a conditioned stimulus paired with acoustic startle stimulus in rats. Journal of Experimental Psychology: Animal Behavior Processes, 16, 279-287.

Lester, L. S., & Fanselow, M. S. (1992). Nocturnality as a defensive behavior in the rat: An analysis in terms of selective association between light and aversive stimulation. The Psychological Record, 42, 221-253.

Lorenzini, C. A., Bucherelli, C., Giachetti, A., & Tassoni, G. (1990). Inhibition of exploratory behavior in the rat by handling. Animal Learning & Behavior, 18, 191-198.

Mackintosh, N. J. (1983). Conditioning and instrumental conditioning. Oxford:

Clarendon Press.

Maes, R. J. H., & Vossen. J. M. H. (1992). One-trial aversive conditioning to contextual cues: Effects of time of shock presentation on freezing during conditioning and testing. Bulletin of the Psychonomic Society, 30, 403-406.

Maier, S. E., Vandenhoff, P., & Crowne, D. P. (1988). Multivariate analysis of putative measures of activity. exploration, emotionality, and spatial behaviour in the Hooded rat (*Rattus norvegicus*. Journal of Comparative Psychology, 102, 378-387.

Maier, S. F. (1990). Role of fear in mediating shuttle escape learning deficit produced by inescapable shock. Journal of Experimental Psychology: Animal Behavior Processes, 16, 137-149.

Maier, S. F. (1991). Stressor controllability, cognition and fear. In J. Madden (Ed.),

Neurobiology of Learning, Emotion and Affect. New York, NY: Raven Press.

Mast, M., Blanchard. R. J., & Blanchard, D. C. (1982). The relationship of freezing and response suppression in a CER situation. The Psychological Record, 32, 151-167.

Masterson, F. A.. Crawford, M., & Bartter, W. (1978). Brief escape from a dangerous place: The role of reinforcement in the rat's one-way avoidance acquisition. Learning and Motivation, 9, 141-163.

Menescal-de-Oleveira, L., & Hoffmann, A. (1993). The parabrachial region as a possible region modulating simultaneously pain and tonic immobility. Behavioural Brain Research, 56, 127-132.

Mineka, S., Cook, M., & Miller, S. (1984). Fear conditioned with escapable and inescapable shock: Effects of a feedback stimulus. Journal of Experimental Psychology: Animal Behavior Processes, 10, 307-323.

Moser, C. G., & Tait, R. W. (1983). Environmental control of multiple defensive responses in a conditioned burying paradigm. Journal of Comparative Psychology, 97, 338-352.

Pare, W. P. (1964). Relationship of various behaviors in the open-field test of emotionality. Psychological Reports, 14, 19-22.

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

Pinel, J. P. J. (1970). Two types of ECS-produced disruption of one-trial training in the rat. Journal of Comparative and Physiological Psychology, 72, 272-277.

Pinel, J. P. J., Corcoran, M. E., & Malsbury, C. W. (1971). Incubation effect in rats: Decline of footshock-produced activation. Journal of Comparative and Physiological Psychology, 77, 271-276.

Pinel, J. P. J., Hoyer, E., & Terlecki, L. J. (1980). Defensive burying and approach-avoidance behavior in the rat. Bulletin of the Psychonomic Society, 16, 349-352.

Pinel, J. P. J., Mana, M. J., & Ward, J. A. (1989). Stretched approach sequences directed at a localized shock source by *Rattus norvegicus*. Journal of Comparative Psychology, 103, 140-148.

Pinel, J. P. J., & Mucha, R. F. (1973a). Activity and reactivity in rats at various intervals after footshock. Canadian Journal of Psychology, 27, 112-118.

Pinel, J. P. J., & Mucha, R. F. (1973b). Incubation and Kamin effects in the rat: Changes in activity and reactivity after footshock. Journal of Comparative and Physiological Psychology, 84, 661-668.

Pinel, J. P. J., Petrovic. D. M., & Jones, C. H. (1990). Defensive burying, nest relocation and pup transport in lactating female rats. The Quarterly Journal of Experimental Psychology, 42, 401-411.

Pinel, J. P. J., Symons. L. A., Christensen. B. K., & Tees, R. C. (1989). Development of defensive burying in *Rattus norvegicus*: Experience and defensive responses. Journal of Comparative Psychology, 103, 359-365.

Pinel, J. P. J., & Treit. D. (1978). Burying as a defensive response in rats. Journal of Comparative and Physiological Psychology, 8, 125-131.

Pinel, J. P. J., Treit. D., Ladak, F., & MacLennan, A. J. (1980). Conditioned defensive burying in rats free to escape. Animal Learning & Behavior, 8, 447-451.

