THE ROLE OF THE DRUG-EFFECT CONTINGENCY
IN THE DEVELOPMENT OF CROSS TOLERANCE
TO ANTICONVULSANT DRUG EFFECTS

By

CHANG KWON KIM

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Department of Psychology

The University of British Columbia
Vancouver, Canada

Date Oct 13, 1989
Abstract

It was recently demonstrated that tolerance develops to the anticonvulsant effect of ethanol on kindled convulsions elicited in rats by electrical stimulation of the amygdala, following each of a series of ethanol injections delivered on a bidaily schedule (once every 48 hr). The tolerance developed only when the convulsive stimulation was administered during the periods of ethanol exposure: subjects that received ethanol 1.5 hr before each convulsive stimulation demonstrated tolerance after just five ethanol injections; whereas, no tolerance was evident in subjects that received ethanol 1.5 hr after each stimulation. Such tolerance, which is not the inevitable product of drug exposure but is contingent upon the expression of the drug effect—the anticonvulsant effect in this case—has been termed contingent tolerance.

In Experiment 1A, tolerance developed to the anticonvulsant effects of bidaily IP injections of phenobarbital (30 mg/kg), trimethadione (270 mg/kg) and clonazepam (0.40 or 0.35 mg/kg) delivered 1 hr before each convulsive stimulation. In Experiment 1B, the rats tolerant to the anticonvulsant effects of phenobarbital, trimethadione, or clonazepam received bidaily injections of carbamazepine (35 mg/kg, IP), administered 1 hr before each stimulation. There was a statistically significant transfer of tolerance from phenobarbital to carbamazepine, but not from either trimethadione or clonazepam to carbamazepine. Thus, cross tolerance appears to be greatest between
anticonvulsant drugs that are effective against a similar profile of clinical and experimental seizures and that have similar mechanisms of action.

In Experiment 2A, tolerance developed to the anticonvulsant effect of bidaily pentobarbital (15 mg/kg, IP) injections only in those rats that received the drug 1 hr before the convulsive stimulation, but not in those rats that had received the drug 1 hr after each stimulation. Furthermore, those rats that had received the convulsive stimulations while under the influence of pentobarbital subsequently displayed a greater degree of cross tolerance to the anticonvulsant effect of ethanol (1.5 g/kg, IP) than those that had received the drug after each stimulation (contingent cross tolerance). Experiment 2B was the converse of Experiment 2A: contingent tolerance was demonstrated to ethanol and contingent cross tolerance to pentobarbital. This study provided the first unambiguous and bidirectional demonstration that the drug-effect contingency plays an important role in the development of cross tolerance.

In Experiment 3, tolerance to the anticonvulsant effect of ethanol dissipated when bidaily pentobarbital (15 mg/kg, IP) injections were delivered 1 hr after each convulsive stimulation (contingent cross-dissipation of tolerance), but did not dissipate when it was delivered 1 hr before each stimulation. Thus, the drug-effect contingency was shown to be important in the dissipation of tolerance to one drug following the administration of another drug.
In Experiment 4, different groups of rats received different doses of pentobarbital (10-50 mg/kg, IP) on a bidaily schedule 1 hr before the convulsive stimulation. Greater tolerance was found to the anticonvulsant effect of pentobarbital in rats that had received successively larger doses of the drug, none of which were large enough to suppress the convulsions, than those rats that were maintained on a high dose of the drug that completely suppressed the convulsions. The greater tolerance in the group that received successively greater doses was attributed to the fact that the convulsions were experienced in the drugged state. This study challenged the generally accepted view that tolerance develops more rapidly and to a greater extent with larger drug doses.

This thesis provides the first unambiguous and systematic evidence of the role of the drug-effect contingency in the transfer of tolerance from one drug to another, and in the dissipation of tolerance to one drug following the administration of another. On the basis of the present experiments, several elaborations were proposed to the drug-effect theory of drug tolerance, which claims that tolerance develops to drug effects and not to drug exposure per se.
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I. General Introduction

Pinel, Colbourne, Sigalet, and Renfrey (1983) demonstrated the development of tolerance to the anticonvulsant effect of ethanol on kindled convulsions elicited in rats by electrical stimulation of the amygdala. The most interesting feature of this demonstration was that the tolerance developed only if convulsive stimulations was delivered during the periods of ethanol exposure. Two groups of rats received an ethanol injection every two days; the rats in one group received the ethanol 1.5 hr before a convulsive stimulation, whereas those in the other group received the ethanol 1.5 hr after a convulsive stimulation. After five injections, the rats in the ethanol-before-stimulation group were almost totally tolerant to the anticonvulsant effect of ethanol, whereas no tolerance at all developed in the ethanol-after-stimulation rats. The difference in the degree of tolerance displayed in these two conditions was attributed to the fact that the anticonvulsant effect of ethanol was repeatedly experienced only in those rats that received ethanol before the stimulation. Such tolerance, which is not the inevitable product of drug exposure but is contingent upon the repeated expression of the drug effect—the anticonvulsant effect in this case—has been termed contingent tolerance (Carlton & Wolgin, 1971). On the basis of numerous demonstrations of contingent tolerance, Pinel, Mana, and Kim (1989) proposed the drug-effect theory of drug tolerance. According to this theory, tolerance develops to drug
effects and not to drug exposure per se.

The main purpose of the present experiments was to assess the role of drug-effects, as opposed to mere drug-exposure, on the development of cross tolerance between anticonvulsant drugs. Does the anticonvulsant drug effect have to be repeatedly experienced for the tolerance to transfer to the anticonvulsant effect of another drug, or is drug exposure without the expression of the anticonvulsant effect sufficient for the transfer of tolerance? Accordingly, the General Introduction to this thesis is divided into nine sections. Sections 1 and 2 introduce the phenomena of drug tolerance and cross tolerance, respectively; Section 3 outlines the traditional drug-exposure theory of drug tolerance; Section 4 outlines the drug-effect theory of drug tolerance; Section 5 briefly reviews the literature on contingent tolerance; and Sections 5 and 6 discuss anticonvulsant drug effects, and the use of the kindled convolution paradigm in their measurement. The General Introduction concludes with the general rationale and the general purpose of the thesis in Sections 8 and 9, respectively.

1. Drug Tolerance: The Basic Phenomenon

Drug tolerance is a decrease in the effect of a drug that occurs as the result of exposure to the drug. It can be manifested as a progressive decrease in the pharmacological effect of a particular dose of a drug with repeated administrations, or as the need for progressively greater doses of a drug to maintain the same pharmacological effect. In effect,
drug tolerance is a rightward shift in the dose response curve of the drug following exposure to the drug.

Although the various mechanisms of drug tolerance are poorly understood, they are considered to be of two general types: dispositional or functional (e.g., Kalant, LeBlanc, & Gibbins, 1971). Dispositional tolerance is tolerance that results from a decrease in the availability of the drug at the site of its action due to changes in the absorption, distribution, metabolism, or excretion of the drug. Functional tolerance is tolerance that results from any change in the sensitivity of the physiological systems affected by the drug; for example, a change in the sensitivity or number of neurotransmitter receptors (Rebec & Lee, 1983; Seeman, 1980), a change in the levels of neurotransmitters (Mechior & Tabakoff, 1981), neuromodulators (Vollicier & Ullman, 1985) or hormones (Wood, 1977; Tabakoff & Yanai, 1979), a change in cell membrane composition (Goldstein, 1983), a change in the activity of secondary messengers (e.g., Siggins, 1979), or a change in ion conductance (Ross, Garrett, & Cardenas, 1979).

Attributing tolerance to either a dispositional or a functional change or to a combination of them is a reasonable first step in the analysis of a particular instance of tolerance, but there are three problems with this approach. First, tolerance is often identified as resulting from a functional change only by the failure to detect an underlying dispositional change (e.g., Dews, 1978). Second, any dispositional or functional change that
is identified is not necessarily causally related to the observed development of tolerance (Demellweek & Goudie, 1983b). Third, the dispositional and functional changes to which specific instances of tolerance are attributed are often too slight to account for the hundred fold or greater decreases in responsiveness that characterize some tolerance effects (e.g., Seevers & Deneau, 1963).

Arguably, the key question that remains to be answered about drug tolerance is why tolerance develops to some drug effects but not others. Tolerance may develop to some effects of a drug while at the same time not develop to other effects of the same drug (e.g., Mansfield, Benedict & Woods, 1983). Moreover, tolerance may develop to a particular drug effect in one study but not develop to the same drug effect in another study in which similar doses of the drug were employed. Moreover, in some cases, sensitization—an increased sensitivity to a drug as the result of previous exposure to it—may develop to some effects of a drug while tolerance develops to other effects of the same drug (Kczenski & Leith, 1981).

2. Cross Tolerance

Cross tolerance is the transfer of tolerance from one drug to another drug. Cross tolerance is common between drugs that have similar mechanisms of action, similar profiles of drug effects, and similar chemical structures. Because cross tolerance is less common between drugs of two different pharmacological classes, demonstrations of cross tolerance between such drugs are
commonly considered to be evidence that they have similar mechanisms of action (Khanna & Mayer, 1982). Like specific instances of tolerance, specific instances of cross tolerance are classified as dispositional or functional (e.g., Coper, 1978).

3. Traditional Drug-Exposure Theory of Drug Tolerance

According to the traditional drug-exposure theory of drug tolerance, the exposure of the organism to the drug is the critical causal factor in the development of tolerance to the drug's effects. Within the context of this theory, the factors that are thought to influence the development of drug tolerance are the dose, the schedule, the duration, and the route of drug exposure. The major shortcoming of this traditional theory is that it cannot explain why the very same regimen of drug administration produces tolerance to a drug in some instances but not in others.

In the last two decades, two serious challenges to the traditional theory of drug tolerance have been issued. Unlike the traditional drug-exposure theory, these new theories are both based on the premise that the experiences of the subjects while they are drugged play a major role in tolerance development. The first of these two theories, the conditioned tolerance theory (e.g., Siegel, 1983), focuses on the role in the development of tolerance of the environment in which the subjects experience the drug effects. The second of these two theories, the drug-effect theory focuses on the role of the behavior of the subject during drug exposure. It is the drug-effect theory that was the focus of
the experiments reported here.

4. Drug-Effect Theory of Drug Tolerance

According to the drug-effect theory, drug tolerance develops to the drug effects, not to drug exposure; tolerance is seen as an adaptation to the repeated expression of a drug's disruptive effect on ongoing neural activity, not to the mere presence of the drug. Accordingly, drug exposure is presumed necessary, but not sufficient for the development of functional tolerance. That is, exposure to the drug per se may not lead to tolerance to a particular drug effect; in order for tolerance to develop to a particular drug effect, that effect must be repeatedly manifested (Pinel et al., 1989)

In order to test the drug-effect theory of tolerance, it is necessary to study drug effects that are not the inevitable consequence of drug exposure. In cases in which drug exposure and the criterion drug effect are inextricably related, both the traditional drug-exposure theory and the drug-effect theory make the same predictions. However, there are drug effects that occur only if the subject engages in a particular response while drugged; for example, the anticonvulsant drug effects can be expressed only when the organism is experiencing a convulsion. In such cases, it is possible to pit the predictions of the traditional drug-exposure theory of tolerance against the predictions of the drug-effect theory.

The drug-effect theory of tolerance can be illustrated with reference to another more well understood form of adaptation, the
adaptation that occurs to the disruptive effects of visual displacement on visual-motor coordination (cf. Poulos & Hinson, 1984). When a subject first wears displacing prisms that shift her or his visual world a few degrees to one side, visual-motor coordination is severely disrupted. But after some experience with the prisms, the subject adapts (i.e., becomes tolerant) to the visual displacement and visual-motor coordination returns to normal. What is the factor that leads to this adaptation? Is it the exposure to displaced vision, which is analogous to drug-exposure, or is it the experience of the disruption of visual-motor coordination, which is analogous to the drug-effect? The evidence overwhelmingly supports the latter view. Little visual-motor adaptation develops to the effects of displacing prisms in subjects that do not perform visual-motor tasks while wearing them (Held, 1972, Rock & Harris, 1972). According to the drug-effect theory of tolerance, the tolerance to the prisms is simply a type of sensory-motor adaptation, and like other forms of sensory-motor adaptation it results from the repeated experience of sensory-motor disruption rather than from the application of the disrupting agent.

