In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of

PSYCHOLOGY

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date October 11, 1986
ABSTRACT

Ethanol blocks the seizures normally elicited in kindled rats by convulsive stimulation. Tolerance to this anticonvulsant effect rapidly develops following a series of ethanol injections delivered at 48-hr intervals only when convulsive stimulation is administered during the periods of intoxication. Subjects receiving ethanol 1 hr before convulsive stimulation demonstrate tolerance after just five tolerance-development trials, whereas there is little tolerance in subjects that receive ethanol 1 hr after each stimulation. Such tolerance is termed contingent tolerance because its development is contingent upon the occurrence of the criterion response (i.e., convulsive activity) during the periods of ethanol exposure.

The purpose of this thesis was to clarify the nature of contingent tolerance to ethanol's anticonvulsant effect. The first three experiments were designed to determine whether any tolerance at all develops in rats that do not receive stimulation during periods of intoxication. The fourth experiment was designed to determine whether the dissipation of tolerance to ethanol's anticonvulsant effect is influenced by the response contingency.

Experiment 1 tested the hypothesis that the development of tolerance in the ethanol-after condition might be
detectable if a smaller treatment dose than that previously studied was used. The subjects that were intubated with ethanol (either 2 g/kg or 5 g/kg) before stimulation on the five tolerance-development trials demonstrated substantial tolerance development, whereas there was little evidence of tolerance in the ethanol-after subjects.

The purpose of Experiment 2 was to determine whether the ethanol-after subjects would develop tolerance if more than the customary number of treatment trials were administered. Rats that received ethanol (2 g/kg, intubated) before each stimulation demonstrated significant tolerance after just 5 tolerance-development trials, whereas there was little evidence of tolerance in the ethanol-after condition even after 20 trials.

In Experiment 3, a sensitive multiple-trial test permitted the detection of tolerance to ethanol's anticonvulsant effect in the ethanol-after group. In the test phase, rats that had received 20 tolerance-development trials in which ethanol (1.5 g/kg, IP) was administered after each stimulation developed tolerance more quickly than rats that had received saline injections. However, even the results of Experiment 3 illustrate the importance of the response contingency in the development of tolerance to ethanol's anticonvulsant effect. The tolerance that developed in the ethanol-after rats was not apparent on the first test trial, and was detectable only as an acceleration
in the development of tolerance when ethanol was administered before stimulation in a series of test trials. In contrast, significant tolerance is typically detectable in the ethanol-before condition after only 5 trials.

In Experiment 4, the response contingency was shown to play a critical role in the dissipation of tolerance to ethanol's anticonvulsant effect. Tolerant rats that received no ethanol over a 14-day retention interval did not lose their tolerance if they were not stimulated during this period, whereas tolerant rats that continued to receive ethanol on the same bidaily schedule associated with tolerance development demonstrated a complete loss of tolerance if they were stimulated before, rather than during, the periods of intoxication. Accordingly, ethanol withdrawal was neither necessary nor sufficient for the dissipation of tolerance; the critical factor was the elicitation of seizures in the absence of ethanol.

Together, the results of these experiments provide unequivocal evidence of the important role that the response contingency plays in both the development and dissipation of tolerance to ethanol's anticonvulsant effect, and they clearly illustrate the inadequacy of two traditional assumptions about drug tolerance: 1) that the development of tolerance to a drug's effects is a sole function of the pattern of drug administration, and 2) that its dissipation is a sole function of drug withdrawal.
# TABLE OF CONTENTS

Abstract ........................................................................... ii  
Table of Contents ......................................................... v  
List of Figures ............................................................... viii  
Acknowledgement .......................................................... ix  

I. General Introduction .................................................... 1  
  1. Drug Tolerance ....................................................... 2  
     Context-Specific Tolerance ....................................... 6  
  2. Contingent Drug Tolerance ......................................... 10  
     The Concept ......................................................... 10  
     The Generality of Contingent Tolerance ..................... 14  
     i. Psychostimulants .................................................. 14  
     ii. Morphine ........................................................... 16  
     iii. Pentobarbital ..................................................... 17  
     iv. Delta-9-THC ...................................................... 17  
     v. Ethanol ............................................................... 18  
  3. Contingent Tolerance to Ethanol's Anticonvulsant Effect ... 20  
  4. General Rationale .................................................... 26  
  5. General Purpose ...................................................... 28  

II. General Background for Experiments 1, 2, and 3 ............. 30  

III. General Methodology .................................................. 38  
    Subjects .................................................................... 38  
    Surgical Procedure ................................................ 38  
    Kindling ................................................................. 38  
    Baseline ................................................................. 39  
    Treatment ............................................................... 40  
    Test ...................................................................... 41  
    Histology ............................................................... 42  
    Statistical Analysis ................................................ 42
LIST OF FIGURES

Figure 1.... Contingent Tolerance to Ethanol's Anticonvulsant Effect (Pinel 1983) .......... 23-24

Figure 2.... Experiment 1. Effect of Dose on the Detection of Tolerance in the Ethanol-After Group ...................... 47-48

Figure 3.... Histology From Experiment 1 ................. 49

Figure 4.... Experiment 2. Effect of Increasing the Number of Tolerance-Development Trials on the Detection of Tolerance in the Ethanol-After Group ...................... 54-55

Figure 5.... Histology From Experiment 2 ................. 56

Figure 6.... Histology From Experiment 3 ................. 60

Figure 7.... Experiment 4. The Effect of the Response Contingency on the Dissipation of Tolerance to Ethanol's Anticonvulsant Effect ............. 67-68

Figure 8.... Histology From Experiment 4 ................. 69
ACKNOWLEDGEMENTS

I thank Dr. John P. J. Pinel for his support and his confidence, and for his contributions in word and thought to this thesis. I thank Dr. W. J. Jacobs for his comments, and J. A. Ward, G. Renfrey, and C. K. Kim for their assistance and insights during the conduct of these experiments. I thank Dr. E. Eich and Dr. A. G. Phillips for their contributions to the final copy. And I thank Janet for making things easier.
I. GENERAL INTRODUCTION

Pinel, Colbourne, Sigalet, and Renfrey (1983) recently demonstrated that tolerance develops to ethanol's anticonvulsant effect on motor seizures elicited in kindled rats. The most interesting feature of their demonstration was that the tolerance developed only if convulsive stimulation was administered during the periods of ethanol exposure. Rats that were not stimulated during the tolerance-development phase of the experiment or that were stimulated prior to, rather than after, each of the five ethanol intubations constituting the tolerance-development phase demonstrated no tolerance whatsoever to the anticonvulsant effect of ethanol. In contrast, rats that received convulsive stimulation during each period of ethanol exposure demonstrated almost complete tolerance. Such tolerance, which is not the inevitable product of drug exposure but is contingent upon the repeated occurrence of the criterion response—convulsive activity, in this case—during the periods of drug exposure, has been termed contingent tolerance (Carlton & Wolgin, 1971).

The general purpose of the present experiments was to clarify the nature of contingent tolerance to ethanol's anticonvulsant effect. Accordingly, the first three sections of this General Introduction deal with: 1) drug tolerance in general, 2) contingent tolerance in general, and 3)
contingent tolerance to ethanol's anticonvulsant effect. The Introduction concludes with statements of: 4) the general rationale and 5) the general purposes of the thesis.

1. Drug Tolerance

Because it is an interesting example of biological adaptation (Cappell & LeBlanc, 1979) and because of its hypothetical relation to the phenomena of drug dependence, withdrawal, and abuse (Cappell & LeBlanc, 1979; Melmon, Gilman, & Mayer, 1980; Siegel & MacRae, 1984); tolerance is one of the most widely studied drug-related phenomena. Yet our understanding of tolerance remains at an elementary level.

Drug tolerance is a decrease in an effect of a drug that occurs as the result of previous exposure to it. There are two ways of measuring drug tolerance, either as a decrease in the effect elicited by a given drug dose or as an increase in the dose required to elicit a particular effect (Schuster, 1978). Although tolerance develops to the effects of many drugs, it does not develop to the effects of all drugs. And it does not necessarily develop to all of the effects of a particular drug; exposure to a particular drug may lead to the development of tolerance to some of its effects, while others may be unchanged or even increased in magnitude (e.g., Woolverton, Kandel, & Schuster, 1978).

Although the basic mechanisms responsible for the
development of drug tolerance are poorly understood, there are two general types of biological change to which tolerance is usually attributed: dispositional change or functional change. Dispositional change refers to any alteration in the absorption, distribution, breakdown, or clearance of a drug; whereas, functional change refers to any alteration in the sensitivity of the physiological systems affected by the drug (Jaffe, 1980; Kalant et al., 1971). Although attributing each instance of tolerance to either a dispositional or a functional mechanism appears to be a reasonable step in the analysis of a particular instance of tolerance, this approach has not proven to be particularly fruitful. In my view, there are two major reasons for this. First, each instance of drug tolerance is not necessarily either dispositional or functional; both types of change can contribute to a particular instance of tolerance (cf. Jaffe, 1980; Kalant, LeBlanc, & Gibbins, 1971; Krasnegor, 1978). Second, instances of functional tolerance are often identified only by the failure to identify an underlying dispositional change (cf. Dews, 1978; Lister, File, & Greenblatt, 1983; Mycek & Breznof, 1976). The reason for this reliance on "negative" evidence is that so many different types of physiological alterations are subsumed under the rubric of functional change that it is difficult to attribute a given instance of tolerance to any one of them. For example, functional change can refer to changes in the sensitivity or number of
neurotransmitter receptors (Rebec & Lee, 1983; Seeman, 1980); changes in the levels of neurotransmitters (Melchior & Tabakoff, 1981), neuromodulators (Vollicier & Ullman, 1985), or hormones (Wood, 1977; Tabakoff & Yanai, 1979); changes in cell membrane composition (Goldstein, 1983); changes in the activity of secondary messengers necessary for many neurotransmitters to have a postsynaptic effect (e.g., Siggins, 1979); or changes in ion conductance (Ross, Garrett, & Cardenas, 1979).

Another reason why researchers have had difficulty determining the mechanisms responsible for a given instance of tolerance—and the one most pertinent to the present experiments—is the fact that they have largely ignored the important role of the subject in the development of tolerance. Implicit in most discussions of tolerance is the assumption that the pattern of drug exposure is the preeminent factor in the development of tolerance, and the majority of the research in the area of tolerance reflects this assumption; most studies of tolerance have focused on the drug dose and on the route and schedule of drug administration. In a sense, the organism receiving the drug has been implicitly regarded as nothing more than a passive recipient of the drug, and the influence of the experiences of the subject during periods of drug exposure on the development of tolerance have thus been largely ignored.

In the last decade, however, it has become obvious that
any account of tolerance that does not acknowledge the importance of the subject's drug-related experiences is grossly inadequate. One of the most exciting recent advances in behavioral pharmacology has been the accumulation of incontrovertible evidence that the development and dissipation of many types of tolerance are often as dependent upon environmental and behavioral variables affecting the organism during periods of drug exposure as they are upon the traditional variables associated with the drug's administration (cf. Balster, 1984). It is this recent recognition of the role of drug-related experiences in drug tolerance that provided the general background and inspiration for the present thesis.