Plappert, C. F., Pilz. P. K., & Schnitzler, H.-U. (1993). Acoustic startle response and habituation in freezing and nonfreezing rats. Behavioral Neuroscience, 107, 981-987.

Prescott, R. G. W. (1967). Diurnal activity cycles and intracranial self-stimulation in the rat. Journal of Comparative and Physiological Psychology, 64, 346-349.

Rodger, R. S., & Rosebrugh. R. D. (1979). Computing a grammar for sequence of behavioral acts. Animal Behavior, 27, 737-749.

Rudy, J. W. (1993). Contextual conditioning and auditory cue conditioning dissociate during development. Behavioral Neuroscience, 107, 887-892.

Shettleworth, S. J. (1978). Reinforcement and the organization of behavior in golden hamsters: Punishment of three action patterns. Learning and Motivation, 9, 99-123.

Shettleworth, S. J. (1993). Varieties of learning and memory in animals. Journal of Experimental Psychology: Animal Behavior Processes, 19, 5-14.

Spetch, M. L., Wilkie, D. M., & Pinel, J. P. J. (1981). Backward conditioning: A reevaluation of the empirical evidence. Psychological Bulletin, 89, 163-175.

Suarez, S. D., & Gallup, G. G. (1981). An ethological analysis of open-field behavior in rats and mice. Learning & Motivation, 12, 342-363.

Thomas, G. V., & Lieberman, D. A. (1990). Commentary: Determinants of success and failure in experiments on marking. Learning and Motivation, 21, 110-124.

Thomas, G. V., Lieberman, D. A., McIntosh, D. C., & Ronaldson, P. (1983). The role of marking when reward is delayed. Journal of Experimental Psychology: Animal Behavior Processes, 9, 401-411.

Urcuioli, P. J., & Kasprow, W. J. (1988). Long-delay learning in the T-maze: Effects of marking and delay-interval location. Learning and Motivation, 19, 66-86.

Van Hooff, J. A. R. A. M. (1982). Categories and sequences of behavior: methods of description and analysis. In K. R. Scherer & P. Ekman (Eds.), Handbook of Methods in Nonverbal Behavior Research (pp.362-439). Cambridge, MA: Cambridge

Press.

Wagner, A. R. (1981). SOP: A model of automatic memory processing in animal behavior. In N. E. Spear & R. R. Miller (Eds.), Information processing in animals: Memory mechanisms. Hillsdale, NJ: Erlbaum.

Wagner, A. R., & Brandon. S. E. (1989). Evolution of a structured connectionist model of Pavlovian conditioning (AESOP). In S. B. Klein & R. R. Mowrer (Eds.), Contemporary learning theories: Pavlovian conditioning and the status of traditional learning theory (pp. 149-190). Hillsdale, NJ: Erlbaum.

Wahlsten, D. (1991). Sample size to detect a planned contrast on a one degree-of-freedom interaction effect. Psychological Bulletin, 110, 587-595.

Wahlsten, D., Cole. M., & Sharp, D. (1968). Facilitation of bar-press avoidance by handling during the intertrial interval. Journal of Comparative and Physiological Psychology, 65, 170-175.

Wahlsten, D., & Sharp. D. (1969). Improvement of shuttle avoidance by handling during the intertrial interval. Journal of Comparative and Physiological Psychology, 67, 252-259.

Walsh, R. N., & Cummins. R. A. (1976). The open-field test: A critical review. Psychological Bulletin, 83, 482-504.

Westbrook, R. F., Good. A. J., & Kiernan, M. J. (1994). Effects of the interval between exposure to a novel environment and the occurrence of shock on the freezing responses of rats. The Quarterly Journal of Experimental Psychology, 47B, 427-446.

Westby, G. W. M., Keay. K. A., Redgrave, R., Dean, P., & Bannister. M. (1990).

Output pathways from the rat superior colliculus mediation approach and avoidance have different sensory properties. Experimental Brain Research, 12, 626-638.

Whimbey, A. E.. & Denenberg, V. H. (1967). Two independent behavioral dimensions in open-field performance. Journal of Comparative and Physiological Psychology, 63, 500-504.

Williams, J. L. (1987). Influence of conspecific stress odors and shock controllability on conditioned defensive burying. Animal Learning & Behavior, 15, 333-341.

Williams, J. L., & Lierle. D. M. (1986). Effects of stress controllability, immunization, and therapy on the subsequent defeat of colony intruders. Animal Learning & Behavior, 14, 305-314.

Wollnik, F. (1989). Physiology and regulation of biological rhythms in laboratory animals: an overview. Laboratory Animals, 23, 107-125.