Most demonstrations of the role of drug-effects in the development of tolerance have used the before-and-after design (Kumer & Stolerman, 1977). In such experiments, there are two groups of subjects: the subjects in the drug-before group receive the drug before each test trial, whereas those in the drug-after group receive the drug after each test trial; thus, the subjects
in the drug-before group experience the drug's effect on the test behavior on each trial, whereas those in the drug-after group do not. On the tolerance test, the subjects in both groups receive the drug before the test trial so that the degree of tolerance that developed in each group can be compared. Because the subjects of both groups have had the same number of test trials and drug administrations at the same dose, on the same schedule, and via the same route of administration, evidence of greater tolerance in the drug-before group can be attributed to the difference in the contingency between the drug exposure and the test response. The numerous demonstrations of significantly greater tolerance in the drug-before condition than in the drug-after condition of a before-and-after experiment have been termed contingent tolerance (Carlton & Wolgin, 1971)—or more ambiguously as behavioral tolerance (e.g., Chen, 1972).

5. Generality of the Drug-Effect Theory of Tolerance

Drug-effect contingencies have been shown to be an important factor in the development of tolerance to a wide variety of drug effects. Reports of contingent drug tolerance are summarized in Table I.

6. Anticonvulsant Drug Effect

In this thesis, the anticonvulsant drug effect was chosen for study for two main reasons. First, with this drug effect there is a clear disassociation between drug-exposure and drug-effect because the drug effect can occur only if a convulsive stimulation is delivered and this is under complete control of
## Table I

### Review of Contingent Tolerance

<table>
<thead>
<tr>
<th>Drug Effect</th>
<th>Specific Drug</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipsia</td>
<td>scopolamine</td>
<td>Poulos &amp; Hinson (1984)</td>
</tr>
<tr>
<td>Analgesia</td>
<td>ethanol</td>
<td>Jorgenson &amp; Hole (1984)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jorgenson et al., (1985)</td>
</tr>
<tr>
<td></td>
<td>morphine</td>
<td>Ferguson &amp; Mitchell (1969)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kayan &amp; Mitchell (1969)</td>
</tr>
<tr>
<td>Anorexigenia</td>
<td>amphetamine</td>
<td>Carlton &amp; Wolgin (1971)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Demellweek &amp; Goudie (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Streather &amp; Hinson (1985)</td>
</tr>
<tr>
<td></td>
<td>cathinone</td>
<td>Foltin &amp; Schuster (1982)</td>
</tr>
<tr>
<td></td>
<td>cocaine</td>
<td>Woolverton et al., (1978)</td>
</tr>
<tr>
<td></td>
<td>quipazine</td>
<td>Rowland &amp; Carlton (1983)</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>ethanol</td>
<td>Alkama et al., (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hjeresen et al., (1986)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Le et al., (1986)</td>
</tr>
<tr>
<td>Disruption of motor coordin-</td>
<td>ethanol</td>
<td>Chen (1968)</td>
</tr>
<tr>
<td>ation and balance</td>
<td></td>
<td>LeBlanc et al., (1976)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mansfield et al., (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wenger et al., (1980)</td>
</tr>
<tr>
<td></td>
<td>phenobarbital</td>
<td>Commissaris &amp; Rech (1981)</td>
</tr>
<tr>
<td>Disruption of operant tasks</td>
<td>amphetamine</td>
<td>Campbell &amp; Seiden (1973)</td>
</tr>
<tr>
<td></td>
<td>delta-9-THC</td>
<td>Carder &amp; Olsson (1973)</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>Chen (1979)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wiggell &amp; Overstreet (1984)</td>
</tr>
<tr>
<td></td>
<td>morphine</td>
<td>Smith (1979)</td>
</tr>
<tr>
<td></td>
<td>pentobarbital</td>
<td>Branch (1983)</td>
</tr>
<tr>
<td></td>
<td>phenobarbital</td>
<td>Tang &amp; Falk (1974)</td>
</tr>
<tr>
<td>Phenomenon</td>
<td>Agent</td>
<td>References</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Acceleration in the decay of</td>
<td>ethanol</td>
<td>Traynor et al., (1981)</td>
</tr>
<tr>
<td>posttetanic potentiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disruption of male sexual</td>
<td>ethanol</td>
<td>Pinel et al., (1989)</td>
</tr>
<tr>
<td>behavior</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticonvulsant effect</td>
<td>carbamazepine</td>
<td>Mana et al., (in press)</td>
</tr>
<tr>
<td></td>
<td>diazepam</td>
<td>Mana et al., (in press)</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>Pinel et al., (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pinel et al., (1985)</td>
</tr>
<tr>
<td></td>
<td>valproate</td>
<td>Mana et al., (in press)</td>
</tr>
</tbody>
</table>
the experimenter. With most drug effects, there is no such clear-cut disassociation between the drug-effect and drug-exposure. For example, the subject experiences many components of a drug's disruptive effect on maze running whether it is running the maze or walking around in its home cage. The clear-cut disassociation between exposure to anticonvulsant drugs and anticonvulsant drug effects is a great advantage in studying contingent tolerance. Second, studies of tolerance to anticonvulsant drug effects may have implications for the pharmacologic treatment of epilepsy, an affliction that affects nearly 1% of the population (Gilman, Goodman, & Gilman, 1980; Katzung, 1987).

7. Kindled Convulsions

In order to study anticonvulsant drug effects, one must have a way of inducing convulsions in experimental subjects. Convulsions can be induced electrically or chemically in all vertebrates and in some species, convulsions can be induced by sound, light, or handling. In this thesis, convulsions were elicited by electrical stimulation of the amygdala in kindled rats. In the broad sense of the term, "kindling" refers to a progressive increase in the severity of motor seizures elicited by a series of periodic convulsive treatments, but in the narrow sense, it refers to Goddard's (e.g., Goddard, McIntyre, & Leach, 1969) observation that periodic (e.g., one every 24 hr) low-intensity electrical brain stimulation through an implanted electrode leads to the development and progressive intensification of elicited convulsions. At first no convulsive
response is elicited, but repeated stimulations gradually come to elicit mild convulsive responses that progressively increase in intensity with each stimulation until each stimulation elicits a generalized clonic convolution that is very stable and changes little from stimulation to stimulation. The progressive increases in the generality of the convulsions has been used as a basis for the topographical classification system developed by Racine (1972) and extended by Pinel and Rovner (1978)—see Table II. Although many sites in the brain can be kindled, the amygdala kindles particularly rapidly and reliably, and thus it has been the preferred stimulation site. Although kindling has been reported in many species, rats have been the most common subjects in kindling experiments.

Kindled convulsions have important advantages over other experimental convulsions when it comes to assessing anticonvulsant drug effects. For example, electroconvulsive-shock-induced and pentylentetrazol-induced convulsions are often associated with subject injury and fatality, and they are extremely variable in form and duration, which makes them difficult to measure. The problem of subject attrition is particularly serious in studies of tolerance in which anticonvulsant effects are repeatedly assessed in the same subjects because any systematic change in the apparent anticonvulsant action of a drug are always confounded by the progressive debilitation and selective attrition of those subjects experiencing the most severe convulsions. In contrast,
Table II

Classification of Kindled Convulsions

<table>
<thead>
<tr>
<th>Class</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>facial clonus</td>
</tr>
<tr>
<td>2</td>
<td>facial clonus, head nodding</td>
</tr>
<tr>
<td>3</td>
<td>facial clonus, head nodding, forelimb clonus</td>
</tr>
<tr>
<td>4</td>
<td>facial clonus, head nodding, forelimb clonus, rearing</td>
</tr>
<tr>
<td>5</td>
<td>facial clonus, head nodding, forelimb clonus, rearing, loss of equilibrium</td>
</tr>
<tr>
<td>6</td>
<td>facial clonus, head nodding, forelimb clonus, multiple sequences of rearing and loss of equilibrium</td>
</tr>
<tr>
<td>7</td>
<td>all of the above plus a running fit</td>
</tr>
<tr>
<td>8</td>
<td>any of the above plus tonus</td>
</tr>
</tbody>
</table>
kindled rats remain healthy and easy to handle for the duration of an experiment, and fatalities are extremely rare. Moreover, in well kindled rats, it is possible to elicit convulsions that vary little from subject to subject in both form and duration, and baselines can be established in individual subjects that display almost no fluctuation from stimulation to stimulation (Pinel, Phillips, & MacNeil, 1973). The importance of such long-term stability in the study of the development of tolerance to anticonvulsant drug effects is obvious, and the stereotyped nature of kindled motor convulsions makes the measurement of their intensity a relatively simple matter.

In most studies of anticonvulsant drug actions on kindled convulsions, the dependent measure has been the class of the convulsion (see Table 2), an ordinal scale of measure. In the present thesis, although convulsion class was always recorded, the primary dependent measure was the duration of forelimb clonus. The duration of forelimb clonus is highly correlated with convulsion class and various other measures of kindled seizure severity, but it has the advantage of being a continuous ratio scale. Experiments from this laboratory have demonstrated the reliability of this measure and its sensitivity to drug manipulations (e.g., Pinel et al., 1989)

8. General Rationale

There were two general rationales for studying the role of the drug-effect contingency on the development of tolerance and cross tolerance. The first was the apparent lack of a complete
understanding of the complex phenomena of drug tolerance and cross tolerance. Despite decades of research and hundreds of research articles, many articles still end with perhaps the most overused cliche' in science, "more research is necessary." Perhaps the reason that progress has been slow is due to the narrowness of the traditional pharmacological drug-exposure theory that has guided most research. With the current understanding that the subjects' drug-related experiences are important in the development of tolerance, the study of tolerance and cross tolerance may yield new and useful information of theoretical and clinical importance. It may lead to a better understanding of why tolerance and/or sensitization occurs or does not occur; increase our understanding of the underlying mechanisms of drug action; provide a better understanding of the relationship between drug tolerance and the phenomena of drug dependence, withdrawal, and abuse; and lead to more effective pharmacological treatment regimens.

The second reason was the apparent gap between the research of mainstream pharmacologists and those that study contingent drug tolerance. Despite the generality of the contingent tolerance phenomenon, many mainstream pharmacologists have ignored this work. By demonstrating that the drug-effect contingency also plays a major role in cross tolerance, the robustness of this phenomenon would be established; and perhaps then it would find more widespread acceptance.

9. General Purpose
The general purpose of the thesis was to assess the role of drug-effects as opposed to mere drug-exposure on the development of cross tolerance to anticonvulsant drug effects. Accordingly, Experiment 1 examined cross tolerance to anticonvulsant drug effects without manipulating the drug-effect contingency; while Experiment 2 examined cross tolerance to anticonvulsant drug effects while manipulating the drug-effect contingency. Experiment 3 examined the role of the drug-effect contingency in the cross-dissipation of tolerance to anticonvulsant drug effects. Experiment 4 determined if different drug administration regimens would differentially influence the degree to which tolerance develops to anticonvulsant drug effects.
II. Background for Experiments 1 and 2

The main focus of the thesis was contingent cross tolerance to anticonvulsant drug effects. Accordingly, the literature on tolerance and cross tolerance to anticonvulsant drug effects and of contingent cross tolerance is reviewed in this section.

1. Tolerance and Cross Tolerance to Anticonvulsant Drug Effects

Tolerance has been demonstrated to the anticonvulsant effect of virtually every antiepileptic drug in current use—see Table III. In contrast, only a handful of studies have examined cross tolerance to anticonvulsant drug effects—see Table IV.

2. Contingent Cross Tolerance

There have been only five studies that have examined the role of the drug-effect contingency on the development of cross tolerance between drugs, and its role is still somewhat uncertain. These experiments either studied a drug effect in which confounding may be a problem, the experimental design was flawed, the data were improperly analyzed, or the results were inconsistent.