The effect of environmental and behavioral variables on tolerance has been demonstrated repeatedly by two types of research: by studies of context-specific drug tolerance (Baker & Tiffany, 1985; Eikelboom & Stewart, 1982; Siegel, 1975; 1977; Siegel & MacRae, 1984; Solomon, 1977; Wikler, 1948; 1973) and by studies of a phenomenon often referred to as contingent drug tolerance (Demellweek & Goudie, 1983b; Goudie & Griffith, 1985). The present thesis is concerned with contingent drug tolerance; however, a brief introduction to context-specific tolerance precedes a more extensive review of the contingent tolerance phenomenon.
Context-Specific Drug Tolerance

The manifestation of many types of drug tolerance has been shown to be dependent upon the drug's being administered in the context in which the subjects had previously experienced the drug's effects (e.g., Dafters & Anderson, 1982; Siegel, 1978). If subjects receive the test dose of a drug in the same context in which it had been previously administered, they display considerable tolerance to its effects; whereas, if the drug is administered on the test trial in a context with no drug-related history, the subjects display little or no tolerance. Such context-specific tolerance has been demonstrated to the effects of: 1) morphine (Siegel, 1975, 1977), 2) ethanol (Mansfield & Cunningham, 1980), 3) pentobarbital (Hinson, Poulos, & Cappell, 1982), 4) scopolamine (Poulos, Wilkinson, & Cappell, 1981), 5) caffeine (Rozin, Reff, Mark, & Schell, 1984), and 6) amphetamine (Poulos & Hinson, 1984). According to Siegel (1975; 1977; 1984), the context specificity of this tolerance is the consequence of Pavlovian conditioning. It is Siegel's view that the context in which a subject repeatedly experiences the drug's effects can be a conditional stimulus (CS) that becomes associated with the unconditional effects of the drug (the unconditional stimuli or UCS's). Siegel argues that as this association is strengthened, the context begins to elicit a conditional compensatory response (CCR),
which opposes the unconditional effects of the drug and increases in magnitude as the association between the context and the drug's effects strengthens. Because the CCR is manifested only when the drug is administered in the usual context, the manifestation of tolerance is context-specific.

Several lines of evidence support Siegel's Pavlovian explanation of context-specific tolerance. First, the development of context-specific tolerance is sensitive to preexposure to the CS; if subjects are repeatedly presented with the context that is to become the CS prior to the regimen of drug exposure, the development of tolerance when the context and the drug's effects are subsequently paired is much slower than it is if subjects have had no prior experience with the context (Siegel, 1977). Second, instances of context-specific tolerance have been found to be sensitive to extinction procedures. When a tolerant subject is repeatedly placed in the context that has become a CS for a particular drug effect, but the drug is not administered, there is a gradual decline in the tolerance that has developed (Siegel, 1975; Greeley, Le, Poulos, & Cappell, 1984).

The third, and most direct, line of evidence supporting Siegel's Pavlovian theory of context-specific tolerance has been provided by studies of the conditional compensatory response, the hypothetical construct on which Siegel's theory is based. The administration of a placebo to tolerant
subjects in the drug-predictive environment has frequently been reported to elicit a CCR, that is a response opposite to the initial effect of the drug. For example, placebo injections in the drug-predictive environment have been shown to elicit hyperalgesia in rats that have developed context-specific tolerance to the analgesic effect of morphine (Krank, Hinson, & Siegel, 1981), hypothermia in rats tolerant to morphine's hyperthermic effect (Siegel, 1978), or hyperactivity in rats tolerant to ethanol's hypoactive effect (Mansfield & Cunningham, 1980). Unfortunately, many attempts to demonstrate a conditional compensatory response have been unsuccessful, and Siegel's Pavlovian model of context-specific tolerance has been criticized by a number of researchers on this basis (e.g., Baker & Tiffany, 1985; Goudie & Griffiths, 1985; Shapiro, Dudek, & Rosellini, 1983; Tiffany, Baker, Petrie, & Dahl, 1983). In what is arguably the most well-developed alternative to Siegel's Pavlovian theory of context-specific tolerance, Baker and his colleagues (Baker & Tiffany, 1985; Kesner & Baker, 1981; Kesner & Cook, 1983) have argued that such tolerance can be attributed to a cued habituation to the drug's effects (see also Solomon, 1977; Wagner, 1978; 1981, for earlier versions of this idea). According to this theory of context-specific tolerance, repeated administration of a drug in a particular environment leads to the development of an association between the contextual cues and the drug's effects. As a
result of this association, subsequent presentation of the contextual cues leads to the retrieval from long-term memory of a representation of the drug's effects. This "associatively generated priming result[s] in decreased neural processing of the drug stimulus. Such decreased processing of drug stimulus information results in [sic] attenuated behavioral effect and constitutes tolerance."

(Baker & Tiffany, 1985, p. 83).

The fact that reasonable alternatives to Siegel's Pavlovian theory of context-dependent tolerance exist illustrates the inappropriateness of using the terms Pavlovian tolerance or conditional tolerance to refer to tolerance that is affected by the context present during periods of drug exposure. Because these terms refer to a possible explanation of context-specific tolerance, their use as labels for the tolerance itself is circular, and places the individual using such terms in the position of debating whether Pavlovian (conditional) tolerance is in fact controlled by Pavlovian (conditioning) mechanisms. Because it is always important to maintain a conceptual distinction between the phenomenon itself and the explanations of it, the less common, but more neutral, term context-specific tolerance is used here to refer to the phenomenon.

Regardless of the specific mechanisms underlying context-specific tolerance, the wide recognition the phenomenon itself has received represents a major advance in
the study of drug tolerance. In studies of context-specific tolerance, various groups of subjects with identical drug histories display markedly different levels of tolerance depending on the contextual cues present during periods of drug exposure. This finding has, more than any other, been responsible for focusing the attention of researchers on the importance of subject-related variables in drug tolerance.

2. Contingent Drug Tolerance

The Concept

Studies of contingent drug tolerance have also provided evidence that subject-related variables play an important role in the development of drug tolerance. Contingent tolerance is tolerance that develops preferentially to a drug's effects on those responses that occur during the periods of drug exposure. It is usually demonstrated in terms of the difference in tolerance observed between the two groups of subjects in what has been termed the before-and-after design (Kumar & Stolerman, 1977). In this design, the subjects in one group (the drug-before group) receive the drug before engaging in a particular response (the criterion response) on each tolerance-development trial, so that the response is performed while the subject is under the influence of the drug. The subjects in the second group (the drug-after group) receive the drug after engaging in the criterion response. On the test trial, all subjects receive
the drug before the performance of the criterion response so that the drug's effects on it can be assessed. Any evidence of greater tolerance in the drug-before subjects is attributed to the response contingency because the subjects in the two groups do not differ in either their exposure to the drug or in their opportunity to perform the criterion response.

Chen (1968) was the first to demonstrate the importance of the response contingency in a study of the development of tolerance to ethanol's disruptive effects on maze running. He trained rats to perform a maze task and then assigned them to one of two groups. The rats in one group received ethanol before running the maze on each tolerance development trial; whereas, the rats in the other group ran the maze before receiving ethanol. Chen found that only those subjects that had the opportunity to practice the maze while under the influence of ethanol subsequently demonstrated tolerance to its disruptive effects. The rats in the ethanol-after group demonstrated no tolerance to ethanol's effect on maze performance even though they had received the same number of ethanol injections and had the same amount of exposure to the maze.

The key to understanding the concept of contingent drug tolerance lies in the idea that tolerance develops not to the systemic presence of a drug but to its effects (cf. Demellweek & Goudie, 1983b; Okamoto, Boisse, Rosenberg, &
Rosen, 1978). Many drug effects are, under normal circumstances, an inevitable consequence of drug exposure (e.g., the hypothermic effect of ethanol). In such cases, the role of the response contingency in the development of tolerance is not readily apparent because the systemic presence of the drug and its effects are inextricably related. However, there are other drug effects that occur only if the drug is administered when the recipient is engaged in a particular response (the criterion response). In such cases, it is possible to show that the drug effect, rather than exposure to the drug per se, is the critical factor in the development of tolerance. For example, in Chen's experiment ethanol's disruptive effect on maze performance could manifest itself only when the rats were exposed to the maze while intoxicated, and it was only in this condition that tolerance developed.

Poulos and his colleagues (Poulos & Hinson, 1984; Poulos et al., 1981) have illustrated the importance of the criterion response to the development of contingent tolerance with an interesting analogy to a well-known perceptual phenomenon. According to these authors, to expect tolerance to develop in the absence of the criterion response is "like expecting adaptation to the effects of laterally displacing prisms to develop in an organism maintained in the dark. Without an adequate instigating stimulus to provide the basis for perceptual adaptation, none can occur" (Poulos et al.,
1981, p. 745). Although their allusion to the "displaced vision" phenomenon is insightful, it requires a slight but significant modification. It is not light per se, but the subject's visual perception of "self-produced movement... with its contingent refferentiation [sic] stimulation [that] is the critical factor in compensating for displaced visual images" (Held, 1972, p. 375; see also Rock & Harris, 1972). That is, adaptation to the disruptive effects of visual displacement on visuomotor responding does not occur unless such responding occurs under the influence of the displaced vision. In the same way, tolerance to a drug's effects does not develop unless the effects are manifested. In instances of contingent tolerance, performance of the criterion response is necessary for the manifestation of the drug effect of interest, and thus for tolerance to develop to that effect.

The term behavioral tolerance has also been used to refer to what I have labelled contingent tolerance (e.g., Chen, 1972; Dews, 1978; Hayes & Mayer, 1978). However, the term behavioral tolerance is also commonly used to refer to any tolerance that develops to the effects of a drug on behavior (e.g., Kumar & Stolerman, 1977), and when used in this fashion, it has no implications whatsoever for the conditions underlying the development of the tolerance. Therefore, the term contingent tolerance is used throughout the present thesis to avoid this ambiguity.
The Generality of Contingent Tolerance

Response contingencies have been shown to be an important, if not crucial, factor in the development of tolerance to a wide variety of drug effects. This subsection briefly reviews reports of contingent drug tolerance to: i) the effects of amphetamine and several other psychostimulants; ii) the effects of morphine; iii) the effects of delta-9-THC; iv) the effects of pentobarbital; and v) the effects of ethanol. The focus of the present thesis, that is contingent tolerance to ethanol's anticonvulsant effect, is introduced in more detail in the next section.

i. Contingent Tolerance to the Effects of Psychostimulants

The term contingent tolerance was first used by Carlton and Wolgin (1971) to describe their observation that the development of tolerance to the anorexigenic effect of d-amphetamine in rats is contingent upon providing the subjects with an opportunity to eat during each period of drug exposure. Using a before-and-after design, Carlton and Wolgin found that rats allowed to drink a sweet milk solution while they were under the influence of d-amphetamine developed tolerance to the drug's anorexigenic effects within just a few treatment sessions. In contrast, rats receiving each of their amphetamine injections after they had consumed the milk solution demonstrated no tolerance when they
subsequently received the drug prior to the milk. Furthermore, the rats in the drug-after condition developed tolerance to amphetamine's anorexigenic effect no faster than drug-naive control rats when they subsequently received a series of amphetamine injections before milk consumption. The results of Carlton and Wolgin's original study have been replicated by a number of researchers (e.g., Demellweek & Goudie, 1982; 1983a; Emmett-Oglesby, Spencer, Wood, & Lal, 1984; Poulos et al., 1981).

Contingent tolerance has also been demonstrated to the anorexigenic effects of psychostimulants other than amphetamine: cocaine (Woolverton et al., 1978), cathinone (Foltin & Schuster, 1982), methylphenidate (Emmett-Oglesby & Taylor, 1981), and guipazine (Rowland & Carlton, 1983). Furthermore, the tolerance that develops to the effects of psychostimulant drugs on a variety of operant tasks has also been shown to be influenced by a response contingency (e.g., Campbell & Seiden, 1973; Emmett-Oglesby et al., 1984). For instance, Emmett-Oglesby et al. (1984) found that rats in a differential-reinforcement-for-low-rates-of-responding (DRL) paradigm developed tolerance to the acceleration of bar-press responding caused by amphetamine only when they had the opportunity to engage in the DRL task during periods of amphetamine exposure. This study is particularly interesting because Emmett-Oglesby and his colleagues found that the rats that had developed tolerance to amphetamine's effect on DRL
responding showed no loss of sensitivity to the drug's anorexigenic effect, and conversely that contingent tolerance developed to amphetamine's anorexigenic effect without affecting the drug's effect on the DRL response rate. In each condition, tolerance developed only to the effect of the drug that was allowed to manifest itself.