Two studies used the anorexigenic drug effect to study contingent cross tolerance; unfortunately, this drug effect is susceptible to a serious experimental confound. Tolerance to anorexia that is seen after chronic drug treatment may be a consequence of motivational changes and/or body weight loss in the rats that received the drug before the feeding sessions; and the amount of "true" tolerance that develops may be negligible.
### Table III

**Review of Tolerance to Anticonvulsant Drug Effects**

<table>
<thead>
<tr>
<th>Specific Drug</th>
<th>Convulsant Model</th>
<th>Species</th>
<th>Tolerance</th>
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Table IV

Review of Cross Tolerance to Anticonvulsant Drug Effects

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Streather and Hinson (1985) controlled for this and demonstrated that cross tolerance to the anorexigenic effect of apomorphine was greater in rats allowed to eat while under the influence of amphetamine than those that were not; however, contingent cross tolerance was evident at only one test dose of apomorphine, and there was no evidence of contingent cross tolerance from amphetamine to fenfluramine. Wooverton, Kandel, and Schuster (1978) demonstrated that cross tolerance to the anorexigenic effect of amphetamine was greater in rats allowed to eat while under the influence of cocaine; but this study was confounded, making interpretation of the results difficult. Two other studies used the motor-coordination-and-balance task, a poor choice of drug effect for the study of the drug-effect contingency because there is no clear disassociation between drug-exposure and drug-effect. The subject may experience components of a drug's disruptive effect whether performing the criterion task or walking around in its home cage. Commissaris and Rech (1981) found that under one condition, there was no significant difference in the degree of tolerance to pentobarbital in those rats that were allowed to practice while drugged and those that were not, but there was a greater degree of cross tolerance to ethanol in rats allowed to practice while under the influence of pentobarbital. Under another set of conditions, there was a difference in the degree of tolerance to pentobarbital, but no significant difference in the degree of cross tolerance to ethanol. Le, El-Ghundi, Khanna, and Kalant (1986) found no
significance difference in the degree of tolerance to ethanol in those rats allowed to practice while drugged and those that were not, but found that cross tolerance to pentobarbital was greater in rats allowed to practice while under the influence of ethanol. Jorgensen, Fasmer, and Hole (1986) reported that cross tolerance to the inhibitory effect of clonidine on the tail-flick reflex of spinally transacted rats was greater when tested while under the influence of ethanol; but there was a problem with the way the data was analyzed, making the findings somewhat questionable. Thus, the few studies of contingent cross tolerance have not clearly indicated whether or not the drug-effect contingency is important in the development of cross tolerance.
III. General Method

This section describes the methods common to most of the present experiments. Specific additions or changes to this general methodology are described in the Methods section of each experiment.

Subjects

The subjects in all the experiments were adult, male, hooded Long-Evans rats (from Charles River, Canada) weighing 300 to 500 g at the time of surgery. Each rat was individually housed in standard stainless steel wire mesh hanging cages with continuous access to Purina rat chow and water. All experimental manipulations occurred during the light phase of the 12/12-hr light/dark cycle.

Surgery

Following the administration of sodium pentobarbital (65 mg/kg, IP) anesthesia, and atropine sulphate (0.04 mg, IP) to help prevent respiratory failure, a single chronic bipolar electrode (Plastic Products Company, MS 303/2) was directed at the left basolateral amygdala of each rat. It was secured to the skull with stainless steel screws and dental acrylic. The tip of each electrode was positioned using one of two coordinate systems: 2.8 mm posterior, 5.0 mm lateral, and 8.7 mm ventral to the bregma skull surface, with the incisor bar set at +3.3 mm (coordinates from Paxinos & Watson, 1982) for Experiments 1 and 4; or 1.2 mm anterior, 5.0 mm lateral, and 10 mm ventral to the
bregma skull surface, with the incisor bar set at +5.0 mm (coordinates from Pellegrino, Pellegrino, & Cushman, 1979) for Experiments 2 and 3. Tetracycline was sprinkled over the incision before suturing to help prevent infections.

**Kindling Phase**

After at least 5 days of postsurgical recovery, each of the rats was stimulated (400 uA, 60 Hz, 1 sec) three times per day, 5 days a week for 3 weeks, with at least 2 hr separating consecutive stimulations. For each stimulation, the rat was removed from its home cage, the stimulation lead was connected, and then the rat was 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 convulsive activity ceased. By the end of this regimen of 45 kindling stimulations, each stimulation produced generalized clonic convulsions.

**No-Drug Baseline Phase**

This phase began 48 hr following the last of the 45 kindling stimulations, and it involved four stimulations delivered one every 48 hr (+2 hr). This bidaily stimulation schedule once initiated during the no-drug baseline phase was maintained for the duration of each experiment. For each convolution, the latency and duration of forelimb clonus and the convolution class was recorded, but the dependent measure in all except Experiment 4 was the duration of forelimb clonus elicited by each stimulation. The isotonic saline vehicle (0.9% saline), matched in volume for
the subsequent drug injections (the injection volume varied for the experiments), was injected 1 hr prior to the fourth and last no-drug baseline stimulation to determine the effects of the injection procedure on the duration of forelimb clonus elicited by the stimulation. All the injections, for the drugs and the saline vehicle, were injected IP at room temperature. Any rats that did not display at least 20 s of forelimb clonus on this no-drug saline baseline trial or displayed running fits (class 7 convulsion according to the classification scheme of Pinel & Rovner, 1978; see Table 2) were not studied further. The rejection criterion was enforced to eliminate any subjects that did not consistently display convulsive activity in the absence of the drug or displayed violent convulsions that are difficult to measure. This criterion was not met by 6.1% of the rats from all the experiments.

**Drug Baseline Test**

Approximately 48 hr after the no-drug saline baseline trial, all rats received the appropriate drug in an isotonic saline vehicle 1 hr prior to the convulsive stimulation. Those rats that did not show a sufficient anticonvulsant response to the drug; that is, those that displayed more than 20 s of forelimb clonus, were not studied further. This rejection criterion was enforced because the development of tolerance to anticonvulsant drug effects cannot be studied if subjects do not display initial sensitivity to the drug's anticonvulsant effect. This criterion led to the rejection of 2.8% of the rats. The particular doses
employed in the present experiments were determined from previous published studies (e.g., Albertson, Peterson & Stark, 1980; Mana & Pinel, 1987; Minabe, Tanii & Kurache, 1987; Pinel et al., in press) and from pilot observations. In all but Experiment 4, an attempt was made to select doses that were the minimum required to completely suppress the forelimb clonus in all rats.

Treatment and Test Phase

The treatment and test phases differed for the experiments, but each had several things in common: the rats were assigned to the experimental groups in such a way that the mean duration of forelimb clonus elicited on the no-drug saline baseline trial and the drug baseline test, and their body weights were approximately equal for the groups; it began 48 hr after the drug baseline test; the treatment and test trials occurred on a bidaily schedule (every 48 ± 2 hr) for the duration of the experiment; and on each treatment and test trial, the subject was removed from its home cage, weighed, and the drug or the saline vehicle was administered either 1 hr before or 1 hr after the convulsive stimulation.

Histology

At the conclusion of each experiment, all of the rats were killed with CO according to Canada Council on Animal Care Guidelines. Their brains were removed, preserved in formalin, frozen, sliced along the coronal plane (approximately 30 um thickness), mounted on slides, and then stained with cresyl violet to confirm the locations of the electrode tips.
Statistical Analysis

In most cases, the statistical significance of the results was evaluated using nonparametric statistical methods (Siegel & Castellan, 1988) because of repeated gross deviations from normality and from homogeneity of variance—it was not uncommon for the scores in an experimental condition to be all zeros. Only in Experiment 4 were the data parametric, and thus parametric methods were used only in this experiment. The basic significance level was set at $p<.05$, but in each study bonferroni adjusted alpha values were used to avoid the problem of elevated alpha values associated with multiple comparisons. Only the data of those rats that completed as experiment were subjected to statistical analysis.
IV. Experiment 1

Tolerance has been demonstrated experimentally to virtually every antiepileptic drug in current use (see Table 3; Frey, 1985, 1986, 1987). But there is a notable lack of research on cross tolerance between anticonvulsant drug effects (see Table 4). The few studies that have examined cross tolerance have usually limited the choice of drugs between those of the same or similar pharmacological classes (Bourgeois, 1986; Rosenberg, Teitz & Chiu, 1985; Vajda, Lewis, Harris, Jarrott, & Young, 1986). In this study, cross tolerance was examined between several drugs that differed in the degree of similarity with each other; the transfer of tolerance was assessed from phenobarbital, trimethadione, and clonazepam to carbamazepine. From among the approximately 20 antiepileptic drugs in current use, these four drugs were selected for study on the basis of three criteria. The first was that they were effective against different clinical seizures (see Figure 1); phenobarbital and carbamazepine are most commonly used in the treatment of partial seizures and generalized tonic-clonic seizures, trimethadione is used exclusively in the treatment of absence seizures, and clonazepam is used in the treatment of the entire spectrum of seizure types, although mostly in the treatment of absence seizures (see Eadie, 1985; Rall & Schleifer, 1980). The second was the fact that they have different putative mechanisms of action; current evidence suggests that phenobarbital and carbamazepine act by decreasing
Figure 1. The classification of the types of epileptic seizures, and the relative efficacy of the antiepileptic drugs—phenobarbital, trimethadione, clonazepam and carbamazepine—at controlling them. Epileptic seizures can be classified into generalized and partial seizures, with further subdivisions within each category; and antiepileptic drugs vary in their effectiveness at controlling the different types of epileptic seizures. Trimethadione is most effective against absence seizures; phenobarbital and carbamazepine is most effective against partial and generalized tonic-clonic seizures; and clonazepam is effective against the whole spectrum of seizures although it is used mostly against absence seizures.
sodium and calcium ion movement across membranes, and that trimethadione and clonazepam act by other mechanisms (see Frey & Janz, 1985; Katzung, 1987). And the third was their different profile of effectiveness against maximal electroshock and pentylentetrazol (metrazol) induced convulsions; phenobarbital and carbamazepine are more effective against maximal electroshock convulsions than against pentylentetrazol convulsions, whereas the reverse is true for trimethadione and clonazepam (see Frey & Janz, 1985).

Based on the similarity among the four drugs in the type of clinical seizures that they most effectively control, their putative mechanism of action, and their relative effectiveness in suppressing electroshock and pentylentetrazol induced convulsions, it was hypothesized that cross tolerance would be greatest from phenobarbital to carbamazepine, less from clonazepam to carbamazepine, and least from trimethadione to carbamazepine. The purpose of Experiments 1A and 1B was to test this hypothesis. The tolerance and cross tolerance were examined without manipulating the drug-effect contingency: the convulsive stimulation always followed the drug injections so that the anticonvulsant drug effects were experienced by the subjects on each trial.

**Experiment 1A**

The purpose of Experiment 1A was to demonstrate that tolerance develops to the anticonvulsant effects of phenobarbital, trimethadione, and clonazepam, and in doing so to
prepare the rats for the cross tolerance test to carbamazepine in Experiment 1B. Previous studies had already demonstrated tolerance to the anticonvulsant effects of these three drugs (e.g., Frey & Kampmann, 1965; Frey & Kretschmer, 1971; Gent, Feely, & Haigh, 1985), but this was the first demonstration of tolerance to the anticonvulsant effect phenobarbital and trimethadione on kindled convulsions.

Method

Subjects. Of the 57 rats completing the kindling phase of Experiment 1, two rats were rejected because they did not meet the criterion on the no-drug saline baseline trial, four rats were rejected because they did not meet criterion on the drug baseline test, and another three were lost due to illness or rejection of their electrode assembly. Thus, 48 rats completed Experiment 1A.

Drugs. The drugs used in Experiment 1A were phenobarbital (30 mg/kg; in a sodium salt form; BDH Chemicals), trimethadione (270 mg/kg; Abbott Pharmaceuticals), and clonazepam (0.40 or 0.35 mg/kg; Hoffmann-La Roche). The vehicle was isotonic saline with 2% Tween 80 (J.T. Baker Chemical) to aid in suspension of the drugs. All drug and saline vehicle injections were delivered in a volume of 5 ml/kg. Sonification was required to get trimethadione and clonazepam into suspension.

Drug Baseline Test. On the drug baseline test, all rats received either phenobarbital, trimethadione, or clonazepam (0.40 mg/kg) 1 hr prior to the convulsive stimulation.
**Tolerance-Development Phase.** During the tolerance-development phase, each group of rats that had been screened on either one of the three drugs on the drug baseline test were divided into two equivalent groups: the drug group (n=9 for phenobarbital; n=8 for both trimethadione and clonazepam) and the corresponding saline group (n=8 for phenobarbital and trimethadione; n=7 for clonazepam). On each tolerance development trial, the appropriate drug—the same drug that was delivered on the drug baseline test—was delivered to the drug group and the saline vehicle to the saline group 1 hr before the convulsive stimulation. There were 10 tolerance development trials for the phenobarbital and trimethadione treated rats, and 21 for the clonazepam treated rats. For clonazepam, the dose administered on the drug baseline test and the first 15 tolerance development trials was 0.40 mg/kg, but the dose was lowered to 0.35 mg/kg for the last six trials.

**Tolerance Test.** On the tolerance test, which occurred 48 hr after the last tolerance development trial, each rat of the drug group and the corresponding saline group received the appropriate drug 1 hr before the convulsive stimulation so that the degree of tolerance to the anticonvulsant effect of the drug could be compared between the drug groups and their respective saline control groups. The test dose of clonazepam was 0.35 mg/kg.

**Statistical Analysis.** The statistical significance of between-subject differences was evaluated with the Wilcoxon-Mann-Whitney U test, and of within-subject differences with the Sign
test. For the analysis of tolerance to phenobarbital and trimethadione, the significance level was set at \( p<0.017 \), one-tailed, because three comparisons were made; for clonazepam, it was set at \( p<0.025 \), one-tailed, because two comparisons were made.

**Results**

As illustrated in the three panels of Figures 2, tolerance developed to the anticonvulsant effects of phenobarbital (Panel A), trimethadione (Panel B), and clonazepam (Panel C), respectively. At the start of the experiment, the drug and the corresponding saline groups did not differ in either their responsiveness to the convulsive stimulation on the no-drug saline baseline trial or to their responsiveness to the anticonvulsant effect of each drug on the drug baseline test, but tolerance developed to each drug in the drug group over the course of the tolerance development trials. For phenobarbital and trimethadione, on the drug baseline test the drugs completely suppressed forelimb clonus in almost every rat in the drug groups; whereas, on the tolerance test, after the 10 drug injections, it had markedly less of an anticonvulsant effect (\( p<0.001 \) for phenobarbital; \( p<0.004 \) for trimethadione). In contrast, the rats of the corresponding saline groups displayed no evidence of tolerance whatsoever: each drug suppressed forelimb clonus on both the drug baseline test and the tolerance test (\( p>0.017 \) for both phenobarbital and trimethadione). Accordingly, the durations of forelimb clonus displayed was much greater for the drug groups then the corresponding saline groups.
Figure 2. Tolerance to the anticonvulsant effects of phenobarbital (30 mg/kg; Panel A), trimethadione (270 mg/kg; Panel B) and clonazepam (0.40 mg/kg on the first 15 tolerance development trials and 0.35 mg/kg on the last six; Panel C) on amygdala-kindled convulsions in rats. For each of the three drugs, there were two groups of rats that did not differ at the start of the experiment on the no-drug saline baseline trial (SB) or the drug baseline test (DB). On tolerance development trial, the drug group received the drug and the saline group received the saline vehicle 1 hr before the convulsive stimulation. On the tolerance test (T) when both groups received the appropriate drug 1 hr before the stimulation, only the drug group displayed tolerance to the anticonvulsant effect of the drug.
PANEL A: TOLERANCE TO PHENOBARBITAL

MEAN FORELIMB CLONUS DURATION (sec)

Saline group

Phenobarbital group

TOLERANCE DEVELOPMENT TRIALS
PANEL B: TOLERANCE TO TRIMETHADIONE

MEAN FORELIMB CLONUS DURATION (sec)

Saline group
Trimethadione group

TOLERANCE DEVELOPMENT TRIALS
PANEL C: TOLERANCE TO CLONAZEPAM

Saline group

Clonazepam group

MEAN FORELIMB CLONUS DURATION (sec)
on the tolerance test (p<.0039 for phenobarbital; p<.0003 for trimethadione).

For clonazepam, tolerance developed to its anticonvulsant effect in the drug group over the 21 trials, but at a slow rate. On the tolerance test, the rats of the drug group displayed markedly more clonus than the rats of the saline group but it just missed statistical significance set at p<.025 (p=.027). However, when the drug and saline groups were compared on the change in duration of forelimb clonus that occurred for each subject between the drug baseline test and the tolerance test, there was a significantly greater increase in the clonus durations of the drug group (p<.015). This type of comparison was used, instead of a direct comparison between the drug baseline test and the tolerance test, because the dose of clonazepam used on these two tests were different.

Discussion

Tolerance developed to the anticonvulsant effects of phenobarbital, trimethadione, and clonazepam when the drug injections occurred before the convulsive stimulation. This demonstration of tolerance to anticonvulsant drug effects was in agreement with other studies that have demonstrated tolerance to the anticonvulsant effects of phenobarbital (e.g., Frey & Kampmann, 1965; Schmidt, Kuperberg, Yonekawa, & Penry, 1980) trimethadione (e.g., DeSalva 1956; Frey & Kretschmer, 1971), and clonazepam (e.g., Gent, Feely, & Haigh, 1985; Scherkl, Kurudi, & Frey, 1988; Scherkl, Scheuler, & Frey, 1985); but this was the
first such demonstration with phenobarbital and trimethadione on kindled convulsions.

**Experiment 1B**

The purpose of Experiment 1B was to demonstrate that the degree of cross tolerance to the anticonvulsant effect of carbamazepine was greatest in rats tolerant to the anticonvulsant effect of phenobarbital, only slight in those tolerant to clonazepam, and the least in those tolerant to trimethadione. Only a small number of other studies have examined cross tolerance between anticonvulsant drug effects (see Table 4), and this was the first examination between these particular drugs.

**Method**

**Subjects.** The subjects in Experiment 1B were the same rats that had completed Experiment 1A: the three drug groups remained intact (n=9 for phenobarbital; n=8 for both trimethadione and clonazepam), but the three saline groups were collapsed into one (n=23) because there were no significant differences among them.

**Cross-Tolerance Phase.** The cross-tolerance phase began 48 hr after the tolerance test in Experiment 1A. The subjects of the three drug groups and the saline group received carbamazepine (35 mg/kg; Geigy Pharmaceuticals) 1 hr before each of 10 bidaily convulsive stimulations. Carbamazepine required sonification to get into suspension; and the injections were delivered in the same volume and the same vehicle as the injections of Experiment 1A. Cross tolerance to carbamazepine was assessed in two ways: 1) by the inability of the first injection of carbamazepine to
suppress forelimb clonus and 2) by the rate at which each rat achieved a criterion of tolerance to carbamazepine, which was set at forelimb clonus durations of at least 50% of that displayed on the no-drug saline baseline trial on two consecutive cross tolerance trials.

**Statistical Analysis.** The Kruskal-Wallis one-way analysis of variance was used to compare the four groups, with the significance level was set at $p<.017$, two-tailed, because three comparisons were made. If the results were statistically significant, post hoc multiple comparisons were made between the individual groups; the significance level was set at $p<.0083$ because six comparisons were made among the four groups. The within-subject comparisons were performed using the Sign test, with the significance level set at $p<.013$, one-tailed, because four comparisons were made.

**Results**

Figure 3 illustrates that the degree of cross tolerance to carbamazepine was greatest with phenobarbital, only slight with clonazepam, and the least with trimethadione. Statistically significant transfer of tolerance was observed only phenobarbital to carbamazepine. The results were similar for both measures of cross tolerance: the Kruskal-Wallis test showed that there were significant differences among the groups on the first cross tolerance trial ($p<.001$) and on the rate at which the criterion of cross tolerance was achieved ($p<.001$). Multiple comparisons revealed that the rats of the phenobarbital group displayed
Figure 3. Cross tolerance from the anticonvulsant effects of phenobarbital, trimethadione or clonazepam to the anticonvulsant effect of carbamazepine on amygdala-kindled convulsions in rats. Carbamazepine (35 mg/kg) was delivered 1 hr before the convulsive stimulation in rats tolerant to phenobarbital, trimethadione or clonazepam and the saline control group on each of 10 cross tolerance trials. There was significant transfer from phenobarbital to carbamazepine, but not from either trimethadione or clonazepam to carbamazepine.
CROSS TOLERANCE TO CARBAMAZEPINE

Legend
○ PHENOBARBITAL GROUP
◇ TRIMETHADION GROUP
□ TULSI/DAP GROUP
● SALINE GROUP

MEAN FORELMB CLONUS DURATION (sec)

CROSS TOLERANCE TRIALS
significantly greater tolerance to carbamazepine than the rats of the saline group (p<.0083 on both measures), and in fact were completely tolerant on the very first trial. The clonazepam group displayed slightly more cross tolerance than the saline group, although not to a significant extent (p>.0083 on both measures). And interestingly, the trimethadione group displayed less forelimb clonus on the first trial and took longer to reach the criterion of tolerance than the saline group, although not to a significant extent (p>.0083 on both measures). Moreover, the phenobarbital group was significantly more tolerant to carbamazepine than the trimethadione group (p<.0083 on both measures). The mean number of trials to reach the criterion of cross tolerance for each group was M=2.0 for phenobarbital, M=3.5 for clonazepam, M=7.5 for trimethadione and M=4.5 for saline.

On the 10th and final cross tolerance trial, the four groups did not differ significantly in their degree of tolerance to carbamazepine (p>.0167).

The within-subject increase in the degree of tolerance to carbamazepine from the first to the last (10th) carbamazepine administration was found to be significant for the trimethadione (p<.008) and the saline (p<.002) groups, but not for the phenobarbital (p>.0125) and the clonazepam (p>.0125) groups.

Histological analysis of the electrode placements revealed that all electrode tips were located in the amygdala complex or near its boundaries (see Figure 4).
Figure 4. Electrode placements in the 48 rats completing Experiment 1.
Discussion

This was the first examination of the transfer of tolerance from phenobarbital, trimethadione, or clonazepam to carbamazepine. And the results confirm the hypothesis that the transfer of tolerance would be greatest from phenobarbital to carbamazepine and the least from trimethadione to carbamazepine. This hypothesis was based on the similarity between the drugs on the type of clinical seizures the drugs most effectively controlled, the putative mechanism of action, and the relative efficacy with which convulsions induced by pentylenetetrazol and electroshock were controlled.

The present results were in agreement with the suggestion that anticonvulsant drugs fall into two general categories (see Vanden Bussche, De Beukelaar, & Wauquier, 1985; Porter & Pitlick, 1987; Rall & Schleifer, 1980). Type 1 drugs control partial seizures and generalized tonic-clonic seizures; work by decreasing the movement of sodium and calcium ions across membranes; are more effective against maximal electroshock than pentylenetetrazol induced convulsions; and work by preventing the spread of the seizure. The drugs of this class include phenobarbital and carbamazepine. Type 2 drugs control generalized seizures, especially absence seizures; are more effective against pentylenetetrazol then maximal electroshock induced convulsions; and work by increasing the seizure threshold. The drugs of this group include trimethadione and clonazepam.

Although this experiment was not designed to study the
development of tolerance to carbamazepine, it was examined, albeit without a control group, by measuring the increase in tolerance from the first to the last (10th) administration of carbamazepine in the saline group. Significant increases in the degree of tolerance to carbamazepine was seen; this is in agreement with Mana et al. (under review) which unequivocally demonstrated the development of tolerance to the anticonvulsant effect of carbamazepine on amygdala-kindled convulsions of rats.
V. Experiment 2

The importance of the drug-effect contingency in the development of tolerance to the anticonvulsant effects of ethanol (Pinel et al., 1983, 1985) and the antiepileptic drugs, carbamazepine, diazepam, and valproate (Mana, Kim, Pinel & Jones, under review) has recently been demonstrated. In each case, tolerance developed rapidly in rats that received the drug injection before each bidaily convulsive stimulation but not at all in those rats injected after the stimulation—tolerance to anticonvulsant drug effects only developed when the convulsive stimulation occurred in the drugged state so that the anticonvulsant drug effects were experienced by the subjects. The importance of the drug-effect contingency in the development of tolerance has been demonstrated to numerous other drug effects.

Its role in the development of cross tolerance is still uncertain (see Background for Experiments 1 and 2). Accordingly, the main purpose of Experiment 2 was to establish without ambiguity that the drug-effect contingency does play a role in the development of cross tolerance to anticonvulsant drug effects. More specifically, the purpose of Experiment 2A was to demonstrate that the expression of the anticonvulsant drug effect was important in the transfer of tolerance from pentobarbital to ethanol; and the purpose of Experiment 2B was to demonstrate this transfer of tolerance from ethanol to pentobarbital. Experiment 2B. These experiments focussed on pentobarbital and ethanol
because cross tolerance has been reported to many effects of these drugs (Khanna & Mayer, 1982), although never to their anticonvulsant effect, and because they had been used in two previous studies of contingent cross tolerance (Commissaris & Rech, 1981; Le, El-Ghundi, Khanna, & Kalant, 1986).