11. Contingent Tolerance to the Effects of Morphine

The response contingency has also been reported to play a role in the development of tolerance to morphine's analgesic effect. In several studies, rats that were placed on a functioning hotplate during periods of morphine exposure demonstrated greater tolerance to its analgesic effect than did rats that were not (e.g., Kayan & Mitchell, 1969; Kayan, Woods, & Mitchell, 1969; Moore, 1983). A similar effect has been demonstrated in human subjects (Ferguson & Mitchell, 1969), using a tourniquet rather than a hotplate to provide the painful stimulation. Because none of these studies of tolerance to morphine's analgesic effect used the before-and-after design, they do not provide unambiguous evidence that the response contingency is an important factor in the development of such tolerance. However, the before-and-after design has been used to show that the response contingency plays an important role in the development of tolerance to the disruptive effects of morphine on operant responding in the rat (Smith, 1979).
iii. Contingent Tolerance to the Effects of Pentobarbital

The response contingency has been implicated in the development of tolerance to the disruptive effects of pentobarbital on operant behavior (Branch, 1983) and rotorod performance (Commissaris & Rech, 1981). The demonstration by Branch (1983) is unique in that a within-subject design was used. He first trained monkeys to perform a bar-press response for a food reward. Once a stable baseline was established, Branch administered pentobarbital to the monkeys immediately after they completed a test session on each of 20 consecutive days. On the twenty-first day, Branch administered pentobarbital to the monkeys before they performed the task, and found no evidence of tolerance to the drug's rate-increasing effects. This administration schedule was maintained for the next 20 consecutive days, and Branch found that tolerance to the drug's effect on the operant response rapidly developed. Branch concluded that drugged performance of the operant response was a critical factor in the development of tolerance to pentobarbital's effects on the task. Unfortunately, because Branch neglected to include the necessary controls (e.g., counterbalancing), his experiment does not provide incontrovertible evidence of contingent tolerance.

iv. Contingent Tolerance to the Effects of Delta-9-THC

Contingent tolerance to the disruptive effects of delta-9-THC on bar-press behavior in the monkey (Carder &
Olson, 1973; Elsmore, 1972), and on bar-press and avoidance behavior in the rat (Manning, 1976a,b) have been demonstrated. In Manning's experiments, the development of contingent tolerance to delta-9-THC's effect on operant responding was not influenced by prior drug history; rats that had previously received the drug after performing the criterion activity developed tolerance no faster than naive controls when both groups of rats received the drug before performing the response.

v. Contingent Tolerance to the Effects of Ethanol

As described earlier in this section, Chen (1968) provided the first report of contingent tolerance to ethanol's effects. Since Chen's seminal demonstration, contingent tolerance has been demonstrated to a variety of ethanol's effects. For example, contingent tolerance has been demonstrated to the effect of ethanol on treadmill running (LeBlanc, Gibbins, & Kalant, 1973; 1975; Wenger, Tiffany, Bombardier, Nicholls, & Woods, 1981), to ethanol's effect on operant responding (Chen, 1979; Wiggell & Overstreet, 1984), and to the ethanol-induced acceleration in the decay of postsynaptic potentiation in the abdominal ganglia of the marine mollusc Aplysia (Traynor, Schlapfer, & Barondes, 1980). Contingent tolerance has also been demonstrated to ethanol's analgesic effect, using the tail-flick test of analgesia (Jorgenson & Hole, 1984; Jorgenson, Berge, & Hole, 1985; Jorgenson, Farmer, & Hole, 1986). The
study by Jorgenson et al. (1985) is particularly interesting, because they demonstrated that contingent tolerance to ethanol's effect on the tail-flick reflex develops even when rats have been spinally transected at the level of the ninth thoracic vertebrae.

The response contingency has also been implicated in the development of tolerance to ethanol's hypothermic effect; Alkana, Finn, and Malcolm (1982) found that tolerance does not develop if subjects are maintained at a constant body temperature during periods of ethanol exposure. This study is particularly interesting because of the methods used by Alkana et al. to manipulate alcohol's hypothermic effect. Contingent tolerance is usually studied to drug effects that can occur only in a particular test situations (e.g., mazes, Skinner boxes, etc.), and the experimenters control the performance of the criterion response by controlling the subjects' exposure to the test environments. In contrast, ethanol-induced hypothermia requires no special test situation to manifest itself; under normal conditions, hypothermia is an inevitable consequence of ethanol exposure. To overcome this problem, Alkana and his colleagues assigned mice to two groups. The mice in one group received a total of six daily ethanol injections. Immediately after each injection, the mice in this group were placed in a heated chamber so that the hypothermic effect of the ethanol was offset by the hyperthermic conditions present in the chamber.
The subjects in the second group received the same number of ethanol injections, but were placed in a chamber maintained at room temperature so that the hypothermic effect of each ethanol injection could be manifested. On the test day, when all of the mice received an ethanol injection and were placed in a chamber maintained at room temperature, Alkana and his colleagues found that only the mice in the group that had experienced ethanol's hypothermic effect demonstrated tolerance on the test day.

3. Contingent Tolerance to Ethanol's Anticonvulsant Effect

Pinel and his colleagues (1983; 1985) have demonstrated that contingent tolerance develops to ethanol's anticonvulsant effect. It is this particular manifestation of contingent tolerance that is the focus of the present thesis. The three experiments in Pinel's original (1983) report are noteworthy for two reasons. First, they provided the first unambiguous evidence that tolerance could develop to ethanol's anticonvulsant effect. Although Allan and Swinyard (1949) had reported tolerance to ethanol's anticonvulsant effects on maximal electroshock seizures, subsequent attempts to confirm this finding had failed (e.g., Chen, 1972; McQuarrie & Fingl, 1972). The second important feature of Pinel et al.'s original report of tolerance to ethanol's anticonvulsant effect, and the one more germane to the present thesis, was that their rats developed tolerance
to ethanol's anticonvulsant effect only if they received convulsive amygdaloid stimulation during each period of ethanol exposure.

In the first experiment of their original (1983) report, Pinel and his colleagues found that kindled rats stimulated once every 24 hr for 5 days rapidly developed tolerance to the anticonvulsant effect of ethanol (1.5 g/kg, IP) injected twice each day, once 12.5 hr before each daily stimulation and again 0.5 hr before. In the second experiment, Pinel et al. found that there was no evidence of tolerance in a group of rats that received the same schedule of ethanol injections that was administered to the ethanol subjects in Experiment 1, but no convulsive stimulation. This suggested that the development of tolerance to ethanol's anticonvulsant effect was contingent upon convulsive stimulation occurring during periods of ethanol exposure.

In the third experiment, Pinel and his colleagues used a before-and-after design to test the hypothesis suggested by the comparison of their first two experiments; that is, that the response contingency was a crucial factor in the development of tolerance to ethanol's anticonvulsant effect. The rats in one group received an ethanol intubation (4.5 g/kg, in a 30% v/v solution) 1.5 hr before each of five bidaily convulsive stimulations; this dose of ethanol completely suppressed convulsive activity on the first treatment trial. The rats in the second group received an
identical number of ethanol intubations, administered 1.5 hr after the stimulations. On the test day, all of the rats received an ethanol injection (1.5 g/kg, IP, in a 25% v/v solution) 1.5 hr before the stimulation was administered. As is evident in Figure 1, Pinel and his colleagues found that the rats in the group that had previously received convulsive stimulation while they were exposed to ethanol (the ethanol-before subjects) displayed substantial tolerance to ethanol's anticonvulsant effect. In contrast, there was no evidence of tolerance in any of the subjects from the ethanol-after group. A subsequent analysis of the levels of ethanol in blood samples taken from the tail of each rat immediately after testing revealed no significant difference between the two groups.

Pinel and Puttaswamaiah (1985) tested the possibility that contingent tolerance to ethanol's anticonvulsant effect was under Pavlovian control. Recall that in instances of context-specific drug tolerance, the development and manifestation of tolerance to a drug's effects are greatly influenced by the history of the contextual cues present during periods of drug exposure. Several authors have proposed that instances of contingent tolerance could be attributed to the same Pavlovian conditioning mechanism used to account for context-specific tolerance (e.g., Hinson & Seigel, 1980; Wenger et al., 1981). In the before-and-after
FIGURE 1. The effect of the response contingency on the development of tolerance to ethanol's anticonvulsant effect. During the treatment phase, ethanol (4.5 g/kg) was intubated at 48-hr intervals, either before or after convulsive stimulation. On the test trial, the group that had received ethanol before stimulation on the treatment days (the prestimulation group) demonstrated substantial tolerance to the anticonvulsant effect of the test dose of ethanol (1.5 g/kg, IP), whereas there was little evidence of tolerance in the rats that had received ethanol after each convulsive stimulation on the treatment days (the poststimulation group). (From Pinel et al., 1983; used with permission of Ankho International).
design, all of the subjects receive the drug before engaging in the criterion response on the test trial. Thus, the test trial is identical to the tolerance-development sessions for the subjects in the before group, but not for the subjects from the after group. According to the Pavlovian explanation of contingent tolerance, the absence of the criterion response prior to drug administration on the test day for the subjects in the after group changes the predrug context enough that the conditional compensatory response necessary for tolerance to occur does not fully manifest itself. As a result, the subjects from the after group do not appear tolerant on the test trial.

Pinel and Puttawamaiah (1985) attacked this view by showing that the manipulation of contextual stimuli associated with the administration of ethanol had no effect on contingent tolerance to ethanol's anticonvulsant effect. Pinel and Puttaswamaiah showed that tolerance to ethanol's anticonvulsant effect was not context-specific; there was no evidence of a conditional compensatory response; and preexposure to the contextual cues associated with ethanol administration had no effect on tolerance development. On the basis of these results, Pinel and Puttaswamaiah (1985) concluded that "tolerance to ethanol's anticonvulsant effect is not amenable to Pavlovian conditioning" (p. 963) and thus that Pavlovian conditioning cannot account for demonstrations of contingent tolerance to ethanol's anticonvulsant effect.
This conclusion is reinforced by Pinel et al.'s earlier (1983) observation that the manifestation of contingent tolerance to ethanol's anticonvulsant effect was unaffected by a change in the route of administration; subjects that had developed tolerance to ethanol's anticonvulsant effect when the drug was intubated displayed substantial tolerance when the ethanol was injected intraperitoneally on the test trial.

4. General Rationale

There were three general reasons for my initial interest in contingent tolerance to ethanol's anticonvulsant effect, as opposed to some other form of contingent tolerance. The first reason has to do with the fact that, although seizures are among the most clinically significant and most frequently studied symptoms of alcohol withdrawal, little attention had been paid to the tolerance that most current theories predict to underlie them. The same physiological adaptations that are presumed to oppose alcohol's unconditional effects, and therefore produce tolerance, are assumed to trigger withdrawal effects opposite to the unconditional effects of the ethanol once it has been eliminated from a subject's body (Cicero, 1980; Goldstein, 1979). Thus, the existence of ethanol withdrawal seizures implies that tolerance develops to ethanol's well-documented anticonvulsant effect. However, until Pinel et al.'s original (1983) report of contingent tolerance to ethanol's anticonvulsant effect, attempts to
demonstrate any kind of tolerance had been equivocal. Accordingly, my initial interest in contingent tolerance to ethanol's anticonvulsant effect was partially stimulated by a general interest in the relation between ethanol tolerance and dependence, and by what was an obvious gap in the knowledge concerning a phenomenon of major theoretical and clinical significance.

The second reason for my interest in contingent tolerance to ethanol's anticonvulsant effect was that it is a particularly robust example of the contingent tolerance. Pinel and his colleagues had used a variety of conditions to demonstrate contingent tolerance to ethanol's anticonvulsant effect, yet in each case the effect of the response contingency was impressively clear. In each of Pinel's experiments, rats in the ethanol-before condition developed almost complete tolerance after just a few treatment sessions; whereas, those in the ethanol-after condition displayed no tolerance whatsoever. Thus, the paradigm used by Pinel and his colleagues seemed particularly suited to study the role of the response contingency in drug tolerance.

The third reason for my initial interest in contingent tolerance to ethanol's anticonvulsant effect was the apparent lack of influence that Pavlovian manipulations have on it (e.g., Pinel & Puttaswamaiah, 1985). Poulos and his colleagues have reported several instances of contingent tolerance that also appear to be partially under Pavlovian
control. For example, Poulos et al. (1981) reported that contingent tolerance to amphetamine's anorectic effect manifested itself only when the drug was administered in the context in which the drug was previously administered; if subjects received the drug in a novel context, there was no evidence of tolerance whatsoever. Similarly, Poulos and Hinson (1984) showed that the response contingency had no effect on the manifestation of tolerance to scopolamine's adipsic effect unless the drug was administered in the usual context on the test day. It seemed advisable, therefore, to study the influence of the response contingency on the development and dissipation of tolerance in a paradigm uncontaminated by the complicating effects of Pavlovian conditioning.

5. General Purposes

The experiments in the present thesis had two general purposes, which were to a large degree independent of one another. The first was to determine whether or not any tolerance at all can develop in the absence of the opportunity to perform the criterion response during periods of ethanol exposure. The second was to determine whether the response contingency plays a significant role in the dissipation of tolerance to ethanol's anticonvulsant effect. The first three experiments dealt with the first issue; whereas, the fourth and final experiment dealt with the
second. Accordingly, the next section reviews the controversy surrounding the question "Can ethanol tolerance develop in the absence of the response contingency?", whereas the literature relevant to the role of the response contingency in the dissipation of tolerance is reviewed prior to Experiment 4.
II. GENERAL BACKGROUND FOR EXPERIMENTS 1, 2, AND 3

Without doubt, the most heated debate generated by the many demonstrations of contingent tolerance to ethanol's effects has been whether any tolerance at all develops in the subjects that do not engage in the criterion response during periods of ethanol exposure. At an empirical level, the question is deceptively simple; "Is there any difference on the test trial in ethanol's effect on the performance of the criterion response between subjects in an ethanol-after group and a group of rats receiving the drug for the first time?". Nevertheless, there has yet to be a satisfactory resolution to the question.

In Chen's original demonstration of contingent tolerance (Chen, 1968), after four tolerance-development trials there was no significant difference between the maze performance of the rats in the ethanol-after group on the test trial after four tolerance-development trials, and the maze performance of rats with prior exposure to ethanol. In a subsequent study, Chen (1972) found no significant tolerance development in the ethanol-after rats after six daily treatment trials. On the basis of these studies, Chen concluded that ethanol per se is not a sufficient stimulus for the development of tolerance to its disruptive effects on maze running.

This conclusion was soon challenged by LeBlanc, Gibbins, and Kalant (1973), who pointed out an obvious flaw in Chen's
experiments. They argued that Chen did not administer ethanol often enough (only four trials in the 1968 study and six trials in the 1972 study) to conclude that tolerance to ethanol's effects could not develop in the absence of intoxicated practice in the maze. LeBlanc et al. argued that tolerance would develop in the absence of any opportunity for intoxicated practice of the maze if enough injections were administered.

To support their claim, LeBlanc et al. (1973) trained three groups of rats in a maze similar to that used by Chen. After the rats had learned to negotiate the maze, they were assigned to one of three groups. The rats in one group received a daily ethanol injection before running in the maze; the rats in the second group received an ethanol injection after running the maze; and those in the third group received daily saline injections before running the maze. Every 4th day was a test trial on which all of the rats received an ethanol injection before performing the maze task so that the development of tolerance could be assessed. LeBlanc et al. found that the rats in the ethanol-before group developed asymptotic tolerance by the 8th day of treatment (i.e., the second test session); that the rats in the ethanol-after group reached the same asymptotic level of tolerance by the 24th day (i.e., by the sixth test session); and that there was no evidence of tolerance in the rats in the saline group despite the fact that these animals had
received 11 ethanol injections, one every 4th day over the course of the experiment. On the basis of these results, LeBlanc et al. (1973) concluded that performance of the criterion response while under the influence of alcohol substantially accelerates the development of tolerance but is not essential for its development.

LeBlanc, Gibbins, and Kalant (1975) provided further support for their view that the response contingency was not necessary for the development of tolerance by demonstrating that the tolerance that rats developed to ethanol's effects on the performance of Chen's maze would generalize to ethanol's effects on the performance of a treadmill task. In this treadmill task, each rat was required to walk on a moving treadmill in order to avoid footshock administered by an electrified grid that surrounded the treadmill. Because there was no evidence of a transfer of learning between the tasks in undrugged subjects, LeBlanc and his colleagues argued that the fact that tolerance to ethanol's effects on one task would generalize to its effects on the other, which had never been performed under the influence of ethanol, meant that the performance of the criterion response was not necessary for the development of tolerance to ethanol's effects on it.

Unfortunately, both lines of evidence offered by LeBlanc and his colleagues to support their view have been discredited. First, Wenger and his colleagues (Wenger,
Berlin, & Woods, 1980; Wenger et al., 1981) have provided evidence in two separate studies that the gradual development of tolerance reported by LeBlanc's group in the rats in the ethanol-after condition was the result of the tests that all of the subjects received every 4 days during the tolerance-development phase—you will recall that on each test trial all of the rats performed the criterion response (either the maze or the treadmill task) while they were intoxicated. Wenger et al. (1980) found that rats from the ethanol-after group that received eight tolerance-development trials, but not the periodic testing, failed to develop any significant tolerance to ethanol's effects on LeBlanc's treadmill task. Wenger et al. (1981) extended these results by demonstrating that rats in the ethanol-after treatment condition did not develop tolerance to ethanol's effects on the treadmill task even after 23 tolerance-development sessions unless they were tested periodically during the tolerance-development phase of the study. Wenger et al. (1981) concluded that:

"the tolerance reported [by LeBlanc and his colleagues] to be a consequence of mere exposure to ethanol is actually due to the practice given the animals every 4 days while they were being tested for the development of tolerance. All of the tolerance established over 23 days appears to be attributable to [the response contingency] (p. 576)."
Unfortunately, careful examination of the experimental bases for Wenger et al.'s criticism of LeBlanc et al.'s conclusions reveals that they are not without their own serious problems. In both papers, there is clear evidence of tolerance development in the subjects from the ethanol-after condition, which was discounted by Wenger and his colleagues because it did not quite reach statistical significance in either case. Thus, if a few more subjects had been tested, if a few more tolerance-development trials had been included in the design of the experiments, if a different dose of ethanol had been administered to the rats during the tolerance-development phase, or if the data from the two studies had been analyzed together; Wenger and his associates would likely have been forced to reach a conclusion completely opposite to the one that they published. The demonstration of an effect that just fails to reach statistical significance in two independent studies is an extremely tenuous basis for concluding that the effect does not exist.

Pinel and Mana (1986) attacked the second line of research used by LeBlanc's group to support the position that tolerance to ethanol's effects on a response can develop in the absence of any opportunity to perform the response while intoxicated. They questioned LeBlanc et al.'s interpretation of their 1975 finding that tolerance to the effects of ethanol on the treadmill task transferred to
a maze task, even though the subjects had never been exposed to the maze while they were intoxicated. Pinel and Mana pointed out that the maze and treadmill tasks share a number of common behavioral elements, such as walking, visuomotor coordination, balancing, etc., which would likely be disrupted by ethanol. As a result, the tolerance that developed to ethanol's effects on these common elements when rats performed the treadmill task while they were intoxicated could also express itself when they were tested on the maze task, even though the rats had never performed the maze task while intoxicated.

Even though the aforementioned studies of Chen, LeBlanc, Wenger, and their respective colleagues failed to determine whether tolerance can develop to ethanol's effects on responses that are not performed during periods of intoxication, each of them provided evidence of the major role of the response contingency in the development of ethanol tolerance. In other words, although they did not accomplish their goal, they each emphasized the importance of the question that they had attempted to answer. Clearly, the response contingency is an important factor in the development of tolerance to ethanol's effects, and until it can be determined whether it plays a permissive or a critical role, our understanding of the phenomenon of drug tolerance, and the mechanisms underlying it, will be incomplete. Accordingly, the first three experiments of
this thesis addressed this issue with a number of innovative approaches.

Perhaps the major innovation was the use of Pinel's model of contingent tolerance to ethanol's anticonvulsant effect to determine whether or not tolerance can develop in the absence of the response contingency. The introduction of a new paradigm seemed obligatory if only to broaden the generality of the data base relevant to the issue; other attempts to assess the necessity of the response contingency had used only the treadmill or maze task. More importantly, Pinel's paradigm has an crucial advantage over the treadmill and maze tasks; in Pinel's paradigm, the experimenter has much greater control of the criterion response than is possible in either the treadmill task or the maze task. It is difficult to control the performance of criterion responses like maze running or treadmill performance, because it is possible for subjects to practice various components of these tasks (e.g., walking, visuomotor coordination) at will. This lack of control makes it impossible to unequivocally demonstrate that tolerance can develop to ethanol's effect on responses that do not occur during periods of intoxication, because it is not possible to be certain that such tolerance did not result from the subjects in the ethanol-after condition performing components of the criterion response (e.g., walking) in their home cages after the ethanol was administered. This
problem does not exist in Pinel's paradigm, in which seizures occur only when they are elicited by electrical stimulation delivered by the experimenter. Clearly, the ability to maintain strict control over the criterion response is important in any attempt to assess the importance of the response contingency to the development of drug tolerance.

Although there was no evidence of tolerance in the rats from the ethanol-after condition in Pinel et al.'s (1983) original studies of contingent tolerance to ethanol's anticonvulsant effect, no effort was made to include conditions in these studies that would be particularly sensitive to the development of tolerance in this condition; their goal was simply to demonstrate that the response contingency influenced tolerance development, not that it was necessary for it. Accordingly, each of the first three experiments in this thesis varied some aspect of the procedure used by Pinel et al. (1983) in order to facilitate the detection of tolerance in the rats from the ethanol-after treatment condition. In Experiment 1, the effects of different treatment doses were assessed; in Experiment 2, the number of tolerance-development trials was increased; and in Experiment 3, a more sensitive, multiple-trial test procedure was employed.
III. GENERAL METHODOLOGY

This section of the paper describes the methods common to the first three experiments. Any specific additions to this general methodology are described in the methods section of each experiment.

Subjects. The subjects in all of the experiments were male hooded rats (Charles River, Canada), weighing 350 to 400 g at the time of surgery. The rats were individually housed in wire mesh cages with continuous access to food and water. Each experiment was conducted during the light phase of the 12/12 hr light/dark cycle.

Surgical Procedure. A single bipolar electrode (Plastic Products MS-303-2) was implanted in the amygdaloid nucleus of each rat, 1.2 mm posterior to bregma, 5 mm lateral and 10 mm ventral to the skull surface at bregma, with the incisor bar set at 0.0. Tetracycline was sprinkled on the incision before suturing, and it was added to the drinking water for 7 days after surgery.