**General Method**

The experimental methodology of the two studies of Experiment 2 was identical except for the order in which the drugs were presented.

**Drugs**

The drugs used were pentobarbital (15 mg/kg; in a sodium salt form; BDH Chemicals) and ethanol (1.5 g/kg in a 25% v/v solution in isotonic saline). The injection vehicle was isotonic saline and all drug and saline injections were delivered in a volume of 7.5 ml/kg. This high volume was required to dilute the concentration of ethanol because high ethanol concentrations can irritate the peritoneal cavity lining.

**Drug Baseline Test**

The subjects received either pentobarbital in Experiment 2A or ethanol in Experiment 2B 1 hr before the convulsive stimulation.

**Tolerance-Development Phase**

The tolerance-development phase comprised 10 bidaily stimulations and 10 bidaily injections of the same drug as on the drug baseline test. The rats were divided into two equivalent groups and on each tolerance development trial, the rats in one
group (the drug-before group) received the drug 1 hr \textit{before} the convulsive stimulation, and the rats in the other group (the drug-after group) received the drug 1 hr \textit{after} the stimulation.

\textbf{Tolerance Test}

The tolerance test occurred 48 hr after the last (10th) tolerance development trial. Each rat received the appropriate drug (pentobarbital in Experiment 2A; ethanol in Experiment 2B) 1 hr \textit{before} the convulsive stimulation so that the development of tolerance to the anticonvulsant effect of the drug could be compared between the drug-before and drug-after groups.

\textbf{Cross-Tolerance Phase}

The cross-tolerance phase began 48 hr after the tolerance test. The subjects received ethanol if they had previously received pentobarbital in the tolerance-development phase (Experiment 2A) and pentobarbital if they had previously received ethanol (Experiment 2B) 1 hr \textit{before} each of 10 bidaily convulsive stimulations. Contingent cross tolerance was assessed in two ways: 1) by the inability of the first injection of the second drug to suppress forelimb clonus and 2) by the rate at which the subjects in the two groups achieved a criterion of tolerance to the second drug set at forelimb clonus durations of at least 50% of the no-drug saline baseline trial on two consecutive trials.

\textbf{Statistical Analysis}

The statistical significance of the results were evaluated with the Wilcoxon-Mann-Whitney \textit{U} test for between subject comparisons and the Sign test for within subject comparisons.
Both the tolerance and cross tolerance data were analyzed at p<.017, one-tailed, because three comparisons were made.

Experiment 2A

The purposes of Experiment 2A were to demonstrate 1) that tolerance develops to the anticonvulsant effect of pentobarbital; 2) that this tolerance occurs only when the convulsive stimulation was delivered in the presence of the drug, and 3) that the transfer of tolerance to the anticonvulsant effect of ethanol is also dependent on the stimulation occurring in the drugged state.

Method

Electrodes were implanted in 30 rats, but the screening criteria were not met by three rats on the no-drug saline baseline trial and one on the drug baseline test, and one other was dropped due to illness. Thus, 25 rats completed the experiment: n=13 in the pentobarbital-before-stimulation group and n=12 in the pentobarbital-after-stimulation group.

Results

The results of Experiment 2A are illustrated in Figure 5, which summarize the results of the two phases of the experiment: the tolerance-development phase and the cross-tolerance phase. It is readily apparent that the two groups did not differ at the start of the experiment in either their responsiveness to the convulsive stimulation on the no-drug saline baseline trial or to their responsiveness to the anticonvulsant effect of pentobarbital on the drug baseline test.
Figure 5. The effect of pentobarbital (15 mg/kg) administration either 1 hr before or 1 hr after bidaily convulsive stimulations, and the subsequent ethanol (1.5 g/kg) administration 1 hr before the stimulations. There were no differences at the start of the experiment between the two groups in either their responsiveness to the convulsive stimulation on the no-drug saline baseline trial (SB) or their responsiveness to the anticonvulsant effect of pentobarbital on the drug baseline test (DB). During the tolerance-development phase, tolerance to the anticonvulsant effect of pentobarbital developed, but only in the pentobarbital-before-stimulation condition. During the cross-tolerance phase, a greater degree of cross tolerance to the anticonvulsant effect of ethanol was apparent in the rats that had previously been on a regimen of pentobarbital-before-stimulation treatments than those that had been on pentobarbital-after-stimulation treatments.
CONTINGENT CROSS TOLERANCE:
PENTOBARBITAL TO ETHANOL

Pentobarbital—after—
stimulation group

Pentobarbital—before—
stimulation group

MEAN FORELIMB CLONUS DURATION (sec)

TOLERANCE DEVELOPMENT TRIALS

CROSS TOLERANCE TRIALS
During the tolerance-development phase, tolerance developed to the anticonvulsant effect of pentobarbital in the pentobarbital-before-stimulation group. On the drug baseline test, pentobarbital completely suppressed forelimb clonus in every rat in this group; whereas, on the tolerance test, after the 10 injections, it had no anticonvulsant effect whatsoever (p<.001). In contrast, the rats of the pentobarbital-after-stimulation group displayed no evidence whatsoever of tolerance to the anticonvulsant effect of pentobarbital: pentobarbital completely suppressed forelimb clonus on both the drug baseline test and the tolerance test (p>.017). Accordingly, although the rats of both groups received the same number of pentobarbital injections and convulsive stimulations, there was a marked difference between the durations of forelimb clonus displayed by the two groups on the tolerance test (p<.001).

During the cross-tolerance phase, the rats that had received the pentobarbital injection before each stimulation during the tolerance-development phase were significantly more tolerant to the anticonvulsant effect of ethanol than were those that had received the pentobarbital injection after each stimulation. The first ethanol injection was significantly less effective at suppressing forelimb clonus of the rats in the pentobarbital-before-stimulation group than of the pentobarbital-after-stimulation group (p<.01). Moreover, the pentobarbital-before-stimulation subjects reached the criterion of tolerance after a mean of only M=2.7 ethanol injections compared to a mean of M=6.2
required by the pentobarbital-after-stimulation subjects (p<.001). Two of the rats in the pentobarbital-after-stimulation group did not reach the criterion of tolerance and were thus assigned a score of 10 (the total number of ethanol injections) for the purposes of calculating the group means.

On the 10th and final ethanol injection trial, there were no significant differences in the degree of tolerance between the groups (p>.017).

Histological analysis of the electrode placements revealed that all electrode tips were located in the amygdaloid complex or near its boundaries (see Figure 7).

Experiment 2B

The purpose of Experiment 2B was the converse of the purpose of Experiment 2A: to demonstrate the transfer of contingent tolerance of the anticonvulsant effect of ethanol to the anticonvulsant effect of pentobarbital.

Methods

Electrodes were implanted in 30 rats, but one rat failed to meet the screening criterion on the no-drug saline baseline trial, and another was excluded because of illness. Thus, 28 rats completed the experiment, n=14 in each of the ethanol-before-stimulation and the ethanol-after-stimulation groups.

Results

The results of Experiment 2B are illustrated in Figure 6, which summarize the results of the tolerance-development phase and the cross-tolerance phase. It can be seen that the two groups
Figure 6. The effect of ethanol (1.5 g/kg) administration either before or after bidaily convulsive stimulations, and the subsequent pentobarbital (15 mg/kg) administration before the stimulations. There were no differences at the start of the experiment between the two groups in either their responsiveness to the convulsive stimulation on the no-drug saline baseline trial (SB) or their responsiveness to the anticonvulsant effect of ethanol on the drug baseline test (DB). During the tolerance-development phase, tolerance to the anticonvulsant effect of ethanol developed, but only in the pentobarbital-before-stimulation condition. During the cross-tolerance phase, a greater degree of cross tolerance to the anticonvulsant effect of pentobarbital was apparent in the rats that had previously been on a regimen of ethanol-before-stimulation treatments than those that had been on ethanol-after-stimulation treatments.
CONTINGENT CROSS TOLERANCE: ETHANOL TO PENTOBARBITAL

MEAN FORELimb CLONUS DURATION (sec)

Ethanol—after—stimulation group

Ethanol—before—stimulation group

TOLERANCE DEVELOPMENT TRIALS

CROSS TOLERANCE TRIALS
Figure 7. Electrode placements in the 53 rats completing Experiment 2.
did not differ at the start of the experiment in either their responsiveness to the convulsive stimulation on the no-drug baseline trial or their responsiveness to the anticonvulsant effect of ethanol on the drug baseline test.

During the tolerance-development phase, complete tolerance developed to the anticonvulsant effect of ethanol in the ethanol-before-stimulation group over the course of the 10 injections: on the drug baseline test, ethanol suppressed forelimb clonus in every rat, but on the tolerance test, it had no anticonvulsant effect (p<.001). In contrast, the rats in the ethanol-after-stimulation group displayed no evidence of tolerance to the anticonvulsant effect of ethanol on either the drug baseline test or the tolerance test (p>.017). Accordingly, the ethanol-before-stimulation group displayed significantly longer forelimb clonus durations on the tolerance test than did the ethanol-after-stimulation group (p<.001).

During the cross-tolerance phase, the rats that had been exposed to ethanol before the stimulations on the tolerance development phase were more tolerant to the anticonvulsant effect of pentobarbital than those that received ethanol after the stimulations. Although on the first injection of pentobarbital, both groups displayed little or no tolerance to its anticonvulsant effect (p>.017), the criterion of tolerance was achieved by the rats of the ethanol-before-stimulation group much faster (M=4.6 pentobarbital injections) than did the rats in the ethanol-after-stimulation group (M=7.7 injections) (p<.005). One
rat in the ethanol-before-stimulation group and three in the ethanol-after-stimulation group did not reach the criterion of pentobarbital tolerance, and thus they were assigned a score of 10, the total number of drug injections, for the purposes of calculating the group means.

On the 10th and final trial of pentobarbital injections, the groups did not differ significantly in their degree of tolerance (p > .017).

Histological analysis of the electrode placements revealed that all electrode tips were located in the amygdaloid complex or near its boundaries (see Figure 7).

Discussion

The two studies of Experiment 2 accomplish several things. First, it confirms previous reports that tolerance does develop to the anticonvulsant effect of ethanol (e.g., Pinel et al., 1983, 1985), and also confirms previous reports that tolerance to the anticonvulsant effect of ethanol developed only when the convulsive stimulations was delivered during the periods of drug exposure (Pinel et al., 1983, 1985). Second, this was the first demonstration of tolerance to the anticonvulsant effect of pentobarbital, and this tolerance was shown to develop only when the convulsive stimulation occurred during the periods of drug exposure. Third, this was the first experimental demonstration of cross tolerance between pentobarbital and ethanol on their anticonvulsant effect. And fourth, the present results provide the first unambiguous and systematic evidence of the role of the
drug-effect contingency in the transfer of tolerance from one drug to another. Unlike previous examinations of contingent cross tolerance, the transfer of contingent tolerance was shown to be bidirectional and to be consistent with the role of the drug-effect contingency in the development of tolerance to the first drug.

Furthermore, the results of Experiment 2 raise an important methodological point. In most studies, cross tolerance has been assessed by only a single test trial, and the failure to observe statistically significant differences on this trial has led to the conclusion that cross tolerance did not develop. In Experiment 2B, the groups did not differ significantly in response to their first injection of pentobarbital, but there was clear evidence of a subsequent differential rate of tolerance development. Accordingly the rate of tolerance development may be a more sensitive measure of tolerance than the response to a single test injection; and thus failure to observe significant tolerance effects on a single trial is not unequivocal evidence that tolerance did not develop.
VI. Experiment 3

The observation of contingent cross-tolerance in Experiment 2 considered in combination with the recent report of Mana and Pinel (1987) that the drug-effect contingency also plays a major role in the dissipation of tolerance suggested an interesting possibility. Remarkably, Mana and Pinel found that the dissipation of tolerance to ethanol was not found to be dependent on the withdrawal of ethanol, but rather on the occurrence of the convulsive stimulation in the absence of ethanol. Thus, the tolerance dissipated in rats given ethanol if it was administered after each of six convulsive stimulations, but maintained when ethanol was given before each stimulation.