Kindling. The kindling phase of each study began at least 7 days after surgery. During the kindling phase, each rat was stimulated (1 sec, 60 Hz, 400 A) three times per day, 5 days per week, for 3 weeks. There was at least 2 hr between consecutive stimulations. Prior to each stimulation, the stimulation lead was connected, and the subject was then
placed in a 58 X 58 X 25 cm opaque plastic chamber containing a layer of San-i-cel bedding material. The stimulation was delivered immediately, and the rat was returned to its home cage once the signs of convulsive activity ceased. As is usual (Pinel & Rovner, 1978; Pinel & Van Oot, 1975; Racine, 1978), the rats' behavior was unaffected by the initial stimulations in the series, but by the end of the kindling phase, each stimulation elicited a clonic seizure characterized in sequence by facial clonus, forelimb clonus, rearing, and a loss of equilibrium.

Baseline. In each study, the baseline phase began 48 hr after the completion of the kindling phase. During the baseline phase, each rat received five amygdaloid stimulations, one every 48 hr. This bidaily stimulation schedule was maintained for the remainder of each experiment. The duration of forelimb clonus elicited by each stimulation was the dependent measure. On the fourth baseline trial, each rat received an IP injection of isotonic saline (room temperature, 7.6 ml/kg, volume matched to that for the subsequent test dose of ethanol) 1 hr before stimulation. This was done to determine the effects of the injection procedure on the duration of forelimb clonus elicited by the stimulation; subjects not displaying at least 20 s of clonus on this baseline trial were dropped from the study at this point. On the fifth and last baseline day, every subject received an IP injection of
ethanol (1.5 g/kg in a 25% v/v solution) 1 hr before convulsive stimulation. This was done to determine each subject's initial sensitivity to ethanol's anticonvulsant effect before they were assigned to treatment groups. Subjects not displaying at least an 80% reduction in the duration of forelimb clonus from the fourth to the fifth baseline stimulation were dropped from the study at this point. The rejection criteria were enforced in each study because the anticonvulsant effect of ethanol and the subsequent development of tolerance to it cannot be readily studied in subjects that do not consistently display substantial convulsive activity in the absence of ethanol and a large initial sensitivity to ethanol's anticonvulsant effect. It should also be pointed out that approximately 15% of the subjects meeting the criteria and beginning the treatment phase of each of the studies did not complete them, usually because their electrode caps became dislodged or because of complications caused by the repeated ethanol administrations.

Treatment. In each experiment, the rats were assigned to treatment groups in such a way that the average duration of forelimb clonus elicited by the fourth baseline stimulation was approximately equal for each group. The treatment trials began 48 hr after the fifth baseline trial, and occurred at about the same time every second day (+/- 2 hr) for the entire treatment phase. During each bidaily
treatment trial, each subject was removed from its home cage, weighed, and the appropriate dose of ethanol or isotonic saline was administered either 1 hr before or 1 hr after convulsive stimulation. The number of treatment sessions varied from study to study.

In Experiments 1 and 2, the treatment dose of ethanol was administered by intubation to reduce the risk of the infection and subject attrition sometimes associated with IP injections. However, the results of a separate series of studies suggested that this was not a problem. Therefore, in Experiment 3 all of the injections of ethanol were administered intraperitoneally.

Test. The test trial always occurred 48 hr after the last treatment trial. On the test trial, every rat in the experiment received an IP ethanol injection 1 hr before convulsive stimulation. In Experiments 1 and 2, the development of tolerance was assessed by comparing the duration of forelimb clonus elicited in a subject on the test trial to that elicited on the fifth baseline day in the same subject. In addition, in Experiment 1 a saline-control group permitted the assessment of tolerance by comparing the duration of clonus elicited on the test trial in the subjects in the two ethanol groups with that elicited in the saline control subjects on the same day. In Experiment 3, a more sensitive measure of tolerance development was used, the specific details of which are provided in the
methodology section of Experiment 3.

**Histology.** At the end of each experiment, all the subjects were sacrificed in a CO2 chamber according to Canada Council on Animal Care guidelines, and their brains were removed and sectioned to permit histological verification of electrode sites using the blue-dot technique (Skinner, 1971) and Paxinos and Watson's atlas of the rat brain (1982).

**Statistical Analyses.** Nonparametric statistics (Siegel, 1956) were used to analyze the clonus-duration data in the first two experiments. Nonparametric statistics were used to analyze these data in order to avoid seriously violating the assumption of homogeneity of variance, which is the basis for more conventional parametric analyses; the duration of forelimb clonus on the fifth baseline trial, when ethanol was administered for the first time, was zero for almost every rat, whereas there was substantial variability in some of the conditions on the test trial. The different measure of tolerance used in Experiment 3 permitted the use of parametric statistical procedures.
IV. EXPERIMENT 1

In Pinel et al.'s original (1983) demonstration of contingent tolerance to ethanol's anticonvulsant effect, there was no significant evidence of tolerance in the ethanol-after group even though a relatively high treatment dose of ethanol (4.5 g/kg, intubated) was administered on the five tolerance-development trials. Although there is a large body of literature indicating that tolerance to a drug's effects develops more rapidly when a high treatment dose is administered (e.g., Kalant et al., 1971; LeBlanc, Kalant, Gibbins, & Berman, 1969; Jorgenson, Fasmer, & Hole, 1986), it has also been suggested that the detection of tolerance can be more difficult in animals that have received a high treatment dose of a drug, because of an accumulation of the drug in a subject or because the high dose resulted in some non-specific change in the subject that interferes with the assessment of tolerance (Kalant et al., 1971). Thus, it is possible to argue that no tolerance was detected in the ethanol-after condition of Pinel et al.'s study because the treatment dose of ethanol was too high. Accordingly, in Experiment 1 contingent tolerance to ethanol's anticonvulsant effect was studied at two treatment doses that approached the lower and upper limits of the paradigm. Pilot studies had indicated that
intubated doses of ethanol less than 2 g/kg would not reliably block the kindled convulsions, and that doses greater than 5 g/kg would lead to subject attrition.

METHOD

Of the 80 rats completing the baseline phase of Experiment 1, 2 rats were rejected because the duration of forelimb clonus elicited on the fourth baseline day did not meet the criterion for inclusion, and 1 rat was rejected because it did not respond adequately to ethanol’s anticonvulsant effect on the fifth baseline day. Of the remaining 77 subjects, 67 completed the study. The data of only these 67 were analyzed.

Tolerance-Development Phase. There were two tolerance-development conditions in Experiment 1: a before condition in which subjects were intubated with either ethanol or isotonic saline 1 hr before each bidaily stimulation and were given a pseudointubation 1 hr after each stimulation, and an after condition in which the subjects received the same treatments but in the reverse order. Nothing was injected during the pseudointubations; the tubes were simply inserted into the stomach for a few seconds and then removed.

After the fifth baseline stimulation, the subjects were assigned to one of six treatment groups. The rats in the three before groups were intubated with 2 g/kg of ethanol
(n=11; ethanol in a 12% v/v solution), 5 g/kg of ethanol (n=12; ethanol in a 30% v/v solution), or an isotonic saline solution (n=11; 21.1 ml/kg). The intubation volume of the solutions was equal for all of the groups. The rats in the three after groups were intubated with 2 g/kg of ethanol (n=11), 5 g/kg of ethanol (n=11), or an isotonic saline solution (n=11) 1 hr after each stimulation.

Test. The test for tolerance development occurred 48 hr after the last tolerance-development trial. Each rat received a 1.5 g/kg IP ethanol injection 1 hr before convulsive stimulation. Immediately after the cessation of the convulsion, a 200 ml sample of blood was drawn from the tail of each rat into a heparinized tube, and blood ethanol levels were subsequently determined using a modification of the head-space gas chromatographic method (Wilkinson, Wagner, & Sedman, 1975).

Analysis. Wilcoxon's Matched-Pairs Ranked-Signs Test's were used to assess the significance of the within-group differences in clonus duration between the fifth baseline trial and the test trial. Mann-Whitney U Tests were used to assess the significance of the between-group differences in clonus duration on the fifth baseline trial. The difference in blood ethanol concentrations between the different treatment groups was assessed with a simple 1-way ANOVA.

Results. Figure 2 illustrates the results of Experiment 1. Regardless of the dose, those subjects intubated with
ethanol before each of the five tolerance-development stimulations displayed significant levels of tolerance on the test trial, whereas those intubated with ethanol after each stimulation did not. Thus, the forelimb clonus displayed by the subjects in the two ethanol-before groups was significantly longer on the test trial than on the fifth baseline trial (both Wilcoxon p's < .01). In contrast, the duration of forelimb clonus on the test trial for the rats in the two alcohol-after groups and the two saline groups was not significantly longer than that elicited on the fifth baseline trial (all Wilcoxon p's > .10). Moreover, the rats in the two ethanol-before groups displayed significantly longer forelimb clonus on the test trial than did the rats from any of the other four groups (all Mann-Whitney p's < .01). There were no significant differences in the duration of the clonus elicited on the test trial between the two alcohol-before groups or between the ethanol-after and saline groups (all Mann-Whitney p's > .10).

There were no significant differences in the blood ethanol levels of the six groups of subjects following the test stimulation, F (2, 6) = 0.02, p > .10. The overall mean blood alcohol level was 1.36 g/l. Examination of the histological data revealed that all of the electrode tips were in the amygdaloid complex, with most terminating in or near the baso-lateral amygdaloid nuclei (see Figure 3).
FIGURE 2. The effect of treatment dose on the development of tolerance to ethanol's anticonvulsant effect. During the treatment phase, kindled rats were intubated with ethanol (2 g/kg or 5 g/kg) either 1 hr before or 1 hr after convulsive stimulation. On the test trial, all subjects received ethanol (1.5 g/kg) 1 hr before stimulation. The rats in the two ethanol-before groups demonstrated substantial tolerance; in contrast, there was little evidence of tolerance development in the ethanol-after or the saline-control groups.
all subjects

saline

ethanol 2 g/kg

ethanol 5 g/kg

MEAN DURATION OF FORELIMB CLONUS (sec)

DAY 4

DAY 5

AFTER GROUP

BEFORE GROUP

BASELINE

TEST
FIGURE 3. Histology from Experiment 1.
Discussion. The results of Experiment 1 provide further evidence of the importance of the response contingency in the development of tolerance to ethanol's anticonvulsant effect. Although a substantial degree of tolerance was evident after the five tolerance-development trials in the rats that received an ethanol injection before each bidaily stimulation during the tolerance-development phase, there was no significant evidence of tolerance in the ethanol-after groups, at either treatment dose.

It should be noted that there was an indication, albeit an insignificant one, of tolerance in the ethanol-after rats in the 2g/kg group (see Figure 2). This raises the possibility that a significant degree of tolerance would have developed in this group if a greater number of tolerance-development trials had been administered. This possibility was tested in Experiment 2.
V. EXPERIMENT 2

It is possible that previous attempts to demonstrate tolerance to ethanol's anticonvulsant effect in rats that receive ethanol after convulsive stimulation during the tolerance-development phase have failed because these studies have been curtailed prematurely. Although the development of tolerance to ethanol's effects can be observed following a single injection (e.g., Mellanby, 1919; Tullis, Sargent, Simpson, & Beard, 1977), in most cases a larger number of tolerance-development trials are required for its development (cf., Hug, 1972; LeBlanc, et al., 1975). The purpose of Experiment 2 was to determine whether tolerance to ethanol's anticonvulsant effect would develop in the ethanol-after condition in rats exposed to 5, 10, or 20 tolerance-development trials.

METHOD

Of the 102 rats completing the baseline phase of Experiment 2, 6 did not display sufficiently long clonus on the fourth baseline day, and 5 more did not display the required reduction in clonus duration required on the fifth baseline day. The remaining 91 rats, only 71 completed the study.

Tolerance-Development Phase. There were two general conditions in the tolerance-development phase: 1) an
ethanol-before condition in which subjects were intubated with ethanol (2 g/kg, in a 25% v/v solution) 1 hr before each bidaily convulsive amygdaloid stimulation; and 2) an ethanol-after condition in which the rats were intubated with the same dose of ethanol 1 hr after each stimulation. The rats in each of these two general conditions were assigned to one of three treatment groups, which received either 5 (before n=11, after n=10), 10 (before n=11, after n=12), or 20 (before n=15, after n=12) bidaily intubations during the tolerance-development phase of the experiment.

Test. On the test trial, which occurred 48 hr after the last tolerance-development trial, every rat received a single test injection of ethanol (IP, 1.5 g/kg) 1 hr before amygdaloid stimulation. Immediately after the seizure subsided, a 200 l sample of blood was taken from the animal's tail, and the blood level of ethanol was determined with a modified head-space gas chromatographic technique (Wilkinson et al., 1975).

Analysis. Wilcoxon's Matched-Pairs Ranked-Signs Test's were used to assess the significance of the tolerance within each treatment group. Mann-Whitney U Tests were used to assess the significance of the differences in clonus duration between groups. The significance of the differences in blood alcohol concentrations between the different treatment groups was assessed with a simple 1-way ANOVA.

Results. It is clear in Figure 4 that the rats receiving
ethanol before stimulation developed a substantial degree of
tolerance to ethanol's anticonvulsant effect, but that there
was little evidence of tolerance in the rats from the
ethanol-after groups. Thus, the rats in all three ethanol-
before groups displayed significantly more forelimb clonus
on the test day than they had on the fifth ethanol baseline
trial (all Wilcoxon p's < .01); whereas, those in the
ethanol-after groups did not (all Wilcoxon p's > .10).
Moreover, there was a significant difference in the duration
of the forelimb clonus displayed by subjects in the
respective ethanol-before and ethanol-after groups in the
5 intubations (Mann-Whitney p < .05), 10 intubations (Mann-
Whitney p < .01), and 20 intubations (Mann-Whitney p < .025)
conditions.

Equipment failure resulted in the loss of most of the
blood samples taken in Experiment 2. Analysis of the 22
samples that were not lost revealed a difference in the
blood alcohol concentrations between the rats in the 5-day
before condition and the 20-day after condition (F
(2,16) = 3.98, p < .05; Neuman Keuls p < .05), but there were no
significant differences between any of the other groups (all
Neuman Keuls p > .05). However, given the unsystematic
nature of the difference and the fact that it has not been
replicated, it should be viewed with caution.
FIGURE 4. Tolerance to the anticonvulsant effects of ethanol (2 g/kg) following 5, 10, or 20 intubations administered either before or after bidaily convulsive stimulation. There was substantial tolerance development in all three of the ethanol-before groups, whereas there was little evidence of tolerance in any of the ethanol-after groups.
DAY 4  DAY 5  AFTER  BEFORE
GROUP  GROUP  BASELINE  TEST

MEAN DURATION OF FORELIMB CLONUS (sec)

- all subjects
- 5 intubations
- 10 intubations
- 20 intubations
FIGURE 5. Histology from Experiment 2.
Histological analysis of the electrode placements revealed that all electrode tips were located in the amygdaloid complex or on its boundaries, with most electrodes terminating in the basolateral nuclei (see Figure 5).

Discussion. The results of Experiment 2 provide further evidence of the important role of the response contingency in the development of tolerance to ethanol's anticonvulsant effect. As in Experiment 1, the rats in the ethanol-before groups demonstrated substantial tolerance after just five treatment trials. In contrast, there was no significant tolerance development in the rats from the ethanol-after conditions. Even after 20 treatment trials, there was only a slight indication of tolerance in the ethanol-after condition, but it was not statistically significant.
VI. EXPERIMENT 3

In Experiment 1, there was a slight indication of tolerance development in the rats from the ethanol-after condition that received the 2g/kg treatment dose of ethanol. In Experiment 2, there was a suggestion of tolerance development in the ethanol-after group that received 20 tolerance-development trials. However, this effect was not statistically significant in either study. Experiment 3 attempted to provide evidence of tolerance development in the ethanol-after group by using a particularly sensitive measure of tolerance development.

The measure of tolerance used in Experiment 3 was a savings measure (cf. Kalant et al., 1971). After 20 bidaily tolerance-development trials, all of the rats in Experiment 3 received a series of bidaily test injections, each 1 hr before convulsive stimulation, and the rate of tolerance development was assessed for each group of rats. The use of this test was based on the assumption that if the rats in the ethanol-after condition were in fact developing some tolerance which was not apparent on the first test trial, it would be expressed as a savings in the rate of subsequent tolerance development (c.f. Kalant et al., 1971).

METHOD

Of the 32 rats that completed the baseline phase of
Experiment 3, 2 rats did not display sufficiently long clonus on the fourth baseline trial and 1 did not display the reduction in clonus duration required on the fifth baseline trial. Of the remaining 29 subjects, 23 completed the study.

Treatment. After the baseline phase, the subjects were assigned to one of two treatment groups. The rats in one group (the ethanol-after group, n=14), received a total of twenty bidaily ethanol injections, each 1 hr after a convulsive amygdaloid stimulation. The rats in the second group, (the saline-after group, n=9), received the same number of injections of an isotonic saline solution 1 hr after each of their bidaily stimulations.

Test. The test phase of the experiment was the same for all subjects and consisted of 10 more bidaily ethanol injections (1.5 g/kg) administered 1 hr before convulsive stimulation. A rat was considered to have developed tolerance when the duration of forelimb clonus elicited in that animal on two consecutive test trials was at least 50% as long as the clonus elicited in the same subject on the fourth baseline day.

Analysis. A single t-test was used to analyze the difference in the number of test trials required for the rats in each group to meet the criterion of tolerance development.
FIGURE 6. Histology from Experiment 3.
Results. The rats from the ethanol-after group required significantly fewer test trials (M=6.2) to meet the criterion of tolerance development than did the rats from the saline group (M=8.1) (t (20)=2.75, p<.01).

Figure 6 illustrates the histological data from Experiment 3. All of the electrode tips were in, or on the boundaries of, the amygdaloid complex, with most terminating in the basolateral nucleus.

Discussion. The results of Experiment 3 provide the first unequivocal evidence of the development of tolerance to ethanol's anticonvulsant effect in the absence of the appropriate response contingency. Although there was no evidence of tolerance development in either group of rats on the first test trial, the rats from the ethanol-after group became tolerant significantly faster than the rats from the saline group once they were exposed to a series of ethanol-before trials. Because this tolerance was not evident on the first test trial, it is referred to here as latent drug tolerance.
VII. EXPERIMENT 4

The Role of the Response Contingency in
The Maintenance and Dissipation
of Tolerance to Ethanol's Anticonvulsant Effect

The response contingency is clearly an important, if not critical, factor in the development of tolerance to a wide variety of ethanol's effects. It is therefore surprising that there are no reports of the response contingency having a role in the dissipation of such tolerance once it has developed. This is especially true in light of several reports in which the response contingency played a crucial role in the dissipation of tolerance to the effects of other drugs. For example, Manning (1976a) demonstrated that tolerance to the disruptive effects of delta-9-tetrahydrocannabinol on operant responding in the rat dissipated over a 14-day retention interval only if the subjects were given the opportunity to perform the operant task in a drug-free state. Similarly, Poulos, Wilkinson, and Cappell (1981) demonstrated that the response contingency was an important factor in the dissipation of tolerance to the anorexigenic effects amphetamine on sweet milk consumption; in their study, rats lost their tolerance only when given access to the sweet milk solution in the absence of the drug. Finally, Poulos and Hinson (1984) found that tolerance to scopolamine's adipsic effect in
water-deprived rats dissipated only when the subjects were given access to water in the absence of the drug.

In Experiment 4, the role of the response contingency in the maintenance and dissipation of tolerance to ethanol's anticonvulsant effect was investigated. Is the relation between ethanol exposure and convulsive stimulation as important in the maintenance and dissipation of tolerance to ethanol's anticonvulsant effect as it is to its development?

Subjects. The subjects were 45 male, 300 to 400 g hooded rats purchased, maintained, and housed as in the first three experiments.

Surgery. The surgical procedure was the same as that employed in the first three experiments.

Kindling and Baseline Phases. The kindling and baseline phases were identical to those in the first three experiments.

Tolerance Development. Beginning 48 hr after the last baseline stimulation, every subject received a series of seven bidaily ethanol injections (1.5 g/kg, IP), each administered 1 hr before a single convulsive stimulation. As usual, the first of these injections completely suppressed the forelimb clonus elicited by amygdaloid stimulation in every rat; and tolerance to this potent anticonvulsant effect developed quickly in most of the rats. Thus, by the end of the tolerance-development phase 42 of the original 45 subjects had met the criterion of tolerance,
which was two consecutive trials on which the forelimb clonus elicited by amygdaloid stimulation was at least 50% as long as that displayed by the same subject on the fourth baseline trial. Because the purpose of Experiment 4 was to study the dissipation of tolerance, the three rats not meeting this criterion were dropped from the experiment at this point.

**Tolerance Dissipation.** The 42 rats meeting the criterion of tolerance development were assigned to five treatment groups. The mean clonus duration on the last two tolerance-development trials was calculated for each subject, and then the rats were assigned to their five treatment groups so that the means of these scores were approximately the same for all five groups. The subjects in each of the five groups were treated in different ways during the ensuing 14-day retention period. The rats in the ETOH-before-STIM group (n=8) continued to receive bidaily ethanol injections 1 hr before amygdaloid stimulation, whereas the rats in the STIM-before-ETOH group (n=9) received the same bidaily treatments but in the reverse order. The rats in the ETOH-noSTIM group (n=8) received the bidaily ethanol injections but no stimulations during the 14-day retention interval, whereas the rats in the STIM-noETOH group (n=9) received the bidaily stimulations but not the ethanol injections. The rats in the latter group received injections of isotonic saline either 1 hr before (n=5) or 1 hr after (n=4) each
bidaily stimulation. The rats in the fifth and final group, the noETOH-noSTIM group (n=8), received neither ethanol injections nor convulsive stimulations during the retention interval. Each rat in this group was weighed and handled every second day.

**Test.** The test for tolerance retention occurred 14 days after the last trial of the tolerance-development phase. The test procedure was identical to that of the tolerance development trials; the effect of a single injection of ethanol on the duration of forelimb clonus elicited by amygdaloid stimulation administered 1 hr later was assessed in each rat.

**Histology.** The histological procedure was identical to that followed in the first three experiments.

**Statistical Analysis.** The retention of tolerance was assessed by comparing the mean duration of the clonus elicited in each rat on the last two tolerance-development trials to the duration of forelimb clonus elicited from that rat on the test trial, and also by comparing the difference in the duration of forelimb clonus on the test day between the different treatment groups. Because the data of Experiment 4 seriously violated the assumption of homogeneity of variance, the data were analyzed using Wilcoxon's Ranked-Signs Matched-Pairs test and the Mann-Whitney U test (Siegel, 1956), as in Experiments 1 and 2.