The main purpose of Experiment 3C was to demonstrate that manipulating the drug-effect contingency during a series of pentobarbital injections can influence the dissipation of a previously developed tolerance to the anticonvulsant effect of ethanol. But before this was examined, the importance of the drug-effect contingency in the development of tolerance to pentobarbital and the transfer of this tolerance to ethanol demonstrated in Experiment 2 was confirmed.

General Method

Drugs

The drugs used were pentobarbital (20 mg/kg in Experiment 3A or 15 mg/kg in Experiments 3B and 3C; BDH Chemicals) and ethanol (1.5 g/kg). All drug and saline injections were in a 7.5 ml/kg
Statistical Analysis

In each experiment, the statistical significance of the results were evaluated with the Wilcoxon-Mann-Whitney U test for between subject comparisons and the Sign test for within subject comparisons: \( p < .017, \) one-tailed, because three comparisons were made.

Experiment 3A

The purpose of Experiment 3A was to demonstrate contingent tolerance to the anticonvulsant effect of pentobarbital; the dose of pentobarbital chosen in this experiment was higher than in the previous demonstration of contingent tolerance in Experiment 2A.

Method

Subjects. Of the 30 subjects completing the kindling phase of Experiment 3, one rat was rejected because it did not meet the criterion on the no-drug saline baseline trial, three because they displayed running fits, and two because of illness or loss of electrode assembly. Thus, 24 rats completed Experiment 3A.

Drug Baseline Phase. All the subjects received pentobarbital (20 mg/kg) 1 hr before the convulsive stimulation.

Tolerance-Development Phase. The tolerance-development phase comprised of 10 bidaily pentobarbital injections and 10 bidaily convulsive stimulations. The rats were divided into two equivalent groups, and on each tolerance development trial, the rats in the pentobarbital-before-stimulation group \((n=12)\) received the drug 1 hr before the stimulation and the rats of the
pentobarbital-after-stimulation group (n=12) received the drug 1 hr after the stimulation.

**Tolerance Test.** The tolerance test occurred 48 hr after the last tolerance development trial. Each rat received pentobarbital 1 hr before the stimulation to assess the degree of tolerance in the two groups.

**Results**

It is clear from Figure 8 that there was no evidence of tolerance to the anticonvulsant effect of 20 mg/kg of pentobarbital in either experimental group. The ability of 20 mg/kg of pentobarbital to block kindled convulsions was not significantly reduced by the 10 treatment injections in either the pentobarbital-before-stimulation group (p > .017) or the pentobarbital-after-stimulation group (p > .017). Accordingly, the difference between the two groups on the tolerance test was not significant (p > .017).

**Experiment 3B**

The purpose of Experiment 3A was to demonstrate contingent tolerance to the anticonvulsant effect of pentobarbital using a lower dose than that used in Experiment 3A; and also to demonstrate contingent cross tolerance to the anticonvulsant effect of ethanol.

**Method**

**Subjects.** The subjects were the same rats that completed Experiment 2A, but one was eliminated because it displayed running fits. Thus, 23 rats completed Experiment 3B.
Figure 8. The effect of pentobarbital (20 mg/kg) administration either 1 hr before or 1 hr after the convulsive stimulation. Tolerance was not apparent after 10 bidaily treatment injections in either the pentobarbital-before-stimulation or the pentobarbital-after-stimulation group.
CONTINGENT TOLERANCE TO PENTOBARBITAL

MEAN FORELIMB CLONUS DURATION (sec)

Pentobarbital—after—stimulation group

Pentobarbital—before—stimulation group

TOLERANCE DEVELOPMENT TRIALS
No-Drug Baseline Phase. Experiment 3B commenced 48 hr after the tolerance test in Experiment 3A. It began, as had Experiment 3A, with the four no-drug baseline stimulations, and again the fourth of these constituted the no-drug saline baseline trial.

Drug Baseline Test. On the drug baseline test, all rats received pentobarbital (15 mg/kg) 1 hr before the convulsive stimulation.

Tolerance-Development Phase. This was similar to Experiment 3A except that there were 15 tolerance development trial rather than 10 and a lower dose of pentobarbital was used. The 23 rats were equally (+1) distributed from the two groups of Experiment 3A to the pentobarbital-before-stimulation (n=12) and the pentobarbital-after-stimulation (n=11) groups.

Tolerance Test. This was similar to Experiment 3A except that the lower dose of pentobarbital was used.

Cross-Tolerance Phase. The cross-tolerance phase began 48 hr after the tolerance test. All subjects received ethanol 1 hr before each of 16 bidaily convulsive stimulations. Contingent cross tolerance was assessed in two ways: 1) by the inability of the first injection of ethanol to suppress forelimb clonus and 2) by the rate at which the subjects in the two groups achieved a criterion of tolerance to ethanol set at forelimb clonus durations of at least 50% of the no-drug saline baseline trial on two consecutive trials.

Results

The results of Experiment 3A are illustrated in Figure 9,
which summarize the results of the two phases of the experiment: the tolerance-development phase and the cross-tolerance phase. It is readily apparent that the two groups did not differ at the start of the experiment in either their responsiveness to the convulsive stimulation on the no-drug saline baseline trial or to their responsiveness to the anticonvulsant effect of pentobarbital on the drug baseline test.

During the tolerance-development phase, tolerance developed to the anticonvulsant effect of pentobarbital in the pentobarbital-before-stimulation group. On the drug baseline test, pentobarbital completely suppressed forelimb clonus in every rat in this group; whereas, on the tolerance test it had no anticonvulsant effect whatsoever (p<.002). In contrast, there was little evidence of tolerance in the pentobarbital-after-stimulation condition; pentobarbital completely suppressed forelimb clonus on the drug baseline test and it suppressed it again on the tolerance test, albeit to a slightly lesser degree (p>.017). Accordingly, although the rats of both groups received the same number of pentobarbital injections and convulsive stimulations, there was a marked difference between the durations of forelimb clonus displayed by the two groups on the tolerance test (p<.001).

During the cross-tolerance phase, the rats that had received the pentobarbital injection before each stimulation during the tolerance-development phase were significantly more tolerant to the anticonvulsant effect of ethanol than were those that had
Figure 9. The effect of pentobarbital (15 mg/kg) administration either 1 hr before or 1 hr after bidaily convulsive stimulations, and the subsequent ethanol (1.5 g/kg) administration 1 hr before the stimulations. There were no differences at the start of the experiment between the two groups in either their responsiveness to the convulsive stimulation on the no-drug saline baseline trial (SB) or their responsiveness to the anticonvulsant effect of pentobarbital on the drug baseline test (DB). During the tolerance-development phase, tolerance to the anticonvulsant effect of pentobarbital developed, but only in the pentobarbital-before-stimulation condition. During the cross-tolerance phase, a greater degree of cross tolerance to the anticonvulsant effect of ethanol was apparent in the rats that had previously been on a regimen of pentobarbital-before-stimulation treatments than those that had been on pentobarbital-after-stimulation treatments.
CONTINGENT CROSS TOLERANCE: PENTOBARBITAL TO ETHANOL
received the pentobarbital injection after each stimulation. The first ethanol injection was significantly less effective at suppressing forelimb clonus of the rats in the pentobarbital-before-stimulation group than of the pentobarbital-after-stimulation group (p<.001). Moreover, the pentobarbital-before-stimulation subjects reached the criterion of tolerance after a mean of only M=3.1 ethanol injections compared to a mean of M=8.5 required by the pentobarbital-after-stimulation subjects (p<.001). One rat in the pentobarbital-after-stimulation group did not reach the criterion of tolerance and was thus assigned a score of 16 (the total number of ethanol injections) for the purposes of calculating the group means.

On the 16th and final ethanol injection trial, there was no significant differences in the degree of tolerance between the groups (p>.017).

Discussion

The drug-effect contingency was shown to play an important role in the development of tolerance to pentobarbital and cross tolerance to ethanol; this confirms the results of Experiment 2A of this thesis. In view of the robust contingent tolerance effects in Experiment 3B as well as in Experiment 2A, its absence in Experiment 3A requires comment. It is possible that pentobarbital injected at 15 mg/kg produces large tolerance effects that are not produced at all by 20 mg/kg injections, but I favor the view that tolerance was developing in Experiment 3A but was not reflected in a significant increase in clonus
duration because 20 mg/kg was well above the threshold dose for complete clonus suppression. This view is supported by the observation of Pinel et al. (1983) that tolerance to the anticonvulsant effect of large doses of ethanol was not apparent until both the ethanol and vehicle control rats were challenged by a smaller test dose. Perhaps tolerance would have eventually been observed in Experiment 3A, even at the 20 mg/kg dose, if more injections had been administered or if a smaller test dose was administered. The results emphasize the dangers of concluding, on the basis of experiments involving a limited range of doses or a limited number of injection trials, that tolerance to a particular drug effect does not develop.

Experiment 3C

The purpose of Experiment 3C was to examine the role of the drug-effect contingency in the dissipation of tolerance to the anticonvulsant effect of ethanol following pentobarbital administrations. It was hypothesized that tolerance to ethanol would dissipate when pentobarbital is administered after the stimulation, but not dissipate when pentobarbital is given before the stimulation.

Method

Subjects. The 22 subjects completing Experiment 3B that had achieved the criterion of tolerance—two consecutive convulsions of forelimb clonus durations of at least 50% of that achieved on the no-drug saline baseline trial—were reassigned to two new groups so as to distribute subjects with differing experimental
histories equally (±1) and to equate the two new groups for tolerance to ethanol on the last cross tolerance trial in Experiment 3B. One of these 22 rats became ill during the course of this experiment; its data were not analyzed.

**Dissipation Phase.** This phase began 48 hr after the last cross tolerance trial of Experiment 3B; it comprised six bidaily pentobarbital injections and six bidaily convulsive stimulations. The subjects in the pentobarbital-before-stimulation group (n=10) received each injection 1 hr before the stimulation and the rats of the pentobarbital-after-stimulation group received the drug 1 hr after each stimulation.

**Tolerance Test.** On the tolerance test, 48 hr after the sixth pentobarbital treatment injection, all subjects received ethanol 1 hr before the convulsive stimulation.

**Results**

The results of Experiment 3C, summarized in Figure 10, were as predicted. The pentobarbital-after-stimulation regimen was associated with a significant decline in the duration of forelimb clonus between the last trial of the ethanol injection in Experiment 3B and the ethanol test injection following the six pentobarbital injections in Experiment 3C (p>.001), whereas the pentobarbital-before-stimulation regimen was not (p>.017). Accordingly, the duration of the forelimb clonus elicited in the pentobarbital-before-stimulation group on the ethanol tolerance test was significantly greater than that elicited in the pentobarbital-after-stimulation group (p<.005).
Figure 10. The dissipation of tolerance to the anticonvulsant effect of ethanol after pentobarbital injections either 1 hr before or 1 hr after the convulsive stimulations. The first points (XT) on the graph represent the initially equal degree of tolerance to ethanol that the two groups displayed on the last session of Experiment 3B; the last points (T) represent the responses of the two groups on the final ethanol tolerance test after the intervening pentobarbital treatments. The pentobarbital-after-stimulation treatments produced a significant decline in ethanol tolerance, but the pentobarbital-before-stimulation treatments did not.
DISSIPATION OF ETHANOL TOLERANCE

Pentobarbital-before-stimulation group

Pentobarbital-after-stimulation group

MEAN FORELIMB CLONUS DURATION (sec)

PENTOBARBITAL TRIALS
Histological analysis of the electrode placements revealed that all electrode tips were located in the amygdaloid complex or near its boundaries (see Figure 11).