**Results.** As is evident in Figure 7, the results of
Experiment 4 were remarkably unambiguous; tolerance dissipated only in the rats that received convulsive stimulation in the absence of ethanol exposure. Although there was no substantial decline of tolerance in the ETOH-before-STIM group, the ETOH-noSTIM group, and the noETOH-noSTIM group (all Wilcoxon p's>.05), it dissipated almost completely in the STIM-noETOH group and the STIM-before-ETOH group (both Wilcoxon p's<.01). Accordingly, on the test trial the clonus of the ETOH-before-STIM group, the ETOH-noSTIM group, and the noETOH-noSTIM group was significantly longer than that of the STIM-noETOH group and the STIM-before-ETOH group (all Mann-Whitney p's<.01).

All of the electrode placements were in or near the amygdaloid complex, with the majority located in the basolateral nucleus (see Figure 8).

Discussion. The results of Experiment 4 clearly establish the importance of the response contingency in the retention of tolerance to ethanol's anticonvulsant effect. Considering the evidence implicating the response contingency in the development of this tolerance, the results of Experiment 4 were not unexpected. What was unexpected, however, was the observation that the withdrawal of ethanol had no effect whatsoever on the maintenance or dissipation of tolerance. Tolerance to ethanol's anticonvulsant effect did not decline at all over the 14-day retention interval when ethanol was completely withdrawn if the rats did not receive convulsive
FIGURE 8. The effect of the response contingency on the dissipation of tolerance to ethanol's anticonvulsant effect. Ethanol withdrawal had no effect on the dissipation of tolerance, as the rats in the noETOH-noSTIM group demonstrated no loss of tolerance even though they were not administered ethanol during the retention interval. Furthermore, the administration of ethanol was not a necessary condition for the maintenance of tolerance, as the rats in the ETOH-afterSTIM group lost their tolerance even though they continued to receive ethanol on the same schedule of administration associated with the development of tolerance. The critical factor in the dissipation of tolerance to ethanol's anticonvulsant effect was the administration of stimulation in the absence of ethanol.
FIGURE 8. Histology from Experiment 4.
stimulation during the interval (the noETOH-noSTIM group). Furthermore, and perhaps more importantly, tolerance dissipated completely in nearly every rat that was maintained on exactly the same regimen of bidaily ethanol injections associated with tolerance development if these subjects (STIM-before-ETOH subjects) were stimulated before, rather than during, the periods of ethanol exposure. This result is especially important because it is the first demonstration that I am aware of where drug withdrawal is not necessary for the dissipation of tolerance. In all of the previous demonstrations in which the dissipation of tolerance was shown to be influenced by the response contingency (e.g., Manning, 1974; Poulos et al., 1981; Poulos & Hinson, 1984), the effect of the response contingency was studied only in subjects that had been withdrawn from the regimen of drug exposure. In Experiment 4, the response contingency was shown to be critical to the dissipation of tolerance even in subjects that continued to receive ethanol on the same schedule associated with the development of tolerance. In Experiment 4, the critical factor in the dissipation of contingent tolerance to ethanol's anticonvulsant effect appeared to be the elicitation of convulsive activity—the criterion response—in the absence of ethanol. If seizures were elicited in the absence of ethanol, tolerance to ethanol's anticonvulsant effect dissipated over the 14-day retention interval; if
this condition was not met, then there was no decline in tolerance.
The general purpose of the four experiments contained in this thesis was to clarify the nature of contingent tolerance to ethanol's anticonvulsant effect. Together, the results of these experiments provide unequivocal evidence of the important role that the response contingency has in both the development and subsequent retention of tolerance to ethanol's anticonvulsant effect. The first two sections of this General Discussion deal with: 1) the first three experiments, which were designed to determine whether tolerance to ethanol's anticonvulsant effect could develop in rats that did not receive convulsive stimulation during periods of intoxication, and 2) the fourth experiment, which examined the importance of the response contingency in the retention of tolerance to ethanol's anticonvulsant effect. The final two sections of the General Discussion deal with: 3) the theoretical explanations that have been advanced to account for contingent drug tolerance and their relevance to contingent tolerance to ethanol's anticonvulsant effect, and 4) the general implications of experiments contained in this thesis.
1. General Discussion of Experiments 1, 2, and 3

The purpose of the first three experiments was to answer the question "Does tolerance to ethanol's anticonvulsant effect develop in kindled rats that do not receive convulsive stimulation during periods of ethanol exposure?". The answer to this question was found to be "Yes, there is a small degree of tolerance development in the rats from the ethanol-after treatment condition." However, this answer requires qualification.

Although each of the first three experiments was designed to facilitate the detection of tolerance in rats in the ethanol-after condition, this proved to be difficult to do. Experiment 1 assessed the possibility that tolerance was not observed in the ethanol-after rats in Pinel et al.'s (1983) original study because the high treatment dose (4.5 g/kg) was too high. However, in Experiment 1, there was little evidence of tolerance in rats from the ethanol-after condition regardless of whether the treatment dose was high (5 g/kg) or low (2 g/kg). In Experiment 2, more treatment trials were administered to facilitate the development of tolerance in the absence of the response contingency; again, there was no significant tolerance in the ethanol-after rats, even after 20 trials. Perhaps the ethanol-after
subjects would have eventually developed substantial tolerance if they had received even more tolerance-development trials; however, pilot studies have indicated that the subjects' health begins to suffer if any more treatment trials are administered. Such subject debilitation makes the results difficult to interpret and the experiments difficult to justify on ethical grounds.

Experiment 3 provided evidence that statistically significant levels of tolerance can develop to ethanol's anticonvulsant effect in the absence of the response contingency. In Experiment 3, a sensitive multiple-trial, savings, test of tolerance facilitated the detection of what I have called latent tolerance to ethanol's anticonvulsant effect in the ethanol-after condition. It should be emphasized, however, that even the results of Experiment 3 provide evidence of the important role of the response contingency in the development of tolerance to ethanol's anticonvulsant effect. In Experiment 3, the tolerance that developed in the ethanol-after rats was not apparent on the first test trial--hence the label latent tolerance--and was detectable only as an acceleration in the subsequent development of tolerance when the subjects were switched to the ethanol-before condition. In contrast, significant levels of tolerance were detectable in the ethanol-before rats after only 4 or 5 trials.
2. General Discussion of Experiment 4

The purpose of Experiment 4 was to determine whether the response contingency was as important in the dissipation of tolerance to ethanol's anticonvulsant effect as it was to its development. The results unequivocally establish the response contingency as a key factor in the dissipation of this type of tolerance, and in so doing raise some intriguing questions about the traditional assumption that the dissipation of tolerance is totally dependent on drug withdrawal. In Experiment 4, the cessation of ethanol administration was neither necessary nor sufficient for the dissipation of tolerance to ethanol's anticonvulsant effect, and the continuation of ethanol administration was neither necessary nor sufficient for the retention of this tolerance once it had developed. Instead, the critical factor in the dissipation of tolerance was the elicitation of the criterion response—convulsive activity—in the absence of ethanol; if seizures were elicited in the absence of ethanol, tolerance to ethanol's anticonvulsant effect dissipated over the 14-day retention interval; if this condition was not met, then there was no decline in tolerance. In Experiment 4, there was complete retention of
tolerance to ethanol's anticonvulsant effect when ethanol was completely withdrawn over the entire 14-day retention interval, if the rats did not receive convulsive stimulation. More importantly, there was little retention of tolerance in rats that continued to receive ethanol on the same bidaily schedule associated with the development of tolerance development if these subjects were stimulated before, rather than during, the periods of ethanol exposure.

In the introductory description of the contingent tolerance phenomenon, the importance of the response contingency in the development of tolerance was illustrated with an analogy to a perceptual phenomenon. The point was made that to expect tolerance to develop to a drug's effects on a response that is not performed during periods of drug exposure is like expecting adaptation to the disruption in visuomotor performance caused by laterally displacing prisms to occur in the absence of an opportunity to perceive the disruption. Neither the adaptation to the disruptive effects of visual displacement on visuomotor coordination nor the adaptation to the disruptive effects of ethanol on kindled seizures can occur unless the disruptive effects are actually experienced.

This displaced-vision analogy is also relevant to the role of the response contingency in the dissipation of tolerance. Once a subject has adapted to the effects of displacing prisms, removing them produces another change in
the relation between visual and motor feedback to which the subject must adapt. Just as the visual perception of self-produced movement with the lenses on is necessary for adaptation to the introduction of displacing lenses, the visual perception of self-produced movement with them off is necessary for adaptation to their removal. Similarly, Experiment 4 demonstrates that the critical event in the dissipation of tolerance to ethanol's anticonvulsant effect is the experience of kindled seizures in the absence of ethanol. The dissipation of tolerance is not a simple consequence of drug withdrawal; the dissipation of tolerance requires that the absence of the drug effect on the criterion response (the convulsive response) can be experienced.

3. Theoretical Explanations of Contingent Tolerance

A number of different explanations have been advanced to account for the phenomenon of contingent drug tolerance. Three of these models are reviewed in this subsection, and their ability to account for the importance of the response contingency to the development and dissipation of tolerance to ethanol's anticonvulsant effect is discussed. The three explanations that are considered are: i) the reinforcement-density model; ii) the state-dependency model; and iii) the homeostatic-conditioning model of tolerance.
1). The Reinforcement-Density Model of Contingent Tolerance. The reinforcement-density model of contingent tolerance (Demellweek & Goudie, 1983; Kumar & Stolerman, 1977) assumes that tolerance develops to the effects of drugs that decrease the density of appetitive reinforcement or increase the density of aversive reinforcement, but not to the effects of drugs that have no effect on the density of reinforcement. For example, Schuster, Dockens, and Woods (1966) found that tolerance to the effects of amphetamine on a bar-press response developed only when the increase in response rate produced by amphetamine led to a decrease in appetitive reinforcement. In Schuster et al.'s study, rats that were trained to bar-press on a combination fixed interval/differential-reinforcement-for-low-rates-of-responding (FI/DRL) schedule developed tolerance to the effects of amphetamine in the DRL portion of the schedule (where the increase in response rate would decrease the number of reinforcements) but not in the FI portion of the schedule (where the increase in response rate would not affect, or even increase, the number of reinforcements). Based upon the principles of operant conditioning, the idea is that subjects learn to assume behavioral strategies that counteract disruptive drug effects because these strategies lead to an increase in positive reinforcement or a decrease in negative reinforcement. Thus, according to the reinforcement-density model of contingent tolerance only the
subjects in the drug-before condition develop tolerance to the drug's effects on the criterion response because only these subjects experience the change in the density of reinforcement caused by performing the criterion response while under the influence of the drug.

The reinforcement-density hypothesis can account for those examples of contingent drug tolerance in which operant reinforcement principles can be readily applied. For example, it is possible that tolerance to ethanol's disruptive effect on treadmill performance develops only in subjects that receive ethanol before they perform the task because only these subjects experience the increase in footshock associated with poor performance on the treadmill. The problem is that the hypothesis is totally incapable of accounting for examples of contingent tolerance which are not easily fitted into an operant conditioning framework. For example, the reinforcement-density hypothesis cannot readily account for the development of tolerance to ethanol's anticonvulsant effect because the initial effect of ethanol on the criterion response (convulsions) appears to be a beneficial one for the subject (a diminution in seizure severity), and the development of tolerance thus represents a decline in the magnitude of a beneficial effect. It is difficult to explain how the reinforcement-density hypothesis could account for the development of tolerance to the therapeutic effect of any drug.
Furthermore, the reinforcement-density hypothesis makes no prediction whatsoever about the role of the response contingency in the dissipation of tolerance.

ii. The State-Dependency Model of Contingent Tolerance. The term state-dependency refers to situations in which the efficient performance of a response is dependent upon a subject being tested in the same psychological state that existed when the response was acquired (Overton, 1966; 1984). According to the state-dependency hypothesis of drug tolerance (Chen, 1972; Cicero, 1980; Feldman & Quenzer, 1984), a response that was acquired by a subject in a drug-free state is poorly performed following the administration of a drug because the drug-induced change in the subject's psychological state impairs the subjects' ability to retrieve the information necessary to perform the task; the development of tolerance to this drug-induced impairment is presumed to reflect the acquisition of the response in the drugged state. Furthermore, withdrawal symptoms are attributed in part to an impairment in the subjects' ability to perform the response in an undrugged state that is caused by an inability to retrieve the information relevant to the task in a drug-free state. This impairment is presumed to last until the task is reacquired in the drug-free state. Thus, according to the state-dependency model of contingent tolerance, only the subjects in the drug-before group develop tolerance to the drug's effects on the criterion
response because only these subjects get the opportunity to perform (i.e., acquire) the criterion response while under the influence of the drug.

The utility of the state-dependency model explanation of contingent tolerance to ethanol's anticonvulsant effect is limited for two reasons. The first is its explicit prediction that the performance of the criterion response by tolerant subjects should be impaired when the drug is withdrawn until the subject reacquires the response in the drug-free state. However, in the present experiments there was no evidence whatsoever that the convulsions elicited in tolerance rats were affected in any way by the withdrawal of ethanol. The second reason is the fact that the state-dependency model is restricted to paradigms in which the state of the subject can be reasonably expected to interfere with the retrieval of information required for the efficient performance of a response (e.g., a maze task); it is difficult to account for an anticonvulsant effect in terms of a failure to retrieve the information necessary to perform the response.

iii. The Homeostatic-Conditioning Model of Tolerance. The homeostatic-conditioning model of drug tolerance (Poulos et al., 1981; Poulos & Hinson, 1984) represents an important advance in the study of the effects of behavioral variables on drug tolerance because it attempts to integrate the
phenomena of context-specific tolerance and contingent tolerance into a single theory. According to Poulos and his associates, the development of tolerance represents a homeostatic adaptation to a drug's effects on the criterion response, and thus the development of tolerance is contingent upon the performance of the criterion response during periods of drug exposure. However, the manifestation of the homeostatic changes responsible for the tolerance is context-specific; that is, the manifestation of the tolerance is dependent upon the drug being administered in the context in which the subject previously experienced the drug's effects. For example, Poulos et al. (1981) found that the development of tolerance to amphetamine's anorexigenic effect was contingent upon the subjects having access to food during periods of amphetamine exposure, and that the manifestation of this tolerance was restricted to the context in which the subjects normally received the drug. Poulos and his colleagues contend that this contextual specificity is the product of Pavlovian conditioning.

Although the synthesis of the areas of contingent and context-specific tolerance offered by the homeostatic-conditioning model of drug tolerance is appealing, it cannot account for contingent tolerance to ethanol's anticonvulsant effect for two reasons. First, Pinel and Puttaswamaiah (1985) have shown that the manifestation of contingent
tolerance to ethanol's anticonvulsant effect is not context-specific. The results of Experiment 3 offer support for this view--albeit indirect. In Experiment 3, the rats in the ethanol-after group received a total of 20 tolerance-development trials, in which ethanol was administered after convulsive stimulation, before the test trials began. The contextual stimuli (especially the cue properties of the ethanol; Greeley et al., 1984) that should serve as conditional stimuli according to a Pavlovian theory of context-specific tolerance were presented a total of 20 times before the test trials began. According to a Pavlovian model of tolerance, this extensive CS preexposure should have lead to latent inhibition and therefore a slower rate of tolerance development. This was not the case--recall that in Experiment 3 the rats from the ethanol-after group developed tolerance faster than did the saline-control rats.

The second reason that the homeostatic-conditioning model of tolerance cannot account for contingent tolerance to ethanol's anticonvulsant effect is that such an extension of the model would necessarily assume that seizures are homeostatically regulated and that their inhibition reflects a deviation from--rather than a return to--homeostasis. To my knowledge, there is no evidence whatsoever to support such a counter-intuitive view. One possible way around this problem is to argue that the development of tolerance to
ethanol's anticonvulsant effect does not represent a homeostatic adaptation to the anticonvulsant effect itself, but to the general depressant effects of ethanol on the central nervous system. Although this sounds plausible, this argument cannot account for the importance of the response-contingency to the development of tolerance to ethanol's anticonvulsant effect--homeostatic adaptation to ethanol's general depressant effects should be just as prevalent in the rats from the ethanol-after group as the ethanol-before group, and therefore tolerance should readily develop in both groups of subjects. This is clearly not the case.

It is obvious that the contingent tolerance phenomenon cannot be convincingly accounted for by any of the three existing theories. One possible reason for this is that each theory has been generated to explain the phenomenon of contingent tolerance within a limited number of paradigms. For example, the generality of the reinforcement-density hypothesis of contingent tolerance is limited to paradigms in which the rules of operant conditioning can be easily applied (e.g., maze running, bar-press responding). Similarly, the homeostatic-conditioning model of contingent tolerance is restricted to paradigms in which the criterion response is under homeostatic regulation and the manifestation of tolerance is context-specific. Finally, the state-dependency model of contingent tolerance is
restricted to paradigms in which the state of the subject can be reasonably expected to interfere with the retrieval of information required for the efficient performance of a learned response. Although it is considerably easier to generate a model of contingent tolerance that is based upon only a few instances of the phenomenon, such a strategy compromises one of the basic features of contingent tolerance—its generality. The response contingency has been found to be a crucial factor in the development of tolerance to the effects of a wide variety of pharmacologically disparate drugs: 1) psychostimulants (e.g., amphetamine, Carlton & Wolgin, 1971; Demellweek & Goudie, 1982; cocaine, Woolverton et al., 1979); 2) cannabinoids (e.g., delta-9-tetrahydrocannabinol, Manning, 1976); 3) sedative-hypnotics (e.g., diazepam, Mana, Pinel, & Kim, in preparation; Tizzano, Bannon, Liberto, Anderson, Roberts, Muchow, & Kallman, 1986; and ethanol, Alkana et al., 1982; Chen, 1968; Pinel et al., 1983; 1985); 4) and opioids (e.g., morphine, Kayan & Mitchell, 1969; Smith, 1979). Equally impressive is the variety of behaviors which have been manipulated as the criterion response in demonstrations of contingent tolerance: 1) barpress responding (Branch, 1979; Woolverton et al., 1979); 2) drinking (Poulos and Hinson, 1984) and 3) feeding (Carlton & Wolgin, 1971); 4) maze running (Chen, 1968); 5) nociception (Jorgenson & Hole, 1984); 6) posttetanic potentiation
(Traynor et al., 1982); 7) thermoregulation (Alkana et al. 1982); 8) treadmill running (LeBlanc et al., 1972); and 9) seizures (Pinel et al., 1983; 1985). The generality of the phenomenon, and the magnitude of the effects generated by manipulating the performance of the criterion response, clearly indicate that the response contingency is a key factor in the development of many forms of drug tolerance—in fact, the response contingency even appears to play a critical role in the adaptation to nonpharmacological disruption such as displaced vision. Clearly, an adequate theory of contingent tolerance must reflect the scope of the phenomenon.

4. Conclusions and Future Directions

Given the inability of any of the present models to account for contingent tolerance to ethanol's anticonvulsant effect, it would be fitting at this point to introduce a new model of contingent tolerance that would explain our current knowledge of the phenomenon and also generate a number of testable predictions about other aspects of tolerance that would be influenced by the response contingency. However, in my view, it would be premature to propose such a model; our knowledge of contingent tolerance is too limited to permit the presentation of an adequate theory of the phenomenon. For example, in spite of almost two decades of research, it is still not known whether the development of
contingent tolerance to a drug's effects is attributable to the same change or changes that underlie the tolerance that develops in the absence of an explicit response contingency. Furthermore, the circumstances in which the response contingency will affect the development of tolerance to drug's effects remain unknown. I have found that the development of tolerance to diazepam's anticonvulsant effect is contingent upon kindled rats receiving convulsive stimulation during periods of drug exposure (Mana, Pinel, & Kim, in preparation); yet Loscher and Schwark (1985) has demonstrated the development of tolerance to diazepam's anticonvulsant effects in kindled rats that were not stimulated at all during chronic exposure to the drug. There are many variables which may have contributed to this difference—for example the dose, the schedule of administration, the stimulation schedule—and until we begin to understand the effects of these variables on the importance of the response contingency any model of contingent tolerance will be seriously deficient.

In order to broaden our understanding of the importance of the response contingency to the development of tolerance, a greater variety of paradigms must be developed to study the phenomenon. One criticism of much of the research to date is that there has been little effort directed at defining its generality: for example, the resources of three different laboratories and almost two decades were spent
attempting to resolve the issue of whether tolerance to ethanol's effects could develop in the absence of the response contingency using only two paradigms—a maze task and a treadmill task. Although it is important to understand the importance of the response contingency in the development of tolerance to a single drug effect, in a single paradigm, it is critical that any attempt to generate a theoretical account of a phenomenon evolve from as diverse a data base as possible. In this vein, one of the distinguishing features of the present thesis was its use of a new and unique paradigm—Pinel's model of contingent tolerance to ethanol's anticonvulsant effect—to study the importance of the response contingency in the development and retention of tolerance to ethanol's anticonvulsant effect. The fact that existing theories cannot account for this type of contingent tolerance illustrates the futility of developing theories without a broader data base.

Two aspects of Pinel's paradigm merit consideration in the development of paradigms to study the contingent tolerance phenomenon. The first is that it permits complete control over the response contingency, a feature that is critical in any paradigm used to study the effects of the response contingency on the development of tolerance, yet one that is available in only a few of the existing paradigms (e.g., the Aplysia ganglia preparation used by Traynor and his colleagues to study contingent tolerance to
ethanol effects on the decay of posttetanic potentiation, and the tailflick preparation used by Jorgenson's group to study contingent tolerance to ethanol's analgesic effects in spinally transected rats). The second aspect of Pinel's paradigm merits consideration is that it involves tolerance to a therapeutic effect. Unlike many drug effects, the suppression of seizures produced by ethanol is a beneficial effect, and the development of tolerance represents a decline in this beneficial effect. As I pointed out in the previous section, evidence of tolerance to the therapeutic effects of a drug provides a critical test of the theories used to explain it. Because most research focuses on adverse drug effects, the theories resulting from them frequently have difficulty accounting for the beneficial consequences of drug exposure.

The present findings raise some interesting questions concerning the role of the response contingency in the eventual development of ethanol withdrawal seizures. According to the usual view of the relation between tolerance and dependence, these phenomena reflect a common adaptation. The physiological change that is presumed to oppose a drug's unconditional effects, and therefore produce tolerance, is also assumed to trigger withdrawal effects opposite to the unconditional effects of the drug once it has been eliminated from the subject's body (Balster, 1984; Cicero, 1980). Thus, the development of tolerance to
ethanol's anticonvulsant effect in the present experiments implies that withdrawal convulsions (e.g., Mucha, Pinel & Van Oot, 1975) should occur once ethanol administration ceases. Although there was no systematic attempt in the present experiments to observe the rats for the full 48-hr interval between ethanol administrations, casual observations were made each day, and there was no indication of spontaneous convulsive activity at any time during any of the experiments. Furthermore, in Experiment 4 there was no evidence of an increase in the duration of elicited seizures when ethanol was completely withdrawn during the retention interval. The study of contingent tolerance has already led to a reevaluation of two of the traditional principles about drug tolerance—that the development of tolerance is solely a function of drug administration, and that the dissipation of tolerance is caused by the cessation of drug administration. Future studies may force a reevaluation of a third fundamental assumption about drug tolerance: the assumption that tolerance and dependence are inextricably related.
References