Discussion

The results of Experiment 3C demonstrate that tolerance that had been established to ethanol dissipated following a series of pentobarbital injections when they occurred after convulsive stimulations (contingent cross-dissipation of tolerance); however, tolerance did not dissipate when the pentobarbital injections occurred before the convulsive stimulation. However, Experiment 3 does not differentiate between the two possible interpretations of the effect. One possibility is that the rats in the pentobarbital-before-stimulation group retained the tolerance to ethanol that they had developed in Experiment 2, whereas the rats in the pentobarbital-after-stimulation group lost theirs because they were stimulated in a drug-free state during the schedule of pentobarbital injections. A second possibility is that both groups of rats lost their tolerance to ethanol's anticonvulsant effect because they were all stimulated in the absence of ethanol, while the rats in the pentobarbital-before-stimulation group developed contingent tolerance to pentobarbital that was manifested in the development of contingent cross-tolerance to ethanol.
Figure 11. Electrode placements in the 21 rats completing Experiment 3.
VII. Experiment 4

A large body of evidence suggests that tolerance to a drug's effect develops to a greater extent and at a faster rate when larger treatment doses are administered (Aston, 1965; Jorgenson, Fasmer, & Hole, 1986; Kalant, LeBlanc, & Gibbons, 1971; LeBlanc, Kalant, Gibbons, & Berman, 1969). The purpose of Experiment 4 was to demonstrate that under certain conditions, greater tolerance would develop in subjects exposed to a lower dose administration regimen. Accordingly, different dose regimens were compared in the extent and rate of tolerance development to the anticonvulsant effect of pentobarbital. The main comparisons were made between two dose regimens: one in which the subjects were maintained on a relatively high dose of the drug that completely suppressed forelimb clonus on every trial for the duration of the experiment; and one in which the subjects were initially given a relatively low dose, that did not completely suppress the convulsions, but had the dose repeatedly increased by small amounts, none of which suppressed forelimb clonus, as tolerance developed to the dose. It was hypothesized that there would be a greater degree of tolerance, as measured by whether forelimb clonus was suppressed or not, in the latter group, despite the fact that this group received a lower dose regimen, because forelimb clonus was experienced in the drugged state, compared to the former group in which forelimb clonus was not experienced in the drugged state.
Method

Subjects

Subjects in this experiment had been previously exposed to anticonvulsant drugs in pilot studies. In order to erase any tolerance that may have developed to the drug effects, each rat received 17 bidaily convulsive stimulations in the absence of any drug. The administration of convulsive stimulations in the absence of an anticonvulsant drug causes the dissipation of tolerance to that drug effect (Experiment 3C of this thesis; Mana & Pinel, 1987).

No-drug Saline Baseline Trial

On the 17th and final stimulation trial, all rats received the saline vehicle (5 ml/kg) 1 hr before the convulsive stimulation to assess the effect of the stimulation in the absence of any drug.

Drug-Baseline Test

All the rats received pentobarbital (30 mg/kg in a 5 ml/kg volume) 1 hr before the convulsive stimulation to assess the anticonvulsant potency of the drug in each rat. In addition to measuring the anticonvulsant effect of the drug, the ataxia demonstrated by the rats in response to the drug was also measured in two ways: 1) the righting test, in which the rat was placed on its back on a San-i-cel bedding covered surface, and the latency to right itself onto all fours was measured; 2) the tube test, in which the rat was placed head first into a 15 x 6 cm plastic tube placed at a 50 degree angle from the vertical, and
the latency to crawl out was measured. The righting and tube tests were conducted 50 and 55 min, after the pentobarbital injection, respectively. On each test, the rat was allowed 5 min to perform the task; rats that failed to perform the task within the time limit were given a latency score of 5 min.

**Tolerance-Development Phase**

The rats were divided into three equivalent groups based on the mean duration of forelimb clonus expressed on the no-drug saline baseline trial and the drug baseline test, the righting and tube test scores on the drug baseline test, drug histories, and body weights. All rats received pentobarbital 1 hr before the convulsive stimulation on each of 20 bidaily trials. One group received a high dose of 50 mg/kg (50 group) throughout; this dose completely suppressed forelimb clonus in every subject on each trial. Another group received a low dose of 10 mg/kg (10 group) throughout; this dose did not completely suppress forelimb clonus in most subjects on most trials. The third group (Increasing group) received 10 mg/kg, that did not suppress forelimb clonus, and then the dose was increased by 1 mg/kg for 13 trials, at which point forelimb clonus became suppressed in over 50% of the subjects. The rats of this group were then maintained at this dose (23 mg/kg) for the remaining six tolerance development trials.

**Tolerance Test**

The tolerance test occurred 48 hr after the last (20th) tolerance development trial. It involved 10 bidaily trials of
pentobarbital (20 mg/kg) administrations, each followed 1 hr later by the convulsive stimulation. Tolerance was assessed in two ways: 1) by the inability of the drug to suppress forelimb clonus on the first test trial and 2) by the rate at which a criterion of tolerance of two consecutive trials of forelimb clonus expression was achieved. In addition, both ataxia tests were administered on the first tolerance trial in the same manner as on the drug baseline test: at 50 and 55 min following the test dose.

Statistical Analysis

Comparisons of tolerance to the anticonvulsant effect of pentobarbital among the three experimental groups were made with the Chi Square test for nominal data (whether forelimb clonus occurred or not), and the Kruskal-Wallis one-way analysis of variance for non-nominal data; the level of significance was set at p<.017, because three comparisons were made. The post hoc comparisons of the nominal data were made using two group Chi Square tests with p<.017. For the effect of pentobarbital on the ataxia measures, the parametric analyses of variance was used because the data were parametric; the level of significance was set at p<.025 because two comparisons were made.

Results

Figure 12 illustrates that the development of tolerance to the anticonvulsant effect of pentobarbital was significantly greater in the group that received the increasingly higher doses of the drug (Increasing group) as compared to the group that
Figure 12. The effect of different dose administration regimens of pentobarbital on the development of tolerance to its anticonvulsant effect. The group that received successively larger doses of the drug (Increasing-group) displayed significantly greater tolerance than the group that was maintained on the high dose throughout (50-group), but did not differ significantly from the group that received the low dose throughout (10-group); and the 10- and 50-groups did not significantly differ.
TOLERANCE TO PENTOBARBITAL

Legend
- 50-GROUP
- 10-GROUP
- INCREASING-GROUP

Percent of subjects displaying forelimb clonus

Tolerance development trials

Tolerance test trials
received the high dose (50 mg/kg) of pentobarbital throughout the tolerance-development phase. On the first tolerance test trial, there were significant differences among the groups (p<.01); and post hoc comparisons revealed that the Increasing group expressed greater tolerance than the 50 group; however there was no significant difference in the degree of tolerance between the Increasing group and the 10 group, or the 10 group and the 50 group. On the rate at which the criterion of tolerance was reached, there was a trend for the Increasing-group to become tolerant more quickly than the other groups but it was not statistically significant (p>.017): the Increasing-group reached it in M=2.3 trials, the 10-group in 4.9 trials, and the 50-group in 5.4 trials. On the last (10th) tolerance test trial, there were no significance differences among the groups in the degree of tolerance to pentobarbital's anticonvulsant effect (p>.017).

Figures 13 and 14 illustrate that the groups did not differ significantly in the degree of ataxia produced by pentobarbital on the first tolerance test trial, as measured by both the righting test (p>.025) and the tube test (p>.025), respectively.

Histological analysis of the electrode placements revealed that all electrode tips were located in the amygdaloid complex or near its boundaries (see Figure 15).

Discussion

The results of this study confirmed the hypothesis that tolerance to the anticonvulsant effect of pentobarbital would be greater in those rats that received successively larger doses of
Figure 13. The effect of the different dose administration regimens of pentobarbital on its effect on the righting test. The groups that received either the progressively increasing dose (Increasing-group), the low (10-group) or the high (50-group) dose of pentobarbital did not differ significantly amongst themselves on either the drug baseline test or the tolerance test.
Figure 14. The effect of the different dose administration regimens of pentobarbital on its effect on the tube test. The groups that received either the progressively increasing dose (Increasing-group), the low (10-group) or the high (50-group) dose of pentobarbital did not differ significantly amongst themselves on either the drug baseline test or the tolerance test.
TUBE TEST

MEAN LATENCY (sec)

<table>
<thead>
<tr>
<th>10-GROUP</th>
<th>50-GROUP</th>
<th>INCREASING-GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>90</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

DRUG BASELINE TOLERANCE TEST
Figure 15. Electrode placements in the 25 rats completing Experiment 4.
the drug, none of which were large enough to suppress forelimb clonus, than those rats that were maintained on a high dose of the drug that suppressed forelimb clonus throughout. The greater tolerance in the Increasing group could be attributed to the fact that convulsions were experienced in the drugged state. The group that received the high dose of the drug, never experienced forelimb clonus in the drugged state. This result was in contrast to the generally accepted view of drug tolerance. For example, Aston (1965) found that the degree of tolerance to sleeping time of a 40 mg/kg test dose of pentobarbital was directly proportional to the size of the treatment dose of pentobarbital that ranged from 25 to 45 mg/kg.

A problem with studies that examine the rate and extent of tolerance development between various doses of a drug is that the detection of tolerance in the subjects that receive the high treatment dose may be masked by the increased drug accumulation in the subject's body 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). However, this was not a problem in this study because, although tolerance was greater in the Increasing group to the anticonvulsant effect, the same trend was not seen with the ataxia measure. If drug accumulation were to account for the less observed tolerance to the anticonvulsant effect of pentobarbital in the higher dose condition, one would also have expected the drug accumulation to produce the most ataxia in the higher dose condition, but this
was no found.
VIII. General Discussion

The general purpose of the thesis was to determine the role of drug-effects as opposed to mere drug-exposure in the development of cross tolerance to anticonvulsant drug effects. The experiments of this thesis provide unequivocal evidence of the importance of the drug-effect contingency in cross tolerance. Accordingly, the General Discussion of this thesis is divided into six sections. Section 1 deals with the first two experiments, which examined cross tolerance to anticonvulsant drug effects with and without the drug-effect contingency present. Section 2 deals with the third experiment which examined the role of the drug-effect contingency in the dissipation of tolerance to the anticonvulsant effect of one drug following exposure to another drug. Section 3 deals with the fourth experiment, which demonstrated that the degree of tolerance to the anticonvulsant drug effect was influenced by the drug administration regimen. Sections 4 and 5 present theoretical and clinical implications of the present research, respectively. And the last section considers future directions of this research.

1. General Discussion of Experiments 1 and 2

The purpose of Experiments 1 and 2 was to determine the role of the drug-effect contingency in the development of cross tolerance to anticonvulsant drug effects. The present results provide the first unambiguous and systematic evidence of the role of the drug-effect contingency in the transfer of tolerance from
one drug to another.

In Experiment 1, tolerance and cross tolerance to anticonvulsant drug effects were examined with the drug-effect contingency present; the drug injections always preceded the convulsive stimulations. Tolerance developed to the anticonvulsant effects of pentobarbital, trimethadione, and clonazepam; significant transfer of tolerance occurred from pentobarbital to clonazepam but not from either trimethadione or clonazepam to carbamazepine. The results confirmed the hypothesis that degree of cross tolerance depended on the similarity between the drugs on three factors: the type of clinical seizures they were most effective at controlling, their putative mechanism of action, and their relative effectiveness at suppressing experimental convulsions induced by electroshock and pentylenetetrazol.

In Experiment 2, tolerance and cross tolerance to anticonvulsant drug effects were examined with and without the drug-effect contingency present; in one condition a drug injection preceded each convulsive stimulation, whereas in another condition a drug injection followed each stimulation. When the contingency was present, tolerance developed quickly to pentobarbital and ethanol, and bidirectional cross tolerance also developed between these drugs. In contrast, in the absence of the drug-effect contingency, there was little evidence of tolerance or cross tolerance. These results were confirmed in Experiment 3B, which demonstrated the transfer of tolerance to
anticonvulsant drug effects from pentobarbital to ethanol. Accordingly, unlike the results of previous examinations of contingent cross tolerance, the transfer of contingent tolerance was shown to be bidirectional and to be consistent with the role of the drug-effect contingency in the development of cross tolerance to the first drug.

2. General Discussion of Experiment 3

The main purpose of Experiment 3 was to examine the role of the drug-effect contingency in the dissipation of tolerance to the anticonvulsant effect of a drug following the administration of another drug. The present results provide the first evidence that the drug-effect contingency was important in the dissipation of tolerance to one drug following the administration of another.

In Experiment 3C, it was found that tolerance that had been established to ethanol dissipated following a series of pentobarbital injections when they occurred after the convulsive stimulation (contingent cross-dissipation of tolerance); however, tolerance did not dissipate when the pentobarbital injections occurred before the convulsive stimulation. The tolerance to ethanol dissipated when the convulsive stimulation occurred in the absence of pentobarbital, despite the administrations of the drug; but tolerance did not dissipate when the stimulation occurred in the drugged state. The key factor in the dissipation of ethanol tolerance was the administration of the convulsive stimulation in the absence of pentobarbital. These results confirm and extend the finding of Mana and Pinel (1986) that
tolerance to one drug (ethanol) dissipated when the convulsive stimulations were delivered in the absence of the same drug.

3. General Discussion of Experiment 4

The purpose of Experiment 4 was to determine if different dose administration regimens of a drug differentially affect the extent of tolerance development to its anticonvulsant effect. Greater tolerance was found to the anticonvulsant effect of pentobarbital in rats that received successively larger doses of the drug, which were not large enough to suppress forelimb clonus, than in those rats that were maintained on a high dose that completely suppressed the forelimb clonus. The greater tolerance in the former group was attributed to the fact that the convulsions were experienced in the drugged state, compared to the latter group that did not experience forelimb clonus in the drugged state. These results challenge the generally accepted view that tolerance develops to a greater extent with larger doses (e.g., Aston, 1965; Jorgenson et al., 1986; Kalant et al., 1971; LeBlanc et al., 1969).

4. Theoretical Implications

According to the drug-effect theory of drug tolerance as proposed in its original form by Pinel et al., (1989), tolerance develops to the drug effects, not to drug exposure; tolerance is seen as an adaptation to the repeated expression of a drug's disruptive effect on ongoing neural activity, not to the mere presence of the drug. Accordingly, drug exposure is presumed necessary, but not sufficient for the development of functional
tolerance.

The following are several elaborations to the drug-effect theory of tolerance, which were derived from the results of the present thesis and from the results of other studies of tolerance (see Table III for a complete review).

1) Tolerance does develop to drug effects whether the criterion drug effect is explicitly expressed, as is the case when the drug administration precedes the criterion response, or when the drug is repeatedly administered without explicitly eliciting the criterion drug effect; however, tolerance develops more rapidly and to a greater extent when the criterion drug effect is explicitly experienced by the subjects.

The question of whether tolerance develops in the absence of the drug-effect contingency has been the most heated debate in the contingent tolerance literature. Several laboratories have attempted to resolve this issue without success (Chen, 1968; Chen, 1972; LeBlanc, Gibbins, & Kalant, 1973; LeBlanc, Gibbins, & Kalant, 1975; Wenger, Berlin, & Woods, 1980; Wenger, Tiffany, Bombardier, Nicholls, & Woods, 1981). The problem with these studies was the inappropriate choice of drug effect; they examined the motor-coordination-and-balance (maze running and treadmill tasks) disrupting effect of ethanol. With this drug effect, there is no clear-cut disassociation between drug-effect and drug-exposure. The subject may experience many components of the drug effect whether performing the maze or treadmill task or walking around in its home cage. Consequently, the most
compelling evidence that tolerance can develop in the absence of the drug-effect contingency is from studies that have examined the anticonvulsant drug effect, because with this drug effect there is a clear-cut disassociation between the drug-effect and drug-exposure. In a yet unpublished study of tolerance to anticonvulsant drug effects revealed that tolerance can develop in the absence of the drug-effect contingency. For example, tolerance was demonstrated to the anticonvulsant effect of ethanol when it was administered daily for 20 days without the convulsive stimulation ever occurring except on the tolerance test trial following the treatment sessions. Similarly, Lippa and Regan (1977) demonstrated tolerance to the anticonvulsant effect of diazepam following seven days of drug treatment without the convulsive stimulation occurring.

And recently, in two yet unpublished studies from this laboratory, tolerance was demonstrated to the anticonvulsant effect of ethanol when convulsive stimulations were delivered before the drug injections so that the drug effect was not expressed. Subjects that received ethanol after each convulsive stimulation did not differ from the saline control group in their response to ethanol on a single tolerance test, and they displayed markedly less tolerance than the subjects that received ethanol before the stimulations. The tolerance was detectable only as an acceleration in the subsequent development of tolerance as compared to the saline control group.

2) Tolerance development to a drug effect is greater in the
absence of the drug-effect contingency with larger drug doses, with shorter intervals between drug administrations, and with longer durations of drug treatment. The factors involved in the development of tolerance and cross tolerance are a complex interaction of the dose of the drug, the schedule of its administration, the duration of its treatment, and possibly the route of its administration (see Kalant et al., 1971). In addition, the experiences of the subjects also play a major role—whether the criterion response occurs during the periods of drug exposure so that the criterion drug effect is experienced by the subject. At lower doses, longer time intervals between drug administrations, and shorter drug treatment durations, the impact of the drug on the organism is relatively more subtle, and thus the experiences of the subjects play a larger role; and thus, a greater difference in the degree of tolerance may be observed between those subjects that experienced the criterion drug effect in the drugged state and those that did not. At higher doses, shorter drug administration intervals, and longer treatment durations, the impact of the drug on the subject is less subtle and the drug-effect contingency factors are overwhelmed by the presence of the drug in the organism; and tolerance is more likely to be observed whether the subjects experience the drug effect in the drugged state or not.

There is only indirect evidence for this elaboration. In a comparison of two studies of tolerance to ethanol's anticonvulsant effect, Pinel et al. (1983) failed to observe
tolerance when a relatively low dose (1.5 g/kg) was administered twice daily for only 5 days; and yet in an unpublished study from this laboratory, complete tolerance developed when a high dose (5 g/kg) was delivered daily for an extended period of 20 days. There is experimental evidence demonstrating that in the development of conditioned tolerance, another type of tolerance in which the experiences of the subject are important—the environment in which the subjects experienced the drug exposure—the environmental cues played a relatively lesser role in the tolerance when high drug doses and shorter drug administration intervals were used (Baker & Tiffany, 1985). And there is the observation that in general, tolerance to a drug's effect is facilitated by higher doses, shorter intervals between drug administrations, and longer treatment durations (see Kalant et al., 1971).

3) The dissipation of tolerance to a drug effect occurs more rapidly when the criterion drug effect is allowed to be experienced by the subjects in the non-drugged state, than when the drug administration is simply terminated without the subjects experiencing the criterion drug effect. Mana and Pinel (1986) recently demonstrated that tolerance to the anticonvulsant effect of ethanol dissipated, despite the administrations of ethanol on the same regimen that produced the tolerance, if the drug injections followed each convulsive stimulation; and tolerance also dissipated when the convulsive stimulations were delivered alone without any drug. And yet tolerance was maintained when
ethanol administration was terminated without applying the convulsive stimulation over the same retention period. The key factor in the dissipation of ethanol tolerance, was not the withdrawal of ethanol, but the administration of the convulsive stimulation in the absence of ethanol; elicitation of the criterion response in the non-drugged state causes the dissipation of tolerance. In Experiment 3C of this thesis, it was shown that this may generalize to the dissipation of tolerance to one drug following the administration of another drug. Tolerance to the anticonvulsant effect of ethanol dissipated when pentobarbital injections were delivered after each convulsive stimulation, but not when it was delivered before each stimulation.

4) The principles of the drug-effect theory of tolerance can be applied to the development of cross tolerance to drug effects. In this thesis, the cross-dissipation of tolerance to one drug following the administration of another drug was also consistent with the drug-effect contingency.

5. Clinical Implications

Before the potentially important clinical implications of this research are discussed, there is a major discrepancy between the studies conducted in experimental and the clinical situation that needs to be addressed. The experimental data overwhelmingly and without any ambiguity whatsoever demonstrate that tolerance and cross tolerance do develop to anticonvulsant effects of antiepileptic drugs (see Table 3; Frey 1985, 1986, 1987), yet
there have been few reports that tolerance is a major problem in the treatment if epileptic patients (e.g., Frey, 1985).

There are two possible explanations for this discrepancy. First, there were differences between the experimental and clinical studies in their methodology: the subjects were animals and not humans; the convulsions were elicited, usually electrically or chemically, and not spontaneously occurring epileptic seizures; the drug administrations were often injected at spaced time intervals and not continual oral ingestion aimed at maintaining a certain blood plasma level of the drug; and the doses used in experimental studies tended to be relatively higher than those used in the clinical situation. It may be possible that the methodological differences could account for the vastly different results. The more likely explanation is that in the clinical setting, any observed decrease in the drug's efficacy during antiepileptic drug treatment has typically been attributed to a number of factors other than tolerance (Brown, 1976): 1) variability of seizure incidence in the epileptic patient, 2) patient noncompliance—the patient does not take the drug as prescribed, 3) growth of the patient that will reduce the relative dosage, and 4) drug metabolites that might interfere with the effect of the parent drug. It is my feeling that in spite of the methodological differences and in light of such overwhelming experimental evidence, tolerance could be demonstrated with human subjects in properly controlled experiments. But this is a situation that is obviously precluded
by ethical constraints, therefore untestable.

The first clinical implication is that the knowledge of tolerance and cross tolerance to anticonvulsant drug effects could be of benefit in the pharmacological treatment of epileptic patients. This is an affliction that affects nearly 1% of the population (Gilman, Goddman, & Gilman, 1980; Katzung, 1987). Quite often in the clinical setting, the epileptic patient is exposed not to one antiepileptic drug, but rather to a variety of them (polypharmacology), sometimes concurrently and sometimes sequentially (e.g., Eadie, 1985). Awareness of the drugs to which cross tolerance does develop would be valuable in making the appropriate choice. For example, phenobarbital, clonazepam, and carbamazepine all are effective against partial seizures and generalized tonic-clonic seizures; however, Experiment 1 has shown that significant cross tolerance was found between phenobarbital and carbamazepine, and not between clonazepam and carbamazepine. Therefore, if a patient displays evidence of tolerance to carbamazepine, a better alternative drug would be clonazepam and not phenobarbital.

The second potential clinical implication of the thesis was the observation in Experiment 4 that tolerance to anticonvulsant drug effects may be facilitated by the expression of the convulsions in the drugged state. In the treatment of epilepsy, the patient is typically given a relatively low dose of the antiepileptic drug, to avoid possible toxic side effects, and when an epileptic seizure occurs, the dose is increased. This may
be done repeatedly until an effective therapeutic dose is obtained. By allowing the patient to experience the convulsions in the drugged state, perhaps the development of tolerance to the antiepileptic effect is facilitated. An alternate therapeutic strategy, which must be weighed against the adverse side effects of high drug doses, would be to give the patient a relatively high initial dose to ensure that the convulsions are less likely to be experienced, and thus ensuring the development of slower and less tolerance to antiepileptic drugs.

The strategy of administering relatively larger drug doses initially is better because the final effective therapeutic dose that is achieved may be lower since less tolerance has developed; and with lower doses, the possibility of adverse side effects are also decreased. This strategy would also bring the seizures under control more quickly, which is important for two reasons. First, there is some evidence suggesting that the occurrence of seizures may result in brain damage (Dam, 1982); this was demonstrated in human epileptics and in animal models. Second, whether actual brain damage occurs or not, there may be some permanent alteration occurring in the brain as the result of the seizure. It has been demonstrated in a variety of experimental convulsant models in animals that repeated elicitation of seizures can lead to progressive intensification of them, and thus make successive seizures more likely to occur; and these changes were permanent (Pinel & Van Oot, 1975). If this can generalize to humans who repeatedly experience seizures, quick effective control of the
seizure would be of prime importance.

6. Conclusions and Future Directions

According to the traditional pharmacological drug-exposure theory of drug tolerance, the exposure of the organism to the drug is the critical factor in the development of tolerance to the drug's effect. This is very simple and succinct, and could account for much of the observations of tolerance. However, this theory cannot explain why the very same regimen of drug administration produces tolerance to a drug effect in some instances but not in others. In the past two decades, two challenges were proposed to the traditional theory. Unlike the traditional theory, these new theories do not view the subject as a passive recipient of the drug, but are based on the premise that the experiences of the subjects while they are drugged play a major role in the tolerance development. The first of these two theories, the conditioned tolerance theory, focused on the role in the development of tolerance of the environment in which the subjects have previously experienced the drug effects. The second of these, the drug-effect theory, focused on the behavior of the subject during drug exposure. The drug-effect theory was the focus of the present thesis.

The thesis has made several contributions: 1) it has established the generality of the drug-effect contingency to the development of cross tolerance to anticonvulsant drug effects and to the cross-dissipation of tolerance; 2) it has determined that different dose administration regimens can influence the extent
of tolerance development to the anticonvulsant drug effects; 3) it has proposed several elaborations to the drug-effect theory to provide a more complete understanding of this phenomenon; 4) it has provided some potentially important clinical implications of this research; and 5) it has demonstrated the utility of the kindled-convulsion model in the study of tolerance and cross tolerance to anticonvulsant drug effects.
